STEREOSELECTIVE SYNTHESIS OF FLUORINATED GLYCOCONJUGATES. TUNING PHYSICOCHEMICAL PARAMETERS FOR THERAPEUTIC DRUG DEVELOPMENT

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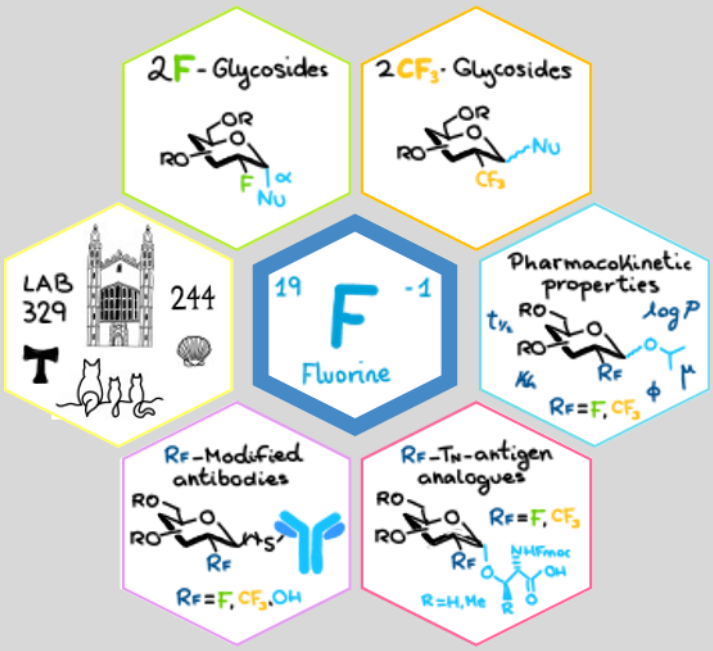

DOCTORAL THESIS
2022

ISABEL BASCUAS JIMÉNEZ DE BAGÜÉS

STEREOSELECTIVE SYNTHESIS OF FLUORINATED GLYCOCONJUGATES. TUNING PHYSICOCHEMICAL PARAMETERS FOR THERAPEUTIC DRUG DEVELOPMENT

DOCTORAL THESIS

Supervised by

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UNIVERSITAT ROVIRA I VIRGILI

Department of Analytical Chemistry and Organic Chemistry

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Dr. Omar Boutureira Martín from the Department of Analytical Chemistry and Organic Chemistry at the University Rovira i Virgili,

I STATE that the present study, entitled "*Stereoselective synthesis of fluorinated glycoconjugates. Tuning physicochemical parameters for therapeutic drug development*", presented by Isabel Bascuas Jiménez de Bagüés for the award of the degree of Doctor and European Mention, has been carried out under my supervision at the Department of Analytical Chemistry and Organic Chemistry of this University.

Tarragona, 29th August 2022

Dr. Omar Boutureira Martín

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ABBREVIATIONS and ACRONYMS

A	**Ab**	Antibody
	Ac	Acetyl
	ACN	Acetonitrile
	ADC	Antibody-drug conjugate
	ADMET	Absorption, distribution, metabolism, excretion, toxicity
	AML	Acute myeloid leukaemia
	APIs	Active principal ingredients
	app	Apparent
	aq	Aqueous
	Ar	Aryl
B	**BCN**	(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-yl
	Bn	Benzyl
	Boc	*Tert*-Butyl carbamate
	Bu	Butyl
	Bz	Benzoyl
	bs	Broad singlet
C	**[c]**	Concentration
	°C	Celsius degrees
	Calc.	Calculated
	CDR	Complementary determining region
	CIP	Close Ion Pair
	Conv.	Conversion
	COSY	Proton homonuclear correlation spectroscopy
	Cys	Cysteine
D	**d**	Doublet
	dd	Doublet of doublets
	ddd	Doublet of doublet of doublets
	2D	Two dimensional
	DBCO	Dibenzocyclooctyne
	DCM	Dichloromethane
	DIBO	4-Dibenzocyclooctynol

	DMF	*N,N*-Dimethylformamide
	DMI	1,3-Dimethyl-2-imidazoline
	DMSO	Dimethyl sulfoxide
E	**EDG**	Electron-donating group
	eq./equiv.	Equivalent(s)
	ESI-TOF	Electrospray ionization-time of flight
	Et	Ethyl
	EWG	Electron withdrawing group
F	**FDG**	2-Deoxy-2-fluoro-glucose
	Fmoc	Fluorenylmethyloxycarbonyl
	FT-IR	Fourier transform infrared spectroscopy
G	**g**	Gram(s)
	Gal	D-Galactose
	Glc	D-Glucose
	GHs	Glycosidases
	GSH	Reduced glutathione
	GTs	Glycosyltransferases
H	**h**	Hours
	HMBC	Heteronuclear multiple bond correlation
	HRMS	High-resolution Mass Spectrometry
	HSQC	Heteronuclear single quantum coherence
	Hz	Hertz
I	**IgG**	Immunoglobulin G
	***i*-Pr**	Isopropyl
	IR	Infrared
J	***J***	Coupling constant
K	k_t	Rate constant
L	**LC**	Liquid chromatography
	LG	Leaving group
M	**m**	Multiplet
	M	Molar
	mAb	Monoclonal antibody

	Man	D-Mannose
	Me	Methyl
	min	Minutes
	m.p.	Melting point
	MRI	Magnetic resonance imaging
	MS	Mass spectrometry
	M.S.	Molecular sieves
	MUC1	Mucine-1
	MW	Molecular weight
	µw	Microwave
	m/z	Mass-to-charge ratio
N	**NIS**	*N*-iodosuccinimine
	NMR	Nuclear magnetic resonance
	NMM	*N*-methyl morpholine
	NOE	Nuclear overhauser effect
	NOESY	Nuclear overhauser effect spectroscopy
	Nu	Nucleophile
P	**PD**	Pharmacodynamics
	PET	Positron emission tomography
	PG	Protecting group
	Ph	Phenyl
	PK	Pharmacokinetics
	pKa	$-\text{Log}_{10}\ Ka$
	ppm	Parts per million
	py	Pyridine
Q	**q**	Quartet
	quint	Quintet
R	$\mathbf{R_F}$	Fluorine-containing groups
	R_f	Retardation factor
	rmAb	Recombinant monoclonal antibody
	r.t.	Room temperature
S	**s**	Singlet

	sat.	Saturated
	Ser	Serine
	SPAAC	Strained-promoted azide-alkyne cicloaddition
	SSIP	Solvent Separated Ion Pair
	STD	Saturation-transfer difference
T	**t**	Triplet
	$t_{1/2}$	Half-life time
	T	Temperature
	TACA(s)	Tumour-associated carbohydrate antigen(s)
	^{t}Bu	*Tert*-butyl
	Tf	Trifluoromethanesulfonyl
	TFA	Trifluoroacetic acid
	TFE	Trifluoroethanol
	TfOH	Trifluoromethanesulfonic acid/triflic acid
	THF	Tetrahydrofuran
	Thr	Threonine
	TLC	Thin layer chromatography
	TS	Transition state
U	**UV**	Ultraviolet
V	***vs***	Versus
	v/v	Volume/volume percentage

SUMMARY

The present PhD work aims to develop new synthetic methodologies for the stereoselective preparation of monofluoro and trifluoromethylated sugars, study their key physicochemical properties, and apply such protocols for the stereoselective synthesis of biologically relevant fluorinated carbohydrates.

CHAPTER I. GENERAL INTRODUCTION

Glycobiology: Carbohydrates are essential biomolecules in living organisms. They normally appear on cell surface and contribute to most interactions between cells and their environment, mediating a wide variety of biological processes, such as, immunological responses, recognition events and disease progression.[1] Their structural diversity allows them to encode information for specific molecular recognition and they are determinant in protein folding, stability, and pharmacokinetics.[2] Carbohydrates have been initially exploited as important tools for studying glycan-mediated interactions and to explore the glycosylation outcome in disease-related processes.[3] Although carbohydrates play essential roles in many biological processes, derived drugs have been a small group of therapeutic compounds and, in contrast to peptides or nucleotides, carbohydrates have been underestimated by the scientific community for long time. However, recent advances in glycochemistry, glycobiology, and glycomedicine have allowed glycans to be considered as key players in pharmacological research, contributing to the development of carbohydrate-based diagnostics, vaccines, and therapeutics.[4]

One of the main reasons making carbohydrate-based drug discovery challenging is the structural diversity that has hampered the efforts to find its correlation with biological function and to define oligosaccharide expression patterns in proteins and cells. Another reason is related to the pharmacokinetic

[1] Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855-867.
[2] Kim, N. A.; Thapa, R.; Jeong, S. H. *Int. J. Biol. Macromol*. **2018**, *109*, 311-322.
[3] Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357-2364.
[4] Fernández-Tejada, A.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur.* **2015**, *21*, 10616-10628.

drawbacks linked to carbohydrates, mainly, the insufficient metabolic stability, poor cell-membrane permeation and the rapid clearance of carbohydrate-based drugs that compromises both their bioavailability and potency. Over the past years, advances in the understanding of carbohydrate-protein interaction allowed the development of a new class of small molecule drugs, known as **glycomimetics**.[5] These compounds mimic the bioactive function of carbohydrates and address the drawbacks of carbohydrate derivatives, which are mainly the low activity and insufficient drug-like properties.[6] Glycomimetics allow the design of carbohydrate-based drugs with improved pharmacological properties, and with the same or better affinity and selectivity towards the desired protein targets.

Importance of fluorine in chemistry and biochemistry: Fluorine plays an important role in diverse areas of medicinal chemistry and drug design. Moreover, introduction of fluorine-containing motifs is commonly addressed during the development and optimization process of new drugs to improve their pharmacological properties, such as metabolic stability and bioavailability, and it can be used to control protein-ligand interactions.[7] The applications of fluorine in drug design are in continuous expansion, favoured by the better understanding of fluorine physicochemical properties and the development of new strategies for the introduction of fluorine motifs and fluoroalkyl modifications. These advances have culminated with the production of high-value drugs during the last century and nowadays, the number of fluorine-containing launched drugs constitutes about 20-30% of all pharmaceuticals, and in the last years this number is exponentially increasing.[8] The introduction of a single fluorine atom can completely change the biological properties of natural products. Moreover, the particular properties of fluorine, named, its high electronegativity, small size, hydrogen bonding patterns, and electrostatic

[5] Tamburrini, A.; Colombo, C.; Bernardi, A. *Med. Res. Rev.* **2020**, *40*, 495-531.
[6] Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Discov.* **2009**, *8*, 661-677.
[7] Gouverneur, V.; Muller, K. Fluorine in Pharmaceutical and Medicinal Chemistry: From Biophysical Aspects to Clinical Applications, 1st ed.; Imperial College Press, London, **2012.**
[8] Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Aceña, J. L.; Soloshonok V.A.; Izawa K.; Liu, H. *Chem. Rev*. **2016**, *116,* 422-518.

interactions, can dramatically impact in the chemical reactivity and the ultimate properties of organic compounds. However, there is not a general rule to determine the effects that fluorine and fluorine-containing substituents can induce.[9] Generally, the introduction of a fluorine atom into a target molecule has been used to increase its metabolic stability, increase its lipophilicity, modulate the acidity of basicity of neighbour functional groups, induce conformational changes, or to affect to specific intermolecular interactions. All these changes, affect to the metabolism, membrane permeability, binding affinity and bioavailability of a given drug candidate.

Importance of fluorosugars: Glycomimetics are small molecules designed to overcome the principal drawbacks of carbohydrates, which are related to the pharmacokinetic properties and their difficult chemical synthesis. One of the most common alterations consists in the introduction of one or more fluorine atoms, and its strategic incorporation allow modulating the physicochemical and ADMET properties of the bioactive molecule. Deoxyfluorination of sugars normally affect to the physicochemical properties, conformation, and molecular interactions, and serve as a stereodirecting strategy to access to modified 2-deoxy-2-fluoro-glycosides.[10] Within the properties induced by deoxyfluorination, the most important are the stabilization of the glycosidic bond and the overall increase of lipophilicity. Fluorinated carbohydrates have played many important roles in medicinal chemistry, including their applications as antiviral and antitumoral agents,[11] mechanistic and structural probes of enzymes, in lectin recognition processes, as diagnostic agents, and as building blocks to construct F-glycoconjugates, including glycopeptides and glycoproteins.[12]

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[9] Gillis, E.; Eastman, K.; Hill, M.; Donnelly, D, Meanwell, N. A. *J. Med. Chem.* **2015**, *58*, 8315-8359.
[10] Bucher, C.; Gilmour, R. *Angew. Chem. Int. Ed.* **2010**, *49*, 8724-8728.
[11] Linclau, B.; Ardá, A.; Reichardt, N. C.; Sollogoub, M.; Unione, L.; Vincent, S. P.; Jiménez-Barbero, J. *Chem. Soc. Rev.* **2020**, *49*, 3863-3888.
[12] a) Salvadó, M.; Amgarten, B.; Castillón, S.; Bernardes, G. J. L.; Boutureira, O. *Org. Lett*. **2015**, *17*, 2836-2839; b) Huo, C. X.; Zheng, X. J.; Xiao, A.; Liu, C. C.; Sun, S.; Lv, Z.; Ye, X. S. *Org. Biomol. Chem*. **2015**, *13*, 3677-3690; c) Johannes, M.; Reindl, M.; Gerlitzki, B.; Schmitt, E.; Hoffmann-Röder, A. *Beilstein J. Org. Chem*. **2015**, *11*, 155-161.

CHAPTER II: GENERAL OBJECTIVES

Results obtained during this PhD work have been divided into five chapters. Chapter III approaches the stereoselective synthesis of 2-deoxy-2-fluoro-α-glycosides and the study of the parameters that affect the stereoselectivity of the microwave-assisted glycosylation step. Chapter IV is focused on the development of a methodology to access to 2-deoxy-2-trifluoromethyl glycosides, exploring the stereodirecting value of the trifluoromethyl group in the glycosylation event and compare it with that of fluorine. The main aim in Chapter V is to study some of the physicochemical properties of the synthetized 2-deoxy-2-fluoro and 2-trifluoromethyl-glycosides and compare the effect of fluorine and the trifluoromethyl group in the properties of these sugars. Chapter VI is focused on the application of the methodologies described in Chapter III and IV to the stereoselective synthesis of fluorinated and trifluoromethylated analogues of the Tn-antigen. Finally, in Chapter VII we explore the selective introduction of native, fluorinated, and trifluoromethylated sugars in antibodies and the study of some of their properties. The work presented in Chapter VII has been carried out in the Bernardes Lab at the University of Cambridge under the supervision of Prof. Gonçalo J. L. Bernardes during a short predoctoral stay.

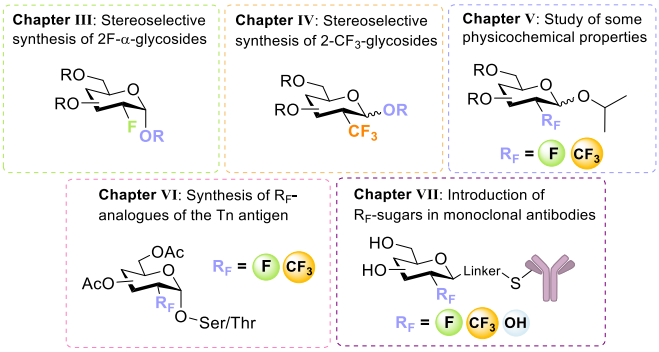


Figure 1. Graphical summary of the chapters in this PhD thesis.

CHAPTER III: STEREOSELECTIVE MICROWAVE-ASSISTED SYNTHESIS OF 2-DEOXY-2-FLUORO-α-GLYCOSIDES

2-Deoxy-2-fluoroglycosides are compounds of increasing importance in biochemistry and medicinal chemistry, and they have found application as antiviral agents, cancer diagnosis probes, and labelling substrates for biological studies.[13] The similar size between fluorine and hydrogen atoms (1.20 Å (H) and 1.47 Å (F)) and the high strength of the C-F bond (and consequent reactive inertness), coupled with the enhanced hydrolytic stability of the glycosidic bond, render the starting 2-fluoro-2-deoxy sugars excellent bioisosteres of 2-deoxy sugars with improved pharmacokinetic properties.[14] The increasing interest on these motifs in chemical biology requires the development of efficient and stereoselective methods for the construction of 2-deoxy-2-fluoroglycosides. However, controlling the stereoselectivity of the glycosylation reaction remains one of the most challenging areas in organic chemistry.[15]

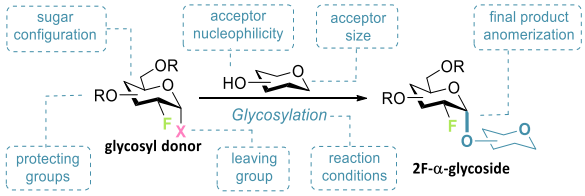
In Chapter III we envisioned a microwave-assisted method in which the activation takes place at high temperatures, favouring the formation of more stable 2-deoxy-2-fluoro-α-glycosides. The methodology involved the use of allyltributyltin and triflic acid as promoters and toluene as solvent, and its applicability was demonstrated using different glycosyl donors (glycosyl bromides and iodides) and acceptors. The effect of different parameters in the glycosylation stereoselectivity was evaluated, such as, the glycosyl donor configuration, the protecting groups and leaving groups, the size and nucleophilicity of the glycosyl acceptor, and the reaction conditions (**Scheme 1**). The stereoselectivity of the glycosylation resulted highly dependent on the temperature, acceptor size, glycosyl donor configuration and protecting groups. High temperatures favoured the formation of the most thermodynamically

[13] a) Boutureira, O.; Bernardes, G. J.; D'Hooge, F.; Davis, B. G. *Chem. Commun.* **2011**, *47*, 10010-10012; b) Maschauer, S., Einsiedel, J., Haubner, R., Hocke, C., Ocker, M., Hübner, H., Kuwert, T.; Gmeiner, P.; Prante, O. *Angew. Chem. Int. Ed.* **2010**, *49*, 976-979.

[14] Meanwell, N. A. *J. Med. Chem*. **2018**, *61*, 5822-5880.

[15] Benito-Alifonso, D.; Galan, M. C. *Selective Glycosylation: Synthetic methods and Catalysis* (Ed.: C.S. Bennet), Wiley-VCH, Weinheim, **2017**, 155-172.

stable α-isomer. *Manno* glycosides gave the best α-selectivities, and strongly inductive protecting groups favour the formation of the α-isomer. Stereoselectivity seemed to be independent of the leaving group at high temperatures, and the nucleophilicity of the acceptor had a modest effect. Finally, the utility of the method was demonstrated by applying it to the stereoselective synthesis of fluorinated analogues of biologically relevant products, obtaining high yields and good α-stereoselectivities.



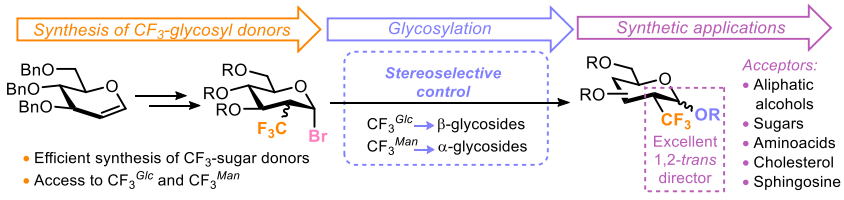
Scheme 1. Glycosylation reaction between a glycosyl donor and a glycosyl acceptor. In boxes, parameters that affect the stereoselectivity and reactivity studied in Chapter III.

CHAPTER VI: TRIFLUOROMETHYL-DIRECTED GLYCOSYLATION: STEREOSELECTIVE SYNTHESIS OF 2-DEOXY-2-CF_3-GLYCOSIDES

There is a growing interest in the preparation of glycomimetics to overcome some of the drawbacks of native carbohydrates, such as the short lifetimes and poor absorption properties (reduced lipophilicity).[16] Among them, F-glycomimetics,[17] resulting from the bioisosteric replacement of H or OH by F, and particularly those including polyfluoroalkyl groups (R_F),[18] such as CF_3[19] (CH_3 isoster) and C_2F_5,[20] have recently attracted considerable interest. However, although the preparation of CF_3-containing active principal ingredients (APIs) has been long appreciated in medicinal chemistry, CF_3-glycosides remain largely understated in g*lycoscience* compared to their monofluorinated congeners.[21]

[16] a) Hevey, R. *Pharmaceuticals* **2019**, *12*, 55; b) Hevey, R. *Chem. Eur. J.* **2021**, *27*, 2240-2253.
[17] Linclau, B.; Ardá, A.; Reichardt, N. C.; Sollogoub, M.; Unione, L.; Vincent, S. P.; Jiménez-Barbero, J. *Chem. Soc. Rev.* **2020**, *49*, 3863-3888.
[18] Uhrig, M. L.; Mora Flores, E. W.; Postigo, A. *Chem. Eur. J.* **2021**, *27*, 7813–7825.
[19] Mestre, J.; Lishchynskyi, A.; Castillón, S.; Boutureira, O. *J. Org. Chem.* **2018**, *83*, 8150-8160.
[20] Mestre, J.; Castillón, S.; Boutureira, O. *J. Org. Chem.* **2019**, *84*, 15087-15097.
[21] Fröhlich, R. F. G.; Schrank, E.; Zangger, K. *Carbohydr. Res.* **2012**, *361*, 100-104.

In Chapter IV the access to previously uncharted 2-deoxy-2-trifluoromehtyl glycosides was achieved from cheap and commercially available glycals. First, 2-trifluoromethyl-D-glucal was obtained in excellent yields from 2-iodo-D-glucal using a convenient and easy-handling trifluoromethylation system ($TMSCF_3$/KF/CuBr) that permits readily scalable synthesis and employs a simple reaction setup. Next, functionalization of the double bond of 2-CF_3-D-glucal was achieved by hydroxymercuration with $Hg(OTFA)_2$ followed by reductive demercuration with $NaBH_4$, affording a mixture of *gluco*- and *manno*-trifluoromethyl pyranoses (**Scheme 2**, left). The utility of 2-CF_3-glycosides as glycosyl donors in the glycosylation reaction proved successful for a range of different alcohols, including primary and secondary aliphatic-OH, sugars, and more complex aglycones. Study of the stereoelectronic properties of the CF_3 group highlighted the selectivity control of glycosylation affording 1,2-*trans* glycosides (**Scheme 2**, middle). Interestingly, conformational analyses of the resulting CF_3-glycosides showed that the typical $^4C_1$ conformation remained unperturbed, which may stimulate the development of new modified 2-CF_3-sugar mimetics. Following a seminal report by Ryan Gilmour,[22] the stereodirecting value of the trifluoromethyl moiety was compared with fluorine, and results suggest a higher contribution of the steric control with CF_3. Finally, the synthetic utility of this method was demonstrated by applying it to the stereoselective synthesis of 2-CF_3-modified natural glycoside mimics, including disaccharides, steroidal aglycones, amino acids and sphingosine analogues (**Scheme 2**, right).



Scheme 2. Scheme of the work developed in Chapter IV.

[22] Bucher, C.; Gilmour, R. *Angew. Chem. Int. Ed.* **2010**, *49*, 8724-8728.

CHAPTER V: STUDY AND COMPARATION OF SOME PHYSICOCHEMICAL PROPERTIES OF 2-DEOXY-2-FLUORO AND 2-TRIFLUOROMETHYL GLYCOSIDES

Primary goals of clinical pharmacokinetics include enhancing the efficiency and decreasing the toxicity of the active principal ingredient (API) and the bioisosteric replacement of functional groups is commonly used in medicinal chemistry as a tool to improve the pharmacokinetic properties of the parent molecule. Deoxyfluorination of sugars has proved to overcome some of the major drawbacks of native carbohydrates as therapeutic drugs, increasing their lipophilicity and improving the binding affinity, bioavailability, stability, and resistance to hydrolysis.[23]

In Chapter V we explored some of the physicochemical properties of the previously synthetised 2-deoxy-2-fluoro-glycosides (Chapter III) and 2-deoxy-2-trifluoromethyl glycosides (Chapter IV). First, experimental and computational evaluation of 2-F and 2-CF_3 glycosides demonstrated that they maintain the $^4C_1$ chair conformation, and minimal distortion is observed for the sterically demanding CF_3 group. Differences between F and CF_3 were more important for *manno* glycosides, in which high differences in dipole moments and dihedral angles were observed. In contrast, *gluco* derivatives presented similar electronic and steric properties. Experimental determination of the lipophilicity was achieved following a ^{19}F NMR protocol developed by Linclau and co-workers.[24] CF_3-sugars presented higher lipophilicity compared to their fluorinated analogues and promising results were obtained for trifluoromethyl glycosides, since they present positive log *P* values. This is of great importance considering the huge increment of lipophilicity (log $P_{glucose}$ = -3.24). The stability of 2-F and 2-CF_3-glycosides was evaluated by incubation in buffers mimicking standard physiological conditions and lysosomal conditions. Finally, resistance to acid hydrolysis was demonstrated by subjecting the fluorinated compounds to extreme acid conditions and following the progress by ^{19}F NMR.

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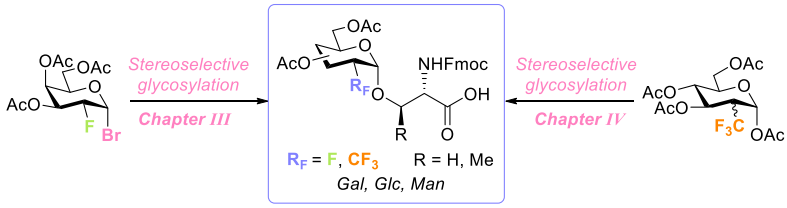
[23] Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. *J. Med. Chem.* **2015**, *58*, 8315-8359.

[24] Linclau, B.; Wang, Z.; Compain, G.; Paumelle, V.; Fontenelle, C. Q.; Wells, N.; Weymouth-Wilson, A. *Angew. Chem. Int. Ed.* **2016**, *55*, 674-678.

CHAPTER VI: STEREOSELECTIVE SYNTHESIS OF FLUORINATED AND TRIFLUOROMETHYLATED TN ANTIGEN ANALOGUES

The Tn antigen (α-*O*-*Gal*NAc-Ser/Thr) is one of the most specific human tumor-associated structures,[25] and it is expressed in approximately 90% of carcinomas, finding a direct correlation between the aggressiveness of the cancer and the occurrence of the Tn antigen.[26] It is considered as an ideal immunotherapy target because it is masked on normal cells and overexpressed on cancer cells. However, it presents some disadvantages, mainly related to the pharmacokinetic drawbacks associated with carbohydrates, which are the low immunogenicity and reduced metabolic stability.

Chapter VI exploits the application of the methodologies described in Chapters III and IV to the stereoselective synthesis of fluorinated and trifluoromethylated analogues of the Tn antigen (**Scheme 3**). The stereoselective synthesis of 2-F and 2-CF_3 analogues of the Tn-antigen using *galacto, gluco,* and *manno* configurations and serine and threonine amino acids, was achieved in good to excellent yields to obtain pure α-isomers of all the products. Fluorinated and trifluoromethylated Tn antigen analogues are interesting tumour associated carbohydrate antigens (TACAs) with improved metabolic stability, and future work will involve studying the effect of the fluorinated moiety in their immunogenicity.

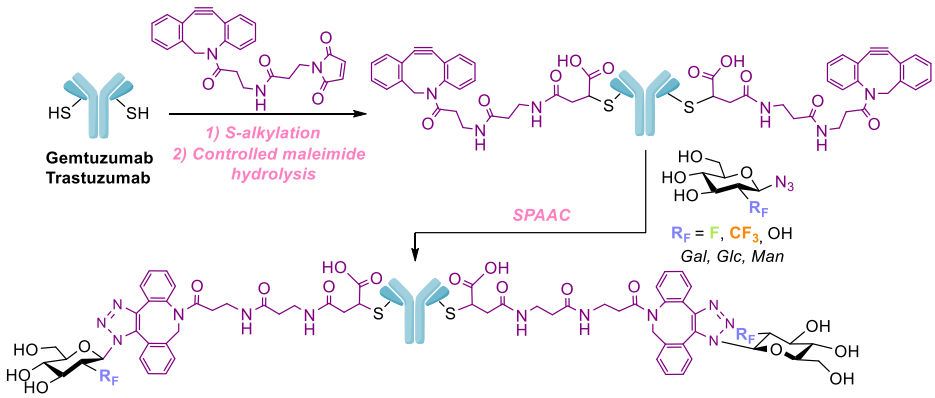


Scheme 3. Scheme of the work developed in Chapter VI.

[25] Ju, T.; Otto, V. I.; Cummings, R. D. *Angew. Chem., Int. Ed.* **2011**, *50*, 1770-1791.
[26] Springer, G. F. *J. Mol. Med.* **1997**, *75*, 594-602.

CHAPTER VII: INCORPORATION OF FLUORINATED AND TRIFLUOROMETHYLATED SUGARS IN RECOMBINANT MONOCLONAL ANTIBODIES

Chapter VII describes the research work carried out in a predoctoral stay in the Bernardes Lab at the University of Cambridge under the supervision of Prof. Gonçalo J. L. Bernardes. In this chapter we describe a two-step protein-modification protocol for the development of synthetic homogeneous fluorinated glycoproteins and antibodies. The protocol involves the incorporation of strained alkynes into proteins by maleimide *S*-alkylation[27] and subsequent copper-free strain-promoted azide-alkyne cycloaddition (SPAAC)[28] using two different antibodies (Trastuzumab and Gemtuzumab) and a variety of native, fluorinated, and trifluoromethylated sugars. The specificity and binding kinetics of the series of modified antibodies were evaluated by flow cytometry and confocal microscopy studies, demonstrating that the modified antibodies maintain the binding properties of the native constructs. This protocol allows to access fluorinated (glyco)peptides and proteins in a simple an efficient way, and we envisioned that will provide valuable information to further explore the biological implications of these new therapeutics and imaging agents in vivo.



Scheme 4. Synthetic route to obtain fluorioglycosylated antibodies.

[27] Renault, K.; Fredy, J. W.; Renard, P. Y.; Sabot, C. *Bioconjug. Chem.* **2008**, *29,* 2497-2513.

[28] a) Pickens, C. J.; Johnson, S. N.; Pressnall, M. M.; Leon, M. A.; Berkland, C. J. *Bioconjug. Chem.* **2018**, *29*, 686-701; b) Oliveira, B. L.; Guo, Z.; Bernardes, G. J. L. *Chem. Soc. Rev.* **2017**, *46*, 4895-4950.

CHAPTER I

GENERAL INTRODUCTION

Chapter I

1.1. GLYCOBIOLOGY

1.1.1. Carbohydrate-based drugs

Carbohydrates are essential biomolecules in living organisms. They normally appear on cell surface and contribute to most interactions between cells and their environment, mediating a wide variety of biological processes, such as immunological responses, recognition events and disease progression.[1] Carbohydrates are present in different molecular forms, such as glycoproteins, glycolipids, or proteoglycans, and their wide diversity is caused by the many possibilities of assembly of monosaccharide building blocks, including pyranose and furanose rings. Additionally, the structural complexity is increased by the two possible configurations of the anomeric centre, α and β isomers, by the linear or branched linkages or the presence of other functional groups.[2] Their structural diversity allows them to encode information for specific molecular recognition and they are determinant in protein folding, stability, and pharmacokinetics.[3]

Carbohydrates have been initially exploited as important tools for studying glycan-mediated interactions and to explore the glycosylation outcome in disease-related processes.[4] Although carbohydrates play essential roles in many biological processes, drugs based on them have been a small group of therapeutic compounds and, in contrast to peptides or nucleotides, carbohydrates have been underestimated by the scientific community for long time. However, recent advances in glycochemistry, glycobiology and glycomedicine have allowed to consider glycans as key players in pharmacological research, contributing to the development of carbohydrate-based diagnostics, vaccines, and therapeutics.[5]

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[1] Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855-867.
[2] Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C. W.; Seeberger, P. H. *ACS Chem. Biol*. **2007**, *2*, 685-691.
[3] Kim, N. A.; Thapa, R.; Jeong, S. H. *Int. J. Biol. Macromol*. **2018**, *109*, 311-322.
[4] Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357-2364.
[5] Fernández-Tejada, A.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2015**, *21*, 10616-10628.

Some of the most successful examples of carbohydrate-based drugs include antiviral compounds Zanamivir (Relenza)[6] and Oseltamivir (Tamiflu), diabetes treatments Acarbose and Miglitol[7], anticoagulant Fondaparinux[8] or epilepsy treatment Topiramate[9] (**Figure 1.1**).

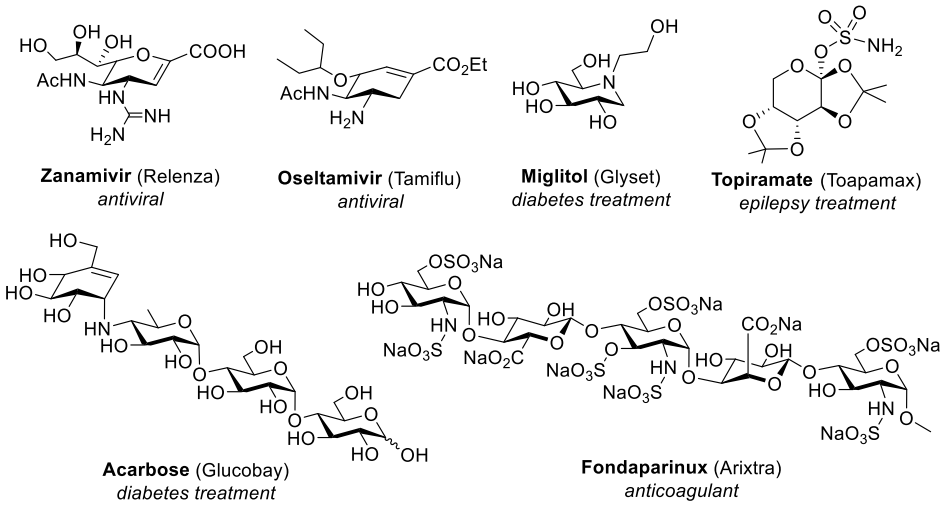

Figure 1.1. Representative examples of carbohydrate-based drugs.

One of the main reasons making carbohydrate-based drug discovery challenging is the structural diversity that has hampered the efforts to find its correlation with biological function and to define oligosaccharide expression patterns in proteins and cells. Another reason is related to the pharmacokinetic drawbacks linked to carbohydrates, mainly, the insufficient metabolic stability, poor permeation properties and the rapid clearance of carbohydrate-based drugs that compromises both their bioavailability and potency.

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[6] Von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W. Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418-423.

[7] a) Asano, N. *Glycobiology* **2003**, *13*, 93R-104R. b) Campbell, L. K.; Baker, D. E.; Campbell, R. K. *Ann. Pharmacother.* **2000**, *34*, 1291-1301.

[8] Petitou, M.; Duchaussoy, P.; Herbert, J. M.; Duc, G.; El Hajji, M.; Branellec, J. F.; Donat, F.; Necciari, J.; Cariou, R.; Bouthier, J.; Garrigou, E. *Semin. Thromb. Hemost.* **2002**, *28*, 393-402.

[9] Maryanoff, B. E.; Nortey, S. O.; Gardocki, J. F.; Shank, R. P.; Dodgson, S. P. *J. Med. Chem.* **1987**, *30*, 880-887.

The poor oral availability of carbohydrate-drugs is caused by their high polarity (due to the high density of hydroxyl groups), which makes difficult the crossing through the enterocyte layer of the small intestine. In addition, carbohydrates are easily degraded by intestinal bacteria, which limits its oral bioavailability. Carbohydrates interact with their biological target principally through weak interactions, such as hydrogen bonding and hydrophobic interactions; therefore, the natural affinity of carbohydrates is usually considered insufficient (**Figure 1.2**).[10] Furthermore, the development of therapeutics based on native glycan structures has been restricted by synthetic limitations. The main challenge on the chemical synthesis of complex carbohydrate molecules is to build glycosidic linkages connecting monomeric units with proper stereo and regiochemical orientation. However, there is a lack of general methods for the preparation of these compounds, usually requiring multiple selective protection and deprotection steps making the chemical synthesis of oligosaccharides a major undertaking.

Over the past years, advances in the understanding of carbohydrate-protein interactions have allowed the development of a new class of small molecule drugs, known as **glycomimetics**.[11] These compounds mimic the bioactive function of carbohydrates and address the drawbacks of carbohydrate derivates, which are mainly the low activity and insufficient drug-like properties (**Figure 1.2**).[12] Glycomimetics allow the design of carbohydrate-based drugs with improved pharmacological properties, and with the same or better affinity and selectivity towards the desired protein targets. Additionally, by designing a prodrug, the oral availability becomes possible. A classic example of a carbohydrate-based glycomimetic is Oseltamivir (**Figure 1.1**), in which drug likeness was achieved by eliminating polar groups and metabolic soft spots[13] that were not required for affinity.

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[10] Jiang, H.; Qin, X.; Wang, Q.; Xu, Q.; Wang, J.; Wu, Y.; Chen, W. Wang, C.; Zhang, T.; Xing, D.; Zhang, R. *Eur. J. Med. Chem.* **2021**, *223*, 113633.
[11] Tamburrini, A.; Colombo, C.; Bernardi, A. *Med. Res. Rev.* **2020**, *40*, 495-531.
[12] Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Discovery*, **2009**, *8*, 661-677.
[13] Trunzer, M.; Faller, B.; Zimmerlin, A. *J. Med. Chem.* **2009**, *52*, 329-335.

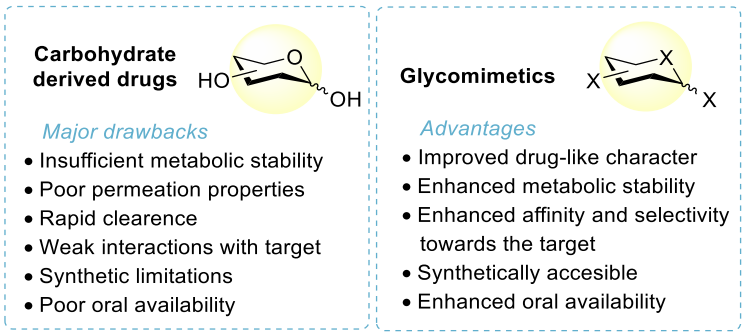


Figure 1.2. Major drawbacks of carbohydrate-based drugs and the development of glycomimetics to overcome such limitations.

The use of carbohydrates as biological targets and drugs has experimented a huge progress as improved methods for carbohydrate synthesis, sequencing, and biological analysis have become more sophisticated.[14] Carbohydrates constitute a relatively untapped source of new drugs, and they offer exciting new therapeutic opportunities. Since the beginning of the 20th century the interest of glycans in therapeutic applications has been increasing (**Figure 1.3**) delivering important advances, focused mainly in glycomimetic drugs, glycoproteins, and glycoengineering of novel agents.[11]

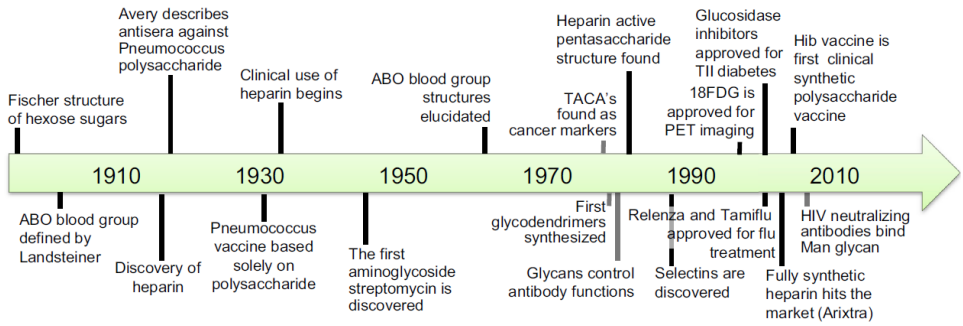


Figure 1.3. Timeline of glycans in medicine during the 20th century.[15]

[14] Wu, C. Y.; Wong, C. H. *Chem. Commun.* **2011**, *47*, 6201-6207.
[15] Hudak, J. E.; Bertozzi, C. R. *Chem. Biol.* **2014**, *21*, 16-37.

The demand for well-defined synthetic carbohydrates for biological research has increased extensively over the past decade. The isolation of carbohydrates from biological material is a tedious process often yielding scarce amounts of oligosaccharide mixtures and limited to organisms that can be cultured. Glycochemistry presents an alternative strategy to obtain pure glycan compounds by a controlled synthesis.[16] Efforts in glycochemistry have been centred on the glycosylation reaction. Despite the promise and potential generality of synthetic approaches, the development of rapid, robust, and preferably simple synthetic methods, remains a critical need in glycoscience.

1.1.2. The glycosylation reaction

Glycosylation is the most important reaction in the field of glycochemistry and has long been a topic of research. Among the variety of glycosidic bonds, the O-glycosidic bonds are of major interest due to their high abundance. The main challenge of the glycosylation reaction is to predict and control the stereoselectivity of the new *O*-glycosidic bond. Currently, there is not a general rule to direct the configuration of the glycosidic bond, as glycosylation reaction often proceeds thought a range of mechanisms involving many different intermediates. Knowledge of the structure of these intermediates is vital for understanding the reactivity and stereoselectivity of the glycosylation reaction.[17]

Over the past few decades, many new sophisticated glycosylation procedures using a variety of glycosyl donors,[18] activation strategies or protecting groups[19] have been developed. Other interesting protocols such as, solid-phase oligosaccharide synthesis, which avoids the purification of intermediates, and automated oligosaccharide synthesis, have also contributed

[16] Bernardes, G. J. L.; Castagner, B.; Seeberger, P. H. *ACS Chem. Biol.* **2009**, *4*, 703-713.
[17] Walvoort, M. T.; van der Marel, G. A.; Overkleeft, H. S.; Codée, J. D. *Chem. Sci.* **2013**, *4*, 897-906.
[18] Yang, Y.; Zhang, X.; Yu, B. *Nat. Prod. Rep*. **2015**, *32*, 1331-1355.
[19] a) Leng, W. L.; Yao, H.; He, J. X.; Liu, X. W. *Acc. Chem. Res.* **2018**, *51*, 628-639; b) Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed*. **2009**, *48*, 1900-1934.

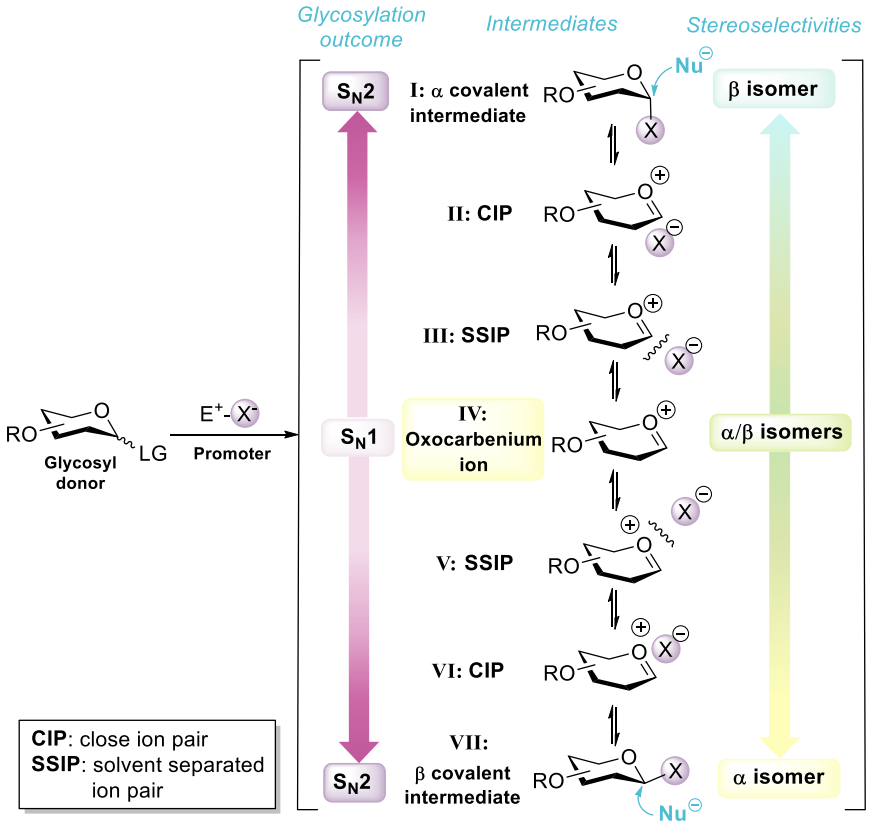
to increase the efficiency of the glycosylation reaction.[20] Many general glycosylation protocols focused on the development of efficient and stereocontrolled methodologies have been developed. The requirement for synthesising two anomeric stereoisomers, α and β, has led to more advanced and detailed studies to introduce variation in the mechanistic pathways that control glycosylation reaction.[21] However, despite the vast efforts made in the area, access to carbohydrate-based products in large quantities with well-defined regio- and stereochemistry is still challenging.

a. Mechanistic considerations

The glycosylation reaction generally involves the nucleophilic attack of the glycosyl acceptor (nucleophile) to the anomeric carbon of the glycosyl donor (electrophile). The reaction starts with the activation of the glycosyl donor, typically using metallic or acid promoters, and the mechanism is believed to involve the cleavage of the leaving group, which leads to an array of electrophilic intermediates (**Scheme 1.1**, **I-VII**). Understanding the structure, conformation, reactivity, and interactions of these electrophilic intermediates is essential to predict the outcome of the glycosylation reaction. These species are formed when the oxocarbenium ion **IV** is intercepted by the counter ion of the promoter $\mathbf{X}^-$ and it can lead to: solvent separated ion pair species (SSIP, **III** and **V**), close ion pair species (CIP, **II** and **VI**) or covalent reactive intermediates (**I** and **VII**).[22] It has been hypothesized that both covalent intermediates and oxocarbenium like species can give the glycosylation product, but in a different stereo-preference. Thus, the relative abundance of these transient species and their kinetic preference determines the outcome of the glycosylation stereoselectivity (**Scheme 1.1**).

[20] Lepenies, B.; Yin, J.; Seeberger, P. H. *Curr. Opin. Chem. Biol.* **2010**, *14*, 404-411.
[21] Chang, C. W.; Lin, M. H.; Chan, C. K.; Su, K. Y.; Wu, C. H.; Lo, W. C.; Lam, S.; Cheng, Y. T.; Liao, P.H.; Wong, C.H.; Wang, C. C. *Angew. Chem. Int. Ed*. **2021**, *60,* 12413-12423.
[22] a) Hansen, T.; Lebedel, L.; Remmerswaal, W. A.; Van Der Vorm, S.; Wander, D. P.; Somers, M.; Overkleeft, H. S.; Filippov, D. V.; Désiré, J.; Mingot, A.; Bleriot, Y.; van der Marel, G. A.; Thibaudeau, S.; Codée, J. D. *ACS Central Science* **2019**, *5*, 781-788; b) Adero, P. O.; Amarasekara, H.; Wen, P.; Bohé, L.; Crich, D. *Chem. Rev.* **2018**, *118*, 8242-8284.

Considering covalent intermediates, **I** and **VII**, the reaction will follow an S_N2 pathway and both the α and β isomers would be obtained in each case. These intermediates can be studied using low-temperature NMR techniques, and over the years, many covalent intermediates have been characterized.[23] In contrast, the oxocarbenium like intermediates will give a S_N1 like pathway, however, due to the low stability and short lifetime of these intermediates, it has been difficult to define a clear structure-reactivity relationship. Recent advances have allowed to study them in a more direct way and provide some new insights about oxocarbenium ions.[24]



Scheme 1.1. General mechanism of the glycosylation reaction.

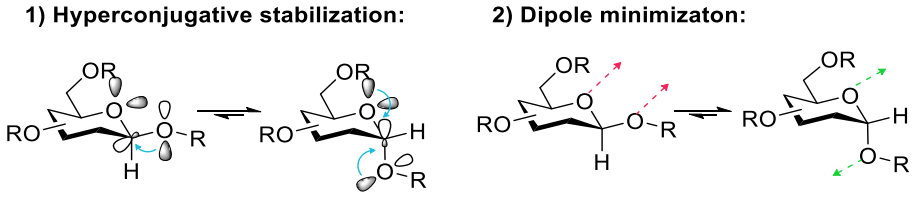
[23] Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.

[24] Franconetti, A.; Ardá, A.; Asensio, J. L.; Blériot, Y.; Thibaudeau, S.; Jiménez-Barbero, J. *Acc. Chem. Res.* **2021**, *54*, 2552-2564.

The reactivity and stereoselectivity are highly affected by glycosyl donor conformation, substituents orientation, and the type, number, and location of protecting groups. The modification of these attributes can lead to a wide variation in the reaction outcome. Other factors, such as glycosyl acceptor, promoters and reaction conditions can also significantly affect the reaction.[25]

b. Anomeric effect

Pyranoses bearing polar substituents in the anomeric centre normally adopt a chair-like conformation and the axial geometry is favoured. This phenomenon was first observed by Edward[26] and later defined as "anomeric effect" by Lemieux[27], and is responsible for the stereochemical outcome of glycosidic bond formations. Pyranose sugars with alkoxy, acetoxy and halogen substituents in the anomeric centre prefer the axial over the equatorial orientation. The preference for the axial position increases with the electron-withdrawing character of the substituent and the most accepted explanations for the anomeric effect are: 1) the existence of a hyperconjugative stabilization due to a periplanar orientation of the non-bonding electron pairs of the cyclic oxygen and the anti-bonding orbital of the glycosidic C-O bond ($\sigma^*_{C1\text{-}O}$) and 2) a favourable dipole-dipole orientation between the cyclic oxygen and the anomeric carbon-heteroatom bond (**Scheme 1.2**).[28]



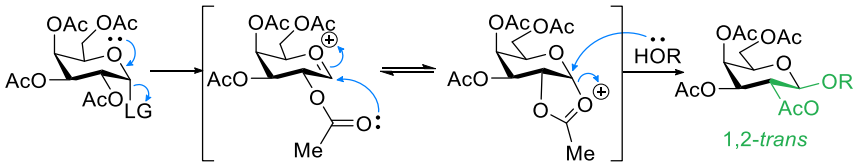
Scheme 1.2. Rationalization of the origin of the anomeric effect.

[25] Chatterjee, S.; Moon, S.; Hentschel, F.; Gilmore, K.; Seeberger, P. H. *J. Am. Chem. Soc.* **2018**, *140*, 11942-11953.
[26] Edward, J. T. *Chem. Ind. (London)* **1955**, 1102-1104
[27] Lemieux, R. U. *Pure Appl. Chem.* **1971**, *25*, 527-548
[28] Juaristi, E.; Cuevas, G. *Tetrahedron*, **1992***, 48,* 5019-5087.

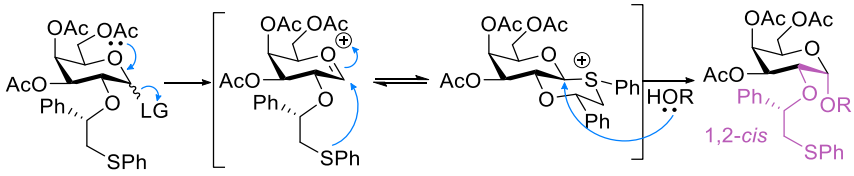
c. *Participation of neighbouring groups*

Despite the preference of the axial orientation of electronegative substituents in the anomeric position, the anomeric effect is not a sufficient stereodirector and therefore the glycosylation reaction often leads to a mixture of α and β glycosides. To achieve high stereoselectivity other modes of stereocontrol are needed, and a typical strategy to control the stereoselectivity is via neighbour group participation. Neighbouring participating groups usually control the stereoselectivity by formation of an intermediate, and remarkable advances have been achieved specially in the formation of 1,2-*trans* glycosidic bonds (**Scheme 1.3, 1**). When using glycosyl donors with an ester protecting group in the C-2 position, glycosyl donor activation leads to the formation of an oxocarbenium ion, which can be further stabilized by the formation of an 1,2-orthoester intermediate. Nucleophilic acceptors can only approach the anomeric carbon from the top face, since the bottom face of the ring is blocked, leading to *1,2-trans* glycosides.[29] This protocol usually provides high or complete stereoselectivity and has been widely and generally used in glycosylation reactions, since it implied the use of common ester protecting groups.

1) Neighbor group assistance to form 1,2-*trans* glycosides:



2) Neighbor group assistance to form 1,2-*cis* glycosides:



Scheme 1.3. Stereoselective control of neighbouring participating groups.

[29] Goodman, L. *Adv. Carbohyd. Chem.* **1967**, *22*, 109-175.

In contrast, the formation of 1,2-*cis* glycosidic bonds is challenging and there is not a general strategy that could be applied to any glycosyl donor. Some methods imply the installation of special neighbour participating groups, for example, using (S)-1-phenyl-2-phenyl-thioethyl group at the C-2 position. The intermediate blocks the top face of the ring, allowing the acceptor to approach from the bottom face giving *1,2-cis* glycosides (**Scheme 1.3, 2**).[30]

d. Protecting groups

Protecting groups are widely used in chemical glycosylation since it usually involves the transformation of a sugar into a fully protected glycosyl donor with a leaving group on the anomeric carbon. Additionally, glycosyl acceptors must have only one free hydroxyl group to achieve efficient selectivity. Among other parameters (e.g., solvent, temperature, acceptors nucleophilicity), protecting groups have the strongest impact on the stereochemistry, and influence the glycosyl donor and acceptor reactivity.

Apart from neighbouring protecting groups adjacent to the anomeric position, protecting groups in other positions have also been used for controlling the stereochemical outcome of the glycosylation reaction. These strategies are mainly focused on the synthesis 1,2-*cis* glycosidic linkages, since 1,2-*trans* glycosides are easily accessible via neighbour group participation (**Figure 1.4**).[31] One example is the use of acyl directing groups at remote positions (C-3, C-4, and C-6) (**Figure 1.4, 1**).[32] This strategy allows obtaining 1,2-*cis gluco* and *galacto* glycosides with high stereocontrol. However, it does not provide the same degree of stereoselectivity as those using directing groups in C-2. Other approaches include the use of conformation-restraining protecting groups (**Figure 1.4, 2**),[33] hydrogen bond-mediated aglycon delivery (**Figure 1.4,**

[30] Kim, J. H.; Yang, H.; Park J.; Boons G. J. *J. Am. Chem. Soc.* **2005**, *127,* 12090-12097.
[31] Ma, X.; Zheng, Z.; Fu, Y.; Zhu, X.; Liu, P.; Zhang, L. *J. Am. Chem. Soc.* **2021**, *143*, 11908-11913.
[32] a) Komarova, B. S.; Tsvetkov, Y. E.; Nifantiev, N. E. *Chem. Rec.* **2016**, *16*, 488-506; b) Nigudkar, S. S.; Demchenko, A. V. *Chem. Sci.* **2015**, *6*, 2687-2704.
[33] a) Crich, D. *J. Org. Chem.* **2011**, *76*, 9193-9209; (b) Imamura, A.; Ando, H.; Korogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. *Tetrahedron Lett*. **2003**, *44*, 6725-6728; (c) Manabe, S.; Ishii, K.; Ito, Y. *J. Am. Chem. Soc.* **2006**, *128*, 10666-10667.

3) and intramolecular aglycon delivery (**Figure 1.4, 4**). However, all these protocols require the use of uncommon protecting groups, that usually are not easy to introduce and need to be eliminated after the glycosylation step.

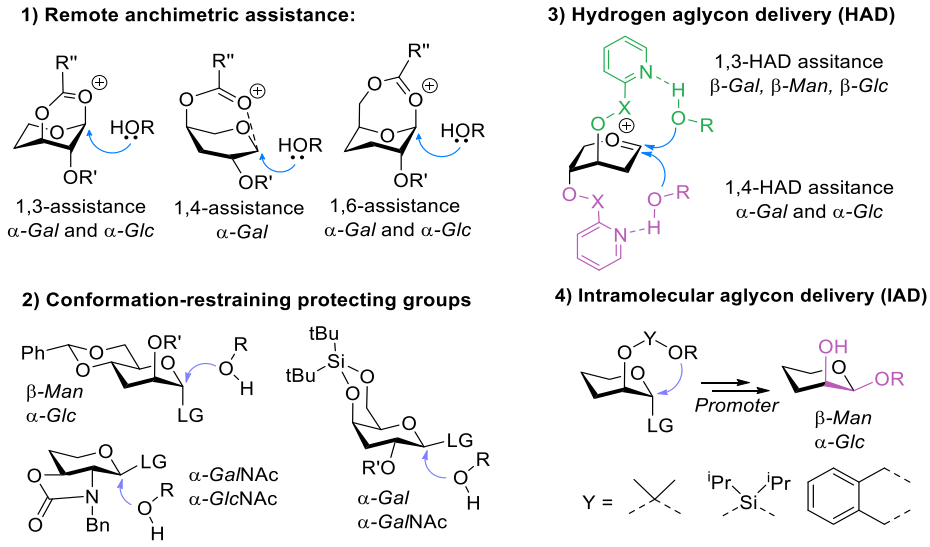


Figure 1.4. Examples of stereocontrol using protecting groups.[34]

Regarding glycosyl donors, protecting groups have great influence in their stability and reactivity. Generally, the mechanism of a glycosylation reaction possesses a partial or complete S_N1 character and the rate determining step typically involves the formation of the oxocarbenium ion. Therefore, the electronic effects of the glycosyl donor substituents can strongly affect its reactivity. Acetyl and benzyl groups are the most used for "temporary" and persistent protection of hydroxyl groups and they affect the reactivity of glycosyl donors following the armed/disarmed principle, which is based on torsional and electronic effects; 1) *Torsional effects:* During the glycosylation reaction, the generated oxocarbenium intermediate adopts a half chair conformation. The energy of this conformation increases when using cyclic protecting groups, that restrict ring flexibility of the molecule and makes difficult the access to the half-chair oxocarbenium ion intermediate (**Figure**

[34] Krasnova, L.; Wong, C. H. *J. Am. Chem. Soc.* **2019**, *141*, 3735-3754.

1.5);[35] 2) *Electronic effects:* Electron withdrawing substituents, such as ester protecting groups, decrease the reactivity of the glycosyl donor, and are termed "disarmed donors". They decrease the electron density of the cyclic oxygen, which results in a destabilization of the oxocarbenium intermediate. In contrast, electron donating substituents, such as ether protecting groups, tent to stabilize the rate-limiting transition oxocarbenium ion, increasing the reactivity, and they are termed as "armed" donors (**Figure 1.5**).[36]

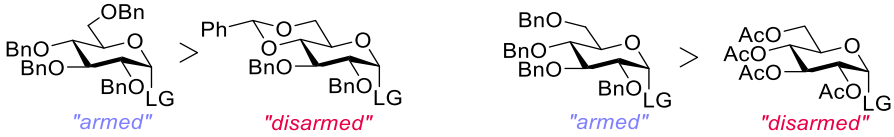


Figure 1.5. Relative reactivity of some glycosyl donors.

e. Glycosyl donor activation

Usually, glycosylation reaction involves the activation of the anomeric carbon of the glycosyl donor. In the literature, a variety of glycosyl donors and activation conditions have been used, such as glycosyl halides, thioglycosides, glycosyl thioimidates, glycosyl phosphates and phosphites, nitroglycals, 1,2-orthoesters or carboxybenzyl glycosides, among others.[37] Usually, a promoter is used to activate the leaving group, which leads to the formation of the oxocarbenium intermediate. Besides substrates and protecting groups, promoters also have influence in the glycosylation selectivity because they affect the formation of the oxocarbenium intermediate. Other parameters, such as the solvent, counterions, additives, time, pressure, temperature, and concentration have significant effects on the glycosylation selectivity. Thus, optimization of the glycosylation reaction is crucial for obtaining good reactivity and selectivity.

[35] Jensen, H. H.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205-9213.
[36] Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068-6070.
[37] Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900-1934.

1.2. IMPORTANCE OF FLUORINE IN CHEMISTRY AND BIOCHEMISTRY

Since the discovery by Fried and Sabo of the first fluorinated drug in 1954, the synthetic 9α-fluoro-substituted corticosteroid *Fludrocortisone* (**Figure 1.6**), fluorine has played an important role in diverse areas of medicinal chemistry and drug design. Few years later, in 1957, Heidelberger and co-workers demonstrated that 5-fluorouracil (**Figure 1.6**) can act as antimetabolite of natural uracil.[38] These two drugs constitute the initial point in the change of fluorine role in biological chemistry.

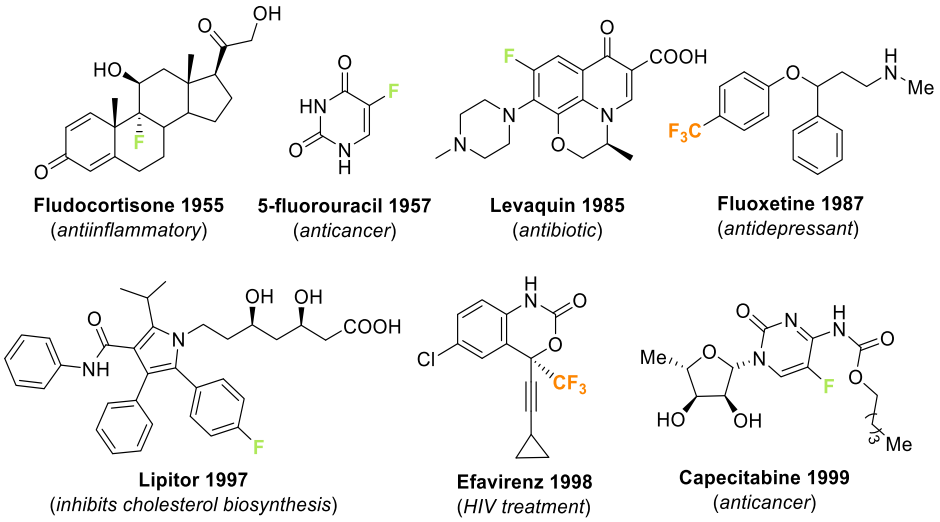


Figure 1.6. Examples of fluorinated drugs and therapeutic applications.

Fluorinated pharmaceuticals are highly appreciated in medicine for its uses as anaesthetics, anti-inflammatory drugs, antiviral and anticancer agents among others.[39] Some examples of the effect of fluorine substitution in the efficiency of a drug are Levaquin (antibiotic), Fluoxetine (antidepressant),[40]

[38] a) Fried, J.; Sabo, E. F. *J. Am. Chem. Soc*, **1954**, *76*, 1455-1456. b) Heidelberger, C.; Chaudhuri, N. K.; Danneberg, P.; Mooren, D.; Griesbach, L.; Duschinsky, R.; Schnitzer, R. J.; Pleven, E.; Scheiner, J. *Nature* **1957**, *179*, 663.

[39] a) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev*. **2008**, *37*, 320-330; b) Filler, R.; Saha, R. *Future Med. Chem*. **2009**, *1*, 777-791.

[40] Wong, D. T.; Bymaster, F. P.; Engleman, E. A. *Life Sci*. **1995,** *57*, 411-441.

Capecitabine (anticancer),[41] Efavienz (used in HIV treatment),[42] and Lipitor which has been the biggest selling pharmaceutical globally in 2008[45b] (**Figure 1.6**). Moreover, fluorine substitution is commonly used during the development and optimization process of new drugs to improve their pharmacological properties and it can be used to control protein-ligand interactions.[43] The applications of fluorine in drug design are in continuous expansion, favoured by the better understanding of fluorine physicochemical properties and the development of new strategies for the introduction of fluorine motifs and fluoroalkyl modifications.[44] These advances have culminated with the production of high-value drugs during the last century (**Figure 1.6**). Nowadays, the number of fluorine-containing launched drugs constitutes about 20-30% of all pharmaceuticals, and in the last years this number is exponentially increasing (**Figure 1.7**).[45]

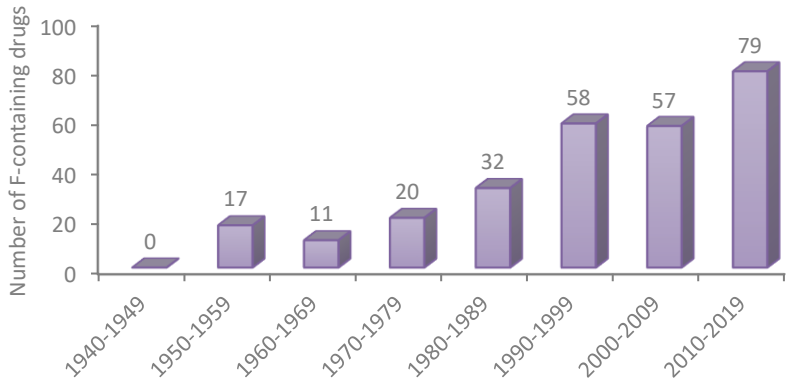


Figure 1.7. Fluorine-containing drugs approved by the FDA during the last century.[46]

[41] Ishitsuka, H.; Shimma, N.; Horii, I. *J. Pharm. Soc. Jpn.* **1999**, *119,* 881-897.
[42] Adkins, J. C.; Noble, S. *Drugs* **1998**, *56*, 1055-1064.
[43] Gouverneur, V.; Muller, K. Fluorine in Pharmaceutical and Medicinal Chemistry: From Biophysical Aspects to Clinical Applications, 1st ed.; Imperial College Press, London, **2012.**
[44] Zhou, Y.; Wang, J.; Gu, Z.; Wang, S.; Zhu, W.; Aceña, J. L.; Soloshonok V. A.; Izawa K.; Liu, H. *Chem. Rev*. **2016**, *116,* 422-518.
[45] a) Hagmann, W. K. *J. Med. Chem*. **2008**, *51*, 4359-4369; b) O'Hagan, D. *J. Fluor. Chem*. **2010**, *131,* 1071-1081.
[46] a) Johnson, B. M.; Shu, Y. Z.; Zhuo, X.; Meanwell, N. A. *J. Med. Chem*. **2020**, *63,* 6315-6386; b) Mei, H.; Han, J.; Fustero, S.; Medio-Simon, M.; Sedgwick, D. M.; Santi, C.; Ruzziconi, R.;

The increasing use of fluorine in modern medicinal chemistry and agrochemicals has induced intensive research in organofluorine area, what gave rise to new fluorinating agents and fluorination methodologies.[47] As demonstrated for the fluorinated drugs in **Figure 1.6**, the introduction of a single fluorine atom can completely change biological properties of natural products. In some cases, it increases the efficiency of the drug, improves the bioavailability and it can enhance the interaction of the drug with its pharmacological target. Fluorine electronegativity, size, lipophilicity, and electrostatic interactions can dramatically influence in the chemical reactivity and properties or organic compounds, however, there is not a general rule determining the effects that fluorine and fluorine-containing substituents can induce in a compound.[48]

1.2.1. Physical and chemical properties of fluorine

Fluorine is the 13th most common element on Earth's crust and the most abundant of the halogens. However, natural fluorine-containing compounds are uncommon in nature. This rarity can be explained considering the chemical and physical properties of fluorine. Most of the fluorine in earth exists as insoluble salts, CaF_2 and Na_3AlF_3, which makes difficult its delivery to aqueous biological systems (its absorption by microorganisms). The high oxidation potential of fluorine (-3.06 V vs -1.36 (Cl), -1.07 (Br), -0.54 (I)) coupled with the extraordinary hydration energy (117 kcal/mol *vs* 84 (Cl), 78 (Br), 68 (I)) makes impossible the biochemical incorporation of fluorine by the most common pathways of enzymatic halogenation.[49]

Soloshonok, V. A. *Chem. Eur. J.* **2019**, *25,* 11797-11819; c) Mei, H.; Remete, A. M.; Zou, Y.; Moriwaki, H.; Fustero, S.; Kiss, L.; Soloshonok, V. A.; Han, J. *Chin. Chem. Lett.* **2020**, *31,* 2401-2413; d) Yu, Y.; Liu, A.; Dhawan, G.; Mei, H.; Zhang, W.; Izawa, K.; Soloshonok, V. A.; Han, J. *Chin. Chem. Lett.* **2021**, *32,* 3342-3354; e) He, J.; Li, Z.; Dhawan, G.; Zhang, W.; Sorochinsky, A. E.; Butler, G.; Soloshonok, V. A.;Han, J. *Chin. Chem. Lett*. **2022**, pre-proof.

[47] a) Mestre, J.; Bernús, M.; Castillón, S.; Boutureira, O. *J. Org. Chem.* **2022**, *87*, 10791-10806; b) Zhu, Y.; Han, J.; Wang, J.; Shibata, N.; Sodeoka, M.; Soloshonok, V. A.; Coelho, J. A. S.; Toste, F. D. *Chem. Rev*. **2018**, *118*, 3887-3964.

[48] Gillis, E.; Eastman, K.; Hill, M.; Donnelly, D, Meanwell, N. A. *J. Med. Chem.* **2015**, *58*, 8315-8359.

[49] Wang, J.; Sánchez-Roselló, M.; Aceña, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Liu, H. *Chem. Rev.* **2014**, *114*, 2432-2506.

Fluorine is the most electronegative element (Pauling constant, χ_P = 4.0) and it is the smallest atom that can substitute the C-H bond with minimal structural change. It has slightly higher size than the hydrogen atom and its electronegativity and van deer Waal radius is comparable to that of the oxygen (**Table 1.1**). Due to its similar size, fluorine (C-F, 1.47 Å) effectively substitutes hydrogen atoms (C-H, 1.20 Å) and hydroxyl groups (C-O, 1.40 Å) altering the electronic properties of the parent molecule without affecting its geometry.

Table 1.1. Atomic parameters of elements of the first period.

Element (E)	Van der Waals radius (Å)	Pauling electronegativity	Atom polarizability (Å^3)	Strength of C-E bond (kcal/mol)
H	1.20	2.1	0.667	98
C	1.70	2.5	1.76	83
N	1.55	3.0	1.10	70
O	1.52	3.5	0.82	84
F	1.47	4.0	0.557	105

Due to the small size and high electronegativity of fluorine, the C-F bond is highly polarized, and it supposes a dramatic change from a C-H bond. In the strongly polarized C-F bond, the fluorine atom bears a partial negative charge, and the carbon bears a partial positive charge ($C^{\delta+}$-$F^{\delta-}$) and they experiment electrostatic attraction, making a strong and short bond. Hence, C-F bonds have an important ionic character and stronger energy than other carbon-halogen bonds. Fluorine atom has three lone pairs, but due to its high electronegativity the lone pairs are highly attracted to the nucleus, provoking the low polarizability of fluorine (**Table 1.1**), which contributes to the inertness of C-F bonds and the decrease of intermolecular interactions. Another consequence of the highly polarized nature of the C-F bond is the presence of a low-energy antibonding orbital σ*, which is located behind the carbon atom in the plane of the bond. This vacant orbital can accept electron density from nearby electron-donating groups, such as, lone pairs or σ-bonds, and it

influences the conformation of the molecule. Overall, the C-F can be defined as a short, strong, polarized, and unreactive bond.[50]

1.2.2. Fluorine effect on bioactive molecules

Many areas of medicine have benefited from advances in fluorine chemistry. In the design of analogues of bioactive molecules, it is usual the replacement of C–H and C–O bonds with fluorine since the peculiar properties of this atom can induce some desired physicochemical changes on bioactive molecules. The introduction of a fluorine atom into a target molecule influences the metabolic stability, lipophilicity, and solubility, it considerably affects to the acidity and basicity of functional groups, and it can change the tendency for hydrogen bonding. All these changes, affect to the metabolism, membrane permeability, binding affinity to the target, and bioavailability of a given drug candidate (**Figure 1.8**).[51] However, the effect of fluorine on the biological activity of organic compounds is difficult to predict.

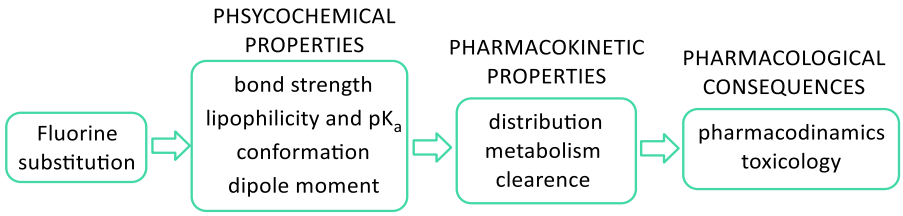


Figure 1.8. Fluorine substitution effects in drug response.[52]

a. *Metabolic stability*

The metabolic stability of a pharmaceutical drug is one of the key features in determining the bioavailability. Lipophilic compounds are normally oxidized by liver enzymes and a common strategy to increase their metabolic

[50] Selected books: a) Ojima, I. *Fluorine in Medicinal Chemistry and Chemical Biology*, Wiley-Blackwell, Chichester, **2009**; b) Bégué, J. P.; Bonnet-Delpon, D. *Bioorganic and Medicinal Chemistry of Fluorine*; Wiley; Hoboken, **2008**; c) Kirsch, P. *Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications*; Wiley-VCH. Weinheim, **2004**.
[51] Hiyama, T.; Shimizu, M. *Angew. Chem. Int. Ed.* **2005**, *44*, 214-231.
[52] Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Ann. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443-470.

stability is to block metabolic labile sites with a fluorine atom. Generally, the introduction of a fluorine atom in the molecule increases the thermal and oxidative stability, which facilitates the development of these molecules as pharmaceuticals. There are many examples in the literature in which fluorine is used for the deactivation of functional groups which are sensible to undesirable hydrolysis.[53] Thus, fluorine substitution in pharmaceuticals is frequently used to block the labile sites of the molecule and increase its stability.

b. Effect on pK_a and lipophilicity

Changes on the pK_a have a strong effect in different parameters, such as, binding affinity (potency, selectivity), physicochemical properties (solubility, log D), bioavailability, and pharmacokinetics of drugs, among others. Fluorine has a strongly electron withdrawing effect on neighbouring functional groups, which affects directly on their acidity. The introduction of a fluorine atom allows the modulation of the pK_a of the molecule, which affects to the absorption, distribution, metabolism, excretion, and toxicity (ADMET) processes of oral drugs and, therefore, in the bioavailability. The introduction of a fluorine atom in the molecule usually increases its lipophilicity, by decreasing the pK_a value and Lewis's basicity of neighbouring functional groups and improves the bioavailability. Basic groups have limited ability to pass through membranes and this feature determines its low bioavailability. A fluorine atom introduced close to a basic group reduces its basicity which results in better cell-membrane permeation (**Table 1.2**, Ethylamine derivates). Moreover, alcohols, carboxylic acids, heterocycles, and phenols become more acidic when they have a fluorine adjacent atom (**Table 1.2**, Acetic acid derivates).

[53] a) Kitazume, T.; Yamazaki. T. *Experimental Methods in Organic Fluorine Chemistry*, Gordon, Breach Publishing Group, Newark, NJ, **1999**. b) Smith, D. H.; van de Waterbeemd, H.; Walker, D. K. *Pharmacokinetics and Metabolism in Drug Design, Methods and Principles in Medicinal Chemistry*, Vol. 13, Wiley-VCH, Weinheim, **2001**.

Table 1.2. Effect of fluorine in the pK_a of acetic acid and ethylamine derivates.[45,54]

Number of fluorine atoms	Acetic acid derivates	pK_a	Ethylamine derivates	pK_a
0	CH_3COOH	4.76	$CH_3CH_2NH_3^+$	10.7
1	CH_2FCOOH	2.66	$CH_2FCH_2NH_3^+$	9.0
2	CHF_2COOH	1.24	$CHF_2CH_2NH_3^+$	7.3
3	CF_3COOH	0.23	$CF_3CH_2NH_3^+$	5.7

c. Effect of fluorine substitution on molecular conformation

In most of the cases, the substitution of oxygen or hydrogen by fluorine atoms is tolerated due to its similar size. However, it supposes a strong electronic modification that can induce changes in the geometry of the molecule. Fluorine atoms can influence the conformation of organic molecules through dipole-dipole interactions, charge-dipole interactions and hyperconjugative effects.[55] Dipole-dipole interactions are responsible of the stabilization of configurations in which the C-F dipole opposes the dipole of other functional groups in the molecule, such as amides, halides, or carbonyl groups. This effect decreases with the decreasing dipole moment of the functional group (**Figure 1.9**, **A**). Electrostatic interactions associated with C-F bonds become more pronounced when a neighbouring group bears a positive charge. In these situations, the favoured conformation is the one in which the partially negative fluorine atoms are close to the positive charge (**Figure 1.9**, **B**). Hyperconjugation stabilization effects are consequence of the vacant low energy antibonding orbital σ* present in the C-F bond, which tent to align periplanar to adjacent groups that can donate electron density to this orbital. Specially interesting is the example of 1,2-difluoroethane, that adopts preferably a *gauche* conformation rather than *anti* conformation which is less sterically hindered. In the *gauche* conformation both fluorine atoms are

[54] Wodzinska, J.; Kluger, R. *J. Org. Chem.* **2008**, *73*, 4753-4754.
[55] Hunter, L. *Beilstein J. Org. Chem* **2010**, *6,* 38.

periplanar to a C-H bond, in which exists a hyperconjugation interaction between the $\sigma_{C\text{-}H}$ bonding orbital and the $\sigma^*_{C\text{-}F}$ anti-bonding orbital (**Figure 1.9, C**). Hyperconjugation can also occur with other electron donors such as lone pairs or π-systems, and in all the cases, the most stable conformations are the ones that align the electron-donating group with the $\sigma^*_{C\text{-}F}$ orbital.

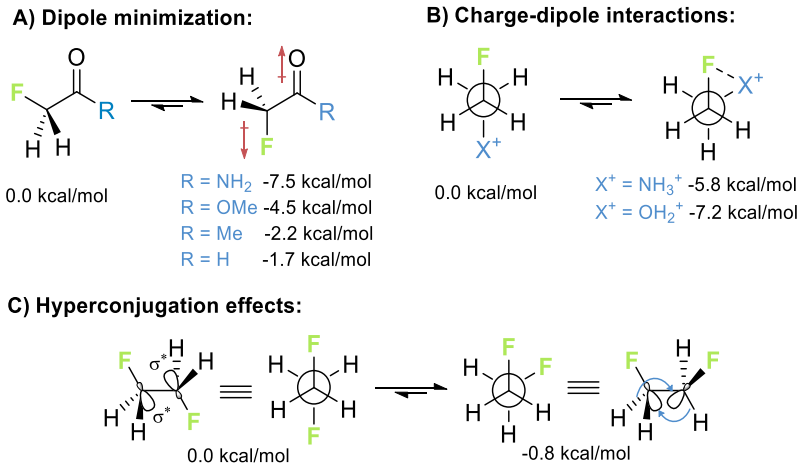


Figure 1.9. Conformational effects associated with C-F bonds.

d. <u>Specific intermolecular interactions</u>

Carbon-fluorine bonds are highly polarized, and due to the high electronegativity of fluorine, the lone electron pairs are highly attached to the nucleus. This fact determines the low polarizability of fluorine and, therefore, the C-F bond is highly non-polarizable, and fluorine can only participate in weak hydrogen bonding interactions. The formation of intermolecular hydrogen bonds with fluorine as acceptor is possible in environments shielded from water and without the competition of other acceptors.[56] It has been found that C(sp^3)-fluorine is better hydrogen bond acceptor than C(sp^2)-fluorine, and the C(sp^3)-F···H-O bond is much weaker than the corresponding C=O···H-O bond (**Table 1.3**).

[56] Dalvit, C.; Invernizzi, C.; Vulpetti, A. *Chem. Eur. J.* **2014**, *20*, 11058-11068.

Table 1.3. Energy of different hydrogen bond interactions.[57]

Hydrogen bond	Energy (kcal/mol)
$C(sp^3)$ –F···H–O	2.0 - 3.2
$C(sp^2)$ –F···H–O	1.48
C=O···H–O	5 - 10

Some examples of other fluorine interactions, such as C-F····C=O dipolar interactions have been observed in some thrombin inhibitors,[58] and short C-F···Metal interactions, which allow the activation of these bonds.[59]

e. Binding affinity and selectivity

The change of properties associated with the substitution of C-H and C-O bonds with fluorine, have placed this strategy as a common practice in drug development. Properties associated with this substitution are well known, however, understanding how fluorine affects to the binding affinity and selectivity to target molecules has been a challenge for many years. Recently, experimental, and computational studies have allowed having a better understanding on these phenomena.[60] It is essential to understand how fluorine substitution affects to the electronic nature and conformation of small molecules, to predict the interaction of fluorinated molecules with proteins and enzymes. It has been established that some enzymes bind preferably the fluorinated analogue of a natural compound, and fluorine participates in electrostatic interactions and contributes to enhance the binding affinity to the active site. The interaction between a fluorine atom and a protein can occur directly, can be bridged by a sphere of solvation or by alterations of the molecule configuration.[45]

[57] Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. *T. Tetrahedron*, **1996**, *52*, 12613-12622.
[58] Olsen, J. A.; Banner, D. W.; Seiler, P.; Obst Sander, U.; D'Arcy, A.; Stihle, M.; Müller, K.; Diederich, F. *Angew. Chem. Int. Ed.* **2003**, *42*, 2507-2511.
[59] Kiplinger, J. L.; Richmond, T. G.; Osterberg, C. E. *Chem. Rev.* **1994**, *94*, 373-431.
[60] Muller, K.; Faeh, C.; Diederich, F. *Science* **2007**, *317*, 1881-1886.

1.2.3. Applications and properties of organofluorine compounds

Organofluorine compounds have experimented a huge progress in many fields of modern chemistry, such as, new materials, pharmaceuticals, agrochemicals, and fine chemicals (**Figure 1.10**). The C-F bond is the fundamental unit in organofluorine chemistry, and its key aspects are the basis on interpreting the behaviour of organofluorine compounds.

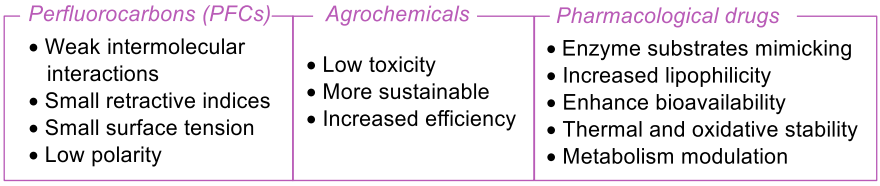


Figure 1.10: Important effects of fluorine in pharmaceuticals and materials.

The development of new fluorination methodologies and protocols have provided access to a variety of new fluorinated motifs.[47,61] In this regard, fluorinated groups have been widely used as bioisosteres. Fluorine has found application as a substitute for lone pairs of electrons, hydrogen atoms and methyl groups. Moreover, it has found application in the mimicking of the carbonyl, carbinol and nitrile functional groups (**Figure 1.11**).[62]

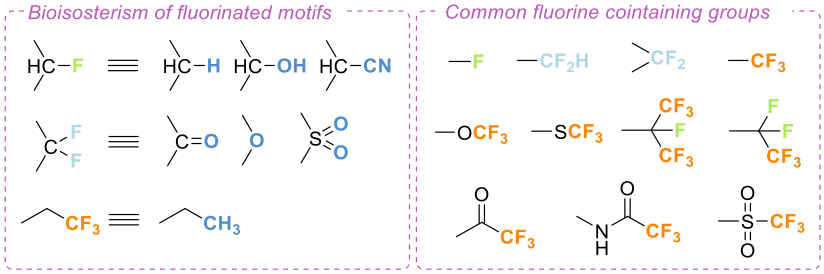


Figure 1.11. Examples of bioisosterism of fluorinated motifs and examples of R_F groups commonly used in medicinal chemistry and agrochemistry.[63]

[61] a) Politanskaya L.V. *et al. Russ. Chem. Rev.* **2019**, *88*, 425-569, b) Ojima, I. *J. Org. Chem.* **2013**, *78*, 6358-6383.
[62] Meanwell, N. A. *J. Med. Chem.* **2018**, *61*, 5822-5880.
[63] Ogawa, Y.; Tokunaga, E.; Kobayashi, O.; Hirai, K.; Shibata, N. *iScience*, **2020**, *23*, 101467.

1.2.4. ^{19}F-NMR probes

^{19}F NMR is considered an invaluable tool due to the unusual combination of properties of fluorinated compounds and the ^{19}F nuclei. The ^{19}F nuclei has 100 % natural abundance and a resonant frequency of 94% of ^{1}H nucleus, thus, this isotope is highly sensitive in NMR measurements. The nuclear spin quantum number of fluorine is ½ and the coupling of fluorine with close ^{1}H and ^{13}C is very similar of the hydrogen couplings. Coupling constants of fluorine with other active nuclei are very variable in magnitude and long range coupling constants between fluorine and other nucleus are normally higher than the ones observed for ^{1}H and ^{13}C, which is very useful for obtaining extensive connectivity information.[64] The chemical shift range of organofluorine compounds is larger than the ^{1}H range (0 to 10 ppm for ^{1}H NMR and >350 ppm for ^{19}F NMR), thus, resonances of different fluorine nuclei are usually well separated, and the spectra obtained are normally of first order. The shielding of ^{19}F varies quite widely, thus, chemical shifts are very sensitive to the environment of the fluorine atom (**Figure 1.12**).[65]

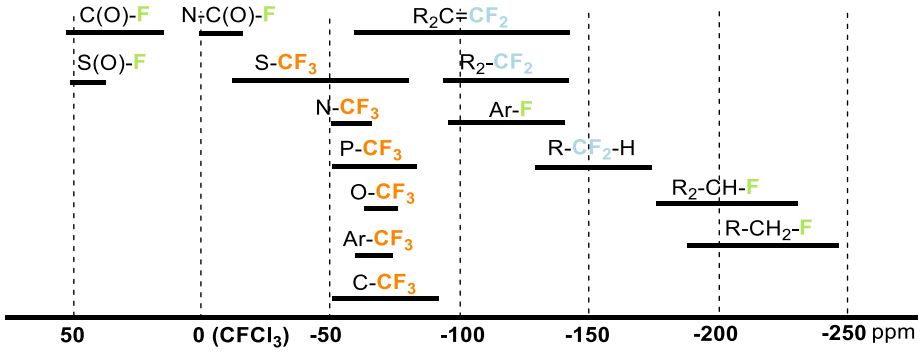


Figure 1.12. Representative chemical shifts of ^{19}F compounds and substituents considering fluorotrichloromethane ($CFCl_3$ = 0 ppm) as internal reference.

The interesting pharmacokinetic properties of fluorine coupled with its absence in natural compounds, makes this atom a highly appreciated motif in

[64] Dolbier, W. R. *Guide to fluorine NMR for organic chemists*. John Wiley & Sons, **2016**.
[65] Brey, W. S.; Brey, M. L. *Fluorine-19 NMR*. eMagRes, **2007.**

the study of biological systems. Additionally, the high sensitivity of fluorine in NMR can provide additional structural and conformational information. Some of the most important applications of ^{19}F NMR are shown in **Figure 1.13**,[66] and these include: drug synthesis and discovery,[67] synthesis and characterization of small molecules,[68] creation of fluorinated fragment libraries,[69] fluorine-based biochemical screening experiments (n-FABS),[70] monitoring of the interactions between fluorinated ligands and proteins,[71] metabolic studies,[66] determination of small molecule-protein interactions,[72] and monitor the interactions and conformations of proteins and nucleic acids.[73]

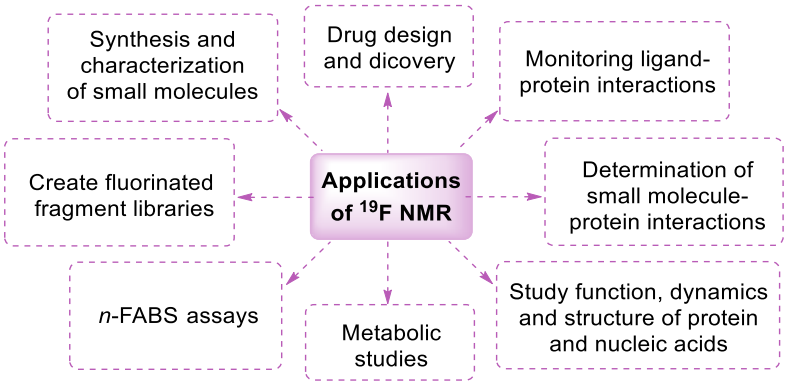


Figure 1.13. Applications of ^{19}F NMR in chemical synthesis and biology.

[66] a) Cobb, S. L.; Murphy, C. D. *J. Fluor. Chem.* **2009**, *130*, 132-143; b) Yu, J. X.; Hallac, R. R.; Chiguru, S.; Mason, R. P. *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *70*, 25.
[67] Dalvit, C.; Vulpetti, A. *J. Med. Chem.* **2019**, *62*, 2218-2244.
[68] Howe, P. W. *Prog. Nucl. Magn. Reson. Spectrosc.* **2020**, *118*, 1-9.
[69] Vulpetti, A.; Dalvit, C. *ChemMedChem* **2013**, *8*, 2057-2069.
[70] a) Dalvit, C.; Veronesi, M.; Vulpetti, A. *J. Biomol. NMR*, **2020**, 74, 613-631; b) Dalvit, C. *Fluorine NMR spectroscopy for biochemical screening in drug discovery* in *NMR of Biomolecules: Towards Mechanistic Systems Biology*. Ed. Bertini, I.; McGreevy, K. S.; Parigi, G. p 314, **2012**.
[71] a) Calle, L. P.; Echeverria, B.; Franconetti, A.; Serna, S.; Fernández-Alonso, M. C.; Diercks, T.; Cañada, F. J.; Ardá, A.; Reichardt N. C.; Jiménez-Barbero, J. *Chem. Eur. J.* **2015**, *21*, 11408-11416; b) Dalvit, C.; Vulpetti, A. *ChemMedChem* **2011**, *6*, 104-114.
[72] Arntson, K. E.; Pomerantz, W. C. *J. Med. Chem.* **2016**, *59*, 5158-5171.
[73] Boeszoermenyi, A.; Ogórek, B.; Jain, A.; Arthanari, H.; Wagner, G. *J. Biomol. NMR*, **2020,** *74*, 365-379.

1.3. IMPORTANCE OF FLUOROSUGARS

Glycomimetics are small molecules designed to overcome the principal drawbacks of carbohydrates, which are related to their poor pharmacokinetic properties and their difficult synthesis. One of the most common alterations consists in the introduction of one or more fluorine atoms, and its strategic incorporation allow to modulate the physicochemical and ADMET properties of the molecule. **Figure 1.14** shows different approaches of fluorinated sugars and their bioisosteres.

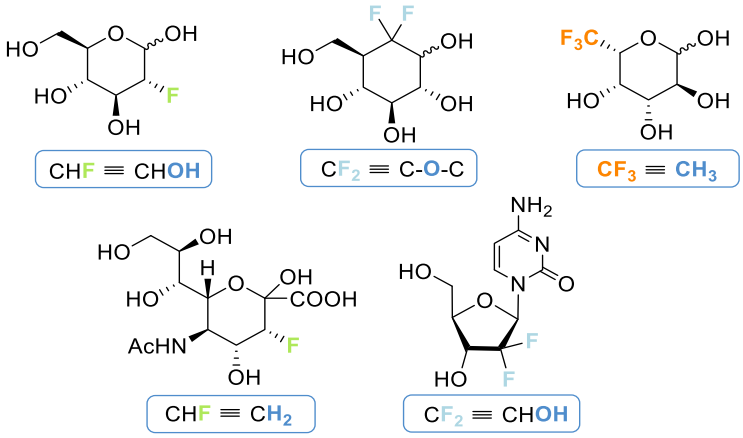


Figure 1.14. Fluorinated sugars with different fluorination motifs and bioisosteres.[74]

1.3.1. Synthesis of fluorinated sugars

Synthetic protocols for the introduction of fluorine and fluorinated motifs into sugars have been extensively developed during past decades.[75] These methodologies can be divided in three main synthetic strategies: 1) "de novo" synthesis involving the construction of the carbohydrate scaffold; 2) nucleophilic fluorination; and 3) electrophilic fluorination sugars (**Figure 1.15**).

[74] Linclau, B.; Ardá, A.; Reichardt, N. C.; Sollogoub, M.; Unione, L.; Vincent, S. P.; Jiménez-Barbero, J. *Chem. Soc. Rev.* **2020**, *49,* 3863-3888.

[75] a) Hein, M.; Miethchen, R. *Advances in Organic Synthesis: Modern Organofluorine Chemistry-Synthetic Aspects;* Atta-ur-Rahman, Ed.; Bentham Sci. Pub. Ltd.: Sharjah, vol. 2, p 381, **2006**; b) Cheng, Y.; Guo, A. L.; Guo, D. S. *Curr. Org. Chem.* **2010**, *14*, 977-999; c) Miethchen, R. *J. Fluor. Chem.* **2004**, *125*, 895-901.

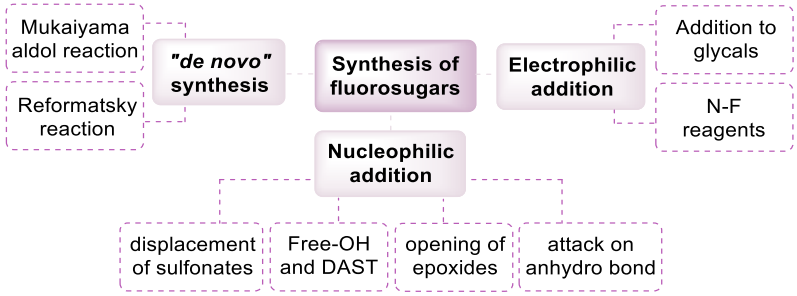


Figure 1.15. Main synthetic strategies for the synthesis of fluorinated sugars.[76]

1.3.2. Properties of fluorinated sugars

Deoxyfluorination of sugars can affect to the molecular interactions, conformation, and properties of the sugar. Among the desired properties, the most important are the stabilization of the glycosidic bond and the increase of the lipophilicity. Further discussion of the effect of fluorine substitution on sugar properties will be analysed in Chapter V.

a. Glycosidic bond stability

Glycosidic bonds are easily hydrolysed under mild acidic conditions or by the activity of the glycosidases, which entails a problem in the development of efficient drugs. An efficient way to increase glycosidic bond stability is to introduce a fluorine atom (or other EWG) adjacent to the anomeric carbon. It destabilizes the formation of oxocarbenium intermediate due to the stronger inductive effect of fluorine in comparison with a hydroxyl group.[77] Fluorine has been used to inactivate glycosidases by forming stable intermediates[78] and to increase the metabolic stability of drugs.[79]

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[76] Delbrouck, J.; Chêne L.; Vincent, S. P. *Fluorosugars as inhibitors of bacterial enzymes*, in *Fluorine in Life Sciences: Pharmaceuticals, Medical Diagnostics, and Agrochemicals*. Ed. Haufe G. and Leroux, F. Academic Press, Elsevier, p. 241, **2018**.

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b. Increased lipophilicity

The lipophilicity value is widely used in medicinal chemistry as a measure of the membrane permeability, and it also affects to the affinity to proteins. Carbohydrates are highly hydrophilic and have low lipophilicity values, which are far from the optimal values for membrane permeability (log *P* values between 1 to 3). The substitution of hydroxyl groups by fluorine induces a great increase of the lipophilicity, and the introduction of various fluorine atoms have proved to increase sugar's lipophilicity close to optimal values.[80]

1.3.3. Applications of fluorosugars

Fluorinated carbohydrates are used for different and diverse purposes including antiviral and antitumoral agents,[74] mechanistic and structural probes of enzymes, lectin ligands, diagnostic agents and as building blocks to construct fluorinated glycoconjugates including glycopeptides[81] and glycoproteins.[82]

a. Therapeutic drugs

Due to the important roles that carbohydrates play in biological systems; fluorinated sugars have been considered valuable targets for developing therapeutic agents. Fluorosugars have been used as antiviral and antitumoral agents[83] and one example are fluorinated analogues of

[80] Linclau, B.; Wang, Z.; Compain, G.; Paumelle, V.; Fontenelle, C. Q.; Wells, N.; Weymouth-Wilson, A. *Angew. Chem. Int. Ed.* **2016**, *55*, 674-678.

[81] a) Johannes, M.; Reindl, M.; Gerlitzki, B.; Schmitt, E.; Hoffmann-Röder, A. *Beilstein J. Org. Chem*. **2015**, *11*, 155-161; b) Lamandé-Langle, S.; Collet, C.; Hensienne, R.; Vala, C.; Chrétien, F.; Chapleur, Y.; Mohamadi, A.; Lacolley, P.; Regnault, V. *Bioorg. Med. Chem*. **2014**, *22*, 6672-6683; c) Lang, C.; Maschauer, S.; Hübner, H.; Gmeiner, P.; Prante, O. *J. Med. Chem*. **2013**, *56*, 9361.

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[83] a) Parrish, J. P.; Lee, S. K.; Boojamra, C. G.; Hui, H.; Babusis, D.; Brown, B.; Shih, I.; Feng, J. Y.; Ray, A. S.; Mackman. R. L. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 3354-3357. b) Horton, D.; Khare, A. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 6179-6181; c) McGuigan, C.; Derudas, M.; Quintiliani, M.; Andrei, G.; Snoeck, R.; Henson, G.; Balzarini, J. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 6264-6267;

nucleosides. These drugs interact with DNA and RNA and are used in the treatment of cancer and antiviral diseases. Fluorine can have different effects in the activity of the drug; the substrate can turn into an inhibitor of a critical enzyme, as in *5-fluorouracil*, it can protect the nucleosidic bond from the hydrolytic cleavage (2-fluoro-nucleoside) and improve the stability of the drug, as *clofarabine,* or it can enable new modes of enzymatic inhibition, as in *trifluridine* (**Figure 1.16**).[84]

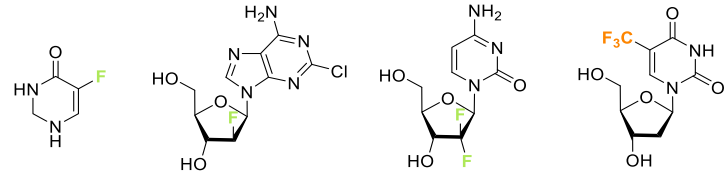

Figure 1.16. Examples of fluorinated drugs including carbohydrate-based drugs.

Fluorinated sugars have been introduced into synthetic antitumor vaccines and therapeutic antibodies to overcome the hydrolysis of the native sugars by enzymes. In addition, the incorporation of fluorine has proved to not affect to the immunogenicity and structure-selectivity of the vaccines[85] and modified antibodies show the same binding ability than the native parents.[86] These facts have aroused the use of fluorinated analogues of tumor-associated carbohydrate antigen (TACA) for the design of synthetic vaccines, and they open new opportunities for the development of therapeutic TACAs with improved pharmacokinetic parameters.[87] Remarkable investigations in the use of fluorinated TACAs were carried out by Hoffmann-Röder and co-workers, who

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d) Lavaire, S.; Plantier, R. R.; Portella, C.; Monte, M.; Kirn, A.; Aubertin A. M. *Nucleosides Nucleotides* **1998**, *17*, 2267-2280.

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[85] a) Hoffmann-Röder, A.; Kaiser, A.; Wagner, S.; Gaidzik, N.; Kowalczyk, D.; Westerlind, U.; Gerlitzki, B.; Schmitt, E.; Kunz, H. *Angew. Chem. Int. Ed.* **2010**, *49*, 8498-8503; b) Wagner, S.; Mersch, C.; Hoffmann-Röder, A. *Chem. Eur. J.* **2010**, *16*, 7319-7330.

[86] Lo, H. J.; Krasnova, L.; Dey, S.; Cheng, T.; Liu, H.; Tsai, T. I.; Wu, K. B.; Wu, C-Y.; Wong, C. H. *J. Am. Chem. Soc*. **2019**, *141*, 6484-6488.

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studied the properties of fluorinated TF and Tn antigens,[88] and some of them generated a very strong immune response in mice.[85]

b. Enzyme substrates

Many enzymes use sugars as substrates and sugar mimetics have been widely used to study the mechanism, structure, and interactions of enzymes. To better understand how enzymes process sugars and the interactions ocurring, sugar substrates are often modified and the subsequent changes in enzyme function recorded. Incorporation of fluorine into sugar analogues provides a strong electron-withdrawing effect which is expected to alter the enzymatic mechanism. Fluorinated sugars have been used as enzyme inhibitors,[89] in recognition processes,[90] as mechanistic proves,[91] and in evaluation of the catalytic activity of enzymes.[92]

Regarding inhibitors, fluorinated sugars have demonstrated to block glycosidases (GHs) and glycosyltransferases (GTs), that catalyse the break and creation of glycosidic bonds in living organisms. These enzymes are involved in crucial biochemical transformations, such as, cell signalling, recognition processes and protein maturation. Particularly important is the work of Withers and co-workers,[77,78] who extensively studied the use of fluorinated sugars as mechanism-based inhibitors of glycosidases (**Figure 1.17**, **A**). Fluorinated sialic acid glycomimetic (**Figure 1.17**, **B**) has shown to block sialyltransferases. Since aberrant high sialic acid expression in cancer cells favour cancer metastasis, interference with sialic acid expression could be of crucial importance in

[88] a) Platen, T.; Schüler, T.; Tremel, W.; Hoffmann-Röder, A. *Eur. J. Org. Chem.* **2011**, 3878-3887; b) Oberbillig, T.; Mersch, C.; Wagner, S.; Hoffmann-Röder, A. *Chem. Commun.* **2012**, *48*, 1487-1489; c) Baumann, A.; Marchner, S.; Daum, M.; Hoffmann-Röder, A. *Eur. J. Org. Chem.* **2018**, *2018,* 3803-3815.

[89] André, S.; Cañada, F. J.; Shiao, T. C.; Largartera, L.; Diercks, T.; Bergeron-Brlek, M.; el Biari, K.; Papadopoulos, A.; Ribeiro, J. P.; Touaibia, M.; Solis, D. Menéndez, M.; Jiménez-Barbero, J.; Roy, R.; Gabius, H. J. *Eur. J. Org. Chem.* **2012**, *23,* 4354-4364.

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preventing it.[93] Fucosatin I (**Figure 1.17**, **C**) has proved to be a potent metabolic inhibitor of cellular protein fucosylation.[94] Moreover, nucleoside diphospho (NDP)-fluoro sugars, which are analogues of donor substrates of GTs, are one of the most general classes of GT inhibitors (**Figure 1.17**, **D**).[95]

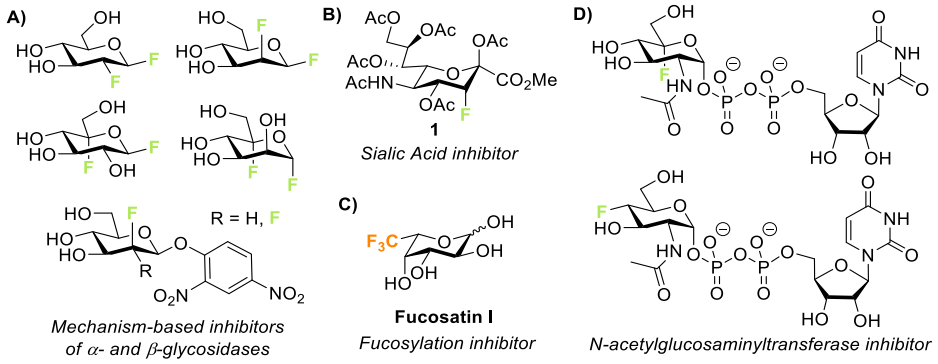

Figure 1.17. Examples of fluorinated sugars as inhibitors of enzymes.

c. Lectin ligands and NMR probes

Fluorinated sugars have been extensively used to study lectin-glycan interactions, in which fluorine can act as a mere analytical probe or as a method to enhance protein interaction. One of the major drawbacks of native sugars is the usually poor affinity towards the protein receptor, which makes it difficult to measure. The strategic design of fluorinated ligands coupled with ^{19}F NMR spectroscopy allows the easy and fast determination of glycan binding, and they can be used in the identification of structural and electronic

[93] a) Büll, C.; Boltje, T. J.; van Dinther, E. A.; Peters, T.; de Graaf, A. M.; Leusen, J. H.; Kreutz, M.; Figdor, C. G.; den Brok, M. H.; Adema, G. J. *ACS Chem. Biol.* **2015**, *10*, 2352-2363; b) Macauley, M. S.; Arlian, B. M.; Rillahan, C. D.; Pang, P. C.; Bortell, N.; Marcondes, M. C. G.; Haslam, S. M.; Dell, A.; Paulson, J. C. *J. Biol. Chem.* **2014**, *289,* 35149-35158; d) Heise, T.; Pijnenborg, J. F.; Büll, C.; van Hilten, N.; Kers-Rebel, E. D.; Balneger, N.; Elferink, H.; Adema, G. J.; Boltje, T. J. *J. Med. Chem.* **2019**, *62*, 1014-1021.

[94] Allen, J. G.; Mujacic, M.; Frohn, M. J.; Pickrell, A. J.; Kodama, P.; Bagal, D.; San Miguel, T.; Sickmier, E. A.; Osgood, S.; Swietlow, A.; Li, V.; Jordan, J. B.; Kim, K.-W.; Rousseau, A.-M. C.; Kim, Y.-J.; Caille, S.; Achmatowicz, M.; Thiel, O.; Fotsch, C. H.; Reddy, P.; McCarter, J. D. *ACS Chem. Biol.* **2016**, *11*, 2734-2743.

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perturbations that occur in the interaction with the protein.[96] Goldstein and co-workers introduced the use of fluorinated sugars as lectin ligands in hapten inhibition assays, as tools to determine the nature of the lectin-glycan interactions and the sugar-binding specificity (**Figure 1.18, A**).[97] Moreover, fluorinated sugars were used to study the nature of hydrogen bonding (**Figure 1.18, B**).[98] Some years later, Jiménez-Barbero, Gabius, and others, incorporated the use of ^{19}F NMR spectroscopy, which allows an easy and fast determination or lectin-glycan interactions by the change of chemical shifts. Recently, Diercks and co-workers developed a ^{19}F-STD NMR technique,[99] which presented an improved sensitivity in the measurements of the binding affinity. These experiments allow the accurate determination of the binding mode of sugars to the protein. For example, the affinity of fluorinated mannose oligosaccharides to Cyanovirin-N was probed by STD-NMR (**Figure 1.18, C**).[100]

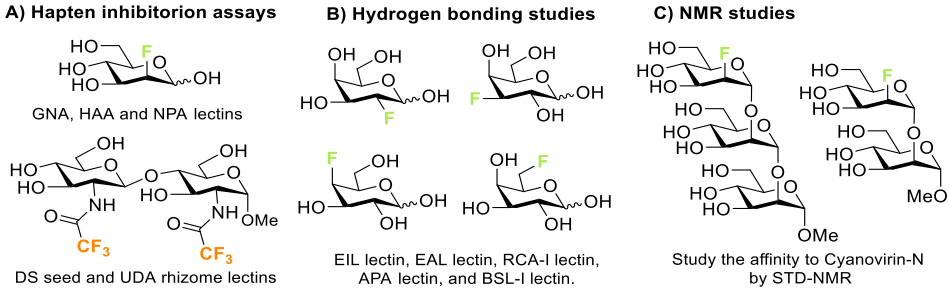

Figure 1.18. Examples of fluorinated sugars used as lectin ligands.

[96] Allman, S. A.; Jensen, H. H.; Vijayakrishnan, B.; Garnett, J. A.; Leon, E.; Liu, Y.; Anthony, D. C.; Subson, N. R.; Feizi, T.; Matthews, S.; Davis, B. G. *ChemBioChem*, **2009,** *10*, 2522-2529.

[97] a) Shibuya, N.; Goldstein, I. J.; Van Damme, E. J.; Peumans, W. J. *J. Biol. Chem.* **1988,** *263*, 728-734; b) Kaku, H.; Van Damme, E. J.; Peumans, W. J.; Goldstein, I. J. *Arch. Biochem. Biophys.* **1990,** *279*, 298-304; c) Crowley, J. F.; Goldstein, I. J.; Arnarp, J.; Lonngren, J. *Arch. Biochem. Biophys.* **1984**, *231*, 524-533; d) Shibuya, N.; Goldstein, I. J.; Shafer, J. A.; Peumans, W. J.; Broekaert, W. F. *Arch. Biochem. Biophys.* **1986**, *249,* 215-224.

[98] Bhattacharyya, L.; Brewer, C. F. *Eur. J. Bochem.* **1988**, *176*, 207-212.

[99] a) Diercks, T.; Ribeiro, J. P.; Cañada, F. J.; André, S.; Jiménez-Barbero, J.; Gabius, H. J. *Chem. Eur. J.* **2009**, *15,* 5666-5668; b) Ribeiro, J. P.; Diercks, T.; Jiménez-Barbero, J.; André, S.; Gabius, H. J.; Cañada, F. J. *Biomolecules*, **2015**, *5*, 3177-3192; c) Diercks, T.; Infantino, A. S.; Unione, L.; Jiménez-Barbero, J.; Oscarson, S.; Gabius, H. J. *Chem. Eur. J.* **2018**, *24,* 15761-15765.

[100] Matei, E.; André, S.; Glinschert, A.; Infantino, A. S.; Oscarson, S.; Gabius, H. J.; Gronenborn, A. M. *Chem. Eur. J.* **2013**, *19*, 5364-5374.

d. Diagnostic agents

The absence of fluorine in biological systems makes this atom attractive to visualize biological phenomena. ^{18}F and ^{19}F isotopes have been used as bioanalytical probes to study and detect fluorinated carbohydrates in a variety of applications and complex matrices. The most used techniques to detect fluorinated carbohydrates are ^{19}F-NMR, ^{19}F-MRI (^{19}F magnetic resonance imaging), and PET (Positron emission tomography).[101] PET imaging has benefitted from the relatively long nuclear half-life of ^{18}F ($t_{1/2}$ = 109.8 min),[102] and 2-deoxy-2-[^{18}F]fluoroglucose (^{18}FDG) is the most widely used radiopharmaceutical for noninvasive imaging. Its usefulness derives from the abnormally increased glucose uptake of cancer cells, which results in an accumulation of the radiotracer.[103] Moreover, ^{19}F MRI has already been applied to cell tracking, molecular imaging of tumour biomarkers in preclinical models, and oxygen sensing,[104] although the low sensitivity of MRI compared to PET, has retarded their application to clinical studies.

[101] Arntson, K. E.; Pomerantz, W. C. *J. Med. Chem.* **2016**, *59*, 5158-5171.

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[103] a) Baschnagel, A. M.; Wobb, J. L.; Dilworth, J. T.; Williams, L.; Eskandari, M.; Wu, D.; Barbara L. Pruetz, B. L.; Wilson, G. D. *Radiother. Oncol.* **2015,** *117*, 118-124; b) Nensa, F.; Tezgah, E.; Poeppel, T. D.; Jensen, C. J.; Schelhorn, J.; Kohler, J.; Heusch, P.; Bruder, O.; Schlosser, T. Nassenstein, K. J. *Nucl. Med.* **2015**, *56*, 255-260.

[104] a) Ahrens, E. T.; Zhong, J. *NMR Biomed.* **2013**, *26*, 860-871; b) Ruiz-Cabello, J.; Barnett, B. P.; Bottomley, P. A.; Bulte, J. W. M. *NMR Biomed.* **2011**, *24*, 114-129; c) Mignion, L.; Magat, J.; Schakman, O.; Marbaix, E.; Gallez, B.; Jordan, B. F. *Magn. Reson. Med.* **2013**, *69*, 248-254.

CHAPTER II

GENERAL OBJECTIVES

Chapter II

GENERAL OBJECTIVES

The present doctoral thesis aims to develop new synthetic methodologies for the stereoselective preparation of fluorinated sugars, to study their key physicochemical properties and to apply the methodologies disclosed herein in the stereoselective synthesis of biologically relevant fluorinated glycosyl derivates. Results obtained during the doctoral studies have been divided into five chapters and are summarize in **Figure 2.1**.

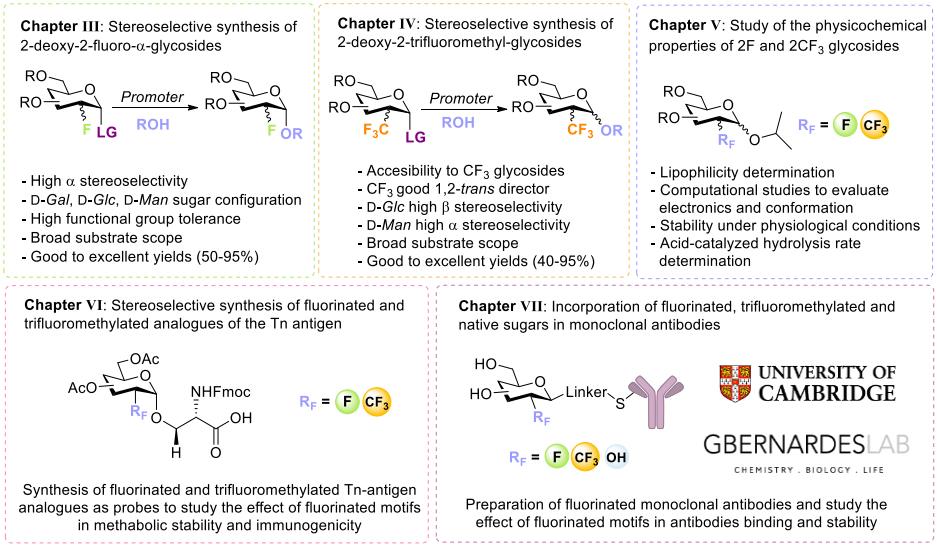

Figure 2.1. Graphical summary of the chapters and main goals in this doctoral thesis.

Chapter III: The main objective of this chapter is to develop a glycosylation method for the stereoselective synthesis of 2-deoxy-2-fluoro-α-glycosides. For this purpose, microwave-assisted heating will be evaluated to reduce reaction times and avoid the formation of by-products. In addition, the effect on glycosylation stereoselectivity and yield of the following aspects will be also evaluated:

i. Screening of different activators, solvents, and temperatures to achieve high yields and α-stereoselectivity in the glycosylation step.
ii. The effect of glycosyl donor configuration, protecting groups and leaving groups on glycosylation stereoselectivity.

iii. The effect of acceptor size and nucleophilicity on glycosylation stereoselectivity.
iv. ^{19}F and ^{119}Sn NMR studies to help to understand the mechanism and stereocontrol outcome of the glycosylation reaction.

Chapter IV: The primary objective of this chapter is to access to 2-deoxy-2-trifluoromehtyl glycosides from cheap and commercially available glycals. Once we have accessed to CF_3-glycosides, we also aim to:

i. Study their utility as glycosyl donors in the glycosylation reaction and to evaluate the directing group effect of the CF_3 group.
ii. Compare the stereodirecting effect of the trifluoromethyl with that of fluorine.
iii. Study the effect of glycosyl donor configuration, protecting groups, and acceptor size and nucleophilicity in the glycosylation stereoselectivity.
iv. Demonstrate the synthetic utility of our method for the stereoselective synthesis of 2-CF_3-modified natural glycoside analogues, including disaccharides, steroidal aglycones, amino acids and sphingosine/phytosphingosine analogs.

Chapter V: The aim of this chapter is to comparatively determine some physicochemical properties of the fluorinated sugars synthetized in Chapter III and the trifluoromethylated sugars synthetized in Chapter VI. For this purpose, the following studies will be performed:

i. Computational and experimental studies to evaluate the electronic and steric changes induced by trifluoromethyl and fluorine groups.
ii. Experimental determination of the lipophilicity of the fluorinated glycosides following a ^{19}F NMR protocol.
iii. Stability probes of the glycosidic linkage under mimic physiological conditions.
iv. Study their resistance against acid-catalyzed hydrolysis and determine the hydrolysis rate and half-life times.

Chapter VI: The aim of this chapter is to apply the methodologies described in Chapter III and IV for the stereoselective synthesis of fluorinated and trifluoromethylated analogues of the Tn antigen as interesting tumor-associated carbohydrate antigens (TACAs) with the aim of studying in the future the effect of the fluorinated group in their metabolic stability and immunogenicity.

Chapter VII: The last chapter of this doctoral thesis describes the research work carried out in a predoctoral stay in the G. Bernardes Lab at the University of Cambridge under the supervision of Prof. Gonçalo J. L. Bernardes. The aim of this chapter is to study the effect of the introduction of different fluorinated sugars on the properties of modified antibodies. For this purpose, the following aspects will be studied:

i. The reparation of fluorinated and trifluoromethylated deprotected glycosyl azides with different sugar configurations.
ii. The study the bioconjugation kinetics using different strained alkynes and selection of the best performer.
iii. The optimization of the bioconjugation and strain-promoted azide-alkyne cycloaddition reactions using antibodies.
iv. The modification of the antibodies and extension of the scope with the synthetized native, fluorinated and trifluoromethylated sugars.
v. The study the specificity of the modified antibodies to the protein receptors by flow cytometry.
vi. The study the specific binding and localization on cells by confocal microscopy.

Chapter II

CHAPTER III

STEREOSELECTIVE MICROWAVE-ASSISTED SYNTHESIS OF 2-DEOXY-2-FLUORO-α-GLYCOSIDES

Chapter III

3.1. INTRODUCTION

Carbohydrate derivatives have found application in the pharmaceutical industry to produce antibacterial, antiviral, antitumor agents, and vaccine components, between others. Among them, 2-deoxy-2-fluoroglycosides are compounds of increasing importance in biochemistry and medicinal research, especially as antiviral agents, cancer diagnosis probes and labelling substrates for biological studies.[1] Replacement of a C-H or C-O bonds by a C-F bond in biologically active compounds drastically perturbs the chemical, physical, and biological properties. Fluorine substitution confers some desired properties, such as, higher metabolic stability, increased lipophilicity and membrane permeability, and often, enhanced binding to target molecules.[2]

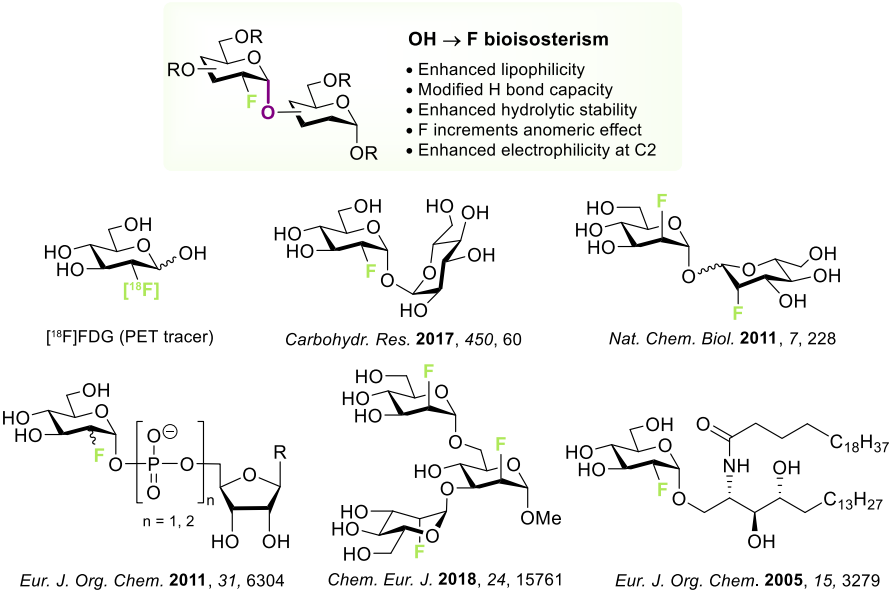

Figure 3.1. Top: Molecular editing at C2 and fluorine effects. Bottom: Selected examples of biomolecules containing 2-deoxy-2-fluoro-glycosides.

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[2] Smart, B. E. *J. Fluorine Chem.* **2001**, *109,* 3-11.

Fluorination can also be exploited to influence the conformation of organic molecules, by electrostatic/dipole interactions, and electronic interactions with neighbour groups or lone pairs.[3] The low steric demand of fluorine coupled with the high strength of the C-F bond (and consequent reactive inertness), and the enhanced hydrolytic stability of the glycosidic bond, render the starting 2-fluoro-2-deoxy sugars excellent bioisosteres of 2-deoxy and 2-OH sugars with improved pharmacokinetic properties (**Figure 3.1**).[4] Fluorinated sugars have been widely used to mimic natural oligosaccharides.[5] Withers and co-workers found 2-fluoro-glycosides particularly useful in the determination of the molecular mechanism of glycosidases and glycosyltransferases by trapping a glycosyl-enzyme intermediate.[6] In other approaches, 2-fluoro-glycosides have been used in the construction of fluoroglycopeptides and glycoproteins,[7] glycosphingolipids for immunostimulant studies,[8] and fluorinated oligosaccharides for adhesion probes,[9] among others. The increasing interest in these motifs in chemical biology requires the development of efficient and stereoselective methods for the construction of 2-deoxy-2-fluoroglycosides. However, as it has been introduced in Chapter I, controlling the stereoselectivity of the glycosylation reaction remains one of the most challenging areas in organic chemistry.[10]

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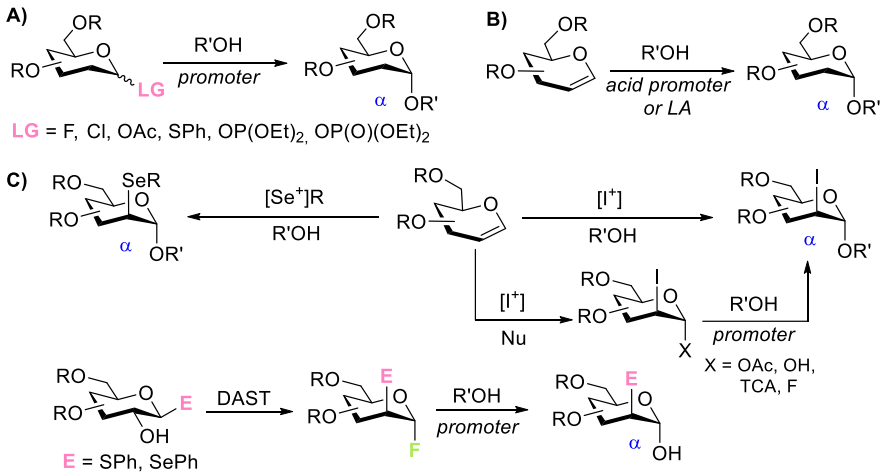
[9] Allman, S. A.; Jensen, H. H.; Vijayakrishnan, B.; Garnett, J. A.; Leon, E.; Liu, Y.; Anthony, D. C.; Sibson, N. R.; Feizi, T.; Matthews, S.; Davis, B. G. *ChemBioChem* **2009**, *10*, 2522-2529.

[10] Benito-Alifonso, D.; Galan, M. C. *Selective Glycosylation: Synthetic methods and Catalysis* (Ed.: C.S. Bennet), Wiley-VCH, Weinheim, **2017**, 155-172.

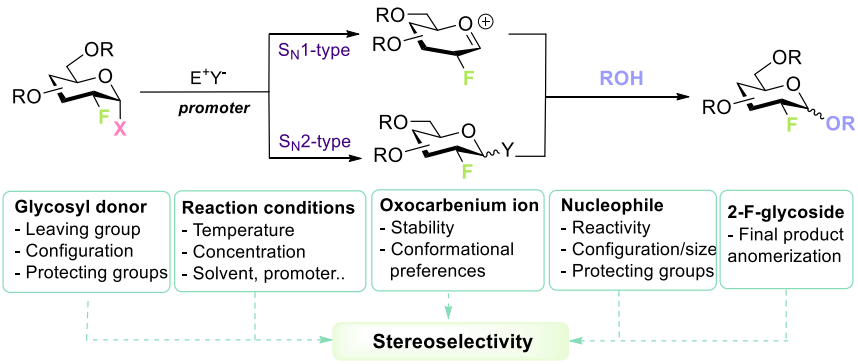
In the absence of C-2 participating groups, glycosylation stereoselectivity is often governed by the anomeric effect.[11] Moreover, there are other strategies that achieve the stereoselective synthesis of 2-deoxy-α-glycosides.[12] One example is the use of glycosyl donors that produce mainly α-glycosides (**Scheme 3.1, A**). 2-deoxy-α glycosides can also be obtained directly from glycals, whose activation, using an acid or metallic promoter, gives an oxocarbenium ion that reacts with the nucleophile (**Scheme 3.1, B**). However, the 2-deoxyglycoside linkage is labile and usually not stable under the acidic conditions of the glycosylation step. Another strategy to override the limited stability and the stereoselective control over the glycosylation event exploits the use of different halogens[13] and chalcogens[14] as temporary directing groups which, upon cleavage, uncover the desired 2-deoxyglycosides (**Scheme 3.1, C**).



Scheme 3.1. Common approaches for the synthesis of 2-deoxy-glycosides.

[11] Hou, D.; Lowary, T. L. *Carbohydr. Res.* **2009**, *344*, 1911-1940.
[12] Meng, S., Li, X., Zhu, J. *Tetrahedron*, **2021**, *88*, 132140.
[13] a) Mestre, J.; Matheu, M. I.; Díaz, Y.; Castillón, S.; Boutureira, O. *J. Org. Chem.* **2017**, *82*, 3327-3333; b) Kimura, T.; Takahashi, D.; Toshima, K. *J. Org. Chem.* **2015**, *80*, 9552-9562; c) Glinschert, A.; Oscarson. S. *Carbohydr. Res*. **2015**, *414*, 65-71; d) Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656-6660.
[14] a) Nicolaou, K. C.; Ladduwahetty, T.; Randall, J. L.; Chucholowski, A. *J. Am. Chem. Soc.* **1986**, *108*, 2466-2467; b) Nicolaou, K. C.; Mitchell, H. J.; Fylaktakidou, K. C.; Suzuki, H.; Rodríguez, R. M. *Angew. Chem. Int. Ed.* **2000**, *39*, 1089-1093; c) Sau, A.; Misra. A. K. *Carbohydr. Res.* **2012**, *361*, 41-48.

As generality, glycosylation reaction can follow two main pathways, either S_N1- or S_N2-type mechanism, and the weight and prevalence of each mechanism determines the stereoselective outcome (**Scheme 3.2**). Additionally, stereoselectivity is largely dependent on several factors, including donor and acceptor nature (configuration, protecting and leaving groups), and reaction conditions (promoters, solvents, counter ions, reactant concentrations, temperature) (**Scheme 3.2**).[15] Considering this, the electronics of the glycosyl donor can be manipulated to control the stereoselectivity and reactivity of the glycosylation event, by modifying factors such as the electron-withdrawing ability of the protecting groups, the lability of the anomeric leaving group and the conformation of the sugar. Accordingly, reaction conditions can also be tuned to control the outcome of the glycosylation reaction. These changes impact on the stereoselectivity and allow the study and control of the stereochemical outcome to obtain high α selectivity.



Scheme 3.2. General S_N1- or S_N2-type pathways and factors that influence in the stereoselectivity.

Woerpel and co-workers presented an important validation of the use of electrostatic stabilisation in stereoselective glycosylation by controlling

[15] a) Yasomanee, J. P.; Demchenko, A. V. *Trend. Glycosci. Glycotechnol.* **2013**, *25*, 13-42; b) Chatterjee, S.; Moon, S.; Hentschel, F.; Gilmore, K.; Seeberger, P. H. *J. Am. Chem. Soc.* **2018**, *140*, 11942-11953; c) Ranade, S. C.; Demchenko, A. V. *Carbohydr. Res.* **2015**, *403*, 115-122.

conformational dynamics and additions to oxonium ions.[16] Of particular interest are the results obtained in C-2, C-3, and C-4 substituted tetrahydropyran acetates, which revealed that the conformational preferences of oxonium ions depend significantly on the electronic nature of the substituent (**Figure 3.2**).[17] It is well known that the tendency of organofluorine compounds to adopt conformations stabilized by hyperconjugative and attractive electrostatic interactions. The highly polarised carbon-fluorine bond can be stabilized by interaction between the low-energetic $\sigma^*_{C\text{-}F}$ antibonding orbital and vicinal σ bonds or non-donating electron pairs.[18] Gilmour and co-workers proved that the strategic OH→ F substitution at C2 of pyranosyl donors, not only induces desired physicochemical properties, also allows fluorine to work as directing-glycosylation group following the Anh-Eisenstein induction model (**Figure 3.2**).[19]

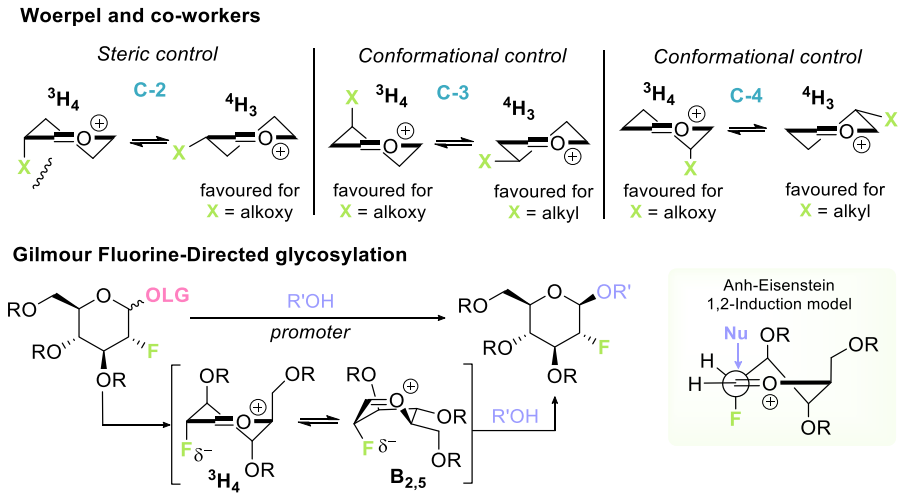
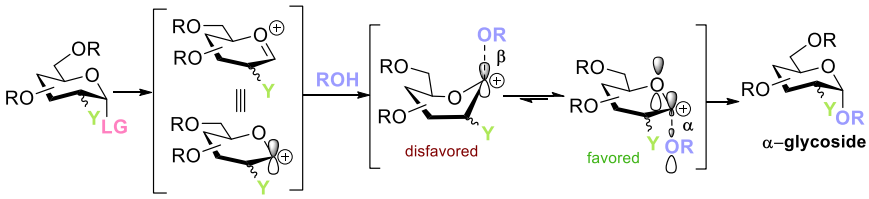


Figure 3.2. Top panel: Stereoselective additions to oxonium ions reported by Woerpel and co-workers. Bottom panel: Fluorine-directed glycosylation reported by Gilmour.

[16] Smith, D. M.; Woerpel, K. A. *Org. Biomol. Chem.* **2006**, *4*, 1195-1201.
[17] Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A. *J. Am. Chem. Soc*. **2000**, *122*, 168-169.
[18] O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308-319.
[19] a) Bucher, C.; Gilmour, R. *Angew. Chem. Int. Ed.* **2010**, *49*, 8724 -8728; b) Bucher, C.; Gilmour, R. *Synlett* **2011**, *8*, 1043-1046; c) Durantie, E.; Bucher, C.; Gilmour, R. *Chem. Eur. J.* **2012**, *18*, 8208-8215; d) Aiguabella, N.; Holland, M. C.; Gilmour, R. *Org. Biomol. Chem.* **2016**, *14,* 5534-5538.

The methodology developed by Ryan Gilmour allow the obtention of 1,2-*trans* 2-deoxy-2-fluoroglycosydes under kinetic control. However, access to α-glycosides under these conditions is not always achieved, especially in for *gluco* and *galacto* configurations, in which the β isomer is obtained favourably. Increasing the stability of the oxocarbenium ion and its various electronic and steric factors may lead to the formation of the more stable α isomer. With these considerations, we hypothesised that a glycosylation method whose activation takes place at high temperatures, will favour an S_N1-pathway obtaining preferentially the α isomer (**Scheme 3.3**).



Scheme 3.3: S_N1-type glycosylation mechanism with non-participating group at the C-2 position favouring the anomeric effect. Y: non-participating group.

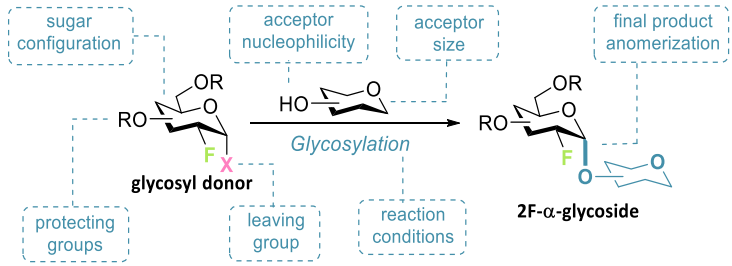
High temperatures must be used to achieve an efficient α-selective control; however, this is usually accompanied by undesired side reactions and can promote equilibration of anomeric glycosides which may erode the stereoselectivity accomplished during the glycosylation reaction. The use of microwave-assisted synthesis allows the acceleration of a variety of chemical reactions, and it presents some advantages over traditional conductive heating, such as, reduced reaction times, good reproducibility, efficient and homogeneous heating, less by-products, better yields and control of temperature, pressure, stirring and potency, among others. Compared with traditional heating, microwave heating is more efficient since microwaves couple directly with the molecules present in the reaction mixture, it allows a rapid increase of the temperature and when the microwave irradiation is stopped, only the latent heat remains.[20]

[20] Hayes, B. L. Microwave synthesis: chemistry at the speed of light. **2002**, *Cem Corporation*.

3.2. OBJECTIVES

The general aim of this chapter is to develop an efficient method for the stereoselective synthesis of 2-deoxy-2-fluoro-α-D-glycosides using microwave heating at high temperatures to favour the anomeric control of the glycosylation reaction. Upon optimization of the method, our aim is to apply it to the synthesis of fluorinated derivates of biologically relevant glycosides. In this context, the specific goals of this chapter are:

1. The efficient synthesis of glycosyl donors with different configurations (D-*Gal*, D-*Glc* and D-*Man*) bearing bromide and iodide as leaving groups.
2. Optimization of the glycosylation conditions to obtain high yields and selectivities. Apply a microwave assisted heating and study the effect of the temperature on selectivity and reactivity.
3. Study the effect of acceptor size and nucleophilicity in the glycosylation stereoselectivity by using different alcohols as acceptors.
4. Study the effect of glycosyl donor configuration together with the influence of different halide leaving groups, and weakly versus strongly inductive protecting groups.
5. Apply the optimized microwave-assisted method for the stereoselective synthesis of α-fluorinated-analogues of biologically relevant glycosides.

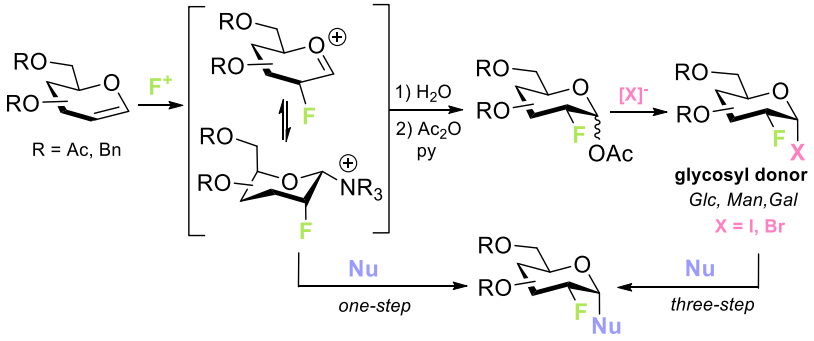


Scheme 3.4. Glycosylation reaction between a glycosyl donor and a glycosyl acceptor. In boxes, parameters that affect to stereoselectivity and reactivity studied in this Chapter.

3.3. RESULTS AND DISCUSSION

3.3.1. Retrosynthetic analysis

Retrosynthetic analysis revealed that 2-deoxy-2-fluoro-glycosides could be obtained following two general strategies: a one-step reaction from glycals or a three-step synthesis from 2-F-glycosyl-1-*O*-Ac derivatives (**Scheme 3.5**). While the one-step strategy is suitable for galactose configurations, the three-step approach gives higher yields and controlled $F_{ax/eq}$ diastereoselectivity for all configurations. In this work, we envisioned a "three-step" approach for the preparation of 2-F-glycosyl halides from commercially available inexpensive D-glycals, enabling an efficient preparation of the fluorinated reagents.



Scheme 3.5. General strategies for the preparation of 2-deoxy-2-F-glycosides. One-step and "three-step" from glycals for the preparation of the glycosyl donor.

3.3.2. Synthesis of 2-deoxy-2-fluoro hexopyranosyl donors

A wide variety of methods have been developed for introducing fluorine into organic compounds,[21] although the most direct and efficient approach reported for preparing 2-deoxy-2-fluoro-glycosides from glycals has been the use of Selectfluor™.[22]

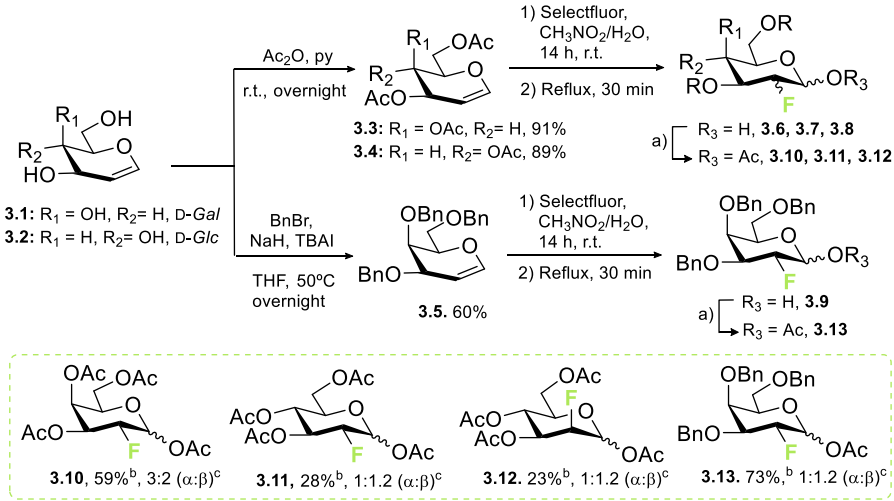
[21] a) Champagne, P. A.; Desroches, J.; Hamel, J. D.; Vandamme, M.; Paquin, J. F. *Chem. Rev.* **2014**, *115*, 9073-9174. b) Yerien, D. E.; Bonesi, S.; Postigo, A. *Org. Biomol. Chem.;* **2016**, *14*, 8398-8427; c) Yang, L.; Dong, T.; Revankar, H. M.; Zhang, C. P. *Green Chem.* **2017**, *19*, 3951-3992.

[22] Uhrig, M. L.; Lantaño, B.; Postigo, A. *Org. Biomol. Chem.* **2019**, *17*, 5173-5189

The enol ether moiety of glycals is susceptible to fluorination by Selectfluor™, and it is converted into the corresponding 2-deoxy-2-fluoroglycoside with excellent yield. The introduction of a fluorine atom in the C-2 position reduces the reactivity of the anomeric carbon as glycosyl donor, and a good leaving group must be introduced in the anomeric position to improve the glycosylation reactivity.[23] Likewise in other fields of synthetic organic chemistry, halides are the most exploited leaving groups to prepare suitable glycosyl donors. Glycosyl iodides usually present some advantages over other glycosyl halides in terms of efficiency, stereochemistry control and reaction times. In the literature, glycosyl iodides have been widely used in the efficient synthesis of glycosides, especially notable is the work of J. Gervay-Hague and co-workers.[24]

First, benzyl- and acetyl- protected glycals were prepared from commercially available deprotected glycals using standard protocols (**Scheme 3.6**). Then, protected glycals were submitted to electrophilic fluorination using Selectfluor™ to obtain the desired 2-deoxy-2-fluoroglycosides. The reaction was carried out in a mixture of nitromethane and water as solvents, stirred at room temperature for 14 hours, and then heated under reflux for 30 min. Without further purification, the anomeric alcohol was protected under acetylation conditions (**Scheme 3.6,** conditions a)), followed by purification using flash column chromatography to obtain protected 2-deoxy-2-fluoroglycosides in good yields. Final yields obtained correspond to the two consecutive steps; electrophilic fluorination and acetylation (**Scheme 3.6**., bottom, 59% for 2F-*Gal,* 28% for 2F-G*lc,* 23% for 2F-*Man* (51% overall yield from D-glucal), and 73% for perbenzylated-2F-*Gal*).

[23] Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900-1934.
[24] a) Gervay-Hague, J.; Nguyen, T. N.; Hadd, M. J. *Carbohydr. Res*. **1997**, *300*, 119-125; b) El-Badry, M. H.; Gervay-Hague, J. *Tetrahedron Lett*. **2005**, *46,* 6727-6728.



General conditions: 1 equiv. of the starting glycal (0.1 M) unless otherwise indicated. [a]General procedure for the acetylation of the anomeric hydroxyl group: 2-deoxy-2-fluoro pyranoglycosides **3.6**, **3.7**+**3.8** and **3.9** (1 mmol), Ac_2O (2 mmol) py (15 mmol). [b]Isolated yields. It is observed the formation of 1,2-deoxy-1,2-difluoro derivate as a non-desired by-product of the electrophilic addition to glycals. [c]α:β ratio determined by ^1H NMR and ^{19}F NMR.

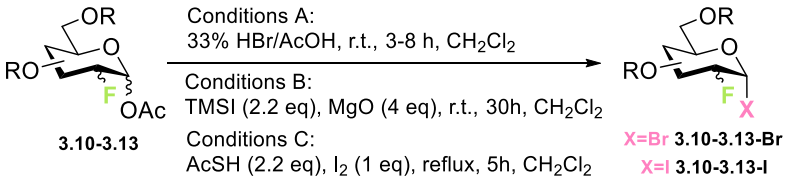
Scheme 3.6. Preparation of 1-OAc-2-F-glycosides from the starting glycals.

We then sought to explore its reactivity to form the corresponding glycosyl halides. Standard bromination conditions A (**Table 3.1**)[25] were applied to all the configurations (*Gal, Glc*, and *Man*) and benzyl and acetyl protecting groups, obtaining 2-F-glycosyl bromides **3.10-Br**, **3.11-Br**, **3.12-Br** and **3.13-Br** (**Table 3.1**, entries 1-4) in excellent yields (80-90%). Regarding the synthesis of 2-F-glycosyl iodides, TMSI was found to be an effective promoter for the preparation of 2-F-galactosyl iodides from the corresponding acetates. In the reaction, trimethylsilyl acetate (TMSOAc) is liberated and it can act as an acceptor of the newly produced glycosyl iodide. The addition of MgO increase the yields because it was proved to be an effective deactivator of the *in situ* generated TMSOAc. These conditions have been found to be very effective in the synthesis of 2-deoxy-2-fluoro-glalactosyl iodides, while recent studies demonstrate that the use of AcSH and I_2 for the nucleophilic iodination of 2-deoxy-2-fluoro glucosyl and mannosyl acetates is more effective than the use of

[25] Kováč, P.; Yeh, H. J.; Glaudemans, C. P. *Carbohydr. Res.* **1987**, *169*, 23-34.

TMSI/MgO conditions. Therefore, two different conditions were used in the synthesis of 2-deoxy- 2-fluoro glycosyl iodides, conditions B and C (**Table 3.1**).[26]

Table 3.1. Glycosyl donor halogenation scope:



Entry	2-F-pyranose	R	Conditions	Product	X	Yield (%)
1	**3.10** (*Gal*)	Ac	A	**3.10-Br**	Br	90
2	**3.11** (*Glc*)	Ac	A	**3.11-Br**	Br	80
3	**3.12** (*Man*)	Ac	A	**3.12-Br**	Br	82
4	**3.13** (*Gal*)	Bn	A	**3.13-Br**	Br	81
5	**3.10** (*Gal*)	Ac	B	**3.10-I**	I	76[b]
6	**3.11** (*Glc*)	Ac	C	**3.11-I**	I	81[b]
7	**3.12** (*Man*)	Ac	C	**3.12-I**	I	71[b]
8	**3.13** (*Gal*)	Bn	B	**3.13-I**	I	ND

[a]General conditions: 2-F-α/β-pyranose (1:0–1:3 α/β) (1 equiv) in dry CH_2Cl_2 (0.1 M).
[b]Isolated yield after two consecutive reaction cycles. ND= not detected.

Conditions B (TMSI/MgO) were applied to the synthesis of 2-F-galactosyl iodides with acetyl (**3.10-I**) and benzyl (**3.13-I**) protecting groups. Acetyl-protected 2-F-α-galactosyl iodide **3.10-I** was obtained in good yield after two consecutive cycles (entry 5, 76%). However, nucleophilic iodination of the benzylic derivate **3.13** gave a mixture of products. Moreover the desired product was unstable and difficult to purify. Conditions C ($AcSH/I_2$) were applied to 2-F-α/β-pyranoses of *Glc* **3.11** and *Man* **3.12** configuration affording glycosyl iodides **3.11-I** and **3.12-I** in good yields (entries 7 and 8). Synthesised 2-F-glycosyl iodides and bromides were purified by flash column chromatography and obtained as sole α anomers regardless of the pyranoside configuration (*Gal*, *Glc*, and *Man*). Acetyl protecting groups proved to be stable under the

[26] Salvadó, M.; Amgarten, B.; Castillón, S.; Bernardes, G. J. L.; Boutureira, O. *Org. Lett*. **2015,** *17*, 2836-2839.

whole sequence of the synthetic route (electrophilic fluorination → halogenation → glycosylation). However, benzyl protecting groups were sensitive to strong acids (HBr) and deprotection of sugar hydroxyl groups was observed in the reaction mixture. Under iodination conditions B, decomposition of the benzylic 2-F-pyranose **3.13** was observed, and the desired product **3.13-I** was not obtained. For all the configurations, synthetized glycosyl donors were obtained as sole isomers and yields correspond to the isolated α anomer. NMR analysis indicated the presence of the α isomer in $^4C_1$ conformation and results were compared with the ones obtained by Michalik and co-workers[27] for the NMR spectra of fluorinated carbohydrates (**Figure 3.3**).

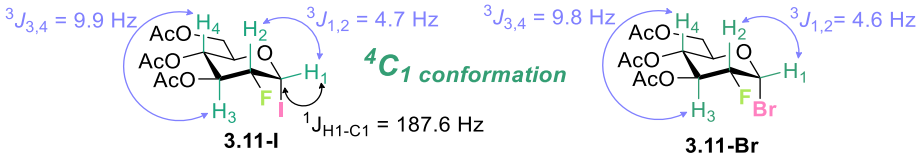


Figure 3.3. Conformational analysis of 2-F-glucosyl iodide **3.11-I** and bromide **3.11-Br.**

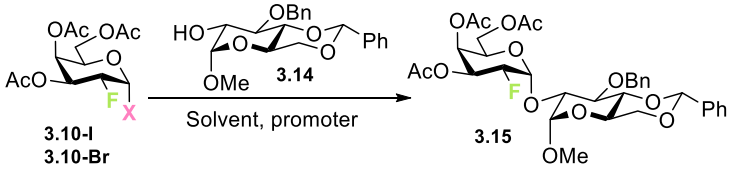
3.3.3. Optimization of the glycosylation reaction

Glycosylation conditions were screened with conventional heating for the reaction of 2-deoxy-2-fluorogalactosyl iodide **3.10-I** and bromide **3.10-Br** with acceptor **3.14** (**Table 3.2**). First, different promoters were evaluated as activators. In a first attempt (entry 1), Koenigs–Knorr[28] conditions were tested using silver triflate as promoter in a mixture of dioxane and diethyl ether as solvents, and in the presence of silver carbonate. However, low glycosylation yield was observed (12%, entry 1). Using silver perchlorate as promoter, glycosylation yield slightly increases (31%, entry 2) and finally, better yield (51%, entry 5) and high stereoselectivities were achieved using a combination of allyl tributyltin and triflic acid as promoters in toluene. However, reaction times were excessively long, and high temperatures were needed. To improve glycosylation yields and reduce the reaction time, reactants were submitted to

[27] Michalik, M.; Hein, M.; Frank, M. *Carbohydr. Res.* **2000**, *327*, 185-218.
[28] W. Koenigs, E. Knorr, Berichte der deutschen chemischen Gesellschaft, **1901**, *34*, 957-981.

microwave heating. Delightfully, good glycosylation yield (65%, entry 6) was obtained for galactosyl iodide **3.10-I** in 2 hours of reaction. Similar result was obtained for the corresponding galactosyl bromide **3.10-Br** (55%, entry 10), and only the α isomer was observed.

Table 3.2. Optimization of the glycosylation of 2-F-galactosyl halides and acceptor **3.14**.



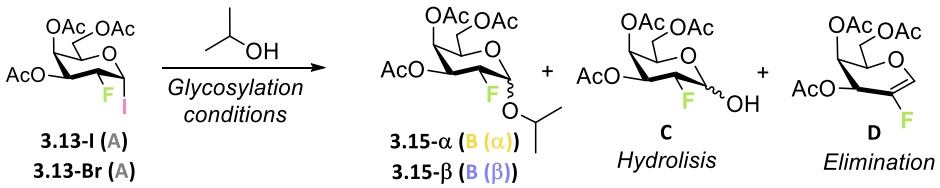
Entry	Glycosyl donor	Reaction conditions[a,b] (equiv.)	Yield (%)
1	**3.10-I**	AgOTf (1), Ag_2CO_3 (2.2), DrieriteR, 12:1 dioxane/Et_2O, rt, 22 h	12[c,d,e]
2	**3.10-I**	$AgClO_4$ (1), Ag_2CO_3 (2.2), DrieriteR, 12:1 dioxane/Et_2O, rt, 17 h	31[c,d,e]
3	**3.10-I**	1) $Bu_3SnAllyl$ (1.3), TfOH (0.3), toluene, rt, 2 h 2) Reflux, 20 h	51
4	**3.10-I**	$Bu_3SnAllyl$, TfOH, toluene, 4 Å MS, 100 ºC (μw), 2 h	65[c,d,e]
5	**3.10-I**	$Bu_3SnAllyl$, toluene, 4 Å MS, 100 ºC (μw), 2 h	0
6	**3.10-I**	TfOH, toluene, 4 Å MS,100 ºC (μw), 2 h	0
7	**3.10-I**	toluene, 4 Å MS, 100 ºC (μw), 2 h	0
8	**3.10-Br**	$Bu_3SnAllyl$, TfOH, toluene, 4 Å MS, 100 ºC (μw), 2 h	55[c,d,e]
9	**3.10-Br**	$Bu_3SnAllyl$, toluene, 4 Å MS, 100 ºC (μw), 2 h	0
10	**3.10-Br**	TfOH, toluene, 4 Å MS,100 ºC (μw), 2 h	0
11	**3.10-Br**	toluene, 4 Å MS, 100 ºC (μw), 2 h	0

[a]General conditions: donor (1 equiv.), acceptor **3.14** (1.2 equiv.) [c] = 0.2 M; [b]General conditions of control experiments: donor (1 equiv.), acceptor **3.14** (1.2 equiv.), $Bu_3SnAllyl$ (1.3 equiv.), TfOH (0.3 equiv), toluene [0.2 M]. [c]Calculated yield by ^{19}F NMR using 1,4-difluoro-benzene as internal standard.[d]10-20% of the corresponding 2-fluoro-galactal obtained. [e]5-20% of the corresponding 2-deoxy-2-fluorogalactose obtained.

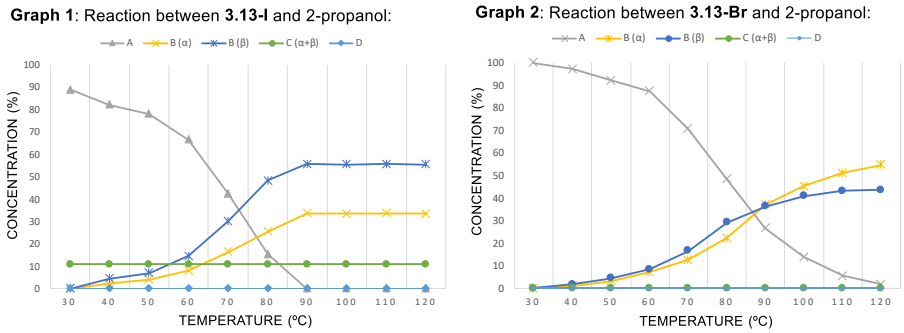
Control experiments for the glycosylation of galactosyl iodide **3.10-I** (entries 5-7) and bromide **3.10-Br** (entries 9-11) showed that all the reactants play an essential role in the glycosylation reaction, and no glycosylation product was obtained when one is absent.

3.3.4. Variable-temperature fluorine (^{19}F) NMR experiments

Variable-temperature fluorine (^{19}F) NMR experiments were performed to study the kinetics of the glycosylation reaction, to obtain information about the formation of by-products and identify possible mechanistic intermediates (**Scheme 3.7**). Glycosylation reaction of 2-deoxy-2-fluoro-galactosyl bromide **3.10-Br** and iodide **3.10-I** with 2-propanol was carried out at different temperatures and followed by ^{19}F NMR (**Scheme 3.8**). As expected, results showed that galactosyl donors have similar yet distinct observable activation temperatures (30 °C for galactosyl iodide **3.10-I** and 40 °C for galactosyl bromide **3.10-Br**), finding galactosyl iodide more reactive than the corresponding bromide. Formation of the hydrolysed product was observed at the lower temperatures (30 °C) and only when a small amount of water was present due to the impossibility of use molecular sieves in the NMR reaction tube. In both experiments no elimination product was observed even at higher temperatures. Regarding the stereochemistry of the reaction, the formation of the β isomer was favoured at lower temperatures and the α isomer at higher temperatures. Galactosyl iodide reacted at lower temperatures (between 50-80 °C) and enriched β anomer was obtained. Interestingly, galactosyl bromide afforded preferentially the β isomer at lower temperatures (from 30 to 70 °C), but at higher temperatures the α isomer was mainly obtained. As we anticipated, above experiments allowed to conclude that high temperatures are necessary to obtain high α stereoselectivity.



Scheme 3.7: Representation of the glycosylation and side reactions.



Scheme 3.8: Graph 1: Results obtained in the glycosylation of galactosyl iodide **3.13-I** (**A**) and 2-propanol followed by ^{19}F NMR. **Graph 2:** Results obtained in the glycosylation of galactosyl bromide **3.13-Br** (**A**) and 2-propanol followed by ^{19}F NMR. Glycosylation products are indicated as **B** (α/β), elimination product **D**, and hydrolyzation product **C.**

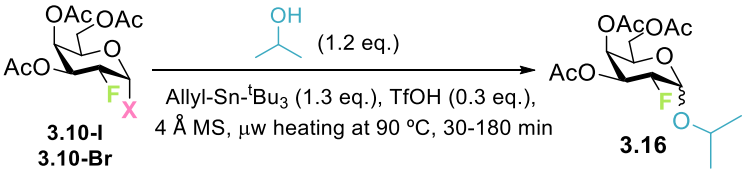
These results led us to carry out a more exhaustive study of the glycosylation reaction mechanism and evaluate the dependence of the stereoselectivity on the leaving group.

3.3.5. Effect of glycosylation temperature on stereoselectivity

Next the effect of temperature in the stereoselectivity of glycosylation of galactosyl bromide **3.10-Br** and iodide **3.10-I** with isopropanol (**Table 3.3**) was evaluated. Each experiment was carried out in an NMR tube under identical reaction conditions and equal reactants amounts, only changing the temperature (from 40 to 120 °C). Reactions were maintained at constant temperature until total consumption of the glycosyl donor, and then analysed by ^{19}F NMR to determine the α:β ratio outcome and the possible presence of by-products. Results showed that between temperatures ranging from 40 to 90 °C benefited the formation of the β isomer for both glycosyl halides (**Table 3.3**, entries 1-6 for iodide and 10-15 for bromide). At 100°C, the ratio was inverted and the α isomer was obtained favourably with similar α:β ratios for both glycosyl halides (**Table 3.3**, entries 7 and 16). Surprisingly, at low temperatures the α:β ratio is dependent on the employed halide and differences were higher at lower temperatures (**Table 3.3**, entries 1 and 10). These results may indicate that the leaving group is involved in the glycosidic bond formation at low

temperatures. However, at higher temperatures the stereoselectivity does not depend on the halide. These observations illustrate that the anomeric effect governs the outcome of the glycosylation reaction at high temperatures (>100ºC). The differences obtained in the stereoselectivity at higher and lower temperatures for iodide and bromide leaving groups could indicate that two different mechanisms are operative. At lower temperatures (up to 70 °C), a competitive S_N2-like mechanism involving α-glycosyl donors cannot be discounted. On the other hand, at higher temperatures the outcome of the glycosylation reaction is not dependent on the halide, which could imply an S_N1-like mechanism is undergoing.

Table 3.3. Study of the influence of the leaving group in the stereoselectivity of the reaction.



3.10-I
3.10-Br

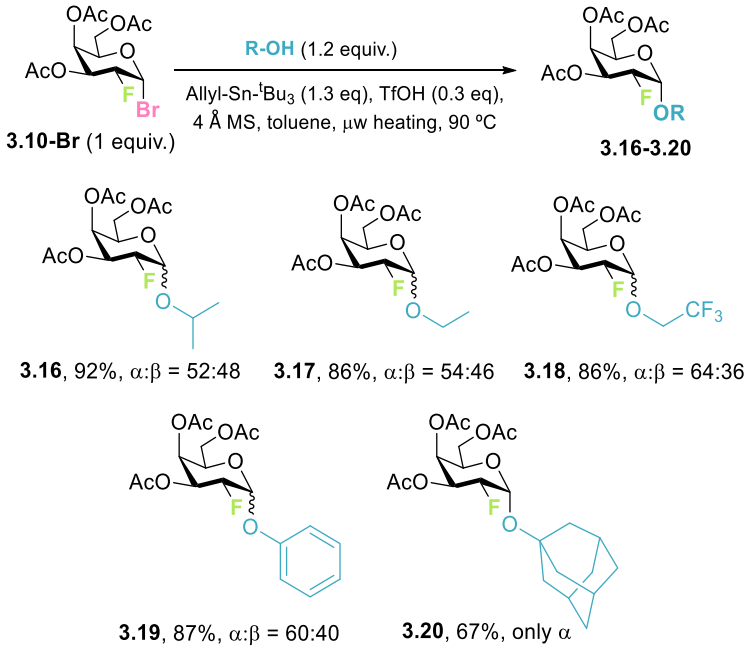
3.16

Temperature (Cº)	3.10-I		3.10-Br	
	Entry	α:β ratio	Entry	α:β ratio
40	1	1:6	10	1:4
50	2	1:5.5	11	1:3.8
60	3	1:4.6	12	1:3.5
70	4	1:3.1	13	1:2.9
80	5	1:2	14	1:2.1
90	6	1:1.5	15	1:1.4
100	7	1:0.9	16	1:1
110	8	1:0.5	17	1:0.5
120	9	1:0.3	18	1:0.3

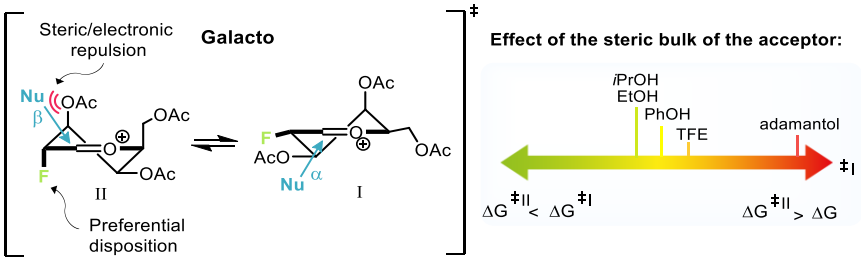
[a]General conditions: donor **3.10-I,-Br** (1 equiv.), isopropanol (1.2 equiv.), allyltributyltin (1.3 equiv.), triflic acid (0.3 equiv.), [c] = 0.2 M; [b]Calculated stereoselectivities by ^{19}F NMR using 1,4-difluorobenzene as internal standard.

3.3.6. Study of the effect of acceptor size and nucleophilicity

To evaluate the effect of the size and nucleophilicity of the acceptor in the stereoselectivity, glycosylation reaction of 2-F-galactosyl bromide **3.10-Br** with different alcohols was studied (**Scheme 3.9**). Reactions were carried out under optimized conditions and yields and stereoselectivities were calculated by ^{19}F NMR using 1,4-difluorobenzene as internal standard. Results showed that small acceptors such as 2-propanol and ethanol give the desired glycosylation products (**3.16** and **3.17** respectively) in good yields but, due to the low steric demand and high nucleophilicity, no stereocontrol was observed. On the other hand, when using a bulkier acceptor such as 1-adamantanol, only the α isomer of **3.20** was obtained. Employing weaker nucleophiles, such as CF_3CH_2OH and phenol, good yields and slightly higher α selectivity were observed. Considering the results obtained with ethanol and trifluoroethanol, and assuming almost the same steric hindrance, the differences in stereoselectivity can be explained according to a Curtin-Hammett scenario, in which intermediate I is slightly favoured in the attack of the less nucleophilic trifluoroethanol (**Scheme 3.10**).



Scheme 3.9. Glycosylation of 2-F-galactosyl bromide **3.10-Br** and alcohols.



Scheme 3.10. Stereoselectivity rationalization using **3.10-Br** and alcohols as acceptors.

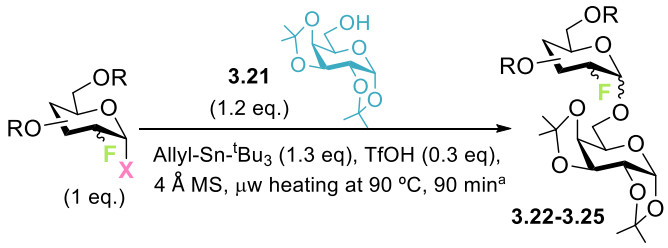
These results indicate that the α-selectivity is compromised by increasing the steric bulk of the acceptor (**Scheme 3.10**) and that nucleophilicity has a weak impact at high temperatures (assuming a S_N1-like scenario). To eliminate the possibility of product epimerization that could influence the selectivity, product **3.16** was submitted again to glycosylation conditions. No anomerization of the final product was observed, indicating that the process in under kinetic control.

3.3.7. Study of the effect of glycosyl donor protecting groups, leaving group and sugar configuration

To accomplish this objective, reaction between different glycosyl donors (**3.10-3.13**) and glycosyl acceptor **3.21** was studied (**Table 3.4**). Mannosyl halides (entries 6 and 7) gave the highest α-selectivity, followed by galactosyl (entries 1 and 2) and then glucosyl analogues, which presented moderate selectivities (entries 4 and 5). These results can be explained considering the steric hindrance of the "two-faces" of the oxocarbenium intermediate. In *manno* configurations, fluorine is in the *axial* position, blocking the "top face" and disfavouring the *equatorial* attack of the acceptor, which leads to the β isomer (**Scheme 3.11**). Thus, the α isomer is steric- and thermodynamically favoured in *manno* configurations. In *gluco* and *galacto* configurations, fluorine is in the *equatorial* position, and both "faces" of the sugar are accessible. The slightly differences obtained in the selectivity of *galacto* and *gluco* configurations could be explained by the configuration of the C4 protecting group, which in galactosides is in axial position and hinders the top face disfavouring the formation of the β isomer.

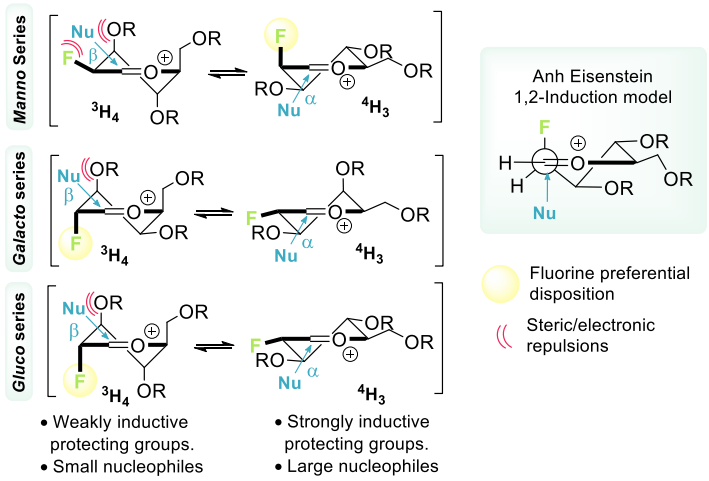
Considering the inductive effect of benzyl and acetyl protecting groups, results obtained for *galacto* sugars (entries 2 and 3) were in concordance with the studies developed by Gilmour and co-workers (**Scheme 3.11**).[19] Strongly inductive acetyl protecting groups favoured the formation of the α-isomer (entry 2) and, on the contrary, weakly inductive benzyl protecting groups favoured the formation of the β-isomer (entry 3). Finally, the effect of the leaving group on the stereochemistry of the reaction was evaluated, but similar stereoselectivities were obtained for all the sugar configurations regardless of the halide, and no correlation between the leaving group and the stereoselectivities were observed. This is in concordance with the results obtained in **Table 3.4**, which suggest that the leaving group does not affect the stereoselectivity at high temperatures.

Table 3.4. Study of the effect of the sugar configuration, protecting groups and leaving groups in the glycosylation stereoselectivity.



Entry	Donor	X	R	Product	Yield (%)[b]	Ratio α:β[b]
1	**3.10-I** *(Gal)*	I	OAc	**3.22**	67	7.3:1
2	**3.10-Br** *(Gal)*	Br	OAc	**3.22**	83	5:1
3	**3.13-Br** *(Gal)*	Br	OBn	**3.23**	96	1:3
4	**3.11-I** *(Glc)*	I	OAc	**3.24**	74	4.4:1
5	**3.11-Br** *(Glc)*	Br	OAc	**3.24**	79	3.8:1
6	**3.12-I** *(Man)*	I	OAc	**3.25**	77	12.5:1
7	**3.12-Br** *(Man)*	Br	OAc	**3.25**	90	1:0

[a]General conditions: donor **3.10-3.12** (1 equiv.), glycosyl acceptor **3.21** (1.2 equiv.), allyltributyltin (1.3 equiv.), triflic acid (0.3 equiv.), [c] = 0.2 M; [b]Calculated yields and stereoselectivities by ^{19}F NMR using 1,4-difluoro-benzene as internal standard.



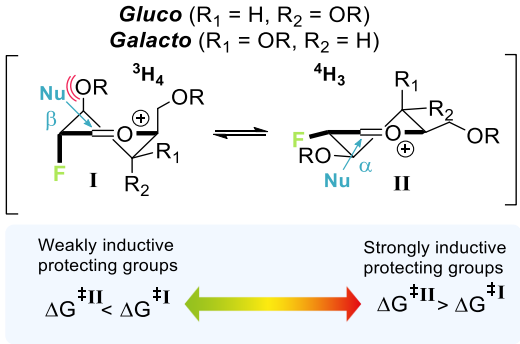
Scheme 3.11. Stereoselectivity rationalization for *manno, gluco* and *galacto* glycosides considering the effect of the protecting groups, the size of the acceptor and the Anh-Eisenstein 1,2-induction model.

3.3.1. Proposed stereochemical model

The remarkable stereoselectivities obtained in the glycosylation of 2-deoxy-2-fluoro glycosyl donors with glycosyl acceptor **3.21** (**Table 3.4**) require some discussion of the possible oxonium ion conformations implicit in the glycosylation event. Considering the findings of Woerpel and co-workers,[16] electrostatic effects exert strongly stabilizing influences on cations, and the lowest energy conformers are the ones where the positive charge is close to substituents bearing partial negative charges. According to this fact, intermediate oxonium ions would adopt conformations in which the electron-dense fluorine atom is located close to the positive oxygen. Due to the conformational fluidity of pyranose-derived oxocarbenium ions, this analysis was restricted to the two half chair intermediates ($^4H_3$ and $^3H_4$). Considering the various trajectories of an incoming nucleophile to approach the oxonium ion centre, and assuming the attack occurs to the half-chair conformation, only those that lead to chair conformation products have been considered (**Scheme 3.11**).

Considering sugars with *manno* configuration, it seems reliable that the transient oxonium ions resemble to the $^{4}H_{3}$ half chair conformation, which is stabilized by electrostatic interaction between fluorine and the positive oxygen ring. It is also likely that the sterically demanding protecting groups favours the stabilization of the $^{4}H_{3}$ intermediate, in which they are in a pseudo-equatorial orientation, favouring the nucleophilic approach to the oxonium centre. The inductive effect of fluorine amplifies the electrophilicity of the oxonium ion, and strongly inductive protecting groups (esters) that destabilize the oxocarbenium ion, will be oriented in an equatorial position, favouring the $^{4}H_{3}$. In contrast, stabilizing interactions are possible when using weakly inductive protecting groups (ethers) in axial positions, favouring the $^{3}H_{4}$ conformation. According to these hypotheses, it is envisaged that the nucleophile will approach the oxocarbenium ion in a manner consistent with the Anh-Eisenstein 1,2-Induction model, which is consistent with the $^{3}H_{4}$ conformation (**Scheme 3.11**, top).

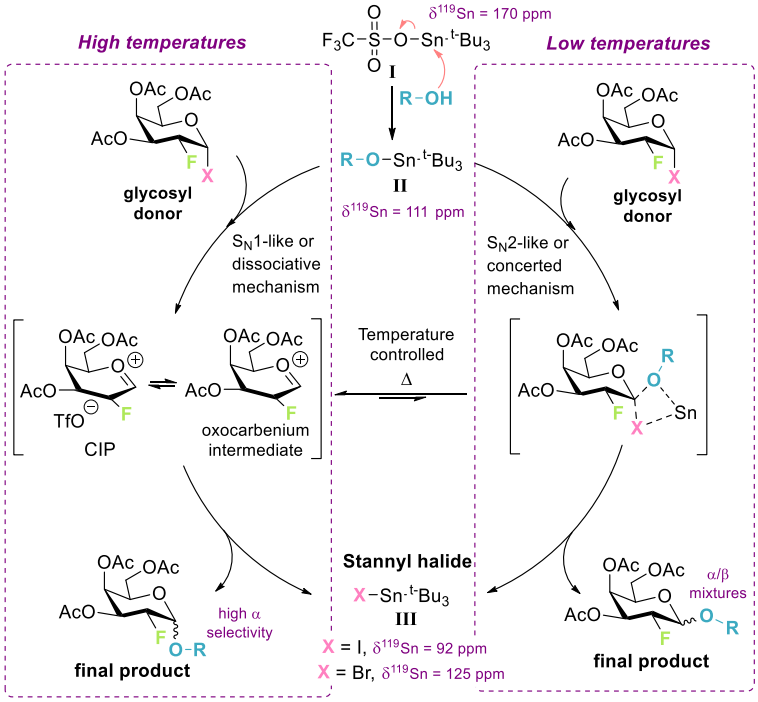
In *galacto* and *gluco* configurations results are consistent with a Curtin-Hammett scenario where the stereoselectivities are reinforced or diminished depending on the combination of C-2 fluorine configuration and the inductive nature of the protecting groups. The reactivity of the intermediates **II** bearing fluorine in axial position, that led to the formation of the α-product in *galacto* and *gluco* configurations, depend highly on the electronic nature of the protecting groups and the acceptor size and nucleophilicity (**Scheme 3.12**).



Scheme 3.12. Proposed stereocontrol model.

3.3.2. Mechanistic studies and hypothesis formulation

Control experiments presented in **Table 3.2** allowed to conclude that all the reactants play an essential role in the glycosylation reaction. Moreover, previous ^{19}F NMR experiments (**Table 3.3**) demonstrate the influence of the glycosyl donor leaving group in the stereochemistry of the reaction. The differences obtained in the stereoselectivity when iodide and bromide leaving groups were used could indicate that two different mechanisms are operative depending on the temperature. At low temperatures, a S_N2-like mechanism involving α-glycosyl donors cannot be discounted, in which the selectivity of the final product is dependent on the leaving group. In contrast, at higher temperatures the outcome of the glycosylation reaction is not dependent on the leaving group, which could suggest that a S_N1-like mechanism is operative. With these premises, the glycosylation reaction between galactosyl bromide and iodide with isopropanol was followed by ^{119}Sn and ^{19}F NMR. ^{19}F NMR measurements were useful to determine the outcome of the glycosylation reaction, since starting material and final product are easily detectable. ^{119}Sn NMR experiments allowed to observe the changes in the Sn species (**Scheme 3.13**). Results demonstrated that allyl tributyl tin (^{119}Sn NMR δ = -18 ppm) reacts with triflic acid to form triflic tributyl tin **I** (^{119}Sn NMR δ = 170 ppm). By adding the acceptor to this mixture, it can be observed the formation of a different tin(IV) specie with ^{119}Sn NMR δ = 111 ppm, that could correspond to a stannyl ether **II**. This tin(IV) specie reacts immediately with the glycosyl donor, and generates the corresponding tributyl tin halide **III** (^{119}Sn NMR δ = 92 ppm for I-Sn$(Bu)_3$ and δ = 125 ppm for Br-Sn$(Bu)_3$). At the same time, ^{19}F NMR provided information about the formation of the glycosylation product.

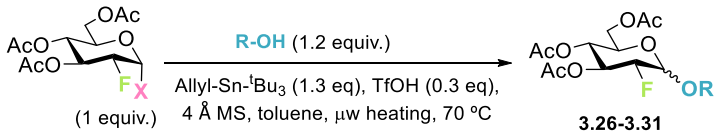


Scheme 3.13. Proposed glycosylation mechanism at high and low temperatures and species based on ^{19}F NMR and ^{119}Sn NMR studies.

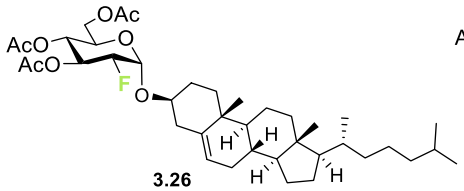
The proposed glycosylation mechanism (**Scheme 3.13**) is in concordance with the experimental results obtained in this study. At low temperatures, stereoselectivity was dependent on the leaving group, which is consistent with a S_N2-like mechanism involving the glycosyl donor. Differences obtained in stereoselectivity could be explained by the different performance of the leaving group. The higher size of iodide compared with bromide results in a higher hinderance of the bottom face, which explains the higher β selectivity obtained for glycosyl iodides at low temperatures. In contrast, at high temperatures, the reaction could follow a S_N1-like mechanism, the anomeric effect governs the outcome of the glycosylation reaction, and when higher temperatures were reached the more effective was the α-selective control. In *galacto* and *gluco* configurations, the more stable α-isomer is obtained with good stereoselectivity. In contrast, in the *manno* configuration the 4H3 intermediate which leads to the α isomer is both electronically and sterically favoured.

3.3.3. Stereoselective synthesis of fluorinated analogues of biologically relevant glycosides

Having demonstrated that our method is efficient for the stereoselective synthesis of 2-deoxy-2-fluoro-α-glycosides, it was applied for the construction of biologically relevant molecules (**Scheme 3.14**). For this, 2-deoxy-2-fluoro-α-glycosyl halides were reacted with cholesterol and protected monosaccharides, to afford cholesteryl glycosides and disaccharides. When using more sterically demanding acceptors, such as the ones mentioned, α-stereoselectivity is favoured and lower temperatures can be used in the glycosylation step without compromising the control of the stereoselectivity. Reactions were conducted at 70ºC under microwave heating to avoid the formation of undesired by-products and the decomposition of the starting materials. The desired F-mimetic glycosides were obtained in good yields and high α-selectivities. *Gluco* glycosyl donors gave lower yields and stereoselectivities in comparison with other sugar configurations. However, the desired products (**3.26** and **3.27**) were obtained in fair to good yields (47-68%) and good stereoselectivities (up to 5:1 α:β ratio). Excellent results were obtained for the reaction of *manno* glycosyl donors with the different glycosyl acceptors (products **3.28**, **3.29** and **3.30**) obtaining high yields (75-95%) and exclusive α selectivities. Good results were also obtained for the *galacto* glycoside (**3.31**), with high yields (64% for Br and 71% for I), and good stereoselectivity (up to 7:1 α:β ratio), which are in concordance with the results obtained in the glycosyl donor scope (**Table 3.4**).

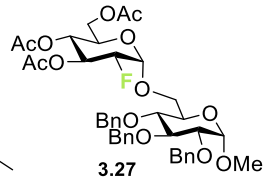


Gluco-derivatives



Cholesterol (H. Pylori)

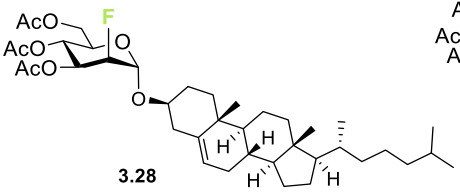
66% α:β = 2.7:1, from **3.11-I**
68% α:β = 3.5:1, from **3.11-Br**



βGlc(1,6)Glc branch
(yeast and fungi β-glucans)

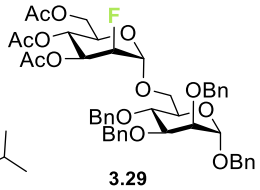
47% α:β = 5:1, from **3.11-I**
61% α:β = 3:1, from **3.11-Br**

***Manno*-derivates**



Cholesterol (H. Pylori)

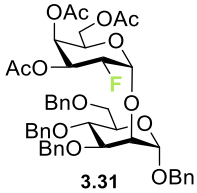
85% α:β = 1:0, from **3.12-I**
90% α:β = 1:0, from **3.12-Br**



αMan(1,6)Man

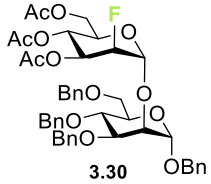
95% α:β = 1:0, from **3.12-I**
75% α:β = 1:0, from **3.11-Br**

***Galacto*-derivates**



αGal(1,2)Man

71% α:β = 7:1, from **3.10-I**
64% α:β = 5:1, from **3.11-Br**



αMan(1,2)Man

88% α:β = 1:0, from **3.12-I**
88% α:β = 1:0, from **3.12-Br**

[a]General conditions: donor (1 equiv.), glycosyl acceptor (1.2 equiv.), allyltributyltin (1.3 equiv.), triflic acid (0.3 equiv.), [c] = 0.2 M. [b]Calculated yields and stereoselectivities by ^{19}F NMR using 1,4-difluoro-benzene as internal pattern.

Scheme 3.14. Stereocontrolled synthesis of biologically relevant fluorinated glycosides.

3.4. CONCLUSIONS

In this chapter, a stereoselective method for the synthesis of 2-deoxy-2-fluoro-α-glycosides with *galacto, gluco* and *manno* configurations has been disclosed. This methodology is complementary to the work developed by Ryan Gilmour in the stereoselective synthesis of 1,2-*trans* 2-deoxy-2-fluoro-glycosides, which allow the obtention of *gluco* and *galacto* β-glycosides under kinetic control. These methodologies permit the ready and easy access to α and β isomers of relevant 2-deoxy-2-fluoroglycosides in a stereoselective manner.

Optimization of the glycosylation reaction revealed that microwave heating reduces reaction times and avoids the formation of undesired by-products. Study of the glycosylation reaction and the different parameters that affect to stereoselectivity and reactivity revealed that:

- The increase of the temperature reduces reaction times and favours the formation of the α-anomer for *galacto*, *gluco* and *manno* configurations. However, at 100°C the formation of elimination product was observed.
- Regarding the reactivity of glycosyl donors, glycosyl iodides proved to be more reactive than glycosyl bromides. The leaving group seemed to affect the stereoselectivity only at low temperatures, and at high temperatures no differences were observed between bromide and iodide glycosyl donors.
- The glycosyl donor configuration has a crucial effect in the stereoselectivity. *Manno* glycosides gave higher α-selectivities, followed by *galacto* and then *gluco* glycosides, which is in concordance with the Anh-Eisenstein 1,2-induction model.
- Glycosyl donor protecting groups have a significant effect on stereoselectivity, and strongly inductive protecting groups favour the formation of the α-isomer.

- Glycosyl acceptor size has a crucial effect in stereoselectivity and large nucleophiles favour the formation of the α-isomer. However, nucleophilicty seemed to have modest impact on stereoselectivity.

Finally, the method was applied to the synthesis of fluorinated derivatives of biologically important glycosides which were obtained in high yields and good α-stereoselectivies, achieving the isolation of the α-isomer in all the cases.

^{19}F NMR variable-temperature and ^{19}Sn NMR experiments allowed to understand the outcome of the glycosylation reaction under the optimized conditions. Results revealed that two mechanisms could be operating depending on the temperature. At low temperatures, a S_N2-like mechanism could be favoured, since stereoselectivity is dependent on the glycosyl donor halide. On the other hand, at high temperatures, a S_N1-like mechanism could be operative, letting the anomeric effect govern the outcome of the glycosylation reaction.

Regarding the stereocontrol of the reaction, and assuming the formation of an oxocarbenium ion, results obtained for *manno* glycosides were consistent with a $^4H_3$ half chair conformation, which is stabilized by electrostatic interactions between fluorine and the positive oxygen of the oxocarbenium ion, yielding the α-isomer favourably. These results are in concordance with the Anh-Eisenstein 1,2-induction model. However, for *gluco* and *galacto* configurations, the $^3H_4$ half chair conformation is favoured, which leads to the formation of the β-isomer. The obtention of the α-isomer can be explained considering a Curtin-Hammett scenario where the stereoselectivities are reinforced or diminished depending on the combination of C-2 fluorine configuration and the inductive nature of the protecting groups.

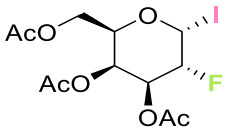
3.5. EXPERIMENTAL SECTION

3.5.1. General considerations

Proton (^{1}H NMR), carbon (^{13}C NMR) and fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (400 MHz for ^{1}H, 100.6 MHz for ^{13}C and 376.5 MHz for ^{19}F). Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^{1}H NMR: $CDCl_3$ = 7.26 and ^{13}C NMR: $CDCl_3$ = 77.00) and $CFCl_3$. Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent. Infrared (IR) spectra were recorded on a Jasco FT/IR-600 Plus ATR Specac Golden Gate spectrophotometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra (HRMS) were recorded on an Agilent 1100 Series LC/MSD mass spectrometer with electrospray ionization (ESI) by the Servei de Recursos Científics (URV). Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using commercial aluminium backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (max = 254 nm) and 6% H_2SO_4 in EtOH. Flash column chromatography was carried out using silica gel 60 A CC (230–400 mesh). Mobile phases are reported in relative composition (e.g. 1:1 EtOAc/hexane v/v). All solvents were used as supplied (Analytical, synthesis or HPLC grade), without prior purification. All reagents were used as received from commercial suppliers. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulphate ($MgSO_4$) and anhydrous sodium sulphate (Na_2SO_4) were used as drying agents after reaction work-up, as indicated.

3.5.2. Synthesis of 2-deoxy-2-fluoro-hexopyranosyl donors

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl iodide (3.10-I):

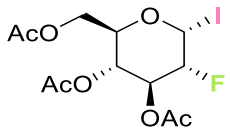


To a mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-galactopyranose (2:1 α/β) (939 mg, 2.68 mmol) and MgO (432 mg, 10.72 mmol) in dry CH_2Cl_2 (22 mL, 8.4 mL/mmol) was added TMSI (1.18 g, 5.90 mmol) at room temperature. The reaction mixture was stirred at room temperature for 24 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. The organic layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The obtained residue is filtered through a short path of silica using Hex:EtOAc (1:1) and then concentrated under reduced pressure. MgO (432 mg, 10.72 mmol) and TMSI (1.18 g, 5.90 mmol) were added to the residue in dry CH_2Cl_2 (22 mL, 8.4 mL/mmol) at room temperature and the reaction mixture was stirred for 24 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. The organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash chromatography (from hexane to 1:1 EtOAc/hexane) to afford **3.10-I** (921.6 mg, 78%) as a yellowish oil.

R_f (1:1 EtOAc/hexane): 0.44; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.98 (dd, $J_{1,2}$ = 4.5 Hz, $J_{F,1}$ = 1.6 Hz, 1H, H-1), 5.49 (td, $J_{3,4}$ = $J_{4,5}$ = 3.3 Hz, $J_{4,F}$ = 1.3 Hz, 1H, H-4), 5.35 (td, $J_{F,3}$ = $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 4.27 (m, 1H, H-5) 4.21–4.03 (m, 3H, H-2, H-6a, H-6b), 2.15, 2.07, 2.06 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –188.4 (ddd, $J_{F,2}$ = 50.4 Hz, $J_{F,3}$ = 10.0 Hz, $J_{F,1}$ = 1.6 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.0, 169.6 (3C=O, Ac), 83.6 (d, $J_{2,F}$ = 194.5 Hz, C-2), 73.6 (C-5), 72.7 (d, $J_{1,F}$ = 26.7 Hz, C-1), 70.8 (d, $J_{3,F}$ = 17.5 Hz,

C-3), 68.9 (d, $J_{4,F}$ = 7.6 Hz, C-4), 60.4 (C-6), 20.5, 20.4 ($3CH_3$, Ac). Spectroscopic data was identical to that previously reported.[29]

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl iodide (3.11-I)



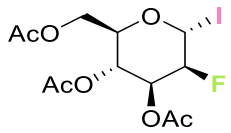
To a mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (1:3 α/β) (472 mg, 1.35 mmol) and I_2 (341 mg, 1.35 mmol) in dry CH_2Cl_2 (13.5 mL) was added AcSH (0.22 mL, 2.96 mmol) at room temperature. Reaction mixture was then heated under reflux for 5 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. Combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was filtered through a short path of silica (1:1 EtOAc/hexane) and the crude was subsequently treated with I_2 (341 mg, 1.35 mmol) and AcSH (0.22 mL, 2.96 mmol) in dry CH_2Cl_2 (13.5 mL) at room temperature and then heated under reflux for 5 h. The crude was then diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. Combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (from hexane to 1:1 EtOAc/hexane) to afford **3.11-I** (454.4 mg, 80.7%) as a yellowish syrup.

R_f (1:1 EtOAc/hexane): 0.60; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.91 (d, $J_{1,2}$ = 4.7 Hz, 1H, H-1), 5.54 (ddd, $J_{F,3}$ = 11.3 Hz, $J_{3,4}$ = 9.9 Hz, $J_{2,3}$ = 9.4 Hz, 1H, H-3), 5.13 (appt, $J_{3,4} = J_{4,5}$ = 9.9 Hz, 1H, H-4), 4.34 (dd, $J_{6a,b}$ = 12.8 Hz, $J_{5,6a}$ = 4.2 Hz, 1H, H-6a), 4.10 (appdd, $J_{6a,b}$ = 12.6 Hz, $J_{6b,5}$ = 1.9 Hz, 1H, H-6b), 4.07 (m, 1H, H-5), 3.93 (ddd, $J_{F,2}$ = 50.0 Hz, $J_{2,3}$ = 9.2 Hz, $J_{1,2}$ = 4.6 Hz, 1H, H-2), 2.09, 2.07 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –181.96 (dd, $J_{F,2}$ = 50.0 Hz, $J_{F,3}$ = 11.3 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 169.8, 169.5 (3C=O, Ac), 85.8 (d, $J_{2,F}$ = 197.6 Hz, C-2), 74.8 (C-5), 72.8 (d, $J_{3,F}$ = 18.5 Hz, C-3), 70.5 (d,

[29] Salvadó, M.; Amgarten, B.; Castillón, S.; Bernardes, G. J. L.; Boutureira, O. *Org. Lett.* **2015,** *17*, 2836-2839.

$J_{1,F}$ = 26.1 Hz, C-1), 66.2 (d, $J_{4,F}$ = 6.9 Hz, C-4), 60.7 (C-6), 20.7, 20.6, 20.5 ($3CH_3$, Ac); spectroscopic data was identical to that previously reported.[29]

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl iodide (3.12-I):

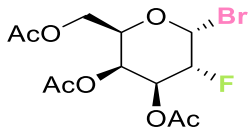


To a mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-mannopyranose (3:1 α/β) (498 mg, 1.42 mmol) and I_2 (360 mg, 1.42 mmol) in dry CH_2Cl_2 (14.2 mL) was added AcSH (0.23 mL, 3.12 mmol) at room temperature. Reaction mixture was then heated under reflux for 5 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was filtered through a short path of silica (1:1 EtOAc/hexane) and the crude was subsequently treated with I_2 (360 mg, 1.42 mmol) and AcSH (0.23 mL, 3.12 mmol) in dry CH_2Cl_2 (14.2 mL) at room temperature and then heated under reflux for 5 h. The crude was then diluted with CH_2Cl_2 and washed with saturated aqueous $Na_2S_2O_5$. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (from hexane to 1:1 EtOAc/hexane) to afford **3.12-I** (1.01 g, 71%) as a yellowish syrup.

***R*$_f$** (1:1 EtOAc/hexane): 0.44; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.84 (d, $J_{F,1}$ = 12.1 Hz, 1H, H-1), 5.73 (ddd, $J_{F,3}$ = 26.0 Hz, $J_{3,4}$ = 10.3 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-3), 5.44 (appt, $J_{3,4}$ = $J_{4,5}$ = 10.3 Hz, 1H, H-4), 5.03 (dd, $J_{F,2}$ = 50.4 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-2), 4.33 (dd, $J_{6a,b}$ = 12.5 Hz, $J_{5,6a}$ = 4.4 Hz, 1H, H-6a), 4.13 (dd, $J_{6a,b}$ = 12.5 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.93 (ddd, $J_{4,5}$ = 10.3 Hz, $J_{5,6a}$ = 4.4 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 2.10, 2.09, 2.07 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –174.4 (ddd, $J_{F,2}$ = 50.4 Hz, $J_{F,3}$ = 26.0 Hz, $J_{F,1}$ = 12.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 170.0, 169.3 (3C=O, Ac), 89.8 (d, $J_{2,F}$ = 190.7 Hz, C-2), 75.1 (C-5), 69.0 (d, $J_{3,F}$ = 17.6 Hz, C-3), 65.7 (d, $J_{1,F}$ = 23.7 Hz, C-1), 64.9 (d, $J_{4,F}$ = 1.5 Hz,

C-4), 60.9 (C-6), 20.63, 20.57 ($3CH_3$, Ac); spectroscopic data was identical to that previously reported.[29]

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide (3.10-Br):

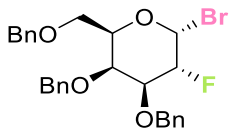


To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α/β-D-galactopyranose (2:1 α/β) (401.6 mg, 1.15 mmol) in dry CH_2Cl_2 (8.1 mL) was added 33% HBr in AcOH (4.01 mL, 3.5 mL/mmol) and the mixture was stirred at room temperature for 4 h. Then, it was diluted with CH_2Cl_2 and washed with sat. aq. solution of $NaHCO_3$. The organic combined layers were dried over Na_2SO_4, filtered, and concentrated under vacuum to afford **3.10-Br** (383.3 mg, 90%) as a yellowish solid. It was used in the next step without further purification.

R_f (Hex/EtOAc 1:1): 0.55; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.61 (d, $J_{1,2}$ = 4.2 Hz, 1H, H-1), 5.53 (td, $J_{3,4}$ = $J_{4,5}$ = 3.3 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 5.47 (td, $J_{F,3}$ = $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 4.76 (ddd, $J_{F,2}$ = 50.2 Hz, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 4.2 Hz, 1H, H-2), 4.51 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-5), 4.17 (dd, $J_{6a,b}$ = 11.5 Hz, $J_{5,6a}$ = 6.4 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,b}$ = 11.5 Hz, $J_{5,6b}$ = 6.4 Hz, 1H, H-6b), 2.14, 2.06, 2.05 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –195.0 (ddd, $J_{F,2}$ = 50.1 Hz, $J_{F,3}$ = 9.9 Hz, $J_{F,4}$ = 3.0 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 169.8, 169.8 (3C=O, Ac), 87.0 (d, $J_{F,1}$ = 25.6 Hz, C–1), 84.2 (d, $J_{F,2}$ = 194.9 Hz, C-2), 71.3 (C-5), 69.0 (d, $J_{F,3}$ = 18.0 Hz, C-3), 67.5 (d, $J_{F,4}$= 7.53 Hz, C-4), 60.7 (C-6), 20.7, 20.7, 20.6 ($3CH_3$, Ac); spectroscopic data was identical previously reported.[30]

[30] Albert, M.; Dax, K.; Ortner, J. *Tetrahedron*, **1998**, *54*, 4839-4848.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-fluoro-α-D-galactopyranosyl bromide (3.13-Br)

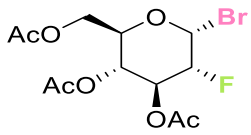


To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-α/β-D-galactopyranose (2:1 α/β) (56.1 mg, 0.114 mmol) in dry CH_2Cl_2 (3.3 mL) was added 33% HBr in AcOH (0.20 mL, 1.75 mL/mmol) at 0 °C. The reaction is stirred at r.t. for 3 hours. The crude was diluted with CH_2Cl_2 and washed with sat. aq. solution of $NaHCO_3$, first at 0 °C, and then at r.t. since neutral pH is obtained. The aqueous phase is successively extracted with CH_2Cl_2. The organic combined layers were dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash column (from hexane to 1:1 EtOAc/Hex) to afford **3.13-Br** (41.0 mg, 70%) as yellowish syrup.

R_f (hexane/EtOAc 9:1): 0.25; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.40-7.25 (m, 15H, ArH), 6.62 (d, $J_{1,2}$ = 4.1 Hz, 1H, H-1), 4.99-4.83 (appddd, $J_{F,2}$ = 50.6 Hz, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 4.2 Hz, 1H, H-2), 4.94 (d, *J* = 11.2 Hz, 1H, CH-Ph), 4.84 (d, *J* = 11.9 Hz, 1H, CH-Ph), 4.70 (d, *J* = 11.8 Hz, 1H, CH-Ph), 4.55 (d, *J* = 11.2 Hz, 1H, CH-Ph), 4.50 (d, *J* = 11.8 Hz, 1H, CH-Ph), 4.43 (d, *J* = 11.8 Hz, 1H, CH-Ph), 4.22 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, 1H, H-5), 4.08 (td, $J_{F,3}$ = $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 2.9 Hz, 1H, H-3), 4.03 (appt, $J_{F,4}$ = $J_{3,4}$ = 3.0 Hz, 1H, H-4), 3.62 (dd, $J_{6a,b}$ = 9.4 Hz, $J_{5,6a}$ = 7.3 Hz, 1H, H-6a), 3.57 (dd, $J_{6a,b}$ = 9.4 Hz, $J_{5,6b}$ = 5.9 Hz, 1H, H-6b); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –194.1 (ddd, $J_{F,2}$ = 50.5 Hz, $J_{F,3}$ = 9.2 Hz, $J_{F,4}$ = 4.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 138.1, 138.0, 137.7 (CH, Ar), 128.7, 128.7 128.5, 128.4, 128.1, 128.0, 127.8 (CH, Ar), 90.0 (d, $J_{F,1}$ = 26.0 Hz, C-1), 88.3 (d, $J_{F,2}$ = 192.6 Hz, C-2), 77.8 (d, $J_{F,3}$ = 15.3 Hz, C-3), 75.4, 74.8 ($2CH_2$-Ph), 74.7 (C-4), 74.4 (C-5), 73.7, 73.4 73.4 ($3CH_2$-Ph), 67.6 (C-6); spectroscopic data was identical previously reported.[31]

[31] Yu, F.; Dickson, J. L.; Loka, R. S.; Xu, H.; Schaugaard, R. N.; Schlegel, H. B.; Luo, L.; Nguyen, H. M. *ACS Catal.* **2020**, *10,* 5990-6001.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide (3.11-Br):

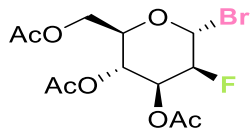


To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranose (1:3 α/β) (501.1 mg, 1.43 mmol) in dry CH_2Cl_2 (10.13 mL) was added 33% HBr in AcOH (5 mL) at r.t. The reaction mixture was stirred at the same temperature for 5 h. Then, the crude was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford **3.11-Br** (422.3 mg, 80%) as a yellowish oil. Used in the next step without further purification.

R_f (1:1 EtOAc/hexane) : 0.63; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.51 (dd, $J_{1,2}$ = 4.6 Hz, $J_{F,1}$ = 1.1 Hz, 1H, H-1), 5.59 (ddd, $J_{F,3}$ = 11.3 Hz, $J_{3,4}$ = 9.8 Hz, $J_{2,3}$ = 9.4 Hz, 1H, H-3), 5.09 (appt, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.52 (ddd, $J_{F,2}$ = 49.3 Hz, $J_{2,3}$ = 9.4 Hz, $J_{1,2}$ = 4.6 Hz, 1H, H-2), 4.37–4.21 (m, 2H, H-5, H-6a), 4.09 (dd, $J_{6a,b}$ = 10.6 Hz, $J_{5,6b}$ = 3.0 Hz, 1H, H-6b), 2.07, 2.06, 2.03 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –188.5 (ddd, $J_{F,2}$ = 49.3 Hz, $J_{F,3}$ = 11.3 Hz, $J_{F,1}$ = 1.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 169.8, 169.5 (3C=O, Ac), 86.3 (d, $J_{F,2}$ = 199.3 Hz, C-2), 85.6 (d, $J_{F,1}$ = 25.0 Hz, C-1), 72.2 (C-5), 71.1 (d, $J_{F,3}$ = 18.7 Hz, C-3), 66.6 (d, $J_{F,4}$ = 7.4 Hz, C-4), 60.9 (C-6), 20.7, 20.7, 20.6 ($3CH_3$, Ac); spectroscopic data was identical to that previously reported.[3234]

[32] Kovac, P.; Yeh, H. J.; Glaudemans, C. P. *Carbohydr. Res.* **1987**, *169*, 23-34.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl bromide (3.12-Br):



To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranose (3:1 α:β) (160.0 mg, 0.457 mmol) in dry CH_2Cl_2 (3.25 mL) was added 33% HBr in AcOH (3.25 mL) at room temperature. The reaction mixture was stirred at the same temperature for 5 h. Then, the crude was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford **3.12-Br** (138.6 mg, 82%) as a yellowish oil. Used in the next step without further purification.

***R*$_f$** (1:1 EtOAc/hexane): 0.60; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.44 (dd, $J_{F,1}$ = 9.6 Hz, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 5.63 (ddd, $J_{F,3}$ = 26.5 Hz, $J_{3,4}$ = 10.2 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-3), 5.42 (appt, $J_{3,4}$ = $J_{4,5}$ = 10.2 Hz, 1H, H-4), 4.99 (ddd, $J_{F,2}$ = 49.5 Hz, $J_{2,3}$ = 2.8 Hz, $J_{1,2}$ = 1.5 Hz, 1H, H-2), 4.34 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 4.3 Hz, 1H, H-6a), 4.24–4.21 (m, 1H, H-5), 4.18 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 2.10, 2.09, 2.06 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –181.6 (ddd, $J_{F,2}$ = 49.5 Hz, $J_{F,3}$ = 26.6 Hz, $J_{F,1}$ = 9.7 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.9, 169.3 (3C=O, Ac), 88.8 (d, $J_{F,2}$ = 188.7 Hz, C-2), 82.4 (d, $J_{F,1}$ = 26.4 Hz, C-1), 72.8 (C-5), 68.6 (d, $J_{F,3}$ = 16.9 Hz, C-3), 64.9 (C-4), 61.1 (C-6), 20.7x2, 20.6 ($3CH_3$, Ac); spectroscopic data was identical to that previously reported.[29]

3.5.3. Microwave-assisted glycosylation system

Glycosylation reaction was submitted to the microwave heating using a CEM Discover Microwave Digestion System. Reaction mixtures were prepared in a microwave reaction vessel with a stir bar and closed with a microwave cap. Then, the vial was inserted in the Microwave System and the parameters set up. Parameters of the Microwave heating were; Power = 330 W, Temperature = between 70 and 100 °C, Pressure = 250 psi, Ramp time = 5 minutes, Hold time = 60 to 90 minutes, Cooling = ON, Stirring = HIGH.

3.5.4. General glycosylation procedure

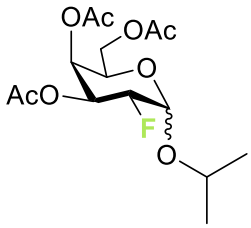
To a solution of the corresponding **donor** (1 mmol) and **acceptor** (1.2 mmol) in dry toluene (20 mL/mmol) were added preactivated 4 Å molecular sieves, allyl tributyltin (1.3 mmol) and TfOH (30% molar) to the microwave reaction vessel under an Argon atmosphere. The mixture was submitted to the µW conditions using a CEM Discover Microwave Digestion System. When the reaction has finished, the crude was diluted with EtOAc and washed with sat. aq. solution of $NaHCO_3$ and KF. The organic combined layers were dried over Na_2SO_4, filtered, and concentrated under vacuum.

3.5.5. ^{19}F NMR Variable temperature experiments

Donor A (1 mmol) and preactivated 4 Å MS were added to an NMR tube under Argon atmosphere and submitted to three cycles of vacuum/refilling. Then the mixture was dissolved in dry toluene (20 mL/mmol), cooled down to 0 °C and then 2-propanol (1.2 mmol), allyl tributyltin (1.3 mmol), TfOH (30% molar) and 1,4-difluorobenzene as internal standard were added, then the NMR tube was closed using a septum and mixed at 0 °C. The mixture was submitted to the corresponding temperature for 10 min then cooled down to 0 °C and analyzed by ^{19}F NMR. This step was repeated for all the temperatures (30 to 120 °C).

3.5.6. Acceptor scope

1-*O*-isopropyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranose (3.16)



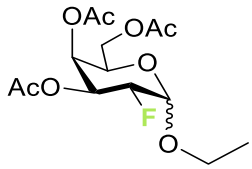
The general procedure was applied, the residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.16** as colourless oil.

Data for **3.16α:** R_f (2:8 EtOAc/hexane): 0.30; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.47 (t, $J_{3,4} = J_{4,F}$ = 3.2 Hz, 1H, H-4), 5.38 (td, $J_{2,3} = J_{3,F}$ = 10.3 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 5.18 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.72 (ddd, $J_{2,F}$ = 50.3 Hz, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.31 (t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, 1H, H-5), 4.08 (m, 2H, H-6a, H-6b), 3.92 (sept., $J_{7,CH3-8} = J_{7,CH3-8'}$ = 6.2 Hz, H-7), 2.12 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 1.26 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.20 (d, $J_{7,8'}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –207.8 (ddd, $J_{F,2}$ = 50.3 Hz, $J_{F,3}$ = 10.6 Hz, $J_{F,4} = J_{F,1}$ = 3.2 Hz, F-2); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 170.1, 170.0 (3C=O, Ac), 95.3 (d, $J_{F,C1}$ = 20.6 Hz, C-1), 85.1 (d, $J_{F,C2}$ = 190.9 Hz, C-2), 71.8, 68.8 (d, $J_{F,C4}$ = 7.7 Hz, C-4), 68.3 (d, $J_{F,C3}$ = 18.8 Hz, C-3), 66.4, 61.6, 23.1, 21.7, 20.7, 20.6, 20.5 (3CH_3, Ac).

Data for **3.16β:** R_f (2:8 EtOAc/hexane): 0.25; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.39 (appt, $J_{3,4} = J_{4,F}$ = 3.2 Hz, 1H, H-4), 5.08 (ddd, $J_{3,F}$ = 13.3 Hz, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.60 (dd, $J_{1,2}$ = 7.7 Hz, $J_{1,F}$ = 3.8 Hz, 1H, H-1), 4.45 (ddd, $J_{2,F}$ = 51.1 Hz, $J_{2,3}$ = 9.7 Hz, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 4.16 (dd, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 6.6 Hz, 1H, H-6a), 4.08 (dd, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6b}$ = 6.8 Hz, 1H, H-6b), 4.01 (sept., $J_{7,CH3-8} = J_{7,CH3-8'}$ = 6.2 Hz, H-7), 3.90 (t, $J_{5,6a} = J_{5,6b}$ = 6.7 Hz, 1H, H-5), 2.11 (s, 3H, CH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 1.27 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.22 (d, $J_{7,8'}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –207.8

(ddt, $J_{F,2}$ = 51.3 Hz, $J_{F,3}$ = 13.3 Hz, $J_{F,4}$ = $J_{F,1}$ = 3.1 Hz, F-2); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 170.1, 170.0 (3C=O, Ac), 99.5 (d, $J_{F,C1}$ = 22.7 Hz, C-1), 87.9 (d, $J_{F,C2}$ = 186.6 Hz, C-2), 73.1, 71.3 (d, $J_{F,C3}$ = 19.2 Hz, C-3), 70.6, 67.7 (d, $J_{F,C4}$ = 8.2 Hz, C-4), 61.2, 23.29 21.9, 20.6, 20.6, 20.6 ($3CH_3$, Ac). Spectroscopic data was identical to that previously reported.[33]

1-*O*-ethan-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranose (3.17)



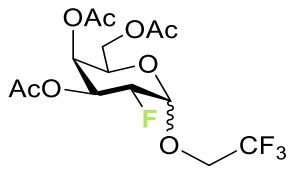
The general procedure was applied, the residue was purified by flash column chromatography (SiO_2, from hexane to 2:8 EtOAc/Hex) to afford **3.17** as a colourless oil.

Data for **3.17α**: R_f (2:8 EtOAc/hexane): 0.20; **$[\alpha]^D_{25}$**: + 129.7 (0.33, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.48 (td, $J_{3,4}$ = $J_{4,F}$ = 3.4 Hz, $J_{4,5}$ = 1.7 Hz, 1H, H-4), 5.42 (td, $J_{2,3}$ = $J_{3,F}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 5.12 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.75 (ddd, $J_{2,F}$ = 50.1 Hz, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.25 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, $J_{4,5}$ = 1.7 Hz, 1H, H-5), 4.11-3.05 (m, 2H, H-6a, H-6b), 3.79 (dq, $J_{7,7'}$ = 9.7 Hz, $J_{7,CH3}$ = 7.1 Hz, 1H, H-7), 3.62 (dq, $J_{7,7'}$ = 9.7 Hz, $J_{7,CH3}$ = 7.1 Hz, 1H, H-7'), 2.13 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.28 (t, $J_{7,8}$ = $J_{7',8}$ = 7.1 Hz, 3H, CH_3-8); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -208.4 (ddd, $J_{F,2}$ = 50.2 Hz, $J_{F,3}$ = 10.5 Hz, $J_{F,4}$ = 3.4 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 170.1, 170.0 (3C=O, Ac), 96.26 (d, $J_{F,2}$ = 20.6 Hz, C-1), 85.2 (d, $J_{F,2}$ = 190.9 Hz, C-2), 68.7 (d, $J_{F,4}$ = 7.6 Hz, C-4), 68.2 (d, $J_{F,3}$ = 18.9 Hz, C-3), 66.3, 64.4, 61.5(x2), 20.7(x2), 20.6 ($3CH_3$, Ac), 15.0; **FT-IR (neat)** ν in cm^{-1}: 2957, 2916, 2849, 1746, 1372, 1217, 1169, 1135, 1073, 1049, 980, 944, 918; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{14}H_{21}FNaO_8^+$ (m/z): calc. 359.1113; found 359.1117.

[33] Durantie, E.; Bucher, C.; Gilmour. R. *Chem. Eur. J.* **2012,** *18*, 8208-8215.

Data for **3.17β**: R_f (2:8 EtOAc/hexane): 0.18; **[α]$^D_{25}$**: + 14.7 (0.41, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.44-5.40 (m, 1H, H-4), 5.12-5.05 (m, 1H, H-3), 4.59-4.55 (m, 1H, H-1), 4.44 (ddd, $J_{2,F}$ = 48.6 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 4.18 (dd, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 6.6 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6b}$ = 6.9 Hz, 1H, H-6b), 3.99 (dq, $J_{7,7'}$ = 9.7 Hz, $J_{7,8}$ = 7.1 Hz, 1H, H-7), 3.92 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.8 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-5), 3.68 (dq, $J_{7,7'}$ = 9.7 Hz, $J_{7',8}$ = 7.1 Hz, 1H, H-7'), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.30 (t, $J_{7,8}$ = $J_{7',8}$ = 7.1 Hz, 3H, CH_3-8).**^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -206.8 (dddd, $J_{F,2}$ = 50.4 Hz, $J_{F,3}$ = 10.5 Hz, $J_{F,4}$ = 6.4 Hz, $J_{F,1}$ = 2.3 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 170.1, 170.0 (3C=O, Ac), 100.6 (d, $J_{F,3}$ = 19.1 Hz, C-1), 87.9 (d, $J_{F,2}$ = 186.8 Hz, C-2), 71.1 (d, $J_{F,3}$ = 19.1 Hz, C-3), 70.5, 67.6 (d, $J_{F,4}$ = 8.3 Hz, C-4), 66.0, 61.1, 29.7, 20,65, 20,6, 20.55 ($3CH_3$, Ac), 15.1; **FT-IR (neat)** ν in cm^{-1}: 2955, 2917, 2849, 1747, 1636, 1371, 1220, 1173, 1138, 1075, 1053, 950, 916; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{14}H_{21}FNaO_8^+$ (m/z): calc. 359.1113; found 359.1119.

1-*O*-trifluoroethan-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranose (3.18)



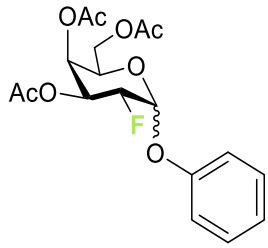
The general procedure was applied, the residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.18** as a colourless oil.

Data for **3.18α**: R_f (3:7 EtOAc/hexane): 0.17; **[α]$^D_{25}$**: + 118.3 (0.12, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.51 (td, $J_{3,4}$ = $J_{4,F}$ = 3.4 Hz, $J_{4,5}$ = 1.4 Hz, 1H, H-4), 5.41 (td, $J_{3,F}$ = $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.20 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.79 (ddd, $J_{2,F}$ = 49.4 Hz, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.27 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.3 Hz, $J_{4,5}$ = 1.4 Hz, 1H, H-5), 4.15-4.06 (m, 2H, H-6a, H-6b), 4.04 (q, $J_{7,CF3}$ = 8.5 Hz, 2H, CH_2-7'), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -74.10 (t, $J_{CF3,7}$ = 8.5 Hz, 3F, CF_3), -209.2

(ddd, $J_{F,2}$ = 49.4 Hz, $J_{F,3}$ = 10.7 Hz, $J_{F,4}$ = 3.4 Hz, 1F, F-2-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 169.9(x2) (3C=O, Ac), 97.3 (d, $J_{F,2}$ = 21.0 Hz, C-1), 84.7 (d, $J_{F,2}$ = 192.8 Hz, C-2), 68.3 (d, $J_{F,4}$ = 7.7 Hz, C-4), 68.2 (d, $J_{F,3}$ = 18.8 Hz, C-3), 67.4, 65.8 (q, $J_{CF3,7}$ = 35.4 Hz, CH_2-7), 61.3, 20.6, 20.5 ($3CH_3$, Ac); **FT-IR (neat)** ν in cm^{-1}: 2919, 2850, 1747, 1372, 1279, 1226, 1159, 1075, 1049; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{14}H_{22}F_4NO_8^+$ (m/z): calc.408.1276; found 408.1290.

Data for **3.18β**: ***R*$_f$** (3:7 EtOAc/hexane): 0.15; Selected signals: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43 (td, $J_{3,4}$ = $J_{3,F}$ = 3.6 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-4), 5.11 (ddd, $J_{3,F}$ = 13.4 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 4.67 (dd, $J_{1,2}$ = 7.6 Hz, $J_{1,F}$ = 4.0 Hz, 1H, H-1), 4.53 (ddd, $J_{2,F}$ = 51.1 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 4.23-3.99 (m, 4H, H-6a, H-6b, CH_2-7), 3.93 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-5), 2.14 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -74.22 (t, $J_{F,7}$ = 8.4 Hz, 3F, CF_3), -207.5 (dddd, $J_{F,2}$ = 51.1 Hz, $J_{F,3}$ = 13.5 Hz, $J_{F,1}$ = 4.0 Hz, $J_{F,4}$ = 3.6 Hz, 2-F-β-anomer); **FT-IR (neat)** ν in cm^{-1}: 2959, 2917, 2849, 1748, 1437, 1372, 1279, 1226, 1159, 1075, 1049; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{14}H_{22}F_4NO_8^+$ (m/z): calc. 408.1276; found 408.1289.

1-*O*-phen-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-galactopyranose (3.19)



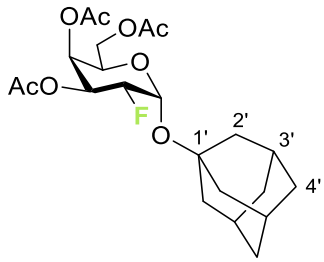
The general procedure was applied, the residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **19** as colourless oil.

Data for **3.19α**: ***R*$_f$** (3:7 EtOAc/hexane): 0.30; **[α]$^D_{25}$**: + 178.0 (0.29, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.35-7.28 (m, 2H, Ph), 7.13-7.01 (m, 3H, Ph), 5.75 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 5.63 (td, $J_{2,3}$ = $J_{3,F}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 5.57 (td, $J_{3,4}$ = $J_{4,F}$ = 3.4 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4), 4.93 (ddd, $J_{2,F}$ = 49.5

Hz, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.39 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, $J_{4,5}$ = 1.5 Hz, 1H, H-5), 4.17-4.04 (m, 2H, H-6a, H-6b), 2.17 (s, 3H, Ac), 2.08 (s, 3H, Ac), 1.96 (s, 3H, Ac); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -208.1 (ddd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 10.7 Hz, $J_{F,4}$ = 3.2 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.3, 170.0(x2) (3C=O, Ac), 156.3, 129.6(x2), 123.2, 116.9(x2), 95.3 (d, $J_{F,2}$ = 21.0 Hz, C-1), 84.8 (d, $J_{F,2}$ = 192.6 Hz, C-2), 68.4 (d, $J_{F,4}$ = 7.6 Hz, C-4), 68.2 (d, $J_{F,3}$ = 18.8 Hz, C-3), 67.3, 61.3, 61.5(x2), 20.7, 20.6(x2) ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2961, 2922, 1747, 1597, 1591, 1493, 1372, 1216, 1130, 1075, 1048; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{18}H_{25}FNO_8^+$ (m/z): calc. 402.1559; found 402.1571.

Data for **3.19β**: R_f (3:7 EtOAc/hexane): 0.23; **$[\alpha]^D_{25}$**: + 6.8 (0.22, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.37-7.28 (m, 2H, Ph), 7.13-7.06 (m, 3H, Ph), 5.48 (ddd, $J_{3,4}$ = 3.5 Hz, $J_{4,F}$ = 2.5 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-4), 5.20 (ddd, $J_{3,F}$ = 13.4 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.6 Hz, 1H, H-3), 5.14 (dd, $J_{1,2}$ = 7.6 Hz, $J_{1,F}$ = 3.9 Hz 1H, H-1), 4.77 (ddd, $J_{2,F}$ = 51.3 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 4.23 (dd, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 6.9 Hz, 1H, H-6a), 4.14 (dd, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6b}$ = 6.9 Hz, 1H, H-6b), 4.07 (td, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, $J_{4,5}$ = 1.1 Hz, 1H, H-5), 2.17 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.06 (s, 3H, Ac); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -206.6 (dddd, $J_{F,2}$ = 51.3 Hz, $J_{F,3}$ = 13.4 Hz, $J_{F,1}$ = 3.8 Hz, $J_{F,4}$ = 2.5 Hz, β-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.3, 170.0, 169.9 (3C=O, Ac), 156.6, 129.6 (x2), 123.5, 117.2(x2), 99.2 (d, $J_{F,1}$ = 23.7 Hz, C-1), 87.4 (d, $J_{F,2}$ = 188.4 Hz, C-2), 71.00 (d, $J_{F,3}$ = 18.8 Hz, C-3), 70.9, 67.4 (d, $J_{F,4}$ = 8.3 Hz, C-4), 61.1, 20.62, 20.6, 20.55 ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2959, 2924, 1750, 1491, 1370, 1222, 1075, 1055; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{18}H_{25}FNO_8^+$ (m/z): calc. 402.1559; found 402.1574.

1-*O*-adamanthan-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranose (3.20)

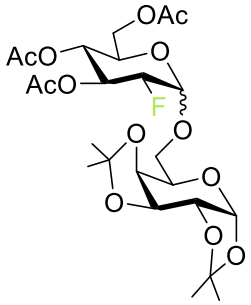


The general procedure was applied, the residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **20** as white powder.

R_f (2:8 EtOAc/hexane): 0.20; **$[\alpha]^{D}_{25}$**: + 16.2 (0.72, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.50-5.45 (m, 2H, H-1, H-4), 5.41 (td, $J_{2,3} = J_{3,F}$ = 10.4 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.71 (ddd, $J_{2,F}$ = 50.8 Hz, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.43 (t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, 1H, H-5), 4.14-4.01 (m, 2H, H-6a, H-6b), 2.16 (m, 3H, 3xH-3'), 2.13 (s, 3H, Ac), 2.03 (s, 6H, 2xAc), 1.82 (m, 6H, 3xCH_2-4'), 1.82 (m, 6H, 6xCH_2-2'), 1.65 (m, 6H, 6xH-4'); **^{19}F NMR** ($CDCl_3$, 375.6 MHz) δ in ppm: -206.35 (ddd, $J_{F,2}$ = 50.7 Hz, $J_{F,3}$ = 10.4 Hz, $J_{F,1}$ = 3.4 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4 (x2), 170.1 (3C=O, Ac), 84.9 (d, $J_{F,2}$ = 190.8 Hz, C-2), 75.4, 68.9 (d, $J_{F,4}$ = 7.2 Hz, C-4), 68.5 (d, $J_{F,3}$ = 19.1 Hz, C-3), 66.0, 61.7, 42.3, 36.1, 30.6, 29.7, 20.7 (3CH_3, Ac); **FT–IR (neat)** ν in cm^{-1}: 2916, 2850, 1751, 1372, 1231, 1072, 1044, 1029; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{22}H_{31}FNaO_8^+$ (m/z): calc. 465.1895; found 465.1904.

3.5.7. Glycosyl donor scope

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-glucosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.22):



The general procedure was followed using glucosyl iodide **3.11-I** (10.2 mg, 0.024 mmol), acceptor **3.21** (7.6 mg, 0.03 mmol), allyl tributyltin (10 µL, 0.032 mmol) and TfOH (0.8 µL, 0.009 mmol) in 0.5 mL of toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 74% yield of **3.22** obtaining an α:β ratio of 4.4:1.

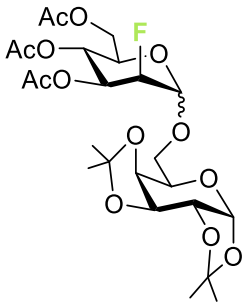
The general procedure was followed using glucosyl bromide **3.11-Br** (25 mg, 0.06 mmol), acceptor **3.21** (19.7 mg, 0.075 mmol), allyl tributyltin (24.3 µL, 0.078 mmol) and TfOH (1.6 µL, 0.018 mmol) in 1.2 mL of dry toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 79% yield of **3.22** obtaining an α:β ratio of 3.8:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **21** as colorless crystals (14.5 mg, 31%).

Data for **3.22α:** R_f (2:8 EtOAc/hexane): 0.21; **^{1}H NMR** ($CDCl_3$, 400 MHz): δ in ppm: 5.54 (dt, $J_{F,3}$ = 12.1 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.50 (d, $J_{1,2}$ = 5.1 Hz, 1H, H-1'), 5.13 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 5.02 (appt, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.61 (dd, $J_{3\text{-}4}$ = 7.9 Hz, $J_{2,3}$ = 2.3 Hz, 1H, H-3'), 4.49 (ddd, $J_{F,2}$ = 49.2 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.28 (m, 3H, H-4', H-2', H-6a), 4.16 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.0 Hz, $J_{5,6b}$ = 2.1 Hz, 1H, H-5), 4.07 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.0 Hz, 1H, H-6b), 4.01 (td, $J_{5,6a}$ = 7.9 Hz, $J_{4,5}$ = 1.7 Hz, 1H, H-5'), 3.82 (m, 2H, H-6a', H-6b'), 2.08, 2.06, 2.03 (s, 9H, 3CH_3, Ac), 1.56 (s, 3H, CH_3'), 1.42 (s, 3H, CH_3'), 1.33 (s,

3H, CH_3'), 1.32 (s, 3H, CH_3'); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -200.8 (dd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 11.8 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 168.8, 168.2, 167.8 (3C=O, Ac), 107.4, 106.8, 94.6 (d, $J_{1,F}$ = 20.4 Hz, C-1), 94.3, 85.4 (d, $J_{2,F}$ = 195.1 Hz, C-2), 68.9 (d, $J_{3,F}$ = 19.5 Hz, C-3), 68.8, 68.7, 68.6, 66.2, 66.0 (d, $J_{4,F}$ = 7.5 Hz, C-4), 65.2, 64.7, 59.8, 24.2, 24.0, 23.0, 22.4, 18.9, 18.8, 18.7. Spectroscopic data was identical to that previously reported.[34]

Data for **3.22β: ^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -199.4 (ddd, $J_{F,2}$ = 50.5 Hz, $J_{F,3}$ = 14.4 Hz, $J_{F,4}$ = 2.7 Hz, β-anomer).

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-mannosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.23):



The general procedure was followed using glucosyl iodide **3.12-I** (22.5 mg, 0.054 mmol), acceptor **3.21** (17.5 mg, 0.067 mmol), allyl tributyltin (21.7 µL, 0.070 mmol) and TfOH (1.5µL, 0.016 mmol) in 1 mL of toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 77% yield of **3.23** obtaining an α:β ratio of 12.5:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.23** as colorless crystals (15.7 mg, 43%).

The general procedure was followed using glucosyl bromide 3.12-Br (9.25 mg, 0.025 mmol), acceptor **3.21** (7.8 mg, 0.03 mmol), allyl tributyltin (10 µL, 0.033 mmol) and TfOH (0.8 µL, 0.008 mmol) in 0.5 mL of dry toluene. After

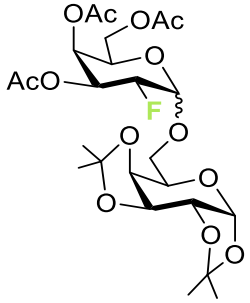
[34] Vincent, S. P.; Burkart, M. D.; Tsai, C. Y.; Zhang, Z.; Wong, C. H. *J. Org. Chem.* **1999**, *64*, 5264-5279.

90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 90% yield of **22** obtaining only the α anomer.

Data for **3.23α:** R_f (2:8 EtOAc/hexane): 0.17; **^{1}H NMR** ($CDCl_3$, 400 MHz): δ in ppm: 5.51 (d, $J_{1,2}$ = 5.0 Hz, 1H, H-1'), 5.35 (appt, $J_{3,4}$ = 10.0 Hz, 1H, H-4), 5.24 (ddd, $J_{3,F}$ = 28.4 Hz, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 2.3 Hz, 1H, H-3), 5.05 (dd, $J_{F,1}$ = 7.2 Hz, $J_{1,2}$ = 2.1 Hz, 1H, H-1), 4.77 (appdt, $J_{2,F}$ = 49.8 Hz, $J_{2,3}$ = $J_{1,2}$ = 2.2 Hz, 1H, H-2), 4.62 (dd, $J_{3,4}$ = 7.9 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-3'), 4.35-4.27 (m, 2H, H-6a, H-2'), 4.22 (dd, $J_{3,4}$ = 7.9 Hz, $J_{4,5}$ = 1.8 Hz, 1H, H-4'), 4.16-4.05 (m, 2H, H-5, H-6b), 3.96 (td, $J_{5,6a}$ = $J_{5\text{-}6b}$ = 6.3 Hz, $J_{4,5}$ = 1.7 Hz, 1H, H-5'), 3.84 (dd, $J_{6a,6b}$ = 10.3 Hz, $J_{5,6b}$ = 6.5 Hz, 1H, H-6a'), 3.75 (dd, $J_{6a,6b}$ = 10.3 Hz, $J_{5,6b}$ = 6.4 Hz 1H, H-6b'), 2.10, 2.09, 2.04 (s, 9H, 3CH_3, Ac), 1.55 (s, 3H, CH_3'), 1.43 (s, 3H, CH_3'), 1.32 (s, 6H, 2xCH_3'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –204.35 (ddd, $J_{F,2}$ = 49.8 Hz, $J_{F,3}$ = 28.6 Hz, $J_{F,1}$ = 7.3 Hz, F-2, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.9, 170.2, 169.6 (3C=O, Ac), 109.5, 108.8, 97.2 (d, $J_{1,F}$ = 29.5 Hz, C-1), 96.3, 86.9 (d, $J_{2,F}$ = 179.3 Hz, C-2), 70.9, 70.6, 70.5, 70.0 (d, $J_{3,F}$ = 16.8 Hz, C-3), 68.6, 67.0, 66.1, 65.9, 65.6, 62.0, 26.1, 26.0, 24.9, 24.5, 20.81, 20.71, 15.30; spectroscopic data was identical to that previously reported.[34]

Data for **3.23β:** **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –203.8 (ddd, $J_{F,2}$ = 49.2 Hz, $J_{F,3}$ = 27.4 Hz, $J_{F,1}$ = 6.5 Hz, F-2, β-anomer).

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-galactosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.24):



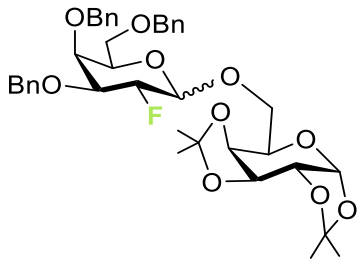
The general procedure was followed using galactosyl iodide **3.10-I** (21.3 mg, 0.051 mmol), acceptor **3.21** (16.6 mg, 0.064 mmol), allyl tributyltin (19.7 µL, 0.066 mmol) and TfOH (1.3 µL, 0.015 mmol) in 1 mL of toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 67% yield of **23** obtaining an α:β ratio of 7.3:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.23** as colorless crystals (11.5 mg, 41%).

The general procedure was followed using galactosyl bromide **3.10-Br** (25 mg, 0.067 mmol), acceptor **3.21** (24.5 mg, 0.095 mmol), allyl tributyltin (38.4 µL, 0.124 mmol) and TfOH (2.5 µL, 0.028 mmol) in 1.4 mL of dry toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 83% yield of **3.23** obtaining an α:β ratio of 5:1.

Data for **3.24α:** R_f (2:8 EtOAc/hexane): 0.15; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.52-5.49 (m, 2H, H-1', H-4), 5.42 (dt, $J_{2,3}$ = $J_{3,F}$ = 10.4 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 5.17 (dd, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.76 (ddd, $J_{2,F}$ = 53.4 Hz, $J_{2,3}$ = 10.4 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 4.62 (dd, $J_{3,4}$ = 8.0 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-3'), 4.38 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 5.2 Hz, 1H, H-5), 4.31 (dd, $J_{1,2}$ = 5.2 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-2') 4.27 (dd, $J_{3,4}$ = 8.0 Hz, $J_{4,5}$ = 2.1 Hz, H-4'), 4.11-4.09 (m, 2H, H-6a, H-6b), 4.02 (m, 1H, H-5'), 3.86-3.76 (m, 2H, H-6a', H-6b'), 2.14, 2.59, 2.04 (s, 9H, 3CH_3, Ac), 1.56 (s, 3H, CH_3'), 1.44 (s, 3H, CH_3'), 1.33 (s, 6H, 2xCH_3'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –208.9 (ddd, $J_{F,2}$ = 53.4 Hz, $J_{F,3}$ = 10.4 Hz, $J_{F,4}$ = 3.0 Hz, F-2, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.7, 170.3, 170.2 (3C=O, Ac), 109.5, 108.9, 97.0 (d, $J_{1,F}$ = 20.5 Hz, C-1), 96.4, 85.6 (d, $J_{2,F}$ = 190.7 Hz, C-2), 70.9, 70.8, 69.0 (d, $J_{4,F}$ = 7.6 Hz, C-4), 68.4 (d, $J_{3,F}$ = 18.3 Hz, C-3), 67.9, 66.8, 66.7, 61.7, 29.9 26.3, 26.2, 25.2, 24.6, 21.0, 20.9, 20.8. Spectroscopic data was identical to that previously reported.[34]

Data for **3.24β:** **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –206.5 (dd, $J_{F,2}$ = 51.3 Hz, $J_{F,3}$ = 12.3 Hz, F-2, β-anomer).

2-Deoxy-2-fluoro-3,4,6-tri-*O*-benzyl-α-D-galactosyl-(1-6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.25):



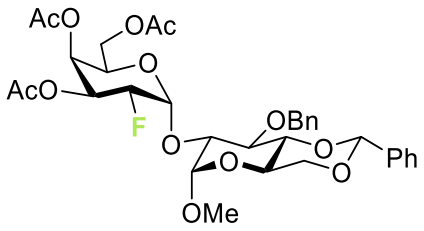
The general procedure was followed using galactosyl bromide **3.13-Br** (16 mg, 0.031 mmol), acceptor **3.21** (9.8 mg, 0.037 mmol), allyl tributyltin (12.5 µL, 0.0403 mmol) and TfOH (0.8 µL, 0.009 mmol) in 0.7 mL of dry toluene. After 90 min of microwave heating at 100 °C quantitative ^{19}F NMR analysis indicated 96% yield of **3.25** obtaining an α:β ratio of 1:3. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.25α** as colorless oil (4.5 mg, 20%) and **3.25β** as colorless oil (11.5 mg, 51%).

Data for **3.25α:** R_f (3:7 Et_2O/hexane): 0.24; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.32-7.17 (m, 15H), 5.50 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1'), 5.10 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.94 (ddd, $J_{2,F}$ = 50.3 Hz, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 4.0 Hz, 1H, H-2), 4.92 (d, J = 11.3 Hz, 1H, CH-Ph), 4.79 (d, J = 11.8 Hz, 1H, CH-Ph), 4.68 (d, J = 11.8 Hz, 1H, CH-Ph), 4.59 (dd, $J_{3,4}$ = 8.1 Hz, $J_{2,3}$ = 2.5 Hz, 1H, H-3'), 4.56 (d, J = 11.3 Hz, 1H, CH-Ph), 4.49 (d, J = 11.8 Hz, 1H, CH-Ph), 4.42 (d, J = 11.8 Hz, 1H, CH-Ph), 4.30 (dd, $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-2'), 4.27 (dd, $J_{3,4}$ = 8.1 Hz, $J_{4,5}$ = 1.8 Hz, 1H, H-4'), 4.09 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 6.7 Hz, 1H, H-5), 4.06-3.97 (m, 3H, H-3, H-4, H-5'), 3.82 (dd, $J_{6a,6b}$ = 10.6 Hz, $J_{5,6a}$ = 6.6 Hz, 1H, H-6a'), 3.74 (dd, $J_{6a,6b}$ = 10.6 Hz, $J_{5,6b}$ = 6.2 Hz, 1H, H-6b'), 3.62-3.50 (m, 2H, H-6a, H-6b), 1.52 (s, 3H, CH_3'), 1.41 (s, 3H, CH_3'), 1.33 (s, 6H, 2 CH_3'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –208.07 (ddd, $J_{F,2}$ = 50.3 Hz, $J_{F,3}$ = 9.7 Hz, $J_{F,4}$ = 4.7 Hz, F-2, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 138.45, 138.4, 137.9, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.5, 127.4, 109.3, 108.6, 96.9 (d, $J_{C1,F}$ = 20.9 Hz, C-1), 96.3, 89.2 (d, $J_{C2,F}$ = 188.3 Hz, C-2), 75.4 (d, $J_{C3,F}$ = 8.0 Hz, C-3), 74.9, 73.3, 72.9 (d, $J_{C4,F}$ = 1.9 Hz, C-4), 70.8, 70.6, 70.5, 69.2, 68.3, 66.9, 66.4, 53.4, 29.7, 26.0, 25.9, 25.0, 24.4.

Data for **3.25β:** R_f (3:7 Et_2O/hexane): 0.16; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.32-7.17 (m, 15H), 5.46 (d, $J_{1,2}$ = 4.9 Hz, 1H, H-1'), 4.85 (d, J = 11.5 Hz, 1H, CH-Ph), 4.71 (d, J = 12.1 Hz, 1H, CH-Ph), 4.70-4.50 (m, 1H, H-2), 4.61 (d, J = 12.2 Hz, 1H, CH-Ph), 4.57-4.46 (m, 3H, CH-Ph, H-3', H-1), 4.38 (d, J = 11.8 Hz, 1H, CH-Ph), 4.34 (d, J = 11.7 Hz, 1H, CH-Ph), 4.22 (dd, $J_{1,2}$ = 5.0 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-2'), 4.19 (dd, $J_{3,4}$ = 7.9 Hz, $J_{4,5}$ = 1.7 Hz, H-4'), 3.99-3.93 (m, 2H, H-5', H-6a'), 3.87 (t, $J_{3,4} = J_{4,F}$ = 3.2 Hz, H-4), 3.70 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 8.0 Hz, 1H, H-6b'), 3.59-3.48 (m, 4H, H-3, H-6a, H-6b, H-5), 1.46 (s, 3H, CH_3'), 1.36 (s, 3H, CH_3'), 1.26 (s, 3H, CH_3'), 1.24 (s, 3H, CH_3'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –205.7 (ddd, $J_{F,2}$ = 51.4 Hz, $J_{F,3}$ = 12.7 Hz, $J_{F,4} = J_{F,1}$ = 3.8 Hz, F-2); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 138.4, 138.1, 137.8, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 109.3, 108.7, 101.0 (d, $J_{1,F}$ = 23.6 Hz, C-1), 96.3, 91.7 (d, $J_{2,F}$ = 183.7 Hz, C-2), 80.3 (d, $J_{3,F}$ = 16.1 Hz, C-3), 74.7, 74.0 (d, $J_{4,F}$ = 9.0 Hz, C-4), 73.6, 73.5, 72.7, 72.7, 71.2, 70.6, 70.5, 68.4, 68.2, 67.6, 26.0, 25.9, 25.0, 24.4. Spectroscopic data was identical to that previously reported.

3.5.8. Stereoselective synthesis of relevant 2-deoxy-2-fluoro-α-glycosides

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-galactosyl-(1,2)-methyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (3.15):

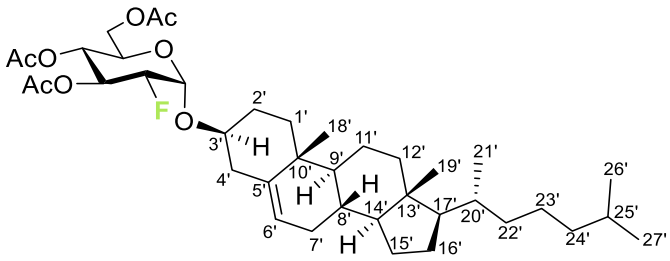


The general procedure was followed using galactosyl iodide **3.10-I** (12 mg, 0.029 mmol), acceptor **3.14** (14.3 mg, 0.036 mmol), allyl tributyltin (11 µL, 0.037 mmol) and TfOH (0.8 µL, 0.009 mmol) in 0.6 mL of dry toluene. After 2h of microwave heating at 100 °C quantitative ^{19}F NMR analysis indicated 65% yield of **3.15** obtaining only the α anomer.

The general procedure was followed using galactosyl bromide **3.10-Br** (30 mg, 0.081 mmol), acceptor **3.14** (36.2 mg, 0.097 mmol), allyl tributyltin (32.6 µL, 0.105 mmol) and TfOH (2 µL, 0.024 mmol) in 1.6 mL of toluene. After 2h of microwave heating at 100 °C quantitative ^{19}F NMR analysis indicated 55% yield of **3.15** obtaining only the α anomer. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.15** as a white powder (17.2 mg, 32%).

Data for **3.15α:** ***R*$_f$** (3:7 EtOAc/hexane): 0.32; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.55-7.23 (m, 10H, Ar-H), 5.60 (s, 1H, H-7'), 5.50 (td, $J_{2,3}$ = $J_{F,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 5.29 (td, $J_{3,4}$ = $J_{4,5}$ = 3.3 Hz, $J_{F,4}$ = 1.2 Hz, 1H, H-4), 5.23 (d, $J_{F,1}$ = 3.7 Hz, 1H, H-1), 4.96 (d, J = 10.6 Hz, 1H, CH-Ph), 4.88 (d, $J_{F,1}$ = 3.6 Hz, 1H, H-1'), 4.79 (ddd, $J_{F,2}$ = 53.3 Hz, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2) 4.70 (d, J = 10.6 Hz, 1H, CH-Ph), 4.45 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 4.8 Hz, 1H, H-5), 4.32 (dd, $J_{6a,6b}$ = 10.1 Hz, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a), 4.09 (appt, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H-3'), 3.83 (appdd, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 3.6 Hz, 1H, H-2'), 3.77 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6}$ = 4.8 Hz, 1H, H-6b), 3.93-3.73 (m, 3H, H-5', H-6a', H-6b'), 3.65 (appt, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, 1H, H-4'), 3.46 (s, 3H, OMe'), 2.10, 2.05, 1.90 (s, 9H, 3CH_3, Ac); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.1, 169.9 (3C=O, Ac), 138.2, 137.4 (CH, Ar), 129.1, 128.7, 128.4, 128.1, 100.6.1 (CH, Ar), 101.5 (C-7'), 97.6 (C-1'), 94.6 (d, $J_{F,1}$ = 21.0 Hz, C-1), 85.4 (d, $J_{F,2}$ = 191.1 Hz, C-2), 83.0, 77.4, 75.9, 75.7, 69.1, 68.6, 68.0, 66.7, 62.4, 61.3, 55.7 (CH_3, OMe), 20.8, 20.7, 20.6 (3CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –208.83 (ddd, $J_{F,2}$ = 50.0 Hz, $J_{F,3}$ = 10.8 Hz, $J_{F,1}$ = 2.8 Hz, F-2).

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-glucosyl-(1-3)-cholesterol (3.26):



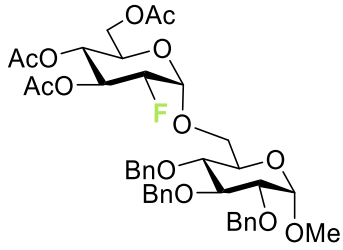
The general procedure was followed using glucosyl iodide **3.11-I** (10.5 mg, 0.025 mmol), cholesterol (11.8mg, 0.03 mmol), allyl tributyltin (10 µL, 0.033 mmol) and TfOH (0.7 µL, 0.008 mmol) in 0.5 mL of toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 66% yield of **3.26** obtaining an α:β ratio of 2.7:1.

The general procedure was followed using glucosyl bromide **3.11-Br** (18.5 mg, 0.05 mmol), cholesterol (23.2mg, 0.06 mmol), allyl tributyltin (20 µL, 0.065 mmol) and TfOH (1.4 µL, 0.015 mmol) in 1 mL of toluene. After 90 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 68% yield of **3.26** obtaining an α:β ratio of 3.5:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.26** as a white solid (15.5 mg, 46%).

Data for **3.26α:** R_f (1:9 EtOAc/hexane): 0.14; $[\alpha]^{D}_{25}$: + 71.9 (0.59, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.53 (dt, $J_{3,F}$ = 11.8 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.34 (d, J = 5.2 Hz, 1H, H-6'), 5.18 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.98 (t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.45 (ddd, $J_{F,2}$ = 49.7 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.24 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a) 4.14 (ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 4.07 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.48 (m, 1H, H-3'), 2.44-2.31 (m, 2H, H-4', H-7'), 2.07 (s, 3H, CH_3, Ac), 2.05 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.10-1.75 (m, 4H, H-4'', H7'', H-15', H-16'), 1.60-0.80 (m, 22H), 1.00 (s, 3H, CH_3-18), 0.90 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21), 0.86 (d, $J_{25,26}$ = 6.6 Hz, 3H, CH_3-26), 0.84 (d, $J_{25,27}$ = 6.6 Hz, 3H, CH_3-27), 0.66 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -200.6 (dd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 11.8 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 170.1, 169.7 (3C=O, Ac), 140.3 (C-5'), 122.2 (C-6'), 94.6 (d, $J_{F,C1}$ = 20.4 Hz, C-1), 87.0 (d, $J_{F,C2}$ = 194.9 Hz, C-2), 79.4 (C-3') 70.82 (d, $J_{F,C3}$ = 19.4 Hz, C-3), 68.27 (d, $J_{F,C4}$ = 7.0 Hz, C-4), 67.3 (C-5), 61.9, 56.7, 56.1, 50.0, 42.3, 40.0, 39.7, 39.5, 37.0, 36.6, 35.1, 35.8, 31.9, 31.8, 28.2, 28.0, 27.8, 24.3, 23.8, 22.8, 22.6, 21.0, 20.8, 20.7, 20.6, 19.3, 18.7, 11.8, 1.0; **FT–IR (neat)** ν in cm^{-1}: 2935, 2867, 2850, 1751, 1465, 1456, 1436, 1374, 1222, 1030; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{39}H_{61}FNaO_8^+$ (m/z): calc. 699.4243; found 699.4249.

Data for **3.26β:** R_f (1:9 EtOAc/hexane): 0.12; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.37 (d, J = 5.2 Hz, 1H, H-6'), 5.31 (dt, $J_{3,F}$ = 14.4 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.01 (t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.67 (dd, $J_{1,2}$ = 7.6 Hz, $J_{1,F}$ = 2.7 Hz, 1H, H-1), 4.45 (ddd, $J_{F,2}$ = 50.5 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 4.26 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a) 4.09 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.69 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 3.48 (m, 1H, H-3'), 2.44-2.31 (m, 2H, H-4', H-7'), 2.08 (s, 3H, CH_3, Ac), 2.07 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.10-1.75 (m, 4H, H-4'', H7'', H-15', H-16'), 1.60-0.80 (m, 31H), 1.00 (s, 3H, CH_3-18), 0.67 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -198.9 (ddd, $J_{F,2}$ = 50.5 Hz, $J_{F,3}$ = 14.4 Hz, $J_{F,1}$ = 2.4 Hz, β-anomer); **FT–IR** (neat) ν in cm^{-1}: 2938, 2867, 1752, 1465, 1456, 1436, 1367, 1227, 1172, 1137, 1031; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{39}H_{61}FNaO_8^+$ (m/z): calc. 699.4243; found 699.4251.

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-glucosyl-(1-6)-methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (3.27):



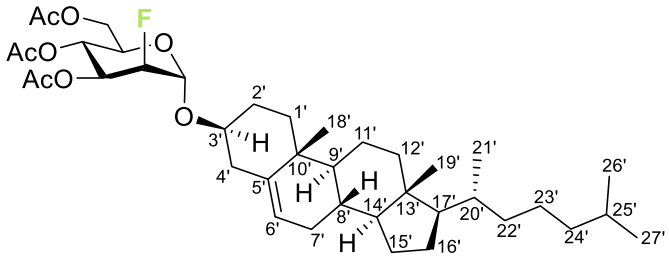
The general procedure was followed using glucosyl iodide **3.11-I** (19.5 mg, 0.047 mmol), methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (27.1 mg, 0.058 mmol), allyl tributyltin (18.8 µL, 0.060 mmol) and TfOH (1.2 µL, 0.014 mmol) in 1 mL of toluene. After 90 min of microwave heating at 90 °C quantitative ^{19}F NMR analysis indicated 47% yield of **3.27** obtaining an α:β ratio of 5:1.

The general procedure was followed using glucosyl bromide **3.11-Br** (21.5 mg, 0.058 mmol), methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (33.7 mg, 0.072 mmol), allyl tributyltin (25 µL, 0.075 mmol) and TfOH (1.5 µL, 0.017 mmol) in 1.2 mL of dry toluene. After 90 min of microwave heating at 90 °C quantitative ^{19}F NMR analysis indicated 63% yield of **3.27** obtaining an α:β ratio

of 3:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **27** as colorless crystals (23.5 mg, 53%).

Data for **3.27α:** ***R*****$_f$** (1:9 EtOAc/hexane): 0.09; **[α]$^D_{25}$**: + 54.8 (1.40, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ in ppm: 7.39-7.23 (m, 15H, Ph), 5.47 (dt, $J_{F,3}$ = 12.0 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.13 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.97-4.87 (m, 3H, H-4, 2CH-Ph), 4.76 (d, J = 11.0 Hz, 1H, CH-Ph), 4.76 (d, J = 12.0 Hz, 1H, CH-Ph), 4.63 (d, J = 12.1 Hz, 1H, CH-Ph), 4.58 (d, J = 11.6 Hz, 1H, CH-Ph), 4.55 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1'), 4.42 (ddd, $J_{F,2}$ = 49.2 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.12 (dd, $J_{6a,6b}$ = 12.6 Hz, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 3.98-3.94 (m, 3H, H-3', H-5, H-6b), 3.78-3.69 (m, 3H, H-5', H-6a', H-6b'), 3.51-3.42 (m, 2H, H-4', H-2'), 3.34 (s, 3H, CH_3-O'), 2.02 (s, 3H, CH_3, Ac), 1.99 (s, 3H, CH_3, Ac), 1.98 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -201.1 (dd, $J_{F,2}$ = 49.3 Hz, $J_{F,3}$ = 12.0 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 170.0, 169.7 (3C=O, Ac), 138.6, 138.3, 138.2, 128.5(x2), 128.4, 128.1(x2), 128.0, 127.9, 127.8, 127.72, 127.7, 97.9, 96.0 (d, $J_{1,F}$ = 20.4 Hz, C-1), 87.2 (d, $J_{2,F}$ = 195.4 Hz, C-2), 82.3, 80.1, 77.6, 75.85, 75.0, 73.5, 70.7 (d, $J_{3,F}$ = 19.5 Hz, C-3), 70.1, 68.0 (d, $J_{4,F}$ = 7.2 Hz, C-4), 67.2, 66.6, 61.6, 55.2, 20.8, 20.7, 20.6 ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2931, 1752, 1455, 1366, 1223, 1164, 1136, 1073, 1027; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{47}FNaO_{13}^+$ (m/z): calc. 777.2893; found 777.2893.

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-mannosyl-(1-3)-cholesterol (3.28):



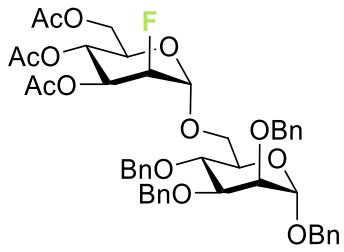
The general procedure was followed using mannosyl iodide **3.12-I** (7.5 mg, 0.018 mmol), cholesterol (11.8 mg, 0.003 mmol), allyl tributyltin (10 µL, 0.035 mmol) and TfOH (0.5 µL, 0.005 mmol) in 0.5 mL of toluene. After 60 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 85%

yield of **3.28** obtaining only the α anomer. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **26** as a white solid (8.5 mg, 70%).

The general procedure was followed using glucosyl bromide **3.12-Br** (6.3 mg, 0.017 mmol), cholesterol (7.9 mg, 0.02 mmol), allyl tributyltin (7.3 µL, 0.022 mmol) and TfOH (0.5 µL, 0.005 mmol) in 0.5 mL of toluene. After 120 min of microwave heating at 70 °C quantitative ^{19}F NMR analysis indicated 90% yield of **3.28** obtaining only the α anomer.

Data for **3.28α:** R_f (1:9 EtOAc/hexane): 0.10; **[α]$^{D}_{25}$**: + 36.0 (0.85, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.38-5.20 (m, 3H, H-3, H-6', H-4), 5.12 (d, $J_{1,F}$ = 7.2 Hz, 1H, H-1), 4.45 (d, $J_{F,2}$ = 50.2 Hz, 1H, H-2), 4.24 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 4.12 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 1.7 Hz, 1H, H-6b), 4.07 (m, 1H, H-5), 3.57-3.43 (m 1H, H-3'), 2.33 (d, $J_{4',3'}$ = 7.8 Hz, 2H, H-4', H-4''), 2.08 (s, 3H, CH_3, Ac), 2.07 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.10-1.75 (m, 4H), 1.60-0.80 (m, 22H), 0.99 (s, 3H, CH_3-18), 0.90 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21), 0.85 (m, 6H, CH_3-26, CH_3-27), 0.66 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -202.7 (dd, $J_{F,2}$ = 50.2 Hz, $J_{F,3}$ = 28.5 Hz, $J_{F,1}$ = 7.0 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.7, 170.2, 169.6 (3xC=O, Ac), 140.2 (C-5'), 122.3 (C-6'), 95.5 (d, $J_{F,C1}$ = 29.0 Hz, C-1), 87.0 (d, $J_{F,C2}$ = 179.9 Hz, C-2), 78.4 (C-3') 70.0 (d, $J_{F,C3}$ = 16.7 Hz, C-3), 68.5 (C-5), 66.0 (C-4), 62.3, 56.7, 56.1, 50.0, 42.3, 39.9, 39.7, 39.5, 39.9, 36.6, 36.1, 35.8, 31.9, 31.8, 28.2, 28.0, 27.7, 24.25, 23.8, 22.8, 22.6, 21.0, 20.8, 20.75, 20.65, 19.3, 18.7, 11.8; **FT–IR (neat)** ν in cm^{-1}: 2951, 2867, 1750, 1456, 1436, 1373, 1228, 1134, 1091, 1050, 981, 801; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{39}H_{61}FNaO_8^+$ (m/z): calc. 699.4243; found 699.4261.

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-mannosyl-(1-6)-1,2,3,4-tetra-*O*-benxyl-α-D-mannopyranose (3.29):



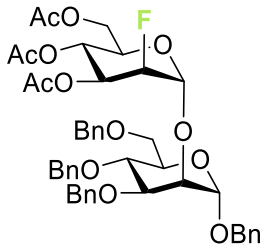
The general procedure was followed using mannosyl iodide **3.12-I** (6.5 mg, 0.015 mmol), 1,2,3,4-tetra-*O*-benxyl-α-D-mannopyranose (9.5mg, 0.018 mmol), allyl tributyltin (6.2 µL, 0.020 mmol) and TfOH (0.5 µL, 0.005 mmol) in 0.5 mL of toluene. After 90 min at 70 °C quantitative ^{19}F NMR analysis indicated 95% yield of **3.29** obtaining only the α anomer.

The general procedure was followed using glucosyl bromide **3.12-Br** (8.2 mg, 0.022 mmol), 1,2,3,4-tetra-*O*-benxyl-α-D-mannopyranose (16.5 mg, 0.032 mmol), allyl tributyltin (11 µL, 0.035 mmol) and TfOH (0.6 µL, 0.006 mmol) in 0.6 mL of toluene. After 120 min at 70 °C quantitative ^{19}F NMR analysis indicated 75% yield of **3.29** obtaining only the α anomer. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.29** as a colourless oil (11 mg, 61.2%).

Data for **3.29α:** R_f (1:9 EtOAc/hexane): 0.13; **[α]$^{D}_{25}$**: + 52.5 (1.10, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.14 (m, 20H, 4 × C_6H_5), 5.37-5.18 (m, 2H, H-3, H-4), 5.21 (dd, $J_{1,F}$ = 6.9 Hz, $J_{1,2}$ = 1.8 Hz, H-1), 4.98 (d, J = 11.2 Hz, 1H, CH-Ph), 4.86 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1'), 4.78 (dt, $J_{2,F}$ = 49.7 Hz, $J_{1,2}$ = $J_{2,3}$ = 1.8 Hz, 1H, H-2), 4.73 (d, J = 12.2 Hz, 1H, CH-Ph), 4.68-4.59 (m, 5H, 5×CH-Ph), 4.44 (d, J = 12.0 Hz, 1H, CH-Ph), 4.21 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 4.04 (ddd, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ 2.2 Hz, 1H, H-5), 4.03-3.89 (m, 2H, H-3', H-5'), 3.89 (dd, $J_{5,6a}$ = 12.2 Hz, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a'), 3.81-3.73 (m, 3H, H-2', H-4', H-6b'), 2.07 (s, 3H, CH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -204.2 (ddd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 29.6 Hz, $J_{F,1}$ = 6.9 Hz, α-anomer); **^{13}C NMR** (100.6 MHz,

$CDCl_3$) δ in ppm: 170.8, 179.9, 169.5 (3C=O, Ac), 138.3, 138.2, 138.0, 137.0, 128.4, 128.35, 128.3, 127.9, 127.85, 127.8, 127.7, 127.67, 127.63, 127.61, 97.3 (d, $J_{F,1}$ = 29.1 Hz, C-1), 96.9 (C-1'), 86.94 (d, $J_{F,2}$ = 179.3 Hz, C-2), 80.1 (C-3'), 75.1, 74.7, 74.3, 72.8, 72.1, 71.9, 69.7 (d, $J_{F,3}$ = 16.6 Hz, C-3), 68.9, 68.4, 66.6, 65.9, 62.0, 20.74, 20.71, 20.6 ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2917, 2851, 1748, 1496, 1455, 1366, 1225, 1127, 1092, 1049, 1028, 980, 915; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{46}H_{51}FNaO_{13}^+$ (m/z): calc. 853.3206; found 853.3209

2-Deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-mannosyl-(1-2)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (3.30):



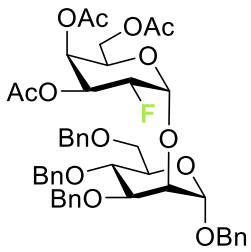
The general procedure was followed using mannosyl iodide **3.12-I** (6.5 mg, 0.015 mmol), 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (9.5 mg, 0.018 mmol), allyl tributyltin (6 µL, 0.019 mmol) and TfOH (0.5 µL, 0.005 mmol) in 0.5 mL of toluene. After 90 min at 70 °C quantitative ^{19}F NMR analysis indicated 88% yield of **3.30** obtaining only the α anomer.

The general procedure was followed using glucosyl bromide **3.12-Br** (16.5 mg, 0.045 mmol), 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (30.1 mg, 0.055 mmol), allyl tributyltin (17.2 µL, 0.058 mmol) and TfOH (1.2 µL, 0.013 mmol) in 1 mL of toluene. After 120 min at 70 °C quantitative ^{19}F NMR analysis indicated 88% yield of **3.30** obtaining only the α anomer. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.30** as a colorless oil (15.5 mg, 46%).

Data for **3.30α:** R_f (1:9 EtOAc/hexane): 0.16; **$[\alpha]^D_{25}$**: + 18.9 (0.85, $CHCl_3$); **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.14 (m, 20H, Ph), 5.40-5.24 (m, 2H, H-3, H-4), 5.15 (dd, $J_{F,1}$ = 7.1 Hz, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.96 (d, $J_{1,2}$ = 1.7 Hz, 1H,

H-1'), 4.89 (dt, $J_{2,F}$ = 50.1 Hz, $J_{1,2}$ = $J_{2,3}$ = 1.7 Hz, 1H, H-2), 4.80 (d, J = 10.8 Hz, 1H, CH-Ph), 4.77-4.55 (m, 5H, 5×CH-Ph), 4.53-4.46 (m, 2H, 2×CH-Ph), 4.20 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 4.6 Hz, 1H, H-6a), 4.11 (m, 1H, H-5), 4.03-3.94 (m, 3H, H-6b, H-2', H-3'), 3.90-3.79 (m, 2H, H-4', H-5'), 3.74 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6a'}$ = 4.0 Hz, 1H, H-6a'), 3.69 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6a'}$ = 1.8 Hz, 1H, H-6b'), 2.10 (s, 3H, CH_3, Ac), 2.06 (s, 3H, CH_3, Ac), 1.99 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -203.4 (ddd, $J_{F,2}$ = 50.2 Hz, $J_{F,3}$ = 29.7 Hz, $J_{F,1}$ = 6.9 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.7, 170.0, 169.6 (3C=O, Ac), 138.3, 138.1, 138.0, 136.9, 128.5, 128.45, 128.35, 128.3, 128.0, 127.95, 127.9, 127.8, 127.7, 127.65, 127.6, 127.5, 98.8 (d, $J_{F,1}$ = 29.7 Hz, C-1), 97.75 (C-1'), 86.74 (d, $J_{F,2}$ = 179.5 Hz, C-2), 79.6 (C-3'), 75.3, 74.8, 73.2, 72.9, 72.0, 69.8 (d, $J_{F,3}$ = 16.9 Hz, C-3), 69.1, 68.9, 68.8, 65.7, 62.0, 20.8, 20.7, 20.6 ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2919, 2851, 1749, 1455, 1366, 1226, 1138, 1085, 1050, 980; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{46}H_{51}FNaO_{13}^+$ (m/z): calc. 853.3206; found 853.3209.

1-*O*-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside-3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-galactopyranoside (3.31).



The general procedure was followed using galactosyl iodide **3.10-I** (10.8 mg, 0.029 mmol), 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (17.4 mg, 0.032 mmol), allyl tributyltin (11.7 µL, 0.038 mmol) and TfOH (0.8 µL, 0.008 mmol) in 0.6 mL of toluene. After 60 min at 70 °C quantitative ^{19}F NMR analysis indicated 71% yield of **3.31** obtaining an α:β ratio of 7:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **3.31** as a colourless oil (13.5 mg, 63%).

The general procedure was followed using glucosyl bromide **3.12-Br** (9 mg, 0.024 mmol), 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (13.9 mg, 0.026 mmol), allyl tributyltin (9.7 µL, 0.031 mmol) and TfOH (0.7µL, 0.007 mmol) in 0.5 mL of toluene. After 90 min at 70 °C quantitative ^{19}F NMR analysis indicated 64% yield of **3.31** obtaining an α:β ratio of 5:1.

Data for **3.31α:** R_f (1:9 EtOAc/hexane): 0.14; **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.14 (m, 20H, Ph), 5.49-5.38 (m, 3H, H-3, H-4, H-1), 5.03 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1'), 4.85-4.67 (m, 5H, H-2, 4×CH-Ph), 4.63 (d, J = 12.2 Hz, 1H, CH-Ph), 4.57 (d, J = 12.2 Hz, 1H, CH-Ph), 4.51-4.44 (m, 2H, 2×CH-Ph), 4.37 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.5 Hz, 1H, H-5), 4.08-3,90 (m, 5H, H-2', H-3', H-4', H-6a, H-6b), 3.83 (ddd, $J_{4,5}$ = 8.3 Hz, $J_{5,6a}$ = 5.5 Hz, $J_{5,6a}$ = 3.2 Hz, 1H, H-5'), 3.76-3.67 (m, 2H, H-6a', H-6b'), 2.12 (s, 3H, CH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 1.98 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -207.1 (ddd, $J_{F,2}$ = 49.8 Hz, $J_{F,3}$ = 10.4 Hz, $J_{F,1}$ = 3.4 Hz, α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.4, 170.0, 169.8 (3C=O, Ac), 138.5, 138.3, 138.15, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.45, 127.4, 97.9 (C-1'), 97.5 (d, $J_{F,1}$ = 20.7 Hz, C-1), 85.0 (d, $J_{F,2}$ = 192.7 Hz, C-2), 79.5, 75.7, 75.2, 74.8, 73.3, 72.3, 72.1, 69.25, 69.23, 68.7 (d, $J_{F,4}$ = 7.4 Hz, C-4), 68.3 (d, $J_{F,3}$= 19.0 Hz, C-3), 66.8, 61.6, 20.7, 20.65, 20.6 ($3CH_3$, Ac); **FT–IR (neat)** ν in cm^{-1}: 2918, 2851, 1751, 1455, 1370, 1228, 1136, 1076, 1052; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{46}H_{51}FNaO_{13}^+$ (m/z): calc. 853.3206; found 853.3211.

Chapter III

CHAPTER IV

TRIFLUOROMETHYL-DIRECTED GLYCOSYLATION:

STEREOSELECTIVE SYNTHESIS OF 2-DEOXY-2-CF_3-GLYCOSIDES

Chapter IV

4.1. INTRODUCTION

4.1.1. Importance of the CF_3 group

Among various fluorinated motifs, the trifluoromethyl group (CF_3) has become an important structural component in the development of active ingredients, mainly due to its electronic influence on vicinal groups and its effect on lipophilicity. This has been exemplified in an increasing number of agrochemicals, bioactive molecules, and marketed drugs containing a CF_3 fragment (**Figure 4.1**).[1] As reported by the FDA during the last four years (2018-2021), at least 50 new approved drugs contain one or more fluorine atoms of whose at least 15 approved drugs include the CF_3 group (**Figure 4.1**).[2]

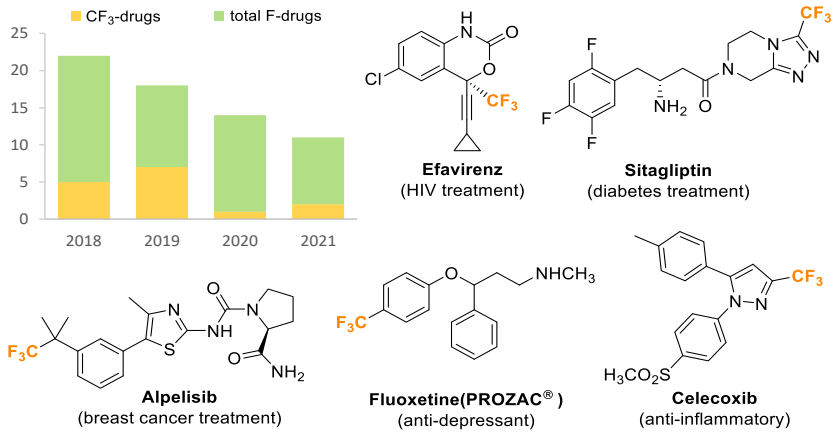


Figure 4.1. Fluorinated drugs approved by the FDA from 2018 to 2021, and some representative CF_3-containing drugs in the market.

However, during many years the CF_3 group has been a subject of controversy regarding its bioisosterism, which has slowed down its use in the

[1] Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320-330.

[2] a) Han, J.; Fustero, S.; Medio-Simon, M.; Sedgwick, D. M.; Santi, C.; Ruzziconi, R.; Soloshonok, V. A. *Chem. Eur. J.* **2019**, *25*, 11797-11819; b) Mei, H.; Remete, A. M.; Zou, Y.; Moriwaki, H.; Fustero, S.; Kiss, L.; Soloshonok, V. A.; Han, J. *Chin. Chem. Lett.* **2020**, *31*, 2401-2413; c) Yu, Y.; Liu, A.; Dhawan G.; Mei, H.; Zhang, W.; Izawa, K.; Soloshonok, V. A.; Han, J. *Chin. Chem. Lett.* **2021**, *32*, 3342-3354; d) He, J.; Li, Z.; Dhawan, G.; Zhang, W.; Sorochinsky, A. E.; Butler, G.; Soloshonok, V. A.; Han, J *Chin. Chem. Lett.* **2022**, *31*, 2401-2413.

optimization of new therapeutic drugs. Nonetheless, many recent reports have demonstrated the bioisosterism of the CF_3 with methyl, ethyl, and isopropyl groups, among others.[3] For instance, by measuring the inhibitory potency of a set of barbiturates it was demonstrated that the CF_3 group is, in terms of size, smaller than isopropyl, larger than methyl, and rather similar to the ethyl group.[4] Considering the effect on bioactive molecules, fluorine and trifluoromethyl group usually tend to increase the thermal and oxidative stability, enhance lipophilicity and bioavailability, and they modulate the pKa of vicinal acidic and basic groups, as well as the conformation of the molecule (**Figure 4.2**). All these features make fluorinated drugs especially useful for trespassing the blood-brain barrier. Additionally, in some cases fluorinated motifs can induce new modes of action. One example is trifluridine, a 2-deoxynucleoside used for the treatment of herpes simplex virus, that produces a covalent inhibition after a series of fluoride elimination.[5]

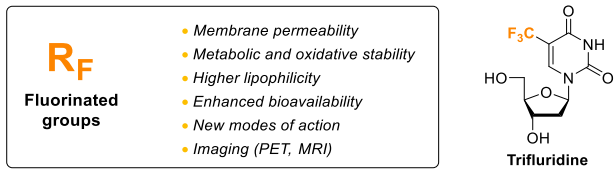


Figure 4.2. Some effects of fluorine and polyfluorinated groups in the properties of bioactive compounds and examples of polyfluorinated drugs.

4.1.2. Polyfluorinated sugars

Methods for the preparation of biomimetic carbohydrates have been of growing interest in medicinal chemistry as a strategy to overcome some of the limitations of native glycans, such as low binding affinity, low stability, and rapid excretion, among others. F-glycomimetics, resulting from the bioisosteric replacement of H and/or OH by F and their polyfluorinated counterparts (particularly those featuring polyfluoroalkyl groups, *e.g.*, CF_3, C_2F_5) have

[3] Meanwell, N. A. *J. Med. Chem.* **2018**, *61*, 5822-5880.
[4] Jagodzinska, M.; Huguenot, F.; Candiani, G.; Zanda, M. *ChemMedChem: Chemistry Enabling Drug Discovery*, **2009,** *4*, 49-51.
[5] Santi D. V.; Sakai, T. T. *Biochemistry*, **1971**, *10*, 3598-3607.

recently received especial attention due to the beneficial properties such polyfluorinated groups impart.[3] It is expected that the number of organofluorine compounds that is investigated as potential drug candidates can depend strongly on the availability of synthetic methods to access this moieties.[6] Although the preparation of CF_3-containing active principal ingredients (APIs)/drugs has been long appreciated, these motifs remain underexplored in glycoscience despite the presence of fluorine is prevalent for both therapeutic and imaging applications (*e.g.*, [^{18}F]FDG). Unlike the state-of-the-art in the synthesis of fluorinated APIs for drug discovery that collects a wide diversity of modifications,[7] strategies for the preparation of fluorinated glycosides beyond monofluorination are scarce. Easily accessible trifluoroacetamides and trifluoroacetoxy groups have been prepared which are the closest-art in the CF_3-containing glycosides (**Figure 4.3**).[8] To date, trifluoromethylated sugars are limited to α-CF_3 alcohols resulting from 1,2-nucleophilic addition of $TMSCF_3$ to ketones and CF_3-glycals produced by photoredox radical addition[9] and via cross-coupling of $CuCF_3$ with iodoglycals.[10] Nonetheless, 2-deoxy-2-CF_3-glycosides are unexplored substrates due to the lack of synthetic protocols for their preparation (**Figure 4.3**).

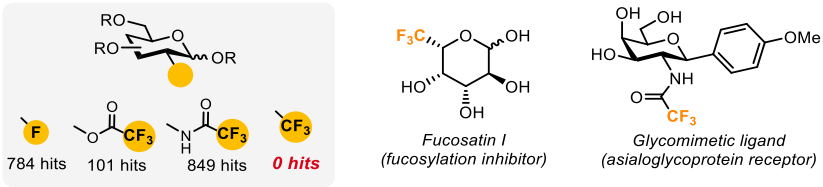


Figure 4.3. Reported fluorinated glycans (hits by structure obtained from Reaxys®) and examples of some bioactive CF_3-containing glycosides.

[6] Inoue, M.; Sumii, Y.; Shibata, N. *ACS Omega* **2020**, *5*, 10633-10640.
[7] Cahard, D.; Ma, J.; Emerging Fluorinated Motifs. Synthesis, Properties and Applications. Wiley-VCH, Weinheim **2020**.
[8] a) Cosimi, E.; Trapp, N.; Ebert, M. O.; Wennemers, H. *Chem. Commun*. **2019**, *55*, 2253-2256; b) Unione, L.; Alcalá, M.; Echeverria, B.; Serna, S.; Ardá, A.; Franconetti, A.; Cañada, F. J.; Diercks, T.; Reichard, N.; Jiménez-Barbero, J. *Chem. Eur. J*. **2017**, *23*, 3957-3965; c) Hevey. R. *Chem. Eur. J.* **2021**, *27*, 2240-2253.
[9] Wang, B.; Xiong, D.-C.; Ye. X.-S, *Org. Lett*. **2015**, *17*, 5698-5701.
[10] Mestre, J.; Lishchynskyi, A.; Castillón, S.; Boutureira. O. *J. Org. Chem.* **2018**, *83*, 8150-8160.

4.1.3. Stereoselective synthesis of 2-deoxy-β-glycosides

The stereoselective synthesis of 2-deoxy-β-glycosides is challenging due to the absence of a C-2 protecting group directing the stereochemistry, and the general preference of the α isomer owing to the anomeric effect. The most extended strategy for the synthesis of 2-deoxy-β-glycosides is the use of large temporary C-2-directing groups such as halogens[11] and chalcogens,[12] which upon removal uncover the corresponding 2-deoxyglycosides.[13] Three-membered rings were initially claimed as responsible for the high 1,2-*anti*-stereoselectivities observed.[14] However, posterior mechanistic investigations supported that the actual preceding intermediates are oxocarbenium species that deliver 1,2-*trans* glycosides as major isomers and the stereoselectivity is mainly governed by steric factors (**Scheme 4.1**, **A**).[15] One example is the use of 2-deoxy-2-iodo and 2-deoxy-2-phenylselenenyl glycosides in the stereoselective synthesis of 2-deoxy-β-glycosides, developed by Castillón and co-workers (**Scheme 4.1, B**).[16]

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[11] a) Mestre, J.; Matheu, M. I.; Díaz, Y.; Castillón, S.; Boutureira, O. *J. Org. Chem.* **2017**, *82*, 3327-3333; b) Kimura, T.; Takahashi, D.; Toshima. K. *J. Org. Chem.* **2015**, *80*, 9552-9562; c) Glinschert, A.; Oscarson. S. *Carbohydr. Res*. **2015**, *414*, 65-71; d) Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656-6660.

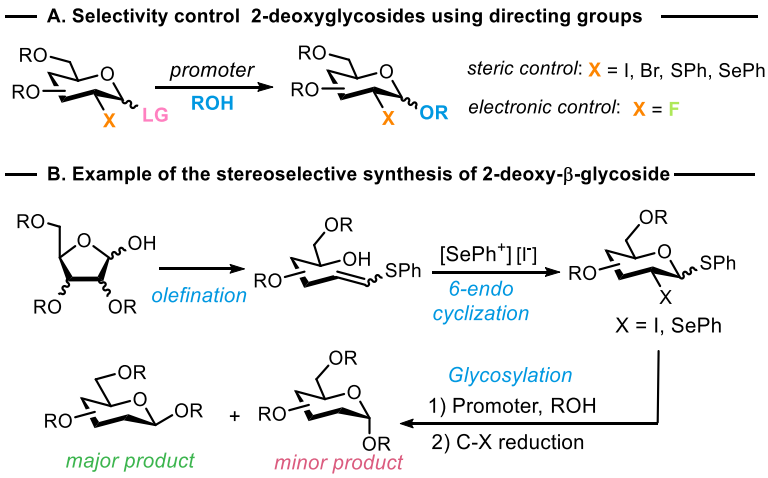
[12] a) Nicolaou, K. C.; Ladduwahetty, T.; Randall, J. L.; Chucholowski. A. *J. Am. Chem. Soc.* **1986**, *108*, 2466-2467; b) Nicolaou, K. C.; Mitchell, H. J.; Fylaktakidou, K. C.; Suzuki, H.; Rodríguez, R. M. *Angew. Chem. Int. Ed.* **2000**, *39*, 1089-1093; c) Nicolaou, K. C.; Fylaktakidou, K. C.; Mitchell, H. J.; van Delft, F. L.; Rodríguez, R. M.; Conley, S. R.; Jin, Z. *Chem. Eur. J.* **2000**, *6*, 3166-3185; d) Sau, A.; Misra. A. K. *Carbohydr. Res.* **2012**, *361*, 41-48.

[13] a) Bennett, C. S.; Galan. M. C. *Chem. Rev.* **2018**, *118*, 7931–7985; b) Ernst, G. W. Hart, P. Sinaý. *Special Problems in Glycosylation Reactions: 2-Deoxy Sugars*, in Carbohydrates in Chemistry and Biology, *Vol. 1* (Eds.: B. Ernst, G. W. Hart, P. Sinaý), Wiley-VCH, Weinheim, **2000**, 367–405.

[14] a) Sirion, U.; Purintawarrakun, S.; Sahakitpichan, P.; Saeeng. R. *Carbohydr. Res*. **2010**, *345*, 2401-2407; b) Smoliakova. I. P. *Curr. Org. Chem.* **2000**, *4*, 589-608; c) Smoliakova, I. P.; Caple, R.; Gregory, D.; Smit, W. A.; Shashkov, A. S.; Chizhov, O. S. *J. Org. Chem.* **1995**, *60*, 1221-1227.

[15] a) Bravo, F.; Viso, A.; Alcázar, E.; Molas, P.; Bo, C.; Castillón, S. *J. Org. Chem.* **2003**, *68,* 686- 691; b) Beaver, M. G.; Billings, S. B.; Woerpel. K. A. *Eur. J. Org. Chem.* **2008**, *2008,* 771-781; c) Lebedel, L.; Ardá, A.; Martin, A.; Désiré, J.; Mingot, A.; Aufiero, M.; Aiguabella Font, N.; Gilmour, R.; Jiménez-Barbero, J.; Blériot, Y.; Thibaudeau, S. *Angew. Chem. Int. Ed.* **2019**, *58*, 13758-13762.

[16] a) Rodríguez, M. Á.; Boutureira, O.; Arnés, X.; Matheu, M. I.; Díaz, Y.; Castillón, S. *J. Org. Chem.* **2005,** *70,* 10297-10310. b) Boutureira, O.; Rodríguez, M. A.; Benito, D.; Matheu, M. I.; Díaz, Y.; Castillón, S. *Eur. J. Org. Chem.* **2007**, *2007*, 3564-3572.

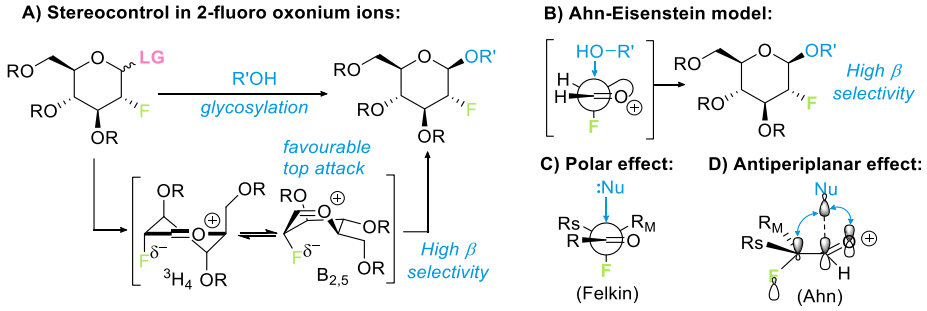


Scheme 4.1. A) Selectivity control in the synthesis of 2-deoxyglycosides using directing groups in C-2. B) Stereoselective synthesis of 2-deoxy-β-glycosides.

Besides its remarkable biophysical properties, fluorine has also proven to be an efficient steering group for the stereoselective preparation of 2-deoxy-2-fluoroglycosides (**Scheme 4.1**). Gilmour and co-workers reported a fluorine-directed glycosylation protocol that achieves the stereoselective synthesis of 2-deoxy-2-fluoro-β-glycosides. In this report, the high 1,2-*anti* selectivity results from the preferred conformation of 2-fluoro-oxocarbenium in the transition state reminiscent to half-chair geometries that are favoured by the high electronegativity of fluorine (**Scheme 4.2, A**). This observation is based on the tendency of organofluorine compounds to adopt conformations that allow the stabilization of hyperconjugative and attractive electrostatic interactions. It was postulated that in a *gluco*-configured 2-fluoro-oxonium ion, the C-F bond, which is highly polarized, would be orientated towards the electropositive centre. The change in oxonium ion conformation induces a perturbation in anomeric carbon charge distribution, leading to a highly β-selective glycosylation event (**Scheme 4.2, A**).[17]

[17] a) Bucher, C.; Gilmour, R. *Angew. Chem. Int. Ed.* **2010,** *49*, 8724-8728; b) Durantie, E.; Bucher, C.; Gilmour, R. *Chem. Eur. J.* **2012,** *18,* 8208-8215.

The stereoselective control of the reaction is consistent with the Anh-Eisenstein model for 1,2-stereo induction (**Figure 4.4, B**), which is an extension of the Felkin model with the incorporation of the antiperiplanar effect, as a consequence of asymmetric induction being controlled by both substituents (**Figure 4.4, C**) and orbital effects (**Figure 4.4, D**). The energy of the transition state is minimized when the nucleophile trajectory is aligned with the σ^*_{C-F} orbital.



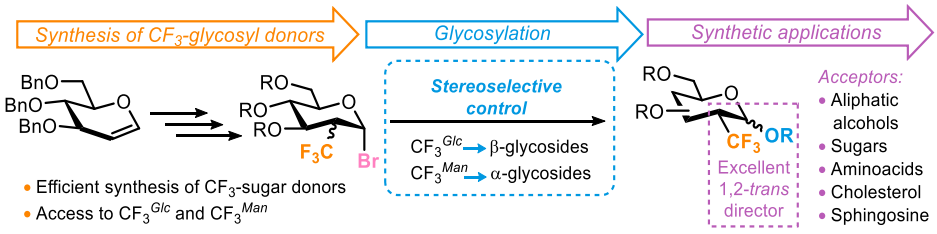
Scheme 4.2. A) Stereocontrol in 2-fluoro oxonium ions. B) Stereocontrol by the Anh-Eisenstein model. C) Polar effect. D) Antiperiplanar effect. Rs = small substituent, R_M = medium substituent.

In this context, we were intrigued on the behaviour of the CF_3 group when placed at the C-2 position of a glycosyl donor, and whether the presence of such strong electron-withdrawing unit may impart stabilizing/destabilizing interactions at the nearby oxocarbenium ion. This scenario could impact on the stereochemical outcome of the glycosylation reaction. Moreover, the bulky trifluoromethyl group could have an impact on the conformation of the final 2-deoxy-2-trifluoromethyl glycosides, that in the case of bioactive compounds, will affect to the recognition process by the corresponding biological receptors.

4.2. OBJECTIVES

The aim of this chapter is to develop a protocol for accessing to 2-deoxy-2-trifluoromehtyl glycosides, unprecedented fluorinated components. Suring the course of our investigations, we will study the glycosylation reaction and the effect of the CF_3 moiety as directing group in the glycosylation step. In particular, it would be interesting to comparatively determine if the electronic factors that govern the stereochemical outcome of the glycosylation of 2-deoxy-2-fluoro-glycosides are also operating in the case of 2-deoxy-2-CF_3 derivatives. For this purpose, the specific objectives are:

1. Development of a new protocol for the preparation of 2-deoxy-2-trifluoromethyl glycosides bearing both electron-donating and electron-withdrawing protecting groups.
2. Optimization of the glycosylation reaction conditions to obtain high yield and selectivity with trifluoromethyl glycosyl donors.
3. Evaluate the stereoselectivity dependence on the sugar configuration (*Glc* and *Man*) with both armed and disarmed glycosyl donors.
4. Study the stereodirecting value of the CF_3 group and compare it with that of fluorine.
5. Study the effect of the nucleophilicity and size of the acceptor in the stereoselectivity of the glycosylation step.
6. Apply the glycosylation protocol for the stereoselective synthesis of trifluoromethylated analogues of biologically relevant glycosides.

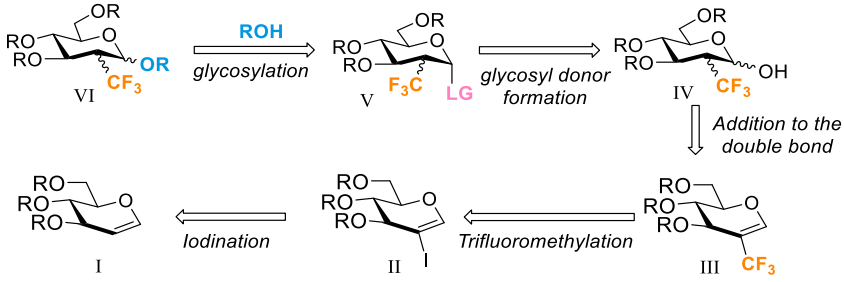


Scheme 4.3. Scheme of the main objectives in this Chapter.

4.3. RESULTS AND DISCUSSION

4.3.1. Retrosynthetic analysis

Retrosynthetic analysis revealed that 2-deoxy-2-trifluoromethyl glycosides could be obtained from the corresponding glycals following the sequence described in **Scheme 4.4**. Preparation of trifluoromethyl glycals **III** could be performed by a two-step iodination/trifluoromethylation sequence, followed by electrophilic activation of the double bond to give the hydroxylated trifluoromethylglycosides **IV**. Next step implies the introduction of a good leaving group in the anomeric position to obtain 2-deoxy-2-trifluoromethyl pyranosyl donors **V**, which under glycosylation conditions and in the presence of an acceptor will give the desired 2-deoxy-2-trifluoromethyl glycosides in a stereocontrolled manner **VI**.

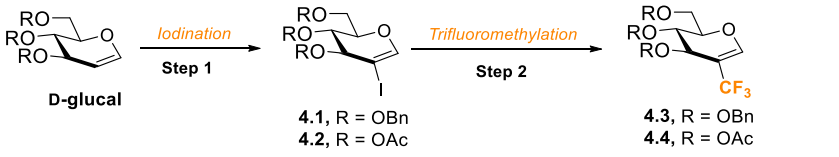


Scheme 4.4. Proposed synthetic route for the synthesis of 2-deoxy-2-trifluoromethyl glycosides.

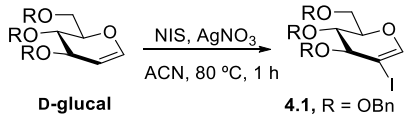
4.3.2. Synthesis of 2-deoxy-2-trifluoromethyl hexopyranosyl donors

The main goal in this chapter is to access 2-deoxy-2-trifluoromethylglycosides in a stereoselective manner and study the directing value of the trifluoromethyl moiety in the glycosylation event. In addition, we aim to explore the effect of sugar configuration and protecting groups in the stereoselectivity. For that purpose, we must synthetise 2-deoxy-2-trifluoromethyl pyranosyl donors with a combination of different CF_3 configurations (*Glc* and *Man*) and protecting groups (acetyl and benzyl).

The first step in the proposed synthetic route is the obtention of trifluoromethylglucal from commercially available glycals. The synthesis of 2-trifluoromethylglucal **4.3** (OBn) and **4.4** (OAc) was performed by a two-step iodination-trifluoromethylation sequence from the corresponding protected D-glucal (**Scheme 4.5**). Optimization of iodination reaction was studied for perbenzylated D-glucal (**Scheme 4.5**, Step 1, entries 1-4) and optimal conditions correspond to the most diluted ones (entry 4). The decrease of the yield in more concentrated media could be explained by the presence of a Ferrier rearrangement side reaction. This reaction involves the cleavage of the benzyl protecting group at the C-3 position favoured by an electron movement from the ring oxygen coordinated to the Lewis acid. It forms an allyloxycarbenium ion which is attacked in the anomeric position by the lost leaving group giving a by-product which has a shift of the double bond to C2-C3 (Ferrier by-product). Optimal conditions were applied for the different protected D-glucals obtaining perbenzylated-iodoglucal **4.3** in 79% yield and peracetylated-iodoglucal **4.4** in 59% yield.



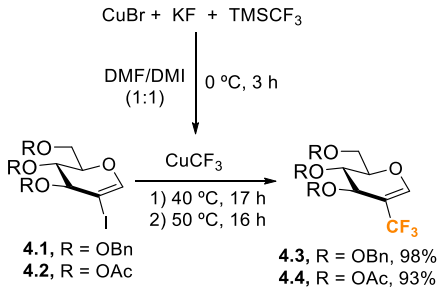
Step 1. Iodination



Entry	OR	D-glucal	Yield
1	OBn	18.2 mmol	42%
2	OBn	9.75 mmol	59%
3	OBn	3.6 mmol	73%
4	OBn	1 mmol	79%
5	OAc	1 mmol	59%

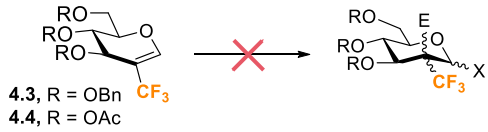
[a] General conditions for iodination: D-glucal (1 mmol), NIS (1.2 mmol), $AgNO_3$ (0.2 mmol), ACN [0.125 M].

Step 2. Trifluoromethylation



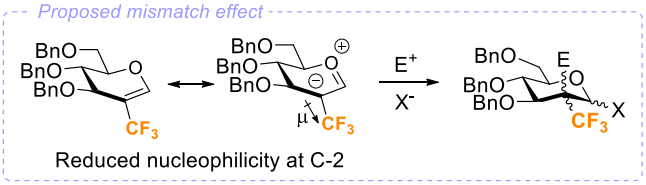
Scheme 4.5. Two-step iodination/trifluoromethylation sequence.

Trifluoromethylation of glycals has been previously studied in our group, and a protocol using CF_3H-derived $CuCF_3$ was recently described.[10] However, this trifluoromethylation process requires the use of a glovebox and manipulation of gaseous reagents. Herein, the overall synthetic sequence was improved by using, first, a more efficient iodination protocol and, second, a new trifluoromethylation protocol (**Scheme 4.5**, step 2, $TMSCF_3$/KF/CuBr) that permits a readily scalable synthesis and employs simpler reaction setup to access to 2-CF_3-glycals in standard laboratories. Next, functionalization of the double bond was studied. Disappointingly, the CF_3-containing vinyl moiety was reluctant to undergo most typical and general transformations, including electrophilic halogenation, hydration, and hydroboration (**Scheme 4.6**). This is due to a mismatch effect between the activating oxygen that increases the electron-density on the alkene through conjugation and the CF_3, which removes electron density by inductive effect, and at the same time, destabilizes the formation of cationic intermediates produced upon electrophilic addition.



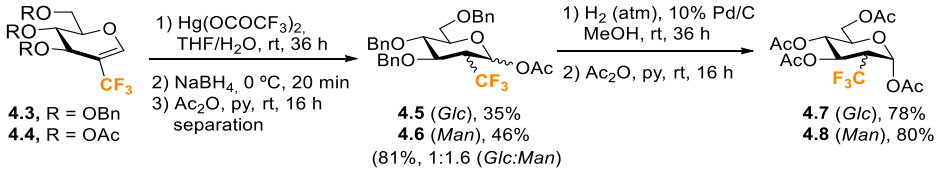
4.3, R = OBn
4.4, R = OAc

1) Epoxidations: a) Oxone, $NaHCO_3$, acetone in CH_2Cl_2/H_2O, b) MCPBA in MeOH, c) Oxone, $NaHCO_3$, CF_3COCH_3 in CH_2Cl_2/H_2O, d) DMDO in acetone.
2) Halogenation: e) NaOCl in H_2O, f) PhIO in MeOH, g) NIS, CH_3CN/H_2O, rt to 80 ºC, h) NIS/TfOH, CH_3CN/H_2O, i) NBS, CH_3CN/H_2O, rt to 80 ºC.
3) Hydroboration: j) BH_3·THF, THF, 0 ºC to rt.
4) Hydroxylation: k) H_2O_2, NaOH, CH_2Cl_2, l) H_2SO_4, THF, rt to 85 ºC.
5) Hydroxymercuration: m) $Hg(OCOCH_3)_2$, THF/H_2O, $NaBH_4$.
6) Hydratation: n) LiBr, Amberlite IR^{-120}, CH_3CN/H_2O (1:1), rt, 40 ºC, 80 ºC, 100 ºC, o) 48% HBr in H_2O, CH_3CN, rt, 40 ºC, p) HBr·PPh_3, CH_3CN/H_2O (4:1), rt, 40ºC, 80ºC, 100 ºC



Scheme 4.6. Top: Attempted reactions in the functionalization of trifluoromethyl glucal. Bottom: Proposed mismatch effect between the oxygen and the CF_3 group, which makes the vinyl moiety considerable unreactive.

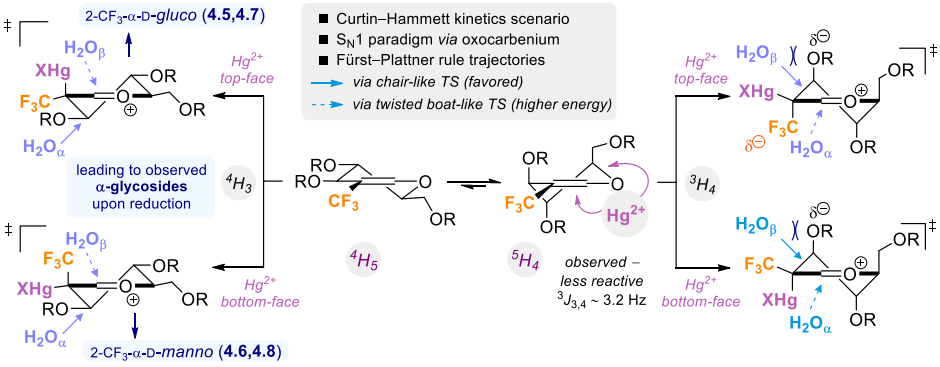
To our delight, complete conversion of the trifluoromethylglucal **4.3** to the desired trifluoromethyl glycosides (*Glc* and *Man*) was observed under hydroxymercuration conditions using $Hg(OCOCF_3)$ in a mixture of THF and H_2O, followed by reductive demercuration with $NaBH_4$ (**Scheme 4.7**). Same conditions were applied for peracetylated trifluoromethyl glucal **4.4**, however, low conversion was observed (34%) along with a mixture of unidentified rearrangement by-products. This can be explained considering that acetyl protecting groups are more electron withdrawing than benzyl groups, which diminish the electron density of the double bond making it less reactive to an electrophilic addition. Hydroxymercuration/demercuration of **4.3** gave an inseparable mixture of *gluco* and *manno* 1-OH-glycosides (1:1.6 *Glc:Man* **4.5-4.6-OH**), in an attempt to separate them, the mixture was submitted to acetylation conditions, which after flash purification gave pure isolated 1-*O*-acetyl-2-deoxy-2-trifluoromethyl *gluco* **4.5** (35%) and *manno* **4.6** (46%) glycosides. Considering that we have accessed to perbenzylated *gluco* **4.5** and *manno* **4.6** trifluoromethyl glycosides in good overall yield (81%), we decided to submit these to hydrogenolysis-acetylation conditions which after purification give the desired peracetylated 2-deoxy-2-trifluoromethyl *gluco* **4.7** and *manno* **4.8** pyranoses (**Scheme 4.7**).



Scheme 4.7. Hydroxymercuration/demercuration/acetylation sequence to obtain 2-CF_3 *gluco* **4.5** and *manno* **4.6** pyranoses, and derivatization to obtain acetylated analogues **4.7** and **4.8**.

Interestingly, hydroxymercuration/demercuration of **4.3** gave only α-isomers of the corresponding *gluco* and *manno* glycosides. To further understand the hydroxymercuration stereocontrol, the reaction was followed by ^{19}F NMR and the results were analysed. Only the starting 2-trifluoromethyl-glucal with $^5H_4$ conformation was observed in the crude, however, electrophilic

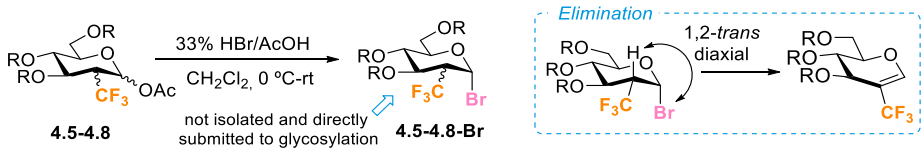
addition of mercury to this 2CF_3-glucal conformation would lead to the formation of $^3H_4$ glycosides, which following the Fürst-Plattner rule trajectories would lead to β-glycosides after water addition and reduction. With these considerations, we proposed a Curtin-Hammet kinetics scenario, in which the more reactive $^5H_4$ trifluoromethyl-glucal reacts with the transition metal leading to the formation of $^4H_3$ *manno* and *gluco* glycosides, which after water addition and reduction lead to α-glycosides (**Scheme 4.8**).



Scheme 4.8. Proposed selectivity model for the hydroxymercuration

Finally, preparation of suitable glycosyl donors was examined. We first explored the synthesis of trichloroacetimidate glycosyl donors by reacting **4.5** with DBU and trichloroacetimidate, but poor yields were obtained (48%) due to the high hydrolysis detected and the difficult purification, which reduced its synthetic utility. Luckily, conversion of 2-deoxy-2-trifluoromethyl pyranosides **4.5-4.8** to the corresponding bromides was successfully achieved using HBr in AcOH (**Scheme 4.9**). Analysis of the reaction crudes demonstrated almost quantitative yield (>95%) in the formation of the peracetylated products (**4.7-4.8-Br**). Reaction control by TLC was essential for obtaining good yields, especially for armed pyranosides **4.5** and **4.6**, since the C–Br bond is easily activated under the reaction conditions. Perbenzylated glycosyl bromides (**4.5-4.6-Br**) were obtained in excellent yields (>90%) by controlling reaction times and using less equivalents of HBr and more diluted reaction conditions. Formation of the glycal **4.3** from **4.5-Br** was favoured due to the 1,2-*trans*diaxial disposition of the eliminating atoms, which coupled to the purification issues

associated with perbenzylated bromopyranosides lead us to perform bromination and glycosylation in two consecutive steps to avoid decomposition of the glycosyl donors (**Scheme 4.9**).



Scheme 4.9. Left: Bromination of **4.5-4.8** to obtain the glycosyl donors, that were directly submitted to glycosylation conditions. Right: side reaction during bromination.

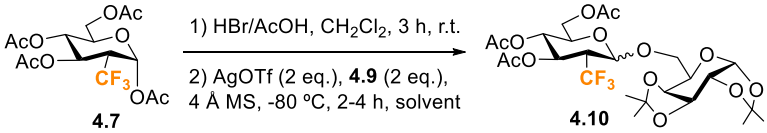
4.3.3. Optimization of the glycosylation reaction

Glycosyl bromides were chosen as glycosyl donors due to their easy preparation, high yields obtained and the unnecesary purification of the crudes. It has been proved that glycosyl bromides are readily activated for glycosylation reaction using silver triflate (AgOTf).[18] Screening of glycosylation solvents was studied for **4.7** as model substrate and primary alcohol **4.9** as glycosyl acceptor in the presence of 4 Å MS and silver triflate as promoter (**Table 4.1**). Preliminary tests showed the importance of conducting the reaction at very low temperature (*ca.* –80 °C) due to 2-CF_3-glycosyl bromides propensity to undergo elimination processes to the parent 2-CF_3-D-glucal (5–20%). Solvent screening showed that reactions in THF afforded low yields and the selectivity was poor (**Table 4.1**, entry 1). Using acetonitrile (at –40 °C), excellent selectivities were obtained but the yield was also low (**Table 4.1**, entry 2). Using either CH_2Cl_2 or toluene, the selectivity was similar, affording **4.10β** as the major isomer, yet in CH_2Cl_2, the yield was poorer, probably due to solubility issues with AgOTf (**Table 4.1**, entries 3 *vs.* 4). To our delight, best yield and selectivities were obtained in a 1:1 (v/v) CH_2Cl_2/toluene mixture (Table 1, entry 5). Treatment of **4.10β** under optimized glycosylation conditions discarded epimerization of final 2-CF_3 glycosides during the reaction.

[18] Singh, Y.; Geringer, S. A.; Demchenko, A. V. *Chem. Rev.* **2022**, *122*, 11701–11758.

Analysis of products **4.7α** and **4.10β** by X-ray diffraction indicated that typical $^4C_1$ conformation is adopted in the solid state despite the presence of sterically demanding CF_3 group. Meaningfully, ^{1}H NMR data showed that the same geometry is also conserved in solution owing to diagnostic $^1J_{C1-H1}$ and $^3J_{H,H}$ coupling constants as well as key NOE contacts. This observation suggests, *a priori,* that 2-deoxy-2-CF_3-glycoside analogues of natural products could be designed to maintain molecular recognition events (unless key specific interactions are operative at C-2) by biological entities.

Table 4.1. Solvent screening of glycosylation reaction.



Entry	solvent	T (ºC)	yield (%)[c][d]	α:β ratio[c]
1	THF	-80	30	29:71
2	ACN	-40	31	6:94
3	CH_2Cl_2	-80	33[e]	13:87
4	toluene	-80	76	18:82
5	CH_2Cl_2/toluene (1:1)	-80	86	8:92

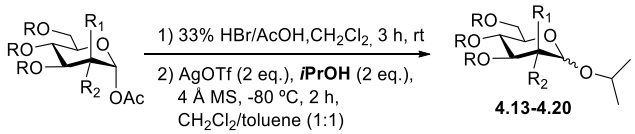
[a] General bromination conditions: **4.7** (1 equiv), 33 wt. % HBr in AcOH, 0 ºC to rt, 1–3 h. [b] General glycosylation conditions: **4.7** (1 equiv), **4.9** (2 equiv), AgOTf (2 equiv), 4 Å MS, indicated temperature, 2-4h, solvent. [c] Determined by ^{19}F NMR of the crude reaction mixture using 1,4-difluorobenzene (DFB) as internal standard (see the SI for details). [d] Yield over two steps from OAc. [e] Poor solubility of AgOTf observed.

4.3.4. Study the directing value of the CF_3 group and the effect of protecting groups and sugar configuration

Aiming to analyse the directing effect of the CF_3 on the stereoselectivity of the glycosylation reaction, different 2-CF_3-configuration, and variation of the electronic nature of protecting groups were evaluated. To facilitate direct comparison of the stereodirecting value of CF_3 *vs.* F, isopropanol was chosen as nucleophile following the seminal report by Gilmour.[17] Stereoselectivities were highly dependent on the configuration of the CF_3 and 1,2-*trans* glycosides were

always obtained. *Gluco*-configured CF_3 glycosyl donors favoured formation of β-products, whereas α-glycosides were preferred for *manno*-CF_3 donors (**Table 4.2**, entries 1–4). Stereoselectivity was also dependant on the protecting groups, and the best selectivities obtained resulted from the combination of acetyl-protecting groups with *manno*-CF_3 **4.8-Br** (95:5 α/β) and benzyl ether groups with *gluco*-CF_3 **4.5-Br** (3:97 α/β).

Table 4.2. Bromination/glycosylation of 2-CF_3 and 2-F-sugars with *i*PrOH.



4.13-4.20

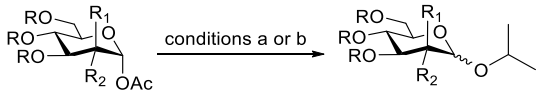
Entry	donor	R	R_1	R_2	product	yield (%)[c][d]	α:β ratio[e]
1	**4.7** *(Glc)*	Ac	H	CF_3	**4.13**	76	20:80
2	**4.8** *(Man)*	Ac	CF_3	H	**4.14**	91	95:5
3	**4.5** *(Glc)*	Bn	H	CF_3	**4.15**	72	3:97
4	**4.6** *(Man)*	Bn	CF_3	H	**4.16**	62	79:21
5	**3.11** *(Glc)*	Ac	H	F	**4.17**	95	24:76
7	**3.12** *(Man)*	Ac	F	H	**4.18**	84	95:5
8	**4.11** *(Glc)*	Bn	H	F	**4.19**	41	4:96
9	**4.12** *(Man)*	Bn	F	H	**4.20**	38	20:80

[a] General bromination conditions: **donor** (1 equiv), 33 wt. % HBr in AcOH, 0 ºC to rt, 1–3 h. [b] General glycosylation conditions: **donor** (1 equiv), ***i*PrOH** (2 equiv), AgOTf (2 equiv), 4 Å MS in 1:1 CH_2Cl_2/toluene, -80 ºC, 2 h. [c] Isolated yield. [d] Yield over two steps from OAc. [e] Determined by ^{19}F NMR of the crude reaction mixture using 1,4-difluorobenzene (DFB) as internal standard (see the SI for details).

Our findings are in line with the results obtained in the glycosylation of 2-fluorosugars, yet in this case trichloroacetimidates were used as glycosyl donors.[17] For the sake of a more accurate comparison, 2-deoxy-2-fluoroglycosyl bromides were synthetized and submitted to the exact reaction conditions of the present study. 2-Deoxy-2-fluoro derivatives **4.15–4.18** were obtained in similar stereoselectivities than trifluoromethylated analogs **4.11-4.14** (**Table 4.2**, entries 1–4 *vs.* 5–9). However, inversion on the selectivity was observed in the benzyl-protected *manno*-sugars **4.14** and **4.18** (**Table 4.2**, entry 4 *vs.* entry 9). Limited stereoselective control was also adverted for benzyl-*manno*

derivatives in the Gilmour report (**Table 4.3**), due to mismatched effects between protecting groups and the 2-fluoro substituent.

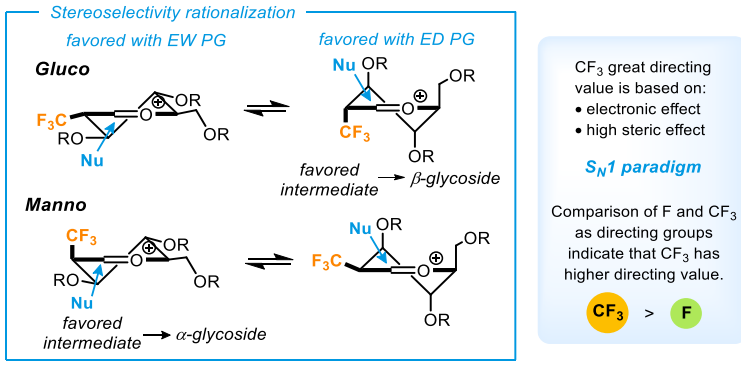
Table 4.3: Comparison of the stereoselectivities obtained for 2-CF_3-sugars and 2-F-sugars in this study and stereoselectivities obtained by Gilmour.[17]



			CF_3-sugars			F-sugars			Gilmour's work		
Entry	donor	R	R_1	R_2	α:β ratio[c]	R_1	R_2	α:β ratio[c]	R_1	R_2	α:β ratio[c]
1	*Glc*	Ac	H	CF_3	20:80	H	F	24:76	H	F	34:66
2	*Man*	Ac	CF_3	H	95:5	F	H	95:5	F	H	only α
3	*Glc*	Bn	H	CF_3	3:97	H	F	4:96	H	F	5:95
4	*Man*	Bn	CF_3	H	79:21	F	H	20:80	F	H	24:76

[a] Conditions a: **glycosyl bromide** (1 equiv), ***i*PrOH** (2 equiv), AgOTf (2 equiv), 4Å MS in 1:1 CH_2Cl_2/toluene, -80º C, 2h. [b] Conditions b: **glycosyl trichloroacetimidate** (1 equiv), ***i*PrOH** (1.2 equiv), TMSOTf (0.1 equiv), CH_2Cl_2, –50 or –30 ºC, 2 h. [c] Determined by ^{19}F NMR of the crude reaction mixture.

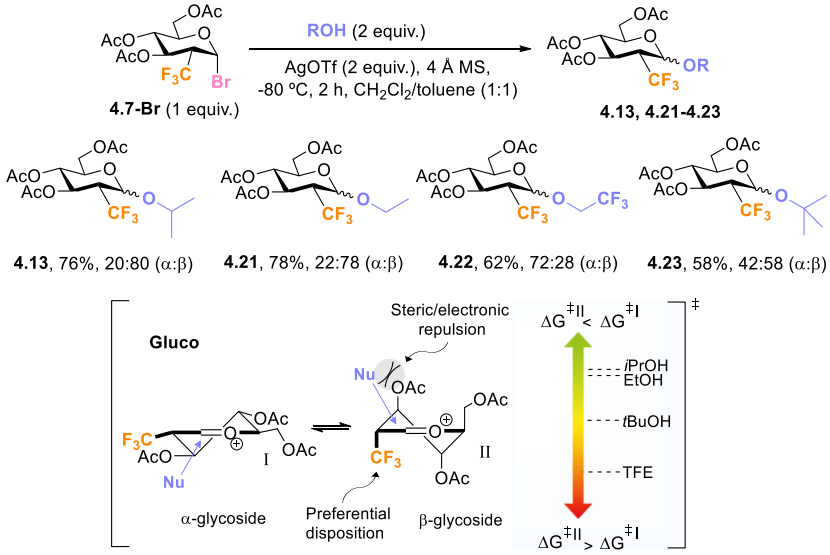
Stereoselectivity in 2-deoxy-2-fluorosugars is rationalized by the Anh-Eisenstein 1,2-induction model (**Scheme 4.10**) in which stereoselective control is reinforced or diminished depending on the electronic nature of protecting groups (EWG or EDG). The higher stereoselectivity observed in trifluoromethyl *O*-benzyl-protected *manno* derivatives may arise from a higher contribution of steric control in comparison with fluorinated analogues.



Scheme 4.10. Stereoselectivity rationalization.

4.3.5. Study of the effect of acceptor size and nucleophilicty

Next, the effect of the acceptor nucleophilicity was examined with donor **4.7-Br** and using different small alcohols as acceptors. To avoid any variation of the yield and selectivities due to the bromination step, we decided to perform the glycosylation step from the same batch of freshly prepared glycosyl bromide **4.7-Br** (**Scheme 4.11**). No remarkable differences were observed when isopropanol and ethanol were used as acceptors and preferred 1,2-*trans* stereoselectivity was obtained (up to 20:80 α:β ratio for **4.13**). On the other hand, low or inverted (1,2-*cis*) stereoselectivities were observed when larger/weaker nucleophilic *tert*-butyl alcohol and trifluoroethanol were used as acceptors as they require donors with substantial oxocarbenium ion character and are engaged in dissociative S_N1-like reactions (**4.22** and **4.23**). The increased steric hindrance of *tert*-butyl alcohol may disfavor its approach by the top face due to steric and electronic repulsions with protecting groups (**Scheme 4.11**, intermediate II). Results are compatible with a Curtin-Hammett scenario, where $\Delta G^{\ddagger II}$ increases energy more than $\Delta G^{\ddagger I}$ for bulky and weak nucleophiles (**Scheme 4.11**, bottom).



Scheme 4.11. Glycosylation between glycosyl bromide **4.7-Br** and small alcohols as acceptors and stereoselectivity rationalization.

4.3.6. Evaluation of the bioisosterism of the CF_3 group

As mentioned in the introduction, the CF_3 group has been controversial regarding its bioisosterism, namely its capacity to replace groups with similar size or shape without altering the structure or conformation of the molecule.[4] Few examples of trifluoromethylated glycosides have been reported in the literature and the effect of the trifluoromethyl group in the geometry and electron density of carbohydrates has not been explored. With these considerations, computational studies were carried out to evaluate the effect of the introduction of a trifluoromethyl group in the geometry and potential energy surface of glycosides and compare with the introduction of a fluorine atom. To this end, the optimised conformations, dihedral angles, dipolar moments, and potential energy surfaces of 2-deoxy-2-fluoro **S1** and 2-trifluoromethyl mannopyranosides **S2** were calculated using Gaussian 9, and results obtained are shown in **Figure 4.5**. Additionally, calculation of the Van der Waals volume (V_{vdW})[19] of these glycosides yields a value of 150.0 Å^3 for 2-F-mannopyranose **S1** and 179.4 Å^3 for 2-CF_3-mannopyranose **S2** (**Figure 4.4**). As expected, trifluoromethyl confers a larger Van der Waals volume.

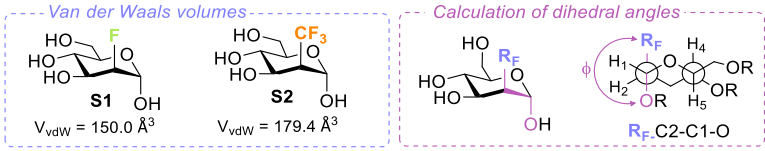


Figure 4.4. Calculated Van der Waals volumes and measurement of dihedral angles.

Regarding the conformation of the sugar, it was observed that the trifluoromethyl group, probably due to repulsion interactions with the C-6 hydroxyl group, slightly distorts the angle of the R_F-C2-C1-O. Measurement of the calculated dihedral angle indicated a distortion of almost 10 degrees in the trifluoromethylated glycoside **S2** in comparation with the fluorinated analogue **S1**. Thus, as expected, the introduction of the large trifluoromethyl group slightly distorts the conformation of the sugar, however, it does not affect to

[19] Zhao, Y. H.; Abraham, M. H.; Zissimos, A. M. *J. Org. Chem.* **2003**, *68*, 7368−7373.

the chair conformation, and the $^4C_1$ conformation is still maintained. In addition, direction and magnitude of the dipole moments were calculated for both glycosides. Results obtained are shown in **Figure 4.5** and no differences between the direction and magnitude of the dipole moments of the 2-F and 2-CF_3 glycosides could be observed. Thus, it can be concluded that the introduction of a trifluoromethyl motif in a glycoside has an effect in the conformation of the molecule but does not have a remarkable effect in the dipole moment magnitude and direction in comparison with fluorine.

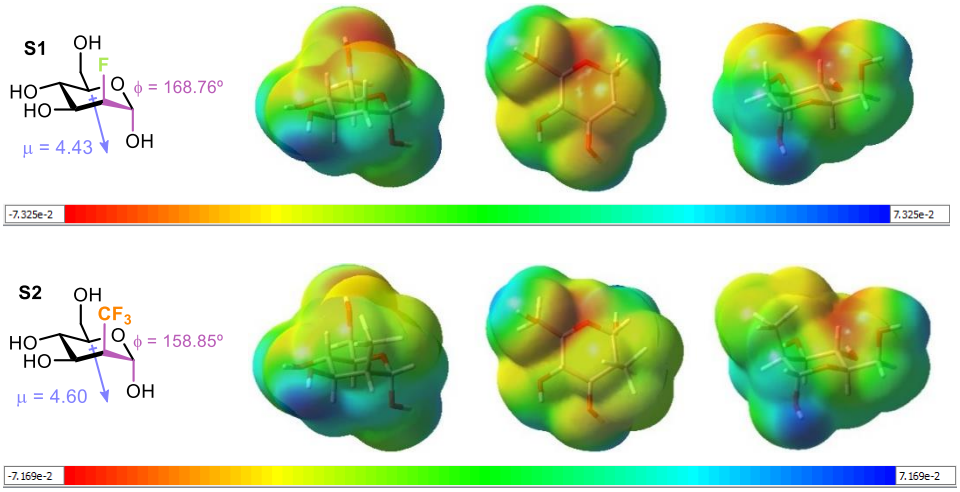


Figure 4.5. Optimized conformations and potential energy surfaces of 2-F-α-D-mannopyranose (**S1**) and 2-CF_3-α-D-mannopyranoside (**S2**).

Examination of the potential energy surfaces indicated that the introduction of the trifluoromethyl group has a strong effect in the volume of the molecule but has not a great effect in the potential energy surface in comparison with fluorine (**Figure 4.5**). Small differences could be observed in 2-F-mannopyranoside **S1,** that has a higher negative region located in the top of the ring oxygen compared to the trifluoromethylated analogue **S2**. Interestingly, fluorine atom slightly contributes to this negative region, however, the trifluoro methyl group, seems to create a shield that reduces the negative charge around the cyclic oxygen. Additionally, optimized conformations and potential energy surfaces were also calculated for 2-deoxy-

2-fluoro-α-D-glucopyranose **S3** and 2-deoxy-2-trifluoromethyl-α-D-glucopyranose **S4,** and results are reported in the experimental section (**Figure 4.8** and **Figure 4.9**). In all the cases, potential energy surfaces for 2-F and 2-CF_3-glycosides were similar, with slightly differences in some regions. Thus, the introduction of a trifluoromethyl group has a great impact in the volume of the glycoside in comparation with fluorine, but the electronic properties and the $^4C_1$ chair conformation remained almost unaltered.

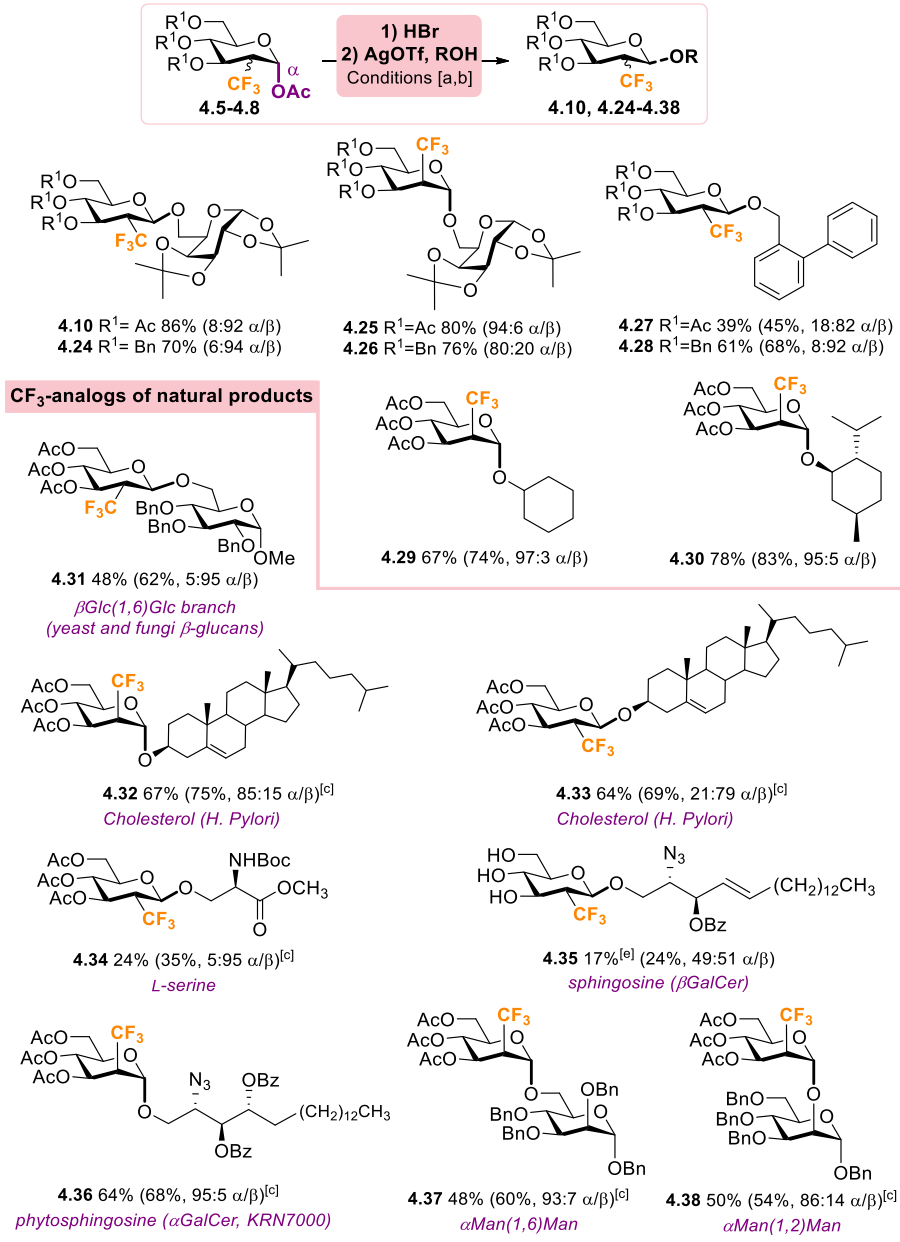
4.3.7. Glycosylation scope and stereoselective synthesis of CF_3-analogues of natural products

Finally, reaction scope was further examined using a diversity of representative acceptors, including primary and secondary glycosides, amino acids, and lipid aglycones such as cholesterol, sphingosine and phytosphingosine derivatives (**Scheme 4.8**). For instance, sequential bromination of **4.5-4.8** and subsequent glycosylation with acceptor **4.9** afforded products **4.10, 4.24, 4.25** and **4.26** in good yields (70-86% isolated yields) with expected 1,2-*trans* stereoselectivities in all the cases. As previously observed in 1-*O*-isopropyl-2-CF_3-glycosides in **Table 4.2**, reinforcement of the stereoselectivities was observed for *gluco* configuration with benzyl protecting groups yielding product **4.26** with a α:β ratio of 6:94, and *manno* configuration with acetyl protecting groups, yielding product **4.24** with a α:β ratio of 94:6. In most cases, the major anomer was obtained as single product after chromatographic purification. When the minor isomer was produced in larger amounts both epimers could be successfully separated and structurally characterized. Conformational analysis of **4.10, 4.24, 4.25** and **4.26** by ^{1}H NMR showed $^4C_1$ conformations dominate in all cases (**Figure 4.10**).

Reaction with biphenyl-2-methanol occurred smoothly with *gluco* donors to afford β-glycosides and selectivity was slightly benefited using benzyl protecting groups (8:92 in **4.27** *vs.* 18:82 in **4.28**). An explanation for the lower yield obtained for the peracetylated biphenyl methyl product **4.28** could be the higher amount of elimination product obtained (29%) in comparison with the perbenzylated counterpart (11%). As expected, CF_3^{manno} afforded preferably α-

glycosides **4.29** and **4.30** with excellent stereoselectivities (up to 97:3 α:β) and slightly higher yields (78% for **4.30** *vs.* 67% for **4.29**) when reacted with secondary alcohols, since elimination side-reactions are significantly less operative from *manno*-configured donors.

The presented protocol was applied to the stereoselective synthesis of 2-CF_3-analogs of some relevant natural products. Despite the higher amount of side-elimination reactions using peracetylated glycosyl donors, these were chosen as reactants in the glycosylation scope, mainly due to their higher flexibility for accessing final unprotected derivatives (*via* Zemplén deacetylation) in comparison to the perbenzylated analogs. Fair yield although excellent selectivity was observed in the synthesis of **4.31** from the mismatched donor **4.7-Br** (62%, 5:95 by ^{19}F NMR). Reaction of cholesterol with both *manno* and *gluco* epimers afforded **4.32** (67% isolated, 75% ^{19}F NMR) and **4.33** (64% isolated yield, 69% ^{19}F NMR yield) with good isolated yields and stereoselectivities and only reduced elimination (10–15%). When using protected L-serine as acceptor, low yield of the desired product **4.34** (24% isolated yield, 35% ^{19}F NMR yield) was obtained due to competing elimination process (35% of 2-CF_3-D-glucal). However, excellent β-selectivity (5:95 α:β) was achieved by virtue of the equatorial CF_3 configuration. Unexpectedly, the stereoselective control was completely eroded using a sphingosine analogue, which reacted with **4.7-Br** producing a racemic mixture of the desired product. Moreover, problematic purification required hydrolysis of acetyl groups to obtain **4.35** in pure form. In stark contrast, when protected azido-phytosphingosine acceptor was reacted with *manno* **4.8-Br**, excellent α-selectivity was obtained (95:5 α:β), and **4.36** was isolated in 63% yield. The same glycosyl donor smoothly reacted with primary and secondary sugars to afford disaccharides **4.37** (48% isolated, 60% ^{19}F NMR) and **4.38** (50% isolated, 54% ^{19}F NMR) with preferred α-configuration (up to 97:3 α:β). It should be noted that 2-CF_3-D-glucal by-product was obtained in variable amounts and detected by ^{19}F NMR (10–35% for *gluco* and <10% for *manno*). Competing elimination process using CF_3^{gluco} donors resulted in reduced overall yields for certain glycosylations.



Reagents and conditions: [a] **4.5-4.8** (1 equiv), 33 wt. % HBr in AcOH, 0 ºC to rt, 4 h. [b] **4.5-4.8-Br** (1 equiv), ROH (2 equiv), and AgOTf (2 equiv) in 1:1 (v/v) CH_2Cl_2/toluene, -80 ºC, 2 h. [c] To facilitate purification, the crude was subjected to acetylation; Ac_2O (6 equiv), 1:1 (v/v) pyridine/CH_2Cl_2, rt, 16 h and the residue purified by flash column chromatography. [d] To facilitate purification, the crude was subjected to Zemplén deacetylation; NaOMe, MeOH, rt, 6 h and the residue purified by flash column chromatography. [e] Yield over two steps from OAc. Isolated yields given for all compounds unless otherwise indicated. In parenthesis yield and diastereomeric ratio calculated by ^{19}F NMR of the crude reaction mixture before isolation using 1,4-difluorobenzene as internal standard (see SI for details).

Scheme 4.12. Scope of the glycosylation reaction and obtention of CF_3-analogues of natural products.

4.4. CONCLUSIONS

In summary, we have disclosed the unprecedented glycosylation of 2-deoxy-2-trifluoromethylsugars that are in turn prepared from readily available D-glycals. The study of the stereoelectronic properties of the CF_3 group highlighted the selectivity control of glycosylation affording 1,2-*trans* glycosides. Structural screening indicated that best stereoselectivities are reinforced by the effect of protecting groups (OBn/CF_3^{gluco} as β-selective; OAc/CF_3^{manno} as α-selective). Glycosylation proved successful for a range of different alcohols including primary and secondary aliphatic-OH, sugars, and complex aglycones. Interestingly, conformational analyses of resulting CF_3-glycosides showed no perturbations in the molecular geometry, which may stimulate the development of new fluorosugar mimetics with interesting physicochemical properties (*e.g.,* logP enhancement and/or increased ^{19}F NMR sensitivity). Indeed, reaction scope also proved successful for the preparation biologically relevant CF_3-glycosides including amino acids, cholesterol, and sphingosine/phytosphingosine analogues.

4.5. EXPERIMENTAL SECTION

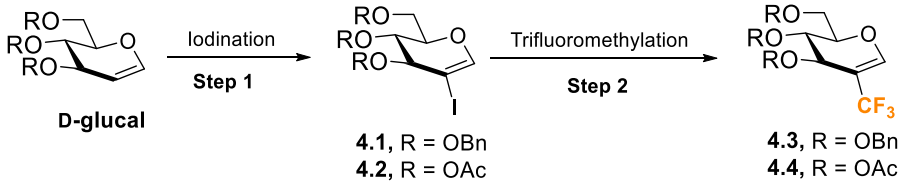
4.5.1. General considerations

Proton (^{1}H NMR), carbon (^{13}C NMR) and fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (400 MHz for ^{1}H, 100.6 MHz for ^{13}C and 376.5 MHz for ^{19}F). Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^{1}H NMR: $CDCl_3$ = 7.26 and ^{13}C NMR: $CDCl_3$ = 77.00) and $CFCl_3$. Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent. Infrared (IR) spectra were recorded on a Jasco FT/IR-600 Plus ATR Specac Golden Gate spectrophotometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (*c*) are given in g/100 mL. High resolution mass spectra (HRMS) were recorded on an Agilent 1100 Series LC/MSD mass spectrometer with electrospray ionization (ESI) by the Servei de Recursos Científics (URV). Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using commercial aluminum backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (max = 254 nm) and 6% H_2SO_4 in EtOH. Flash column chromatography was carried out using silica gel 60 Å (230-400 mesh). Mobile phases are reported in relative composition (e.g. 1:1 EtOAc/hexanes v/v). All solvents were used as supplied (Analytical, synthesis or HPLC grade), without prior purification. All reagents were used as received from commercial suppliers. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulphate ($MgSO_4$) was used as drying agent after reaction work-up, as indicated.

4.5.2. Preparation of $CuCF_3$ reagent

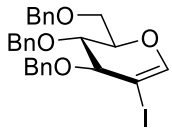
DMF (25 mL) and DMI (25 mL) were added to a flask containing KF (3.56 g, 61.3 mol) and CuBr (8.79 g, 61.3 mmol) under argon. The suspension was vigorously stirred at 0 °C. $TMSCF_3$ (8.8 mL, 59.5 mmol) was slowly added and the mixture stirred for 3 h at 0 °C. An aliquot (0.6 mL) was transferred to an NMR tube under argon and BTB (20 µL, 0.129 mmol) was added and the tube caped with a rubber septum. The $CuCF_3$ was produced in 89% yield as determined by quantitative ^{19}F NMR analysis. The batch solution was transferred to a schlenk under argon and kept at -30 °C.

4.5.3. Preparation of 2-deoxy-2-trifluoromethyl-glycosyl donors



Scheme 4.13. Synthesis of 3,4,6-Tri-*O*-benzyl-2-trifluoromethyl-D-glucal.

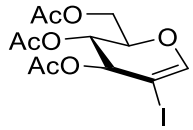
3,4,6-Tri-*O*-benzyl-2-iodo-D-glucal (4.1)



3,4,6-Tri-*O*-benzyl-D-glucal (1 g, 2.40 mmol) was azeotropically dried with toluene and dissolved in ACN (20 mL) under Argon. To the stirring solution, previously dried NIS (630 mg, 2.8 mmol) and AgOTf (81.6 mg, 0.48 mmol) were added, and the reaction is stirred for 1 h at 80 °C. The crude was then diluted with EtOAc and filtered through a short path of silica. The solvent was evaporated under vacuum and the crude was purified by column chromatography EtOAc/Hexanes (1:9) giving **4.1** (1.03 g, 79%) as a yellowish solid.

R_f (EtOAc/hexanes 1:9): 0.40; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.43-7.19 (m, 15H, ArH), 6.74 (bs, 1H, H-1), 4.74-4.48 (m, 6H, $3CH_2Ph$), 4.30 (m, 1H, H-5), 4.09 (d, $J_{3,4}$ = 4.8 Hz, 1H, H-3), 4.00 (dd, $J_{4,5}$ = 7.0 Hz, $J_{3,4}$ = 4.8 Hz, 1H, H-4), 3.79 (dd, $J_{6a,b}$ = 10.8 Hz, $J_{5,6a}$ = 5.5 Hz, 1H, H-6a), 3.71 (dd, $J_{6a,b}$ = 10.8 Hz, $J_{5,6b}$ = 3.8 Hz, 1H, H-6b); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 148.4 (C-1), 137.8, 137.6, 137.6 (C, Ar), 128.5, 128.4, 128.4, 128.1, 127.9, 127.9, 127.7, 127.7 (CH, Ar), 78.9 (C-3), 76.5 (C-5), 73.4 (C-4), 73.4, 73.1, 72.2 ($3xCH_2Ph$), 70.4 (C-2), 67.8 (C-6). Spectroscopic data are in agreement with that reported.[20]

3,4,6-Tri-*O*-acetyl-2-iodo-D-glucal (4.2)

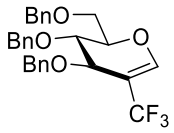


NIS (4.95 g, 22 mmol) and $AgNO_3$ (0.62 g, 3.65 mmol) were added under argon atmosphere to a solution of 3,4,6-Tri-*O*-acetyl-D-glucal (5.0 g, 18.36 mmol) in dry CH_3CN (50 mL) at room temperature. The reaction mixture was warmed up to 80 °C and stirred for 4 h. The crude was filtered through a short path of Silica and the solvent evaporated. The residue was purified by column chromatography using (2:8 EtOAc/hexanes) to afford **4.2** (4.3 g, 59%) as an colourless syrup.

R_f (2:3 EtOAc/hexanes): 0.46; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.69 (d, $J_{1,3}$ = 1.1 Hz, 1H, H-1), 5.36 (d, $J_{3,4}$ = 5.1 Hz, 1H, H-3), 5.10 (appt, $J_{3,4}$ = $J_{4,5}$ = 6.4 Hz, 1H, H-4), 4.35-4.23 (m, 2H, H-6a, H-5), 4.07 (dd, $J_{6a,6b}$ = 14.6 Hz, $J_{5,6b}$ = 5.6 Hz, 1H, H-6b), 2.00, 1.96, 1.96 (s, 9H, $3CH_3$, Ac); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.1, 169.6, 169.1 (3xC=O, Ac), 149.1 (C-1), 73.7 (C-5), 70.3 (C-3), 67.30 (C-4), 66.2 (C-2), 60.7 (C-6), 20.7, 20.6, 20.5 ($3xCH_3$, Ac). Spectroscopic data are in agreement with that reported.[21]

[20] Cobo, I.; Matheu, M. I.; Castillón, S.; Boutureira, O.; Davis, B. G. *Org. Lett.* **2012**, *14*, 1728-1731.
[21] Dharuman, S.; Vankar, Y. D. *Org. Lett.* **2014**, *16*, 1172-1175.

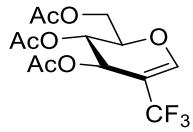
3,4,6-Tri-*O*-benzyl-2-trifluoromethyl-D-glucal (4.3)



A round-bottom flask containing **4.1** (4.1 g, 7.55 mmol) was evacuated and backfilled with argon 4 times. DMI (18 mL) and $CuCF_3$ solution (18.2 mL, 15.1 mmol) were successively added and the mixture was shaken to complete homogeneity. The reaction was heated without stirring at 40 °C for 17 h and then the temperature raised to 50 °C for additional 16 h. Saturated aqueous NH_4Cl was slowly added at 0 °C and the crude extracted with Et_2O dried over $MgSO_4$ and the solvent evaporated. Purification by column chromatography (1:15 EtOAc/hexanes) gave **4.3** (3.61 g, 98%) as a colourless syrup.

R_f (2:8 EtOAc/hexanes): 0.43; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.40-7.21 (m, 15H, ArH), 7.07 (bq, $J_{1,F}$ = 1.5 Hz, 1H, H-1), 4.59-4.44 (m, 7H, $3CH_2Ph$, H-5), 4.10 (bs, 1H, H-3), 3.90 (appt, $J_{3,4}$ = $J_{4,5}$ = 3.2 Hz, 1H, H-4), 3.78 (dd, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6a}$ = 6.9 Hz, 1H, H-6a), 3.67 (dd, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6b}$ = 5.1 Hz, 1H, H-6b); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (s, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 148.1 (q, $J_{1,F}$ = 7.2 Hz, C-1), 137.8, 137.6, 137.4 (C, Ar), 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0 127.9, 127.8 (CH, Ar), 125.0 (q, $J_{C,F}$ = 269.9 Hz, CF_3), 103.6 (q, $J_{2,F}$ = 30.7 Hz, C-2), 76.5 (C-5), 73.4, 72.4, 72.2 ($3xCH_2Ph$), 71.2 (C-4), 68.9 (C-3), 71.2 (C-4), 68.9 (C-3), 67.7 (C-6); Spectroscopic data were in agreement with those reported.[21]

3,4,6-Tri-*O*-acetyl-2-trifluoromethyl-D-glucal (4.4)

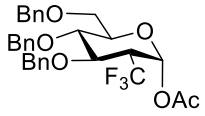


A round-bottom flask containing **4.2** (3.3 g, 8.29 mmol) was evacuated and backfilled with argon 4 times. DMI (20 mL) and $CuCF_3$ solution (20 mL, 16.6 mmol) were successively added and the mixture was shaken to complete homogeneity. The reaction was heated without stirring at 40 °C for 17 h and then the temperature raised to 50 °C for additional 16 h. Saturated aqueous NH_4Cl was slowly added at 0 °C and the crude extracted with Et_2O dried over $MgSO_4$ and the solvent evaporated. Purification by column chromatography (3:7 EtOAc/hexanes) gave **4.4** (2.62 g, 93%) as a colourless syrup.

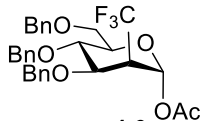
R_f (2:3 EtOAc/hexanes): 0.47; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.17 (q, $J_{1,F}$ = 1.5 Hz, 1H, H-1), 5.57 (d, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.16 (t, $J_{4,5}$ = $J_{4,3}$ = 3.5 Hz, 1H, H-4), 4.54-4.49 (m, 1H, H-5), 4.45 (dd, $J_{6a,b}$ = 12.0 Hz, $J_{6a,5}$ = 7.6 Hz, 1H, H-6a), 4.20 (dd, $J_{6b,a}$ = 12.0 Hz, $J_{6b,5}$ = 4.2 Hz, 1H, H-6b), 2.10, 2.10, 2.08 (s, 9H, 3CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -63.6 (s, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 169.4, 169.4 (3xC=O, Ac), 149.4 (q, J = 6.8 Hz, C-1), 123.9 (q, J = 270.1 Hz, CF_3), 102.1 (q, J = 32.0 Hz, C-2), 74.4 (C-5), 65.8 (C-4), 61.3 (C-3), 60.8 (C-6), 20.9, 20.8, 20.8 (3xCH_3, Ac); Spectroscopic data were in agreement with those reported.[22]

[22] Wang, B.; Xiong, D.-C.; Ye, X.-S. Org. Lett. **2015**, 17, 5698-5701.

1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-D-glucopyranose (4.5) and 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-D-manno-pyranose (4.6)



4.5



4.6

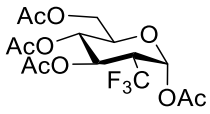
To a stirred solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-D-glucal **4.3** (3.23 g, 6.66 mmol) in THF (40 mL) was added a solution of $Hg(OCOCF_3)_2$ (4.26 g, 10 mmol) in H_2O (20 mL) at 0 °C. The mixture was stirred 36 h at room temperature. Then H_2O (0.2 mL) was added followed by portion wise addition of $NaBH_4$ (1.5 g, 40 mmol) at 0 °C and the mixture stirred at this temperature for 20 min. The crude was concentrated under vacuum and diluted with 1:1 (v/v) EtOAc/H_2O (60 mL) and the aqueous phase extracted successively with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and the solvent evaporated. The crude was dissolved in pyridine (23 mL) and Ac_2O (2.3 mL) was added and the reaction mixture stirred at room temperature for 15 h. The solvent was then evaporated under vacuum and the residue dissolved in EtOAc and washed with saturated aqueous $CuSO_4$, NH_4Cl and NaCl. The organic phase was dried with $MgSO_4$, filtered, the solvent evaporated and the crude purified by flash column chromatography (1:9 EtOAc/hexanes) to give pure fractions of **4.5** (1.26 g, 35%) and **4.6** (1.67 g, 46%).

Data for **4.5**: White solid. R_f (1:4 EtOAc/hexanes): 0.44; **m.p**: 114-116 °C; $[\alpha]^D_{25}$: +74.9 (0.12, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.40-7.15 (m, 15H, ArH), 6.44 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 4.89-4.81 (m, 2H, 2xCH-Ph), 4.77 (d, *J* = 10.2 Hz, 1H, CH-Ph), 4.63 (d, *J* = 12.0 Hz, 1H, CH-Ph), 4.58 (d, *J* = 10.7 Hz, 1H, CH-Ph), 4.50 (d, *J* = 12.0 Hz, 1H, CH-Ph), 4.22 (m, 1H, H-3), 3.90-3.78 (m, 3H, H-4, H-5, H-6a), 3.67 (bd, $J_{6a,6b}$ = 10.9 Hz, 1H, H-6b), 2.85 (m, 1H, H-2), 2.13 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -63.9 (d, $J_{CF3,H2}$ = 8.25 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 168.5 (C=O, Ac), 137.9, 137.8, 137.7 (C, Ar),

128.6, 128.6, 128.5, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9 (CH, Ar), 124.7 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 88.7 (q, $J_{C1,F}$ = 4.7 Hz, C-1), 78.1 (C-4), 76.5 (C-3), 75.5, 75.4, 73.7 (3xCH_2Ph), 73.3 (C-5), 67.9 (C-6), 49.11 (q, $J_{C2,F}$ = 25.0 Hz, C-2), 20.9 (CH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 3063, 3032, 2870, 2361, 2331, 1757, 1455, 1367, 1221, 1156, 1116, 954, 738; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{30}H_{31}F_3NaO_6^+$ (m/z): calc. 567.1965; found 567.1964.

Data for **4.6**: Colourless syrup. R_f (1:4 EtOAc/hexanes): 0.35; **[α]$^D_{25}$**: +41.4 (1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.45-7.15 (m, 15H, ArH), 6.53 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.75 (d, *J* = 11.0 Hz, 1H, CH-Ph), 4.74 (d, *J* = 11.1 Hz, 1H, CH-Ph), 4.63 (d, *J* = 12.0 Hz, 1H, CH-Ph), 4.59 (d, *J* = 11.1 Hz, 1H, CH-Ph), 4.53 (d, *J* = 12.0 Hz, 1H, CH-Ph), 4.47 (d, *J* = 11.0 Hz, 1H, CH-Ph), 4.17 (appt, $J_{2,3}$ = $J_{3,4}$ = 5.6 Hz, 1H, H-3), 4.01-3.90 (m, 2H, H-4, H-5), 3.75 (dd, $J_{6a,6b}$ = 11.0 Hz, $J_{6a,5}$ = 4.2 Hz, 1H, H-6a), 3.69 (dd, $J_{6a,6b}$ = 11.1 Hz, $J_{5,6b}$= 2.3 Hz, 1H, H-6b), 2.99 (m, 1H, H-2), 2.10 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -69.6 (bs, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 168.8 (C=O, Ac), 138.2, 137.8, 137.3 (C, Ar), 128.6, 128.5, 128.4, 128.2, 128.2, 128.1, 127.8, 127.7 (CH, Ar), 125.2 (q, $J_{C,F}$ = 281.2 Hz, CF_3), 89.6 (q, $J_{C,F}$ = 4.4 Hz, C-1), 75.5 (C-3), 74.5 (CH_2Ph), 73.9 (C-5), 73.8 (C-4), 73.5, 72.4 (2xCH_2Ph), 68.9 (C-6), 45.12 (q, $J_{C,F}$ = 24.7 Hz, C-2), 21.0 (CH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 3032, 2870, 2361, 1758, 1222, 1173, 1118, 1010, 954, 740, 698; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{30}H_{31}F_3NaO_6^+$ (m/z): calc. 567.1965; found 567.1964. Spectroscopic data were in agreement with those reported.[23]

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucopyranose (4.7)



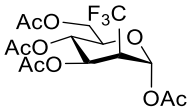
10% Pd/C (33 mg, 0.03 mmol Pd) was added to a solution of **4.5** (180 mg, 0.33 mmol) in dry and deoxygenated methanol (4 mL) at room

[23] Jordi Mestre Ventura (2017). PhD thesis. Universitat Rovira I Virgili, Tarragona.

temperature. The mixture was stirred under H_2 (1 atm) at the same temperature for 16 h, filtered through a short path of Celite® 545, and concentrated under reduced pressure. The crude was dissolved in pyridine (1 mL) and Ac_2O (0.23 mL) was added and the reaction stirred at room temperature overnight. Pyridine was then evaporated under vacuum and the residue dissolved in EtOAc and washed with saturated aqueous $CuSO_4$, NH_4Cl and NaCl. The organic phase was dried with $MgSO_4$, filtered, the solvent evaporated and the crude purified using column chromatography (3:7 EtOAc/hexanes) to give **4.7** (103 mg, 78%) as a white solid.

***R*$_f$** (3:7 EtOAc/hexanes): 0.35; **m.p**: 94-96 °C; **[α]$^D_{25}$** :+111.2 (0.24, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.45 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.70 (dd, $J_{3,2}$ = 11.4 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 5.11 (appt, $J_{4,5}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-4), 4.31 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 3.8 Hz, 1H, H-6a), 4.12-4.01 (m, 2H, H-5 and H-6b), 3.04-2.92 (m, 1H, H-2), 2.16, 2.08, 2.05, 2.04 (s, 12H, 4CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: - 65.3 (d, $J_{2,F}$ = 7.5 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.7, 169.7, 169.7, 168.1 (4xC=O, Ac), 123.7 (q, $J_{C,F}$ = 281.1 Hz, CF_3), 87.7 (q, $J_{C1,F}$ = 4.3 Hz, C-1), 69.7 (C-5), 68.3 (C-4), 66.7 (bq, $J_{C3,F}$ = 2.0 Hz, C-3), 61.5 (C-6), 47.7 (q, $J_{C2,F}$ = 26.2 Hz, C-2), 20.8, 20.7, 20.7 (4xCH_3, Ac); **FT-IR (neat)** *v* in cm^{-1}: 2970, 1759, 1370, 1221, 1178, 1014, 938; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{15}H_{19}F_3NaO_9^+$ (m/z): calc. 423.0873; found 423.0880. Spectroscopic data were in agreement with those reported.[23]

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-mannopyranose (4.8)



10% Pd/C (124 mg, 0.11 mmol Pd) was added to a solution of **4.6** (680 mg, 1.24 mmol) in dry and deoxygenated methanol (12 mL) at room temperature. The mixture was stirred under H_2 (1 atm) at the same temperature for 16 h, filtered through a short path of Celite® 545, and concentrated under reduced pressure. The crude was dissolved in pyridine

(4.20 mL) and Ac_2O (0.9 mL) was added and the reaction stirred at room temperature overnight. Pyridine was then evaporated under vacuum and the residue dissolved in EtOAc and washed with saturated aqueous $CuSO_4$, NH_4Cl and NaCl. The organic phase was dried with $MgSO_4$, filtered, the solvent evaporated, and the crude purified using column chromatography (3:7 EtOAc/hexanes) to give **4.8** (400 mg, 80%) as a colourless syrup.

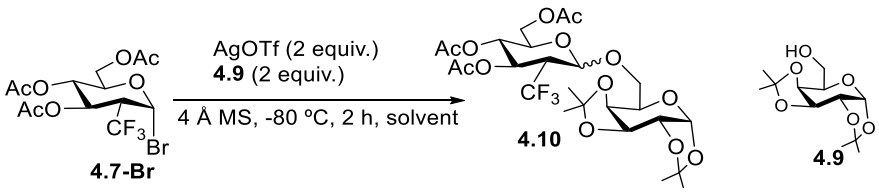
R_f (3:7 EtOAc/hexanes): 0.26; **$[\alpha]^D_{25}$** : +58.2 (0.27, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 6.45 (d, $J_{1,2}$ = 2.0 Hz, 1H, H-1), 5.47-5.31 (m, 2H, H-3, H-4), 4.20-4.10 (m, 2H, H-6a, H-6b), 4.03 (m, 1H, H-5), 3.14 (m, 1H, H-2), 2.16, 2.06, 2.06 (s, 12H, 4CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.8 (d, $J_{2,F}$ = 9.3 Hz, 3F); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.7, 170.2, 169.4, 168.2 (4xC=O, Ac), 124.4 (q, $J_{C,F}$ = 281.3 Hz, CF_3), 88.9 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 70.5 (C-5), 67.4 (C-3), 65.4 (C-4), 62.0 (C-6), 45.2 (q, $J_{C2,F}$ = 25.5 Hz, C-2), 20.9, 20.8, 20.7 (4xCH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 2969, 1749, 1371, 1220, 1159, 1125; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{15}H_{19}F_3NaO_9^+$ (m/z): calc. 423.0873; found 423.0872. Spectroscopic data were in agreement with those reported.[23]

4.5.4. General glycosylation procedure

To a solution of the **donor** (1 mmol) in CH_2Cl_2 (5-30 mL/mmol) was added 30 % HBr in AcOH (1.75-5 mL/mmol) at 0 °C. The reaction mixture was then slowly allowed to warm up to room temperature and stirred for 2-4 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ at 0 °C. The two layers were separated, and the aqueous layer was successively extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated. The resulting crude was used in the next step without further purification. It was azeotropically dried with toluene twice and maintained under vacuum for 3 h, then dissolved in anhydrous CH_2Cl_2 (10 mL/mmol) and transferred via cannula under argon into a Schlenk flask containing the **acceptor** azeotropically dried (3 mmol) and preactivated 4 Å MS. The mixture was stirred and maintained at -80 °C for 30 minutes. A solution of azeotropically dried AgOTf (2 mmol) in toluene (10 mL/mmol) was transferred via cannula under argon. The reaction was stirred at -80 °C for 2 h, then diluted

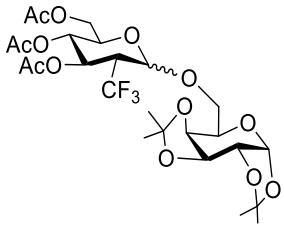
with CH_2Cl_2, filtered through a short path of silica and the solvent evaporated. The α/β ratio and glycosylation yield was determined by ^{19}F NMR using 1,4-difluorobenzene as internal pattern and the residue was purified by flash-column chromatography.

4.5.5. Screening of glycosylation solvent



To a solution of **4.7** (0.10 mmol) in CH_2Cl_2 (0.5 mL) was added 30 % HBr in AcOH (0.5 mL) at 0 °C. The reaction mixture was then slowly allowed to warm up to room temperature and stirred for 4 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ at 0 °C. The two layers were separated, and the aqueous phase was successively extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated to afford the crude bromopyranoside, which was used in the next step without further purification. The resulting crude was azeotropically dried with toluene, maintained under vacuum for 3 h and redissolved with dry solvent (1 mL). The solution was transferred via cannula to a Schlenk flask containing azeotropically dried [1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside **4.9** (52 mg, 0.2 mmol) and 4 Å MS under argon and maintained at -80 °C for 30 minutes. A solution of azeotropically dried AgOTf (51.4 mg, 0.2 mmol) in the indicated solvent (1 mL) was transferred via cannula under argon. The reaction was stirred at -80 °C for 2 h, then diluted with CH_2Cl_2, filtered through a short path of silica and the solvent evaporated. The α/β ratio and reaction yield was determined quantitative ^{19}F NMR analysis using 1,4-difluorobenzene (20 μL, 0.194 mmol) as internal standard.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-α-D-mannopyranosyl-(1→6)-[1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside (4.10)



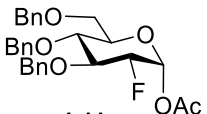
To a solution of **4.7** (97 mg, 0.242 mmol) in CH_2Cl_2 (1 mL) was added 33% HBr in AcOH (1 mL) at 0 °C. The reaction was stirred at room temperature for 3 h. The crude was diluted with CH_2Cl_2 and saturated aqueous $NaHCO_3$ was added at 0 °C. The two phases were separated, and the aqueous layer successively extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated. The resulting crude was azeotropically dried with toluene, maintained under high vacuum for 3 h, dissolved in CH_2Cl_2 (2.5 mL) and transferred under argon to a schlenk containing acceptor [1:2,3:4]-di-O-isopropylidene-α-D-galactopyranoside **4.9** (126 mg, 0.484 mmol) and activated 4 Å MS. The solution was stirred at -80 °C for 30 minutes and azeotropically dried AgOTf (125 mg, 0.484 mmol) was dissolved in dry toluene (0.5 mL) and the solution transferred to the schlenk via cannula under argon. The reaction was stirred at -80 °C for 2 h, diluted with CH_2Cl_2, filtered through a short path of silica and the solvent evaporated. An α/β (8:92) ratio was determined by ^{19}F NMR. The crude was purified by column chromatography (1:4 EtOAc/hexanes) to give **4.10** (124 mg, 86%) as a white solid.

R_f (2:3 EtOAc/hexanes): 0.39; **m.p**: 148-150 °C; **$[\alpha]^{D}_{25}$** : -20.7 (1.21, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.49 (d, $J_{1',2'}$ = 5.0 Hz, 1H, H-1'), 5.42 (dd, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 5.04 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-4), 4.86 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.59 (dd, $J_{3',4'}$ = 7.9 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-3'), 4.30 (dd, $J_{1',2'}$ = 5.0 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-2'), 4.28 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.1 Hz, 1H, H-6a), 4.20 (dd, $J_{3',4'}$ = 7.9 Hz, $J_{4',5'}$ = 1.8 Hz, 1H, H-4'), 4.10 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-6b), 4.01-3.94 (m, 2H, H-6a', H-5'), 3.79 (dd, $J_{6a',6b'}$ =

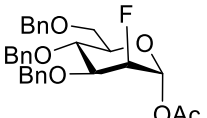
12.8 Hz, $J_{5',6b'}$ = 8.2 Hz, 1H, H-6b'), 3.71 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-5), 2.68 (m, 1H, H-2), 2.07, 2.01, 2.01 (s, 9H, 3CH_3, Ac), 1.50, 1.43, 1.32 (s, 12H, 4CH_3); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.8 (d, $J_{CF3,2}$= 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.8, 169.8, 169.7 (3xC=O, Ac), 126.0 (q, $J_{C,F}$ = 282.0 Hz, CF_3), 109.5, 108.9 (2Cketal), 98.5 (q, $J_{C1,F}$ = 2.5 Hz, C-1), 96.4 (C-1'), 71.5 (C-5), 71.3 (C-4'), 70.7 (C-3'), 70.5 (C-2'), 69.0 (C-4), 68.6 (C-6'), 68.1 (q, $J_{C3,F}$ = 1.6 Hz, C-3), 67.7 (C-5'), 62.3 (C-6), 49.8 (q, $J_{C2,F}$ = 24.5 Hz, C-2), 26.1, 26.0, 25.1, 24.5 (4xCH_3'), 20.9, 20.7 (3xCH_3, Ac); **FT- IR (neat)** ν in cm^{-1}: 2988, 1755, 1375, 1220, 1180, 1121, 1069, 747, 695; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{25}H_{27}F_{35}NaO_{13}^+$ (m/z): calc. 623.1922; found 623.1916. Spectroscopic data were in agreement with those reported.[23]

4.5.6. Screening of CF_3 configuration, protecting groups, nucleophiles and comparation with 2-deoxy-2-fluorosugars

1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-D-glucopyranose (4.11) and 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-D-mannopyranose (4.12)



4.11



4.12

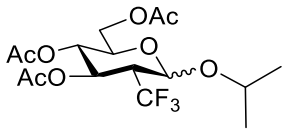
Selectfluor™ (6.12 g, 17.3 mmol) was added to a solution of **3,4,6-tri-O-benzyl-D-glucal** in nitromethane (43.2 mL) and water (8.64 mL) at room temperature and the mixture was stirred for 5 h. After this time, the reaction was put under reflux at 90 °C for 30 min. Once the TLC indicated total conversion of the starting material, solvents were evaporated, and the residue redissolved in EtOAc (250 mL). Then, $NaHCO_3$ was slowly added, and the organic phase was successively extracted with brine (20 mL) and $NaHCO_3$ (10 mLx5). The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4), filtered, evaporated, and concentrated under reduced pressure to afford a yellowish syrup. The crude was directly submitted to acetylation conditions, it was dissolved in CH_2Cl_2 (30 mL) and then pyridine (2.32 mL, 28.8 mmol) and acetic anhydride (2.7 mL, 28.8 mmol) were added to a solution and stirred

overnight at room temperature. Solvents were evaporated and the residue was concentrated under reduced pressure, then washed with 10% HCl, saturated aq. $NaHCO_3$ (3 times) and brine. The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4), filtered, evaporated, and concentrated under reduced pressure. The residue was purified by flash-column chromatography (from hexanes to 9:1 hexanes/EtOAc) to afford separated products, ***gluco* 4.11** (α:β 1:3) as a yellowish syrup and ***manno* 4.12** (only α) as a colourless syrup.

Data for **4.11**: Yellowish syrup. R_f (7:3 hexanes/EtOAc): 0.48; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.45-7.29 (m, 52H, Ph), 7.24-7.16 (m, 8H, Ph), 6.42 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1α), 5.75 (dd, $J_{1,2}$ = 8.1 Hz, $J_{1,F}$ = 3.2 Hz, 3H, H-1β), 5.00-4.78 (13H, m, Bn), 4.70 (ddd, $J_{2,F}$ = 48.8 Hz, $J_{2,3}$ = 9.2 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2α), 4.67-4.42 (m, 14H, H-2β, Bn), 4.12 (dt, $J_{3,F}$ = 12.4 Hz, $J_{2,3}$ = 9.1 Hz, 1H, H-3α), 3.94-3.75 (m, 15H, H-3β, H-4α, H-4β, H-5α, H-6aα, H-6aβ, H-6bβ), 3.71 (dd, $J_{6a,6b}$ = 10.9 Hz, $J_{5,6b}$ = 1.9 Hz, 1H, H-6bα), 3.62 (dt, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 2.7 Hz, 3H, H-5β), 2.19 (s, 9H, β-OAc), 2.17 (s, 3H, α-OAc); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -197.2 (ddd, $J_{F,2}$ = 51.4 Hz, $J_{F,3}$ = 14.6 Hz, $J_{F,1}$ = 3.0 Hz, 3F, β-anomer), -199.4 (dd, $J_{F,2}$ = 48.8 Hz, $J_{F,3}$ = 12.4, 1F, α-anomer);

Data for **4.12**: Colourless syrup. R_f (7:3 hexanes/EtOAc): 0.35; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.43-7.26 (13H, m, Ph), 7.22-7.18 (m, 2H, Ph), 6.28 (dd, $J_{1,F}$ = 6.3 Hz, $J_{1,2}$ = 2.2 Hz, 1H, H-1), 4.88 (d, J = 10.6 Hz, 1H, O-CH_2-Ph), 4.79 (d, J = 11.7 Hz, 1H, O-CH_2-Ph), 4.75 (d, J = 11.7 Hz, 1H, O-CH_2-Ph), 4.69 (dt, $J_{2,F}$ = 48.8 Hz, $J_{1,2}$ = $J_{2,3}$ = 2.4 Hz, 1H, H-2), 4.67 (d, J = 12.2 Hz, 1H, O-CH_2-Ph), 4.56 (d, J = 10.6 Hz, 1H, O-CH_2-Ph), 4.54 (d, J = 12.2 Hz, 1H, O-CH_2-Ph), 4.05 (td, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, $J_{4,F}$ = 0.9 Hz, 1H, H-4), 3.88 (ddd, $J_{3,F}$ = 29.0 Hz, $J_{3,4}$ = 9.4 Hz, $J_{2,3}$ = 2.5 Hz, 1H, H-3), 3.86 (ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 1.8 Hz, 1H, H-5), 3.79 (dd, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 4.1 Hz, 1H, H-6a), 3.70 (dd, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6b}$ = 1.9 Hz, 1H, H-6b), 2.06 (s, 3H, CH_3); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -204.0 (ddd, $J_{F,2}$ = 48.8 Hz, $J_{F,3}$ = 29.0 Hz, $J_{F,1}$ = 6.3 Hz, 1F); spectroscopic data was identical to that previously reported.[17]

1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (4.13)

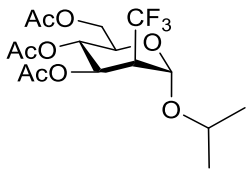


The title compound was prepared following the general procedure **(GP)** using **4.7** (55 mg, 0.137 mmol), CH_2Cl_2 (1 mL) and 30 % HBr in AcOH (1 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.7-Br**, isopropyl alcohol (31 µL, 0.41 mmol), dry CH_2Cl_2 (1.4 mL), dry toluene (1.4 mL), preactivated 4 Å MS and AgOTf (70 mg, 0.27 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (20:80) and yield (76%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.13α** (7 mg, 13% yield) and a fraction containing an anomeric mixture of **4.13** (α:β, 1:7) (29 mg, 53% yield).

Data for **4.13α**: Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.26; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.66 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.21 (d, J = 3.5 Hz, 1H, H-1), 5.01 (dd, $J_{4,5}$ = 10.2 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-4), 4.28 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 4.6 Hz, 1H, H-6a), 4.14 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 4.06 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-6b), 3.88 (hept., $J_{7,8}$ = 6.3 Hz, 1H, H-7), 2.78 (dqd, $J_{2,3}$ = 11.4 Hz, $J_{2,F}$ = 7.7 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.08, 2.04, 2.02 (s, 9H, $3CH_3$, Ac), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, H-8), 1.17 (d, $J_{7,8}$ = 6.2 Hz, 3H, H-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.03 (d, $J_{CF3,2}$ = 8.1 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.9, 169.6 (3xC=O, Ac), 126.0 (q, $J_{C,F}$ = 280.8 Hz, CF_3), 93.9 (q, $J_{C1,F}$ = 4.0 Hz, C-1), 71.9 (C-7), 69.1 (C-4), 67.6 (C-5), 67.1 (q, $J_{C3,F}$ = 2.0 Hz, C-3), 62.0 (C-6), 48.9 (q, $J_{C2,F}$ = 25.6 Hz, C-2), 23.1 (C-8), 21.3 (C-8'), 20.7, 20.65, 20.60 ($3xCH_3$, Ac); **FT-IR (neat)** ν in cm^{-1}: 2975, 2923, 2362, 1752, 1369, 1307, 1223, 1180, 1157, 1114, 1044, 1026, 924, 914; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{16}H_{23}F_3NaO_8^+$ (m/z): calc. 423.1237; found 423.1239.

Data for **4.13β**: Inseparable mixture of α:β (1:7). Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.23; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 5.00 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-4), 4.71 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 4.26 (dd, $J_{6a,b}$ = 12.2 Hz, $J_{5,6a}$ = 5.3 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,b}$ = 12.2 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-6b), 3.96 (sept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.68 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.2 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-5), 2.66 (ddq, $J_{2,3}$ = 10.7 Hz, $J_{1,2}$ = 8.2 Hz, $J_{2,F}$ = 7.7 Hz, 1H, H-2), 2.07, 2.02, 2.02 (s, 9H, 3CH_3, Ac), 1.25 (d, $J_{7,8}$ = 6.2 Hz, 3H, H-8), 1.17 (d, $J_{7,8}$ = 6.2 Hz, 3H, H-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.69 (d, $J_{CF3,2}$ = 7.6 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.7, 169.6 (3xC=O, Ac), 126.0 (q, $J_{C,F}$ = 281.8 Hz, CF_3), 97.1 (q, $J_{C1,F}$ = 2.5 Hz, C-1), 72.9 (C-7), 71.3 (C-5), 69.1 (C-4), 68.1 (q, $J_{C3,F}$ = 1.7 Hz, C-3), 62.2 (C-6), 49.9 (q, $J_{C2,F}$ = 23.9 Hz, C-2), 23.2 (C-8), 21.4 (C-8'), 21.2, 20.7, 20.6 (3xCH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 2977, 1750, 1668, 1433, 1372, 1328, 1223, 1182, 1122, 1072, 1031, 906; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{16}H_{23}F_3NaO_8^+$ (m/z): calc. 423.1237; found 423.1245.

1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α-D-mannopyranose (4.14)

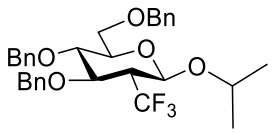


The title compound was prepared following the general procedure **(GP)** using **4.8** (25 mg, 0.062 mmol), CH_2Cl_2 (0.5 mL) and 30 % HBr in AcOH (0.5 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.8-Br**, isopropyl alcohol (4 µL, 0.186 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (31.9 mg, 0.124 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (>95:5) and yield (91%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard. The residue was purified by flash column chromatography

(from hexanes to 1:4 EtOAc/hexanes) to afford **4.14α** (20 mg, 82% yield) as a colourless syrup.

Data for **4.14α**: Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.18; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.41-4.31 (m, 2H, H-3, H-4), 5.26 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1), 4.17 (d, $J_{5,6}$ = 3.9 Hz, 2H, H-6a, H-6b), 4.02 (dt, $J_{4,5}$ = 8.9 Hz, $J_{5,6a}$ = 3.9 Hz, $J_{5,6b}$ = 1H, H-5), 3.92 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.08 (qdd, $J_{2,F}$ = 9.8 Hz, $J_{2,3}$ = 5.5 Hz, $J_{1,2}$ = 1.6 Hz, 1H, H-2), 2.08, 2.07, 2.05 (s, 9H, $3CH_3$, Ac), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.18 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.64 (d, $J_{CF3,2}$ = 9.8 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.7, 170.1, 169.5 (3xC=O, Ac), 124.7 (q, $J_{C,F}$ = 281.5 Hz, CF_3), 93.4 (q, $J_{C1,F}$ = 4.4 Hz, C-1), 70.6 (C-7), 68.2 (C-5), 67.9 (C-4), 66.0 (C-3), 62.4 (C-6), 46.6 (q, $J_{C2,F}$ = 24.6 Hz, C-2), 22.9 (C-8), 21.3 (C-8'), 20.6, 20.6, 20.5 ($3xCH_3$, Ac); **FT-IR (neat)** ν in cm^{-1}: 2976, 1748, 1371, 1306, 1267, 1227, 1160, 1110, 1044, 977, 911; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{16}H_{23}F_3NaO_8^+$ (m/z): calc. 423.1237; found 423.1246.

1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranose (4.15)

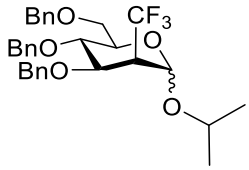


The title compound was prepared following the general procedure **(GP)** using **4.5** (56,5 mg, 0.104 mmol), CH_2Cl_2 (3 mL) and 30 % HBr in AcOH (0.2 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.5-Br**, isopropyl alcohol (32 µL, 0.421 mmol), dry CH_2Cl_2 (1 mL), dry toluene (1 mL), preactivated 4 Å MS and AgOTf (53.5 mg, 0.208 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α:β ratio (5:95) and yield (72%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:9 EtOAc/hexanes), obtaining an inseparable mixture of the

desired product **4.15β** (24 mg, 42% yield) and the elimination product (3,4,6-tri-*O*-benzyl-glucal) as yellowish syrup.

Data for **4.15β**: Inseparable mixture. Yellowish syrup. **R_f** (8:2 hexanes/EtOAc): 0.35; **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.35-7.27 (m, 13H, C_6H_5), 7.22-7.18 (m, 2H, C_6H_5), 4.79 (d, J = 10.2 Hz, 1H, CH-Ph), 4.78 (d, J = 11.0 Hz, 1H, CH-Ph), 4.71-4.65 (m, 2H, CH-Ph), 4.63-4.49 (m, 3H, CH-Ph(x2), H-1), 3.98 (sept, $J_{7,8}$ = 6.1 Hz, 1H, H-7), 3.83 (dd, $J_{4,5}$ = 9.6 Hz, $J_{3,4}$ = 8.6 Hz, 1H, H-4), 3.75 - 3.63 (m, 3H, H-3, H-6a, H-6b), 3.52 (ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 4.4 Hz, $J_{5,6b}$ = 2.9 Hz, 1H, H-5), 2.61 (qdd, $J_{2,CF3}$ = 8.2 Hz, $J_{2,3}$ = 5.3 Hz, $J_{1,2}$ = 2.4 Hz, 1H, H-2), 1.20 (d, $J_{7,8}$ = 6.3 Hz, 3H, CH_3-8), 1.15 (d, $J_{7,8}$ = 6.1 Hz, 3H, CH_3-8'); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -64.63 (d, $J_{CF3,2}$ = 8.2 Hz, 3F, CF_3); **^{13}C NMR** (125 MHz, CDCl3) δ in ppm: 138.1, 137.9, 137.8 (C, Ar), 128.51, 128.48, 128.42, 128.38, 128.08, 127.94, 127.90, 127.87, 127.81, 127.75, 127.65 (CH, Ar), 125.5 (q, $J_{C,F}$ = 282.0 Hz, CF_3), 96.8 (q, $J_{F,1}$ = 2.6 Hz, C-1), 78.7 (C-3), 78.5 (C-4), 74.8 (CH_2Ph), 74.75 (CH_2Ph), 74.7 (C-5), 73.5 (CH_2Ph), 71.9 (C-7), 69.1 (C-6), 51.5 (q, $J_{F,2}$ = 22.9 Hz, C-2), 23.4 (CH_3-8), 21.4 (CH_3-8'); **FT-IR (neat)** ν in cm^{-1}: 3031, 2973, 2921, 2866, 1497, 1454, 1383, 1356, 1329, 1308, 1290, 1245, 1212, 1174, 1124, 1101 1077, 1050, 1027, 912, 735, 697; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{35}F_3NaO_5^+$ (m/z): calc. 567.2329; found 567.2330.

1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose (4.16)



The title compound was prepared following the general procedure **(GP)** using **4.6** (65.5 mg, 0.120 mmol), CH_2Cl_2 (3.6 mL) and 30 % HBr in AcOH (0.2 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.6-Br**, isopropyl alcohol (28 µL, 0.360 mmol), dry CH_2Cl_2 (1.2 mL), dry toluene (1.2 mL), preactivated 4 Å MS and

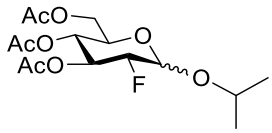
AgOTf (61.7 mg, 0.240 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (80:20) and yield (62%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:9 EtOAc/hexanes) to afford **4.16α** (26 mg, 40% yield) and **4.16β** (9 mg, 14% yield) as yellowish syrups and containing elimination product.

Data for **4.16α**: Inseparable mixture. Yellowish syrup. R_f (9:1 hexanes/EtOAc): 0.45; **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.24 (m, 13H, Ar), 7.19-7.13 (m, 2H, Ar), 5.31 (d, $J_{1,2}$ = 2.4 Hz, 1H, H-1), 4.74 (d, J = 10.9 Hz, 1H, CH-Ph), 4.73 (d, J = 11.2 Hz, 1H, CH-Ph), 4.63 (d, J = 12.1 Hz, 1H, CH-Ph), 4.54 (d, J = 11.2 Hz, 1H, CH-Ph), 4.49 (d, J = 12.1 Hz, 1H, CH-Ph), 4.40 (d, J = 10.9 Hz, 1H, CH-Ph), 4.18 (appt, $J_{2,3}$ = $J_{3,4}$ = 5.4 Hz, 1H, H-3), 3.93 (sept, $J_{7,8}$ = 6.1 Hz, 1H, H-7), 3.88 (m, 2H, H-4, H-5), 3.71 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6a}$ = 3.2 Hz, 1H, H-6a), 3.66 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6b}$ = 1.5 Hz, 1H, H-6b), 2.93 (qdd, $J_{2,CF3}$ = 9.9 Hz, $J_{2,4}$ = 5.3 Hz, $J_{1,2}$ = 2.4 Hz, 1H, H-2), 1.20 (d, $J_{7,8}$ = 6.3 Hz, 3H, CH_3-8), 1.15 (d, $J_{7,8}$ = 6.1 Hz, 3H, CH_3-8'); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -62.2 (d, $J_{CF3,2}$ = 9.9 Hz, 3F, CF_3); **^{13}C NMR** (125 MHz, CDCl3) δ in ppm: 138.3, 138.1, 137.8 (C, Ar), 128.4, 128.35, 128.1, 128.0, 127.85, 127.8, 127.6, 127.5, (CH, Ar), 125.6 (q, $J_{C,F}$ = 281.3 Hz, CF_3), 93.6 (q, $J_{F,1}$ = 4.6 Hz, C-1), 76.5 (C-3), 74.6 (CH_2Ph), 73.3 (CH_2Ph), 72.2 (C-5), 71.3 (CH_2Ph), 69.6 (C-7), 69.2 (C-6), 46.6 (q, $J_{F,2}$ = 23.7 Hz, C-2), 23.2 (CH_3-8), 21.3 (CH_3-8'); **FT-IR (neat)** ν in cm^{-1}: 3031, 2971, 2927, 2864, 1497, 1454, 1375, 1365, 1326, 1297, 1268, 1228, 1220, 1159, 1104, 1048, 1027, 922, 735, 718, 697; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{35}F_3NaO_5^+$ (m/z): calc. 567.2329; found 567.2336.

Data for **4.16β**: Inseparable mixture. Yellowish syrup. R_f (9:1 hexanes/EtOAc): 0.40; **^{1}H NMR** (400 MHz, $CDCl_3$) δ in ppm: 7.39-7.20 (m, 15H, Ar), 4.88 (d, $J_{1,2}$ = 2.0 Hz, H-1), 4.68 (d, J = 11.4 Hz, 1H, CH-Ph), 4.66 (d, J = 11.6 Hz, 1H, CH-Ph), 4.54 (d, J = 11.4 Hz, 1H, CH-Ph), 4.53 (d, J = 11.6 Hz, 1H, CH-Ph), 4.50 (d, J = 12.0 Hz, 1H, CH-Ph), 4.46 (d, J = 12.0 Hz, 1H, CH-Ph), 4.02-3.79 (m, 5H, H-3, H-4, H-5, H-6a, H-7), 3.73 (dd, $J_{6a,6b}$ = 9.9 Hz, $J_{5,6a}$ = 5.3 Hz, 1H, H-6b), 2.93 (qdd, $J_{2,CF3}$ = 9.4 Hz, $J_{2,3}$ = 4.3 Hz, $J_{1,2}$ = 3.2 Hz, 1H, H-2), 1.23 (d, $J_{7,8}$ = 6.2 Hz,

3H, CH_3-8), 1.16 (d, $J_{7,8}$ = 6.1 Hz, 3H, CH_3-8'); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -61,5 (s, 3F, CF_3); **^{13}C NMR** (125 MHz, $CDCl_3$) δ in ppm: 138.3, 137.9, 137.6 (C, Ar), 128.4, 128.35, 128.3, 128.0, 127.8, 127.7 127.65, 127.6, 127.5 (CH, Ar), 125.48 (q, $J_{C,F}$ = 281.8 Hz, CF_3), 94.5 (C-1), 75.7 (C-3), 74.7 (CH_2Ph), 73.4 (CH_2Ph), 73.2 (CH_2Ph), 72.5 (C-4), 71.9 (C-5), 70.7 (C-7), 70.1 (C-6), 44.9 (q, $J_{F,2}$ = 24.4 Hz, C-2), 23.1 (CH_3-8), 21.0 (CH_3-8'); **FT-IR (neat)** ν in cm^{-1}: 3031, 2972, 2924, 2866, 2360, 1496, 1454, 1382, 1361, 1284, 1245, 1225, 1178, 1149, 1111, 1071, 1048, 1027, 1013, 942, 888, 735, 697; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{35}F_3NaO_5^+$ (m/z): calc. 567.2329; found 567.2337.

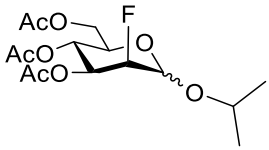
1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (4.17)



The title compound was prepared following the general procedure **(GP)** using 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-glycopyranose **3.11** (100 mg, 0.28 mmol), CH_2Cl_2 (2 mL) and 30 % HBr in AcOH (2 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, isopropyl alcohol (59.4 µL, 0.776 mmol), dry CH_2Cl_2 (2.5 mL), dry toluene (2.5 mL), preactivated 4 Å MS and AgOTf (168.1 mg, 0.576 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (24:76) and yield (95%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard.

Selected data for **4.17α/β: ^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -199.4 (ddd, *J* = 50.6 Hz, *J* = 14.5 Hz, *J* = 2.6 Hz, β-anomer), -200.9 (dd, *J* = 49.6 Hz, *J* = 11.8 Hz, α-anomer). These data were in agreement with those reported.[17]

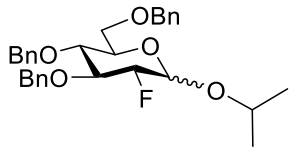
1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-mannopyranose (4.18)



The title compound was prepared following the general procedure **(GP)** using 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-mannopyranose **3.12** (50 mg, 0.14 mmol), CH_2Cl_2 (1 mL) and 30 % HBr in AcOH (1 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, isopropyl alcohol (27.9 µL, 0.363 mmol), dry CH_2Cl_2 (1.2 mL), dry toluene (1.2 mL), preactivated 4 Å MS and AgOTf (62.2 mg, 0.242 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (95:5) and yield (84%) using 1,4-difluorobenzene (10 µL, 0.097 mmol) as internal standard.

Selected data for **4.18α/β: ^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -203.1 to -203.5 (m, α-anomer). These data were in agreement with those reported.[17]

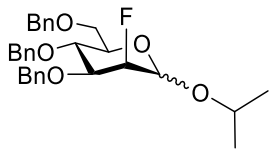
1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-benzyl-α/β-D-glucopyranose (4.19)



The title compound was prepared following the general procedure **(GP)** using 2-deoxy-2-fluoro-3,4,6-tri-*O*-benzyl-glucopyranose **4.11** (13.5 mg, 0.027 mmol), CH_2Cl_2 (0.3 mL) and 30 % HBr in AcOH (82 µL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, isopropyl alcohol (6.4 µL, 0.082 mmol), dry CH_2Cl_2 (0.3 mL), dry toluene (0.3 mL), preactivated 4 Å MS and AgOTf (14.0 mg, 0.054 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (4:96) and yield (78%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard.

Selected data for **4.19α/β: ^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -195.1 (ddd, *J* = 50.8 Hz, *J* = 15.1 Hz, *J* = 2.2 Hz, β-anomer), -198.4 (dd, *J* = 49.8 Hz, *J* = 12.1 Hz, α-anomer). These data were in agreement with those reported.[24]

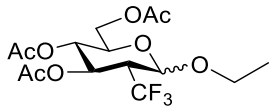
1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose (4.20)



The title compound was prepared following the general procedure **(GP)** using 2-deoxy-2-fluoro-3,4,6-tri-*O*-benzyl-mannopyranose **4.12** (13.2 mg, 0.026 mmol), CH_2Cl_2 (0.27 mL) and 30 % HBr in AcOH (80 µL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, isopropyl alcohol (6.0 µL, 0.080 mmol), dry CH_2Cl_2 (0.27 mL), dry toluene (0.27 mL), preactivated 4 Å MS and AgOTf (13.7 mg, 0.053 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (20:80) and yield (65%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard.

Selected data for **4.20α/β: ^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -219.3 (ddd, *J* = 51.3 Hz, *J* = 28.7 Hz, *J* = 18.8 Hz, β-anomer), -203.2 to -203.5 (m, α-anomer). These data were in agreement with those reported.[17]

1-*O*-Ethyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (4.21)



The title compound was prepared following the general procedure **(GP)** using **4.7** (17 mg, 0.0425 mmol), CH_2Cl_2 (0.25 mL) and 30 % HBr in AcOH (0.25

[24] Santschi, N.; Gilmour, R. *Eur. J. Org. Chem.* **2015**, *2015*, 6983-6987.

mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.7-Br**, ethanol (7.4 µL, 0.126 mmol), dry CH_2Cl_2 (0.4 mL), dry toluene (0.4 mL), preactivated 4 Å MS and AgOTf (21.6 mg, 0.084 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (22:78) and yield (78%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford an inseparable anomeric mixture of **4.21** (α:β, 1:5) (12 mg, 73% yield) along with elimination product (3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucal) as a colourless syrup.

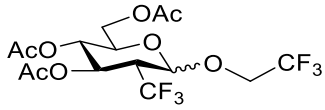
Data for **4.21α/β**: R_f (1:4 EtOAc/hexanes): 0.25; **FT-IR (neat)** ν in cm^{-1}: 2916, 2848, 2369, 2356, 2310, 1220, 1186, 1129, 1037; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{15}H_{21}F_3NaO_8^+$ (m/z): calc. 409.1081; found 409.1088.

Data for **4.21α:** Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.25; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.67 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.11 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.03 (appt, $J_{4,5}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-4), 4.28 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6}$ = 4.1 Hz, 1H, H-6a), 4.12-4.04 (m, 2H, H-5, H-6b), 3.75 (dq, $J_{7,7'}$ = 9.8 Hz, $J_{7,8}$ = 7.1 Hz, 1H, H-7), 3.54 (dq, $J_{7,7'}$ = 9.8 Hz, $J_{7',8}$ = 7.1 Hz, 1H, H-7'), 2.80 (dqd, $J_{2,3}$ = 11.1 Hz, $J_{2,CF3}$ = 7.7 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.09, 2.04, 2.02 (s, 9H, $3CH_3$, Ac), 1.24 (d, $J_{7,8}$ = $J_{7',8}$ = 7.1, 3H, CH_3-8); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.11 (d, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.3, 169.8, 169.3 (3C=O, Ac), 95.0 (q, $J_{C1,F}$ = 3.7 Hz, C-1), 67.5 (C-5), 67.0 (q, $J_{C3,F}$ = 1.5 Hz, C-3), 65.7 (C-4), 64.3 (C-7), 62.0 (C-6), 48.7 (q, $J_{C2,F}$ = 25.6 Hz, C-2), 20.7 (OAc), 20.64 (OAc), 20.63 (OAc), 14.7 (C-8).

Data for **4.21β:** Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.15; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.04 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-4), 4.67 (d, J = 8.0 Hz, 1H, H-1), 4.29 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6}$ = 5.0 Hz, 1H, H-6a), 4.12 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-6b), 3.95 (dq, $J_{7,7'}$ = 9.5 Hz, $J_{7,8}$ = 7.1 Hz, 1H, H-7), 3.71 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6b}$ = 5.0 Hz $J_{5,6b}$ = 2.6 Hz, 1H, H-5), 3.61 (dq, $J_{7,7'}$ = 9.5 Hz, $J_{7',8}$ = 7.1 Hz, 1H, H-7'), 2.69 (ddq, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 8.0 Hz, $J_{2,CF3}$ = 7.7 Hz, 1H, H-2), 2.08, 2.03, 2.02 (s,

9H, 3CH_3, Ac), 1.24 (d, $J_{7,8}$ = $J_{7',8}$ = 7.1, 3H, CH_3-8); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.9 (d, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.7, 169.6 (3C=O, Ac), 126.0 (q, $J_{C,F}$ = 281.5 Hz, CF_3), 98.3 (q, $J_{C1,F}$ = 2.7 Hz, C-1), 71.5 (C-7), 69.0 (C-4), 68.0 (q, $J_{C3,F}$ = 1.7 Hz, C-3), 65.9 (C-5), 62.2 (C-6), 49.8 (q, $J_{C2,F}$ = 24.2 Hz, C-2), 20.7 (OAc), 20.6 (2xOAc), 14.8 (C-8).

1-*O*-Trifluoroethyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (4.22)



The title compound was prepared following the general procedure **(GP)** using **4.7** (45 mg, 0.107 mmol), CH_2Cl_2 (1 mL) and 30 % HBr in AcOH (1 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.7-Br**, trifluoroethanol (23.2 µL, 0.321 mmol), dry CH_2Cl_2 (1.1 mL), dry toluene (1.1 mL), preactivated 4 Å MS and AgOTf (55.0 mg, 0.21 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (72:28) and yield (62%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford an inseparable anomeric mixture of **4.22** (α:β, 10:1) (21 mg, 44% yield) as a colourless syrup.

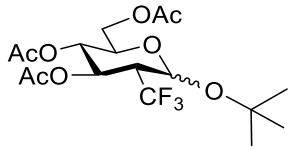
Data for **4.22α/β:** Colourless syrup. Inseparable mixture of α:β (10:1); R_f (3:7 EtOAc/hexanes): 0.25; **FT-IR (neat)** ν in cm^{-1}: 2955, 2917, 2850, 2363, 1748,1368, 1282, 1221, 1181, 1155, 1128, 1078, 1030, 970, 916, 901; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{15}H_{22}F_3NO_8^+$ (m/z): calc. 458.1244; found 458.1249.

Data for **4.22α**: Colourless syrup. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.66 (dd, $J_{2,3}$ = 11.4 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 5.22 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.06 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-4), 4.28 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6}$ = 4.5 Hz, 1H, H-6a), 4.11 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-6b), 4.06 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 3.98 (q, $J_{7',CF3'}$ = 8.2 Hz, 2H, CH_2-7'), 2.88

(ddq, $J_{2,3}$ = 11.1 Hz, $J_{2,CF3}$ = 7.6 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 2.09, 2.05, 2.03 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.22 (d, $J_{CF3,2}$ = 7.5 Hz, 3F, CF_3), -74.08 (d, $J_{CF3,7}$ = 8.4 Hz, 3F, CF_3'); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 169.7, 169.4 (3C=O, Ac), 123.6 (q, $J_{C,F}$ = 280.9 Hz, CF_3), 123.1 (q, $J_{C,F}$ = 278.4 Hz, CF_3'), 95.9 (q, $J_{C1,F}$ = 4.2 Hz, C-1), 68.5 (C-5), 68.45 (C-4), 66.4 (q, $J_{C3,F}$ = 2.0 Hz, C-3), 65.3 (q, $J_{C2,F}$ = 35.8 Hz, C-7'), 61.6 (C-6), 48.3 (q, $J_{C2,F}$ = 26.7 Hz, C-2), 20.65 (OAc), 20.55 (2xOAc).

Data for **4.22β**: Colourless syrup. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.45 (dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 5.08 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-4), 4.82 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1), 4.28 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6}$ = 4.9 Hz, 1H, H-6a), 4.14 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-6b), 4.00 (q, $J_{7,CF3'}$ = 8.3 Hz, 2H, CH_2-7'), 3.75 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-5), 2.76 (dp, $J_{2,3}$ = 10.2 Hz, $J_{2,CF3}$ = $J_{1,2}$ = 7.7 Hz, 1H, H-2), 2.04, 2.04, 2.02 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -66.35 (d, $J_{CF3,2}$ = 7.6 Hz, 3F, CF_3), -74.25 (d, $J_{CF3,7}$ = 8.5 Hz, 3F, CF_3').

1-*O*-Tert-butyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (4.23)



The title compound was prepared following the general procedure **(GP)** using **4.7** (28 mg, 0.07 mmol), CH_2Cl_2 (0.4 mL) and 30 % HBr in AcOH (0.4 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.7-Br**, tert-butyl alcohol (28.5 µL, 0.3 mmol), dry CH_2Cl_2 (1 mL), dry toluene (1 mL), preactivated 4 Å MS and AgOTf (38 mg, 0.15 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard workup, quantitative ^{19}F NMR analysis indicated an α/β ratio (41:59) and yield (58%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.23α** (9 mg, 31%) and a fraction

containing an anomeric mixture of **4.23** (α:β, 1:7) (5 mg, 18% yield) as a colourless syrups.

Data for **4.23α**: Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.25; $[\alpha]^{D}_{25}$: +93.5 (0.29, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.68 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.43 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.00 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-4), 4.29 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 4.9 Hz, 1H, H-6a), 4.23 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6b}$ = 2.1 Hz, 1H, H-5), 4.03 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 2.1 Hz, 1H, H-6b), 2.80 (dqd, $J_{2,3}$ = 11.1 Hz, $J_{2,CF3}$ = 7.6 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.07, 2.04, 2.02 (s, 9H, $3CH_3$, Ac), 1.27 (s, 9H, $3CH_3$, ^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -64.9 (d, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.9, 169.6 (3C=O, Ac), 124.0 (q, $J_{C,F}$ = 281.4 Hz, CF_3), 90.0 (q, $J_{C1,F}$ = 3.9 Hz, C-1), 69.3 (C-4), 67.2 (q, $J_{C3,F}$ = 2.0 Hz, C-3), 67.1 (C-5), 62.1 (C-6), 49.2 (q, $J_{C2,F}$ = 25.1 Hz, C-2), 28.1 (3xC-8), 20.69 (OAc), 20.68 (OAc), 20.64 (OAc); **FT-IR (neat)** ν in cm^{-1}: 2978, 2914, 2370, 2355, 2310, 1750, 1222, 1174, 1138, 1042; 907; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{17}H_{29}F_3NO_8^+$ (m/z): calc. 432.1840; found 432.1851.

Data for **4.23β**: Inseparable mixture of α:β (1:7). R_f (8:2 hexanes/EtOAc): 0.18; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.44 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-3), 4.95 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-4), 4.83 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 4.22 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6}$ = 6.1 Hz, 1H, H-6a), 4.09 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-6), 3.69 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6b}$ = 6.1 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-5), 2.69 (ddq, $J_{2,3}$ = 10.7 Hz, $J_{1,2}$ = 8.2 Hz, $J_{2,CF3}$ = 7.7 Hz, 1H, H-2), 2.06, 2.02, 2.02 (s, 9H, $3CH_3$, Ac), 1.26 (s, 9H, $3CH_3$, ^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.4 (d, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.8, 169.7 (3C=O, Ac), 127.1 (q, $J_{C,F}$ = 281.8 Hz, CF_3), 93.5 (q, $J_{C1,F}$ = 2.2 Hz, C-1), 71.1 (C-5), 69.4 (C-4), 68.3 (q, $J_{C3,F}$ = 1.5 Hz, C-3), 62.5 (C-6), 50.2 (q, $J_{C2,F}$ = 23.4 Hz, C-2), 28.4 (3xC-8), 20.7 (OAc), 20.65 (2xOAc); **FT-IR (neat)** ν in cm^{-1}: 2978, 2917, 2850, 2369, 2356, 2310, 1750, 1367, 1221, 1180, 1131, 1081, 1031, 910; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{17}H_{29}F_3NO_8^+$ (m/z): calc. 432.1840; found 432.1849.

4.5.7. Electrostatic potential surface calculation

DFT calculations were performed using Gaussian 09 software.[25] Geometry optimization was conducted at the CPCM (water) B3LYP/6-311+G(d,p) level of theory. Frequencies were calculated at the same level of theory and used to verify the nature of all stationary points as minima.

2-Deoxy-2-fluoro-α-mannopyranoside (S1)

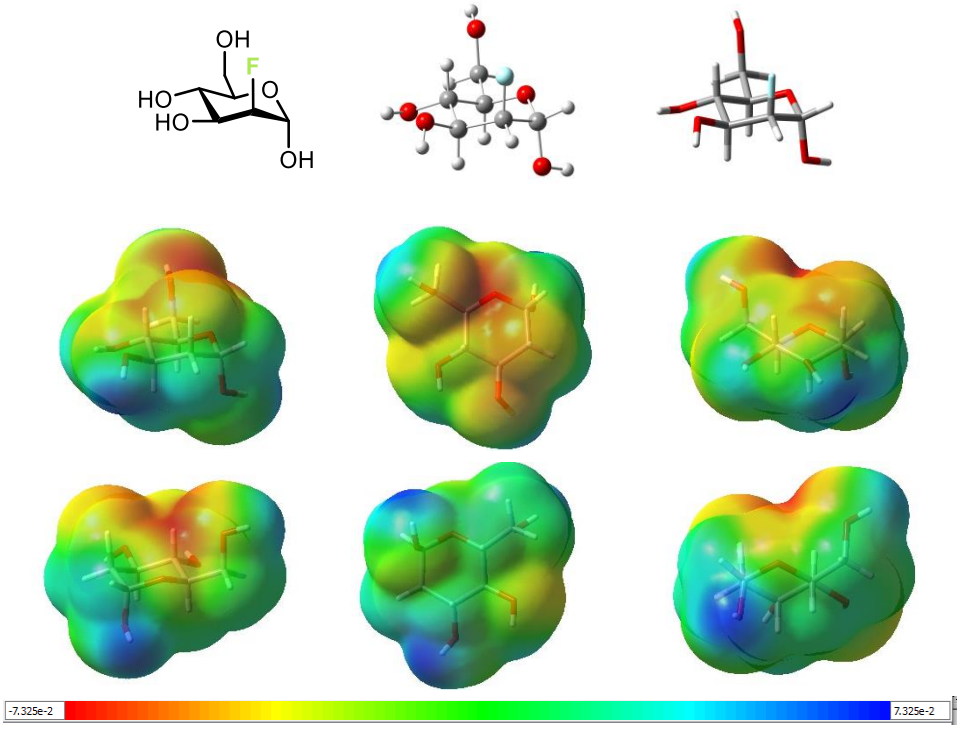


Figure 4.6: Different views of the electrostatic potential surface of 2-deoxy-2-fluoro-α-mannopyranoside (**S1**).

[25] Chalmers, A. A.; Hall, R. H. *J. Chem. Soc. Perkin Trans. 2*, **1974**, 728-732.

2-Deoxy-2-trifluoromethyl-α-mannopyranoside (S2)

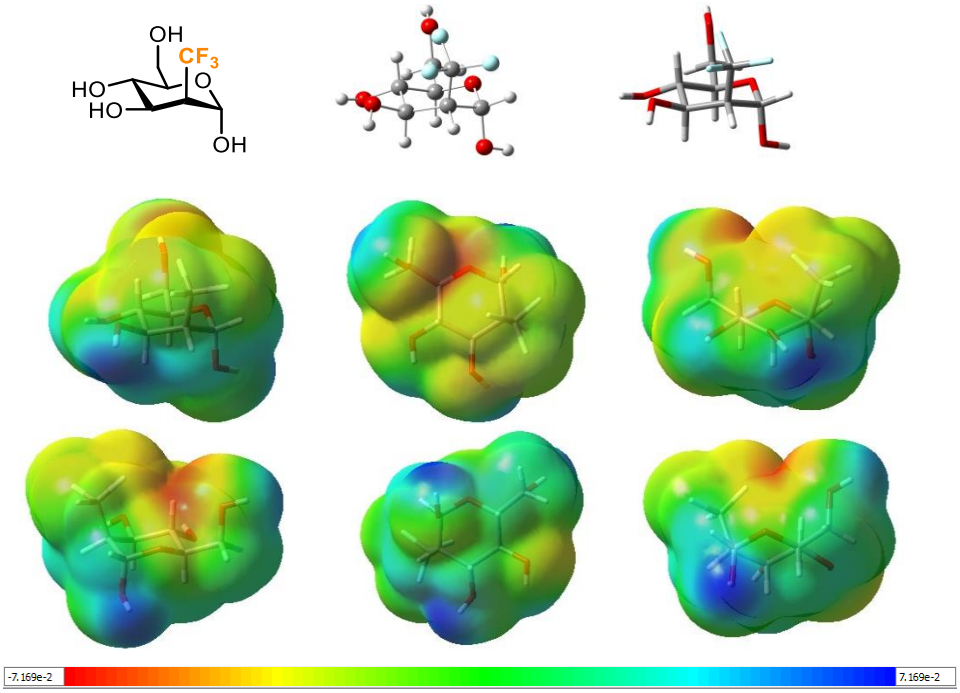
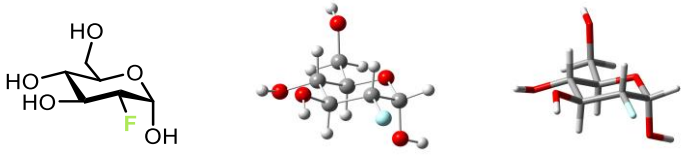


Figure 4.7: Different views of the electrostatic potential surface of 2-CF_3-α-mannopyranoside (**S2**).

2-Deoxy-2-fluoro-α-glucopyranoside (S3)



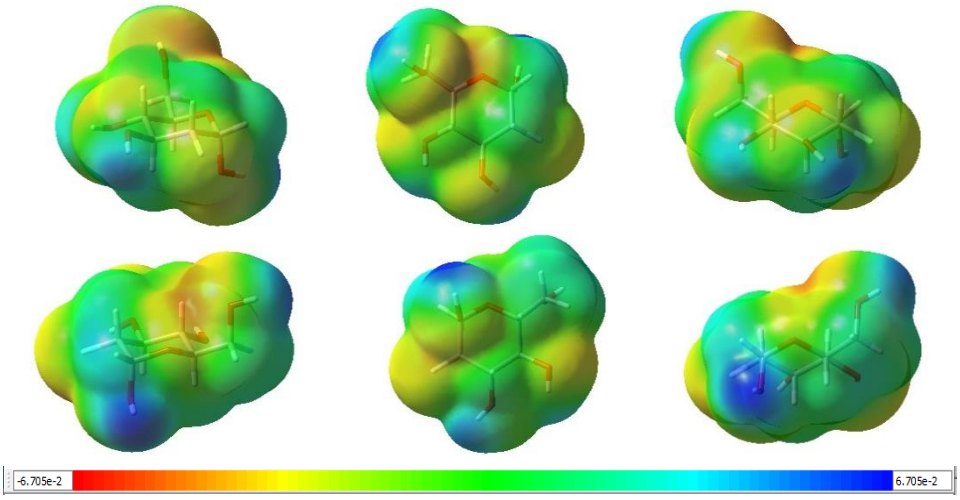


Figure 4.8: Electrostatic potential surface of 2-F-α-glucopyranoside.

2-Deoxy-2-trifluoromethyl-α-glucopyranoside (S4)

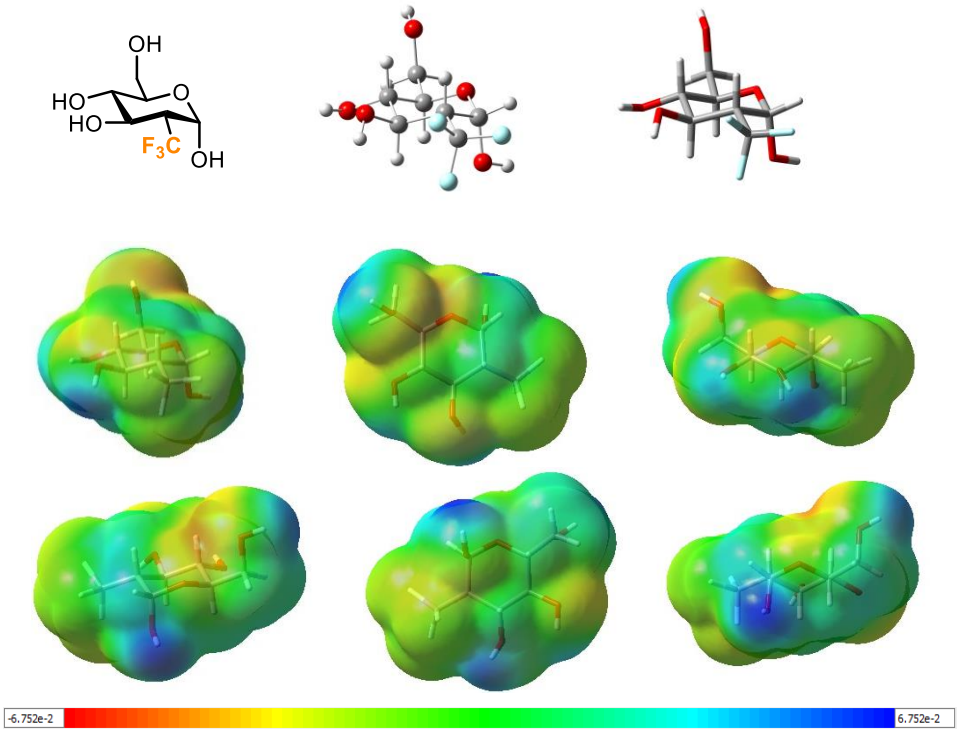
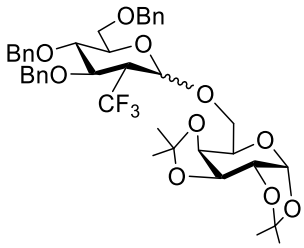


Figure 4.9: Electrostatic potential surface of 2-CF_3-α-glucopyranoside.

4.5.8. Glycosylation scope

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-β-D-glucopyranosyl-(1→6)-[1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside (4.24)

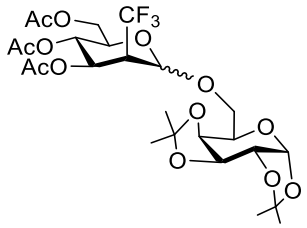


The title compound was prepared following the general procedure **(GP)** using **4.5** (26 mg, 0.0477 mmol), CH_2Cl_2 (1.4 mL) and 30 % HBr in AcOH (83 µL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.5-Br**, [1:2,3:4]-di-*O*-isopropylidene-α-D-galacto-pyranoside **4.9** (37 mg, 0.142 mmol), dry CH_2Cl_2 (0.5 mL), dry toluene (0.5 mL), preactivated 4 Å MS and AgOTf (24 mg, 0.095 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (6:94). The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford pure **4.24β** (25 mg, 70% yield) as a colourless syrup.

R_f (1:4 EtOAc/hexanes): 0.42; **$[\alpha]^{D}_{25}$**: +44.5 (1.76, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.36-7.15 (m, 15H, ArH), 5.52 (d, $J_{1',2'}$ = 5.0 Hz, 1H, H-1'), 4.81 (d, $J_{1,2}$ = 6.9 Hz, 1H, H-1), 4.81-4.52 (m, 7H, 3CH_2Ph and H-3'), 4.30 (dd, $J_{1',2}$ = 4.9 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-2'), 4.12 (dd, $J_{3',4'}$ = 8.0 Hz, $J_{4',5'}$ = 1.7 Hz, 1H, H-4'), 4.03 (dd, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a), 3.96 (m, 1H, H-5'), 3.89-3.80 (m, 2H, H-3 and H-4), 3.77-3.68 (m, 3H, H-6b, H-6a' and H-6b'), 3.56 (m, 1H, H-5), 2.68 (m, 1H, H-2), 1.52, 1.43, 1.33, 1.31 (s, 12H, 4CH_3); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -61.2 (d, $J_{CF3,2}$ = 8.5 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 138.2, 138.1, 137.9 (C, Ar), 128.6, 128.5, 128.5, 128.0, 127.9, 127.9, 127.8, (CH, Ar), 125.6 (q, $J_{C,F}$ = 281.7 Hz, CF_3), 109.4, 108.8 (2Cketal), 98.2 (q, $J_{C1,F}$ = 2.7 Hz, C-1), 96.4 (C-1'), 78.3 (C-3), 78.2 (C-4), 75.0 (C-5), 74.8, 74.5, 73.6 (3xCH_2Ph), 71.3 (C-4'), 70.8 (C-3'), 70.6 (C-2'), 69.1 (C-6), 68.1 (C-6'), 67.6 (C-5'), 51.3 (q,

$J_{C2,F}$ = 23.6 Hz, C-2), 26.1, 26.0, 25.2, 24.5 (4xCH_3'); **FT-IR (neat)** ν in cm^{-1}: 3032, 2987, 2903, 1497, 1455, 1373, 1252, 1210, 1173, 1068, 1005, 900, 738, 698; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{47}F_3NaO_{10}^+$ (m/z): calc. 767.3014; found 767.3008.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α-D-mannopyranosyl-(1→6)-[1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside (4.25)

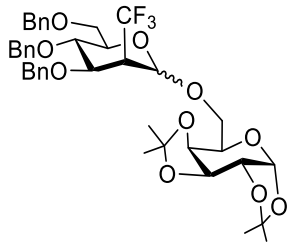


The title compound was prepared following the general procedure **(GP)** using **4.8** (47 mg, 0.117 mmol), CH_2Cl_2 (0.47 mL) and 30 % HBr in AcOH (0.47 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.8-Br**, [1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside **4.9** (91.7 mg, 0.35 mmol), dry CH_2Cl_2 (1.7 mL), dry toluene (1.7 mL), preactivated 4 Å MS and AgOTf (60 mg, 0.235 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (94:6). The crude was purified by column chromatography (1:7 EtOAc/hexanes) to give **4.25α** (56 mg, 80% yield) as a colourless syrup.

***R*$_f$** (2:3 EtOAc/hexanes): 0.41; **[α]$^D_{25}$** : +7.4 (0.59, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.51 (d, $J_{1',2'}$ = 5.0 Hz, 1H, H-1'), 5.42-5.32 (m, 2H, H-4 and H-3), 5.23 (d, $J_{1,2}$ = 1.3 Hz, 1H, H-1), 4.62 (dd, $J_{3',4'}$ = 7.9 Hz, $J_{2',3'}$ = 2.5 Hz, 1H, H-3'), 4.33 (dd, $J_{1',2'}$ = 5.0 Hz, $J_{2',3'}$ = 2.5 Hz, 1H, H-2'), 4.24-4.13 (m, 3H, H-4', H-6a, H-6b), 4.07 (ddd, $J_{4,5}$ = 9.1 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-5), 3.98 (td, $J_{5',6a'}$ = $J_{5',6b'}$ = 6.4 Hz, $J_{4',5'}$ = 1.8 Hz, 1H, H-5'), 3.81 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6a'}$ = 6.4 Hz, 1H, H-6a'), 3.72 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6b'}$ = 6.4 Hz, 1H, H-6b'), 3.19 (m, 1H, H-2), 2.09, 2.06, 2.05 (s, 9H, 3CH_3, Ac), 1.54 (s, 3H, CH_3'), 1.43 (s, 3H, CH_3'), 1.33 (s, 3H, CH_3'), 1.33 (s, 3H, CH_3'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (d,

$J_{CF3,2}$= 9.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.9, 170.3, 169.6 (3xC=O, Ac), 124.8 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 109.6, 108.8 (2Cketal), 96.4 (C-1'), 95.5 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 71.0 (C-4'), 70.7 (C-3'), 70.6 (C-2'), 68.5 (C-5), 68.0 (C-3), 67.1 (C-6'), 66.1 (C-5'), 65.8 (C-4), 62.4 (C-6), 46.2 (q, $J_{C2,F}$ = 24.9 Hz, C-2), 26.2, 26.1, 25.1, 24.5 (4xCH_3'), 20.9, 20.8 (3xCH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 2922, 2850, 1747, 1457, 1372, 1225, 1163, 1119, 1067, 1007; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{25}H_{27}F_{35}NaO_{13}^+$ (m/z): calc. 623.1922; found 623.1923. Spectroscopic data were in agreement with those reported.[23]

3,4,6-Tri-*O*-benzyl-2-deoxy-2-trifluoromethyl-α/β-D-mannopyranosyl-(1→6)-[1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside (4.26)



The title compound was prepared following the general procedure **(GP)** using **4.6** (89 mg, 0.163 mmol), CH_2Cl_2 (4.8 mL) and 30 % HBr in AcOH (260 µL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside **4.6-Br**, [1:2,3:4]-di-*O*-isopropylidene-α-D-galactopyranoside **4.9** (127 mg, 0.489 mmol), dry CH_2Cl_2 (1.6 mL), dry toluene (1.6 mL), preactivated 4 Å MS and AgOTf (83.7 mg, 0.33 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (80:20). The residue was purified by flash column chromatography (from hexanes to 1:9 EtOAc/hexanes) to give **4.26α** (19 mg, 16% yield) and **4.26β** (73 mg, 60% yield).

Data for **4.26α**: Colourless syrup. ***R*$_f$** (1:4 EtOAc/hexanes): 0.37; **[α]$^D_{25}$**: +15.2 (1.21, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.42-7.02 (m, 15H, ArH), 5.53 (d, $J_{1',2'}$ = 4.99 Hz, 1H, H-1'), 5.28 (bs, 1H, H-1), 4.78-4.35 (m, 7H, 3CH_2Ph and H-3'), 4.32 (dd, $J_{1',2'}$ = 5.0 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-2'), 4.21-4.13 (m, 2H, H-4' and H-3), 3.98 (appt, $J_{5,6a}$ = $J_{5,6b}$ = 6.6 Hz, 1H, H-5'), 3.92 (appt, $J_{3,4}$ = $J_{4,5}$ =

8.5 Hz, 1H, H-4), 3.88-3.76 (m, 2H, H-6a', H-5), 3.75-3.62 (m, 3H, H-6b', H-6a, H-6b), 3.03 (m, 1H, H-2), 1.52, 1.44, 1.33 (s, 12H, $4CH_3$); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.2 (bd, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 138.4, 138.2, 137.8 (C, Ar), 128.5, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.8, 127.6 (CH, Ar), 125.6 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 109.5, 108.7 (2Cketal), 97.4 (C-1'), 95.3 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 76.4 (C-3), 74.6 (CH_2Ph), 74.4 (C-4), 73.4, 72.3 ($2CH_2$Ph), 71.5 (C-5), 71.0 (C-4'), 70.7 (C-3'), 70.7 (C-2'), 69.0 (C-6), 65.8 (C-6'), 65.4 (C-5'), 43.9 (q, $J_{C2,F}$ = 24.0 Hz, C-2), 26.1, 26.1, 25.0, 24.5 ($4xCH_3$'); **FT-IR (neat)** ν in cm^{-1}: 3031, 2988, 2934, 1497, 1455, 1382, 1256, 1211, 1160, 1115, 1070, 1005, 906, 737, 698; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{47}F_3NaO_{10}^+$ (m/z): calc. 767.3014; found 767.3024.

Data for **4.26β**: ***R$_f$*** (1:4 EtOAc/hexanes): 0.31; **[α]$^D_{25}$**: -60.2 (0.49, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.38-7.14 (m, 15H, ArH), 5.52 (d, $J_{1',2'}$ = 4.9 Hz, 1H, H-1'), 4.88 (bs, 1H, H-1), 4.70 (d, *J* = 11.2 Hz, 1H, CH-Ph), 4.66 (d, *J* = 11.6 Hz, 1H, CH-Ph), 4.57 (dd, $J_{3',4'}$ = 8.0 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-3'), 4.55-4.45 (m, 4H, $2CH_2$Ph), 4.30 (dd, $J_{1',2'}$ = 4.9 Hz, $J_{2',3'}$ = 2.3 Hz, 1H, H-2'), 4.19 (bd, $J_{3',4'}$ = 8.0 Hz, 1H, H-4'), 4.07-4.01 (m, 2H, H-5',H-6a'), 3.95-3.84 (m, 3H, H-6a, H-3, H-4), 3.80-3.72 (m, 2H, H-6b, H-5), 3.66 (dd, $J_{6a',6b'}$ = 11.9 Hz, $J_{5',6'}$ = 8.2 Hz, 1H, H-6b'), 3.17 (m, 1H, H-2), 1.53, 1.43, 1.34, 1.28 (s, 12H, $4CH_3$); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -60.6 (bs, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 138.5, 138.0, 137.5 (C, Ar), 128.2, 128.4, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6 (CH, Ar), 125.6 (q, $J_{C,F}$ = 282.1 Hz, CF_3), 109.5, 109.0 ($2C_{ketal}$), 97.9 (C-1), 96.4 (C-1'), 76.4 (C-3), 75.1 (C-5), 73.8, 73.3 ($2CH_2$Ph), 72.7 (C-4), 72.0 (CH_2Ph), 71.5 (C-4'), 70.8 (C-3'), 70.7 (C-2'), 69.8 (C-6), 69.1 (C-6'), 68.3 (C-5'), 43.9 (q, $J_{C2,F}$ = 24.4 Hz, C-2), 26.1, 26.0, 25.2, 24.5 ($4xCH_3$'); **FT-IR (neat)** *ν* in cm^{-1}: 3025, 2970, 1483, 1466, 1377, 1220, 1155, 1110, 1080, 1008, 743, 701; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{47}F_3NaO_{10}^+$ (m/z): calc. 767.3014; found 767.3014. Spectroscopic data were in agreement with those reported.[23]

Conformational analysis by NMR of compounds 4.10, 4.24, 4.25 and 4.26.

The configuration and conformation of products **4.10, 4.24, 4.25** and **4.26** were confirmed by ^{1}H, ^{13}C, ^{19}F, HSQC, HMBC, NOESY and COSY. $^{1}J_{C1,H1}$ values of 160–170 Hz are diagnostic of an axial anomeric proton that is associated with a β-glycoside adopting a $^{4}C_{1}$ conformation whereas $^{1}J_{C1,H1} > 170$ Hz indicates an α-glycoside for the same conformation. The vicinal coupling constants and NOE contacts suggested that the products display the $^{4}C_{1}$ conformation.

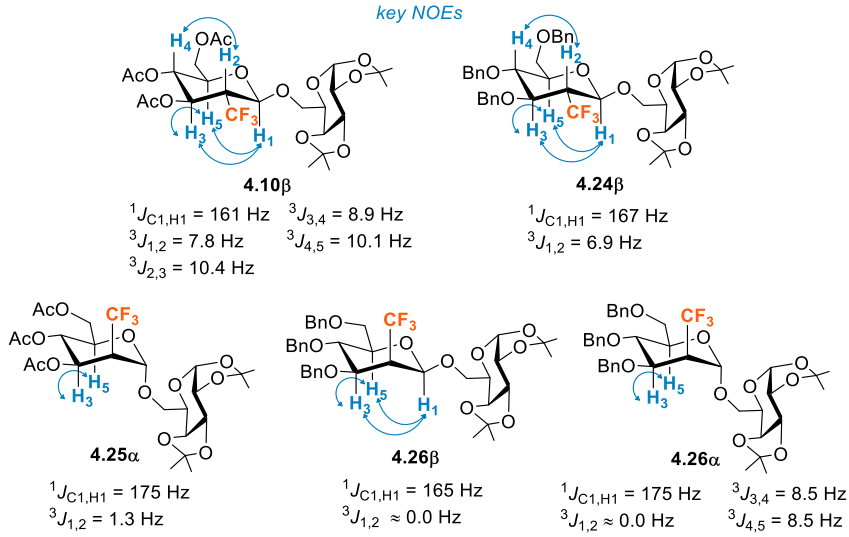
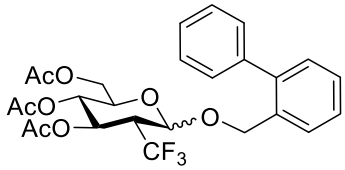


Figure 4.10. Conformational NMR analysis of **4.10, 4.24, 4.25** and **4.26.**

1-*O*-(2-Biphenylmethan)-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (4.27):



The title compound was prepared following the general procedure **(GP)** using **4.7** (44.7 mg, 0.112 mmol), CH_2Cl_2 (0.8 mL) and 30 % HBr in AcOH (0.8 mL). After standard work-up, glycosylation was carried out in a Schlenk flask

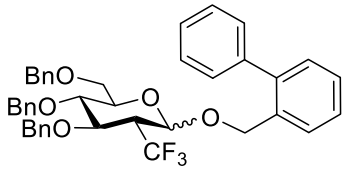
using the crude bromopyranoside, 2-biphenylmethanol (41.4 mg, 0.224 mmol), dry CH_2Cl_2 (1.2 mL), dry toluene (1.2 mL), preactivated 4 Å MS and AgOTf (57.7 mg, 0.224 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (18:82) and yield (45%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.27α** (4 mg, 7% yield) and an inseparable anomeric mixture of **4.27** (α:β, 1:13) (19 mg, 32% yield).

Data for **4.27α:** Colourless syrup. Inseparable mixture. R_f (8:2 hexanes/EtOAc): 0.23; Selected spectroscopic data **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.59-7.51 (m, 1H, Ph), 7.45-7.27 (m, 8H, Ph), 5.63 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.14 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.03 (d, $J_{4,5}$ = 10.2 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-4), 4.67 (d, J = 11.2 Hz, 1H, CH-Ph), 4.38 (d, J = 11.2 Hz, 1H, CH-Ph), 4.17 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 3.87 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.79 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 2.73 (dqd, $J_{2,3}$ = 11.6 Hz, $J_{2,CF3}$ = 7.9 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 2.10 (s, 3H, CH_3, Ac), 2.10 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -64.8 (d, $J_{CF3,2}$ = 7.8 Hz, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 1753, 1455, 1436, 1368, 1323, 1222, 1180, 1131, 1041, 908, 749, 704; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{26}H_{31}F_3NO_8^+$ (m/z): calc. 542.1996; found 542.2007.

Data for **4.27β:** Stinky solid. Inseparable mixture of α:β (1:14). R_f (8:2 hexanes/EtOAc): 0.20; **m.p**: 79-81 °C; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.59-7.51 (m, 1H, Ph), 7.45-7.27 (m, 8H, Ph), 5.39 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.03 (d, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-4), 4.83 (d, J = 11.4 Hz, 1H, CH-Ph), 4.63 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.59 (d, J = 11.4 Hz, 1H, CH-Ph), 4.23 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 3.97 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-6b), 3.67 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-5), 2.73 (dp, $J_{2,3}$ = 10.5 Hz, $J_{2,CF3}$ = $J_{1,2}$ = 7.8 Hz, 1H, H-2), 2.03 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.01 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -65.7 (d, $J_{CF3,2}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.7, 169.6 (C=O, OAc), 142.0, 140.5, 133.3, 130.0, 129.8, 129.3, 128.3, 128.2, 128.1, 127.6,

127.3 (CH, Ar), 124.4 (q, $J_{C,F}$ = 282.0 Hz, CF_3), 97.1 (q, $J_{C1,F}$ = 2.5 Hz, C-1), 71.4 (C-5), 68.9 (C-4), 68.7 (CH_2-Ph), 68.0 (q, $J_{C1,F}$ = 1.8 Hz, C-3), 62.0 (C-6), 49.7 (q, $J_{C,F}$ = 24.4 Hz, C-2), 20.7, 20.6 (x2) (CH_3, OAc); **FT-IR (neat)** ν in cm^{-1}: 1750, 1668, 1369, 1327, 1222, 1180, 1151, 1128, 1025, 911, 754, 704; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{26}H_{31}F_3NO_8^+$ (m/z): calc. 542.1996; found 542.2006.

1-*O*-(2-Biphenylmethan)-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (4.28):



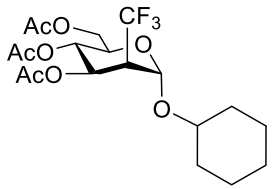
The title compound was prepared following the general procedure **(GP)** using **4.5** (21.9 mg, 0.0402 mmol), CH_2Cl_2 (1.2 mL) and 30 % HBr in AcOH (1.2 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, 2-biphenylmethanol (14.8 mg, 0.0804 mmol), dry CH_2Cl_2 (0.4 mL), dry toluene (0.4 mL), preactivated 4 Å MS and AgOTf (20.7 mg, 0.0804 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (8:92) and yield (68%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard.The crude was purified by column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.28α** (2 mg, 5% yield) and **4.28β** (15 mg, 56% yield).

Data for **4.28α:** Colourless syrup. R_f (9:1 hexanes/EtOAc): 0.15; Selected spectroscopic data: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.54-7.50 (m, 1H, Ph), 7.42-7.24 (m, 21H, Ph), 7.22-7.15 (m, 2H, Ph), 5.12 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.80 (d, *J* = 10.9 Hz, 1H, CH-Ph), 4.78 (d, *J* = 10.3 Hz, 1H, CH-Ph), 4.72 (d, *J* = 10.2 Hz, 1H, CH-Ph), 4.65 (d, *J* = 11.2 Hz, 1H, CH-Ph), 4.58 (d, *J* = 12.1 Hz, 1H, CH-Ph), 4.51 (d, *J* = 11.0 Hz, 1H, CH-Ph), 4.45 (d, *J* = 12.1 Hz, 1H, CH-Ph), 4.46 (d, *J* = 11.3 Hz, 1H, CH-Ph), 4.18 (dd, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 8.5 Hz, 1H, H-3), 3.78-3.64 (m, 3H, H-4, H-5, H-6a), 3.50 (dd, $J_{6a,6b}$ = 10.7 Hz, $J_{5,6b}$ = 1.6 Hz, 1H, H-6b), 2.70 (ddq, $J_{2,3}$

= 11.1 Hz, $J_{2,CF3}$ = 8.2 Hz, $J_{1,2}$ = 3.3 Hz, 1H, H-2); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -63.4 (d, $J_{CF3,2}$ = 8.2 Hz, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 2917, 2883, 2850, 2370, 2339, 2311, 1262, 1173, 1152, 1132, 1122, 1108, 1047, 795, 749, 700; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{41}H_{43}F_3NO_5^+$ (m/z): calc. 686.3088; found 686.3051.

Data for **4.28β:** White solid. ***R$_f$*** (9:1 hexanes/EtOAc): 0.18; **m.p**: 100-102 °C; **$[\alpha]^D_{25}$** : - 23.8 (0.24, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.58-7.50 (m, 1H, Ph), 7.40-7.27 (m, 21H, Ph), 7.22-7.15 (m, 2H, Ph), 4.84 (d, *J* = 11.6 Hz, 1H, CH-Ph), 4.78 (d, *J* = 11.0 Hz, 1H, CH-Ph), 4.77 (d, *J* = 10.4 Hz, 1H, CH-Ph), 4.68 (d, *J* = 10.4 Hz, 1H, CH-Ph), 4.63 (d, $J_{1,2}$ = 6.9 Hz, 1H, H-1), 4.59 (d, *J* = 11.0 Hz, 1H, CH-Ph), 4.54 (d, *J* = 11.6 Hz, 1H, CH-Ph), 4.53 (d, *J* = 12.1 Hz, 1H, CH-Ph), 4.46 (d, *J* = 12.1 Hz, 1H, CH-Ph), 3.88-3.76 (m, 2H, H-3, H-4), 3.65 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 3.59 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-6b), 3.67 (ddd, $J_{4,5}$ = 8.9 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 2.73 (h, $J_{2,3}$ = $J_{2,CF3}$ = $J_{1,2}$ = 8.3 Hz, 1H, H-2); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -65.19 (d, $J_{CF3,2}$ = 8.5 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 141.9, 140.6, 138.0, 137.9, 137.8, 134.1, 130.4, 129.9, 129.6, 128.5, 128.42, 128.39, 128.12, 127.9, 127.8, 127.78, 127.76, 127.66, 127.5, 127.1 (CH, Ar), 125.5 (q, $J_{C,F}$ = 282.2 Hz, CF_3), 97.0 (d, $J_{C1,F}$ = 2.9 Hz, C-1), 78.2 (C-3, C-4), 74.9 (C-5), 74.85 (CH_2-Ph), 74.5 (CH_2-Ph), 73.5 (CH_2-Ph), 68.9 (C-6), 68.4 (CH_2-Ph), 51.4 (q, $J_{C,F}$ = 23.5 Hz, C-2); **FT-IR (neat)** ν in cm^{-1}: 2916, 2881, 2850, 2367, 2347, 2337, 1175, 1099, 1035, 1028, 748, 700; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{41}H_{43}F_3NO_5^+$ (m/z): calc. 686.3088; found 686.3082.

1-*O*-Cyclohexyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α/β-D-mannopyranose (4.29)



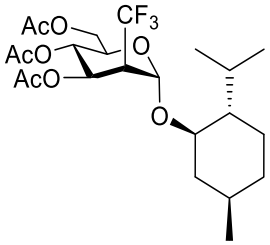
The title compound was prepared following the general procedure **(GP)** using **4.8** (23.5 mg, 0.059 mmol), CH_2Cl_2 (0.3 mL) and 30% HBr in AcOH (0.3 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, cyclohexanol (18.8 µL, 0.18 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (30.8 mg, 0.12 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (97:3) and yield (74%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford pure **4.29α** (18 mg, 67% yield) as a colourless syrup.

Data for **4.29α**: Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.27; $[\alpha]^{D}_{25}$: + 61.7 (0.76, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.42-5.31 (m, 2H, H-3, H-4), 5.30 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.19 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 3.1 Hz, 1H, H-6a), 4.14 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 4.8 Hz, 1H, H-6b), 4.04 (ddd, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 4.8 Hz, $J_{5,6}$ = 3.1 Hz, 1H, H-5), 3.59 (m, 1H, H-7'), 3.09 (qdd, $J_{2,CF3}$ = 9.8 Hz, $J_{2,3}$ = 5.7 Hz, $J_{1,2}$ = 1.6 Hz, 1H, H-2), 2.07, 2.06, 2.04 (s, 9H, $3CH_3$, Ac), 1.92-1.83 (m, 2H, CH_2'), 1.79-1.62 (m, 2H, CH_2'), 1.60-1.48 (m, 1H, CH_2'), 1.46-1.11 (m, 5H, CH_2'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (d, $J_{CF3,2}$ = 9.8 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 170.1, 169.5 (3C=O, Ac), 126.0 (q, $J_{C,F}$ = 281.5 Hz, CF_3), 93.4 (q, $J_{C1,F}$ = 4.6 Hz, C-1), 76.5 (C-7'), 68.3 (C-5), 68.0 (C-4), 66.1 (q, $J_{C3,F}$ = 1.6 Hz, C-3), 62.5 (C-6), 46.8 (q, $J_{C2,F}$ = 24.8 Hz, C-2), 33.1 (CH_2'), 31.3 (CH_2'), 25.4 (CH_2'), 24.1 (CH_2'), 23.8 (CH_2'), 20.72 (OAc), 20.71 (OAc), 20.60 (OAc); **FT-IR (neat)** ν in cm^{-1}: 2934, 2857 1749, 1454, 1369,1267, 1228, 1177, 1159, 1114, 1047; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{19}H_{31}F_3NO_8^+$ (m/z): calc. 458.1996; found 458.2001.

Data for **4.29β**: Colourless syrup. Selected spectroscopic data: R_f (8:2 hexanes/EtOAc): 0.23; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.32 (dd, $J_{3,4}$ = 5.7 Hz, $J_{2,3}$ = 4.2 Hz, 1H, H-3), 5.15-5.10 (m, 1H, H-4), 5.11 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 4.52 (dd, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6a}$ = 6.4 Hz, 1H, H-6a), 4.34 (dd, $J_{6a,6b}$ = 11.5 Hz, $J_{5,6b}$ = 6.1 Hz, 1H, H-6b), 4.04 (appq, $J_{4,5}$ = $J_{5,6a}$ = $J_{5,6b}$ = 6.0 Hz, 1H, H-5), 3.71 (m, 1H, H-7'), 3.09 (m, 1H, H-2), 2.10, 2.09, 2.08 (s, 9H, $3CH_3$, Ac), 1.92-1.83 (m, 2H), 1.79-

1.62 (m, 2H), 1.60-1.48 (m, 1H), 1.46-1.11 (m, 5H); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -63.6 (s, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 2918, 2850, 1748, 1370, 1228, 1179, 1158, 1131, 1046, 1024; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{19}H_{31}F_3NO_8^+$ (m/z): calc. 458.1996; found 458.1994.

1-*O*-Menthyl-2-deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α-D-mannopyranose (4.30)



The title compound was prepared following the general procedure **(GP)** using **4.8** (21.6 mg, 0.054 mmol), CH_2Cl_2 (0.3 mL) and 30 % HBr in AcOH (0.3 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, menthol (25.3 mg, 0.162 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (27.8 mg, 0.108 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{119}F NMR analysis indicated an α/β ratio (95:5) and yield (83%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.30α** (21 mg, 78% yield) as a colourless syrup.

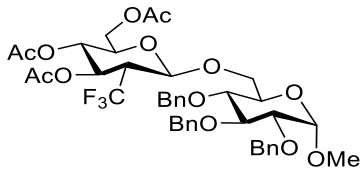
Data for **4.30α**: Colourless syrup. R_f (8:2 hexanes/EtOAc): 0.37; **$[\alpha]^D_{25}$** : + 21.7 (0.96, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43-5.29 (m, 2H, H-3, H-4), 5.31 (d, $J_{1,2}$ = 1.3 Hz, 1H, H-1), 4.25-4.07 (m, 3H, H-5, H-6a, H-6b), 3.40 (td, $J_{1'2'}$ = $J_{1'6'}$ = 10.7 Hz, $J_{1'6''}$ = 4.4 Hz, 1H, H-1'), 3.13 (qdd, $J_{2,CF3}$ = 9.8 Hz, $J_{2,3}$ = 5.7 Hz, $J_{1,2}$ = 1.3 Hz, 1H, H-2), 2.16-2.09 (m, 1H, H-6''), 2.09, 2.08, 2.06 (s, 9H, $3CH_3$, Ac), 2.04-1.97 (m, 1H, H-7'), 1.69-1.60 (m, 2H, H-3', H-4'), 1.48-1.30 (m, 1H, H-5'), 1.34-1.21 (m, 1H, H-2'), 1.05 (q, $J_{5',6'}$ = $J_{1',6'}$ = $J_{6',6''}$ = 12.0 Hz, 1H, H-6'), 1.03-0.94 (m, 1H, H-3''), 0.92 (d, $J_{7',8'}$ = 7.0 Hz, 3H, CH_3-8), 0.90 (d, $J_{5',10'}$ = 6.5 Hz, 3H, CH_3-

10), 1.03-0.94 (m, 1H, H-4''), 0.77 (d, $J_{7',9'}$ = 7.0 Hz, 3H, CH_3-9); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (d, $J_{CF3,2}$ = 9.8 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 170.2, 169.5 (3C=O, Ac), 124.7 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 97.2 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 83.0 (C-1'), 68.5 (C-5), 67.9 (C-4), 66.0 (q, $J_{C3,F}$ = 1.7 Hz, C-3), 62.7 (C-6), 49.3 (C-2'), 46.8 (q, $J_{C2,F}$ = 24.8 Hz, C-2), 42.6 (C-6'), 34.1 (C-3'), 31.6 (C-5'), 25.9 (C-7'), 23.2 (C-4'), 22.3 (CH_3-10), 21.0 (CH_3-8), 20.7 (OAc), 20.67 (2xOAc), 16.0 (CH_3-9); **FT-IR (neat)** ν in cm^{-1}: 2956, 2922, 2871, 1750, 1456, 1370, 1267, 1227, 1163, 1114, 1044; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{23}H_{39}F_3NO_8^+$ (m/z): calc. 514.2622; found 514.2633.

Data for **4.30β**: Colourless syrup. Inseparable mixture. ***R_f*** (8:2 hexanes/EtOAc): 0.2; Selected spectroscopic data: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.32 (t, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, 1H, H-4), 5.12 (dd, $J_{3.4}$ = 8.9 Hz, $J_{2,3}$ = 5.2 Hz, 1H, H-3), 4.94 (t, $J_{1,2}$ = 2.3 Hz, 1H, H-1), 4.29 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6a}$ = 3.6 Hz, 1H, H-6a), 4.29 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 5.7 Hz, 1H, H-6b), 3.70 (ddd, $J_{4,5}$ = 8.5 Hz, $J_{5,6b}$ = 5.7 Hz, $J_{5,6a}$ = 3.6 Hz, 1H, H-5), 3.51 (td, $J_{1'2'}$ = $J_{1'6'}$ = 10.7 Hz, $J_{1'6''}$ = 4.2 Hz, 1H, H-1'), 3.10 (qdd, $J_{2,CF3}$ = 9.3 Hz, $J_{2,3}$ = 5.2 Hz, $J_{1,2}$ = 2.4 Hz, 1H, H-2), 2.28 (hd, $J_{7',8'}$ = $J_{7',9'}$ = 7.0 Hz, $J_{2',7'}$ = 2.3 Hz, 1H, H-7'), 2.07, 2.06, 2.06 (s, 9H, 3CH_3, Ac), 2.07-1.96 (m, 1H, H-6''), 1.71-1.60 (m, 2H, H-3', H-4'), 1.44-1.20 (m, 2H, H-2', H-5'), 1.05-0.70 (m, 3H, H-6'', H-3'', H-4''), 0.94 (d, $J_{5',10'}$ = 6.6 Hz, 3H, CH_3-10), 0.88 (d, $J_{7',8'}$ = 7.2 Hz, 3H, CH_3-8), 0.74 (d, $J_{7',9'}$ = 6.9 Hz, 3H, CH_3-9); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -59.2 (s, 3F, CF_3).

4.5.9. Stereoselective synthesis of 2-deoxy-2-CF_3-glycosides analogues of natural products

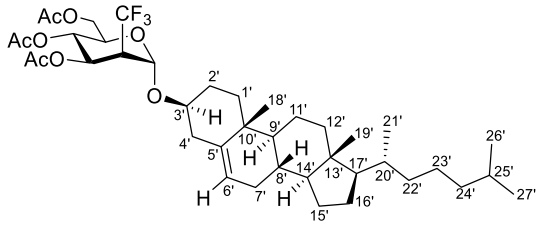
2-Deoxy-2-trifluoromethyl-3,4,6-tri-*O*-acetyl-α-D-glucosyl-(1→6)-1-*O*-methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (4.31)



The title compound was prepared following the general procedure **(GP)** using **4.7** (27.1 mg, 0.067 mmol), CH_2Cl_2 (0.35 mL) and 30 % HBr in AcOH (0.35 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, 1-*O*-methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranose (62.9 mg, 0.135 mmol), dry CH_2Cl_2 (0.7 mL), dry toluene (0.7 mL), preactivated 4 Å MS and AgOTf (34.8 mg, 0.135 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up,^{19}F NMR analysis indicated an α/β ratio (5:95) and yield (62%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. The residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford pure **4.31β** (25 mg, 48% yield) as a colourless syrup.

***R**f* (6:4 hexanes/EtOAc): 0.35; **[α]$^{D}_{25}$** : + 12.8 (0.9, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.39-7.23 (m, 15H, Ph), 5.42 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.04 (d, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-4), 4.99 (d, *J* = 10.8 Hz, 1H, CH-Ph), 4.93 (d, *J* = 11.1 Hz, 1H, CH-Ph), 4.80 (d, *J* = 10.8 Hz, 1H, CH-Ph), 4.79 (d, *J* = 12.2 Hz, 1H, CH-Ph), 4.69-4.60 (m, 3H, H-1, H-1', CH-Ph), 4.53 (d, *J* = 11.1 Hz, 1H, CH-Ph), 4.25 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.9 Hz, 1H, H-6a), 4.15-4.05 (m, 2H, H-6b, H-6a'), 3.99 (t, $J_{3,4}$ = $J_{2,3}$ = 9.3 Hz, 1H, H-3'), 3.78 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 4.0 Hz, $J_{5,6a}$ = 1.8 Hz, 1H, H-5'), 3.71 (dd, $J_{6a,6b}$ = 10.8 Hz, $J_{5,6b}$ = 4.4 Hz, 1H, H-6b'), 3.67 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6a}$ = 2.5 Hz, 1H, H-5), 3.53 (dd, $J_{2,3}$ = 9.7 Hz, $J_{1,2}$ = 3.5 Hz, 1H, H-2'), 3.50 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 8.8 Hz, 1H, H-4'), 3.37 (s, 3H, CH_3-O), 2.76 (dp, $J_{2,3}$ = 10.5 Hz, $J_{2,CF3}$ = $J_{1,2}$ = 7.8 Hz, 1H, H-2), 2.05 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac); **^{19}F NMR** (376.5 MHz, $CDCl_3$) δ in ppm: -65.35 (d, $J_{CF3,2}$ = 7.8 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.6, 169.6 (C=O, OAc) 138.6, 138.3, 138.1, 128.5, 128.4(x2), 128.1, 128.0, 127.9, 127.72, 127.6, 127.5 (CH, Ar), 124.5 (q, $J_{C,F}$ = 281.9 Hz, CF_3), 98.5 (d, $J_{C1,F}$ = 2.6 Hz, C-1), 98.2 (C-1'), 82.1 (C-3'), 79.7 (C-2'), 77.4 (C-4'), 75.8 (CH_2Ph), 74.8 (CH_2Ph), 73.4 (CH_2Ph), 71.5 (C-5), 69.4 (C-5'), 68.8 (C-4), 68.2 (C-6'), 67.9 (C-3), 62.0 (C-6), 55.3 (CH_3-O), 49.5 (q, $J_{C,F}$ = 24.1 Hz, C-2), 20.7, 20.6 (x2) (CH_3, OAc); **FT-IR (neat)** ν in cm^{-1}: 2929, 1753, 1454, 1364, 1223, 1183, 1131, 1086, 1072, 1046, 739, 699; **HRMS (TOF ES^{+})** for $(M+NH_4)^+$ $C_{41}H_{51}F_3NO_{13}^+$ (m/z): calc. 822.3307; found 822.3307.

1-O-Cholesteryl-3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α/β-D-mannoyranose (4.32)



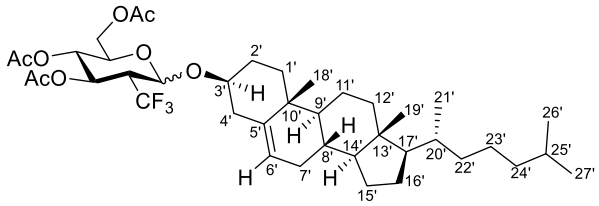
The title compound was prepared following the general procedure **(GP)** using **4.8** (22.2 mg, 0.055 mmol), CH_2Cl_2 (0.4 mL) and 30 % HBr in AcOH (0.4 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, cholesterol (42.6 mg, 0.110 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (28.3 mg, 0.11 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (85:15) and yield (75%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the remaining cholesterol was submitted to acetylation conditions using Ac_2O (62.4 µL, 0.66 mmol) and pyridine (0.6 mL, 6.6 mmol) in CH_2Cl_2 (0.6 mL) for 16h at room temperature. After standard work-up, the residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.32α** (30 mg, 57%) and a fraction containing an inseparable anomeric mixture of **4.32** (α:β, 2.2:1) (5 mg, 10% yield).

Data for **4.32α:** Colourless syrup. $\mathbf{R_f}$ (9:1 hexanes/EtOAc): 0.13; $\mathbf{[\alpha]^D_{25}}$: + 38.5 (1.15, $CHCl_3$) **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43-5.30 (m, 4H, H-1, H-3, H-4, H-6'), 4.20 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.9 Hz, 1H, H-6a), 4.15 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 4.9 Hz, 1H, H-6b), 4.06 (ddd, $J_{4,5}$ = 8.2 Hz, $J_{5,6b}$ = 4.9 Hz, $J_{5,6a}$ = 2.9 Hz, 1H, H-5), 3.48 (m, 1H, H-3'), 2.68 (qdd, $J_{2,CF3}$ = 9.6 Hz, $J_{2,3}$ = 5.7 Hz, $J_{1,2}$ = 1.7 Hz, 1H, H-2), 2.42-2.27 (m, 2H, H-4', H-7'), 2.07 (s, 3H, CH_3, Ac), 2.07 (s, 3H, CH_3, Ac), 2.05 (s, 3H, CH_3, Ac), 2.11-1.79 (m, 4H, H-4'', H7'', H-15', H-16'), 1.64-0.8 (m, 22H), 1.01 (s, 3H, CH_3-18), 0.91 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21), 0.87 (d, $J_{25,26}$ = 6.6 Hz, 3H, CH_3-26), 0.86 (d, $J_{25,27}$ = 6.6 Hz, 3H, CH_3-27), 0.68 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (d, $J_{CF3,2}$ = 9.6 Hz, 3F, CF_3); **^{13}C NMR**

(100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 170.0, 169.5 (3xC=O, Ac), 140.2 (C-5'), 124.7 (q, $J_{C,F}$ = 280.9 Hz, CF_3), 122.2 (C-6'), 93.5 (q, $J_{C1,F}$ = 4.1 Hz, C-1), 78.2 (C-3'), 68.3 (C-4), 67.9 (C-5), 66.0 (C-3), 62.5 (C-6), 56.7, 56.1, 50.0, 48.6 (q, $J_{C2,F}$ = 24.6 Hz, C-3), 42.3, 39.75, 39.7, 39.5, 36.9, 36.6, 36.1, 35.8, 31.9, 31.8, 28.2, 28.0, 27.6, 24.2, 23.8, 22.8, 22.5 (C-26, C-27), 21.0, 20.7 (2xCH_3, Ac), 20.6 (CH_3, Ac), 19.3 (C-18), 18.7 (C-21), 11.8 (C-19); **FT-IR (neat)** ν in cm^{-1}: 2938, 2868, 1751, 1456, 1436, 1370, 1267, 1229, 1177, 1159, 1114, 1047; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{61}F_3NaO_8^+$ (m/z): calc. 749.4211; found 749.4219.

Data for **4.32β:** Inseparable mixture of α:β (2.2:1). White solid. **R_f** (9:1 hexanes/EtOAc): 0.10; Selected spectroscopic data: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.36 (m, 1H, H-6'), 5.30 (dd, $J_{3,4}$ = 6.0 Hz, $J_{2,3}$ = 4.3 Hz, 1H, H-3), 5.15 (t, $J_{3,4}$ = $J_{4,5}$ = 5.8 Hz, 1H, H-4), 5.10 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 4.48 (dd, $J_{6a,6b}$ = 11.6 Hz, $J_{5,6a}$ = 6.1 Hz, 1H, H-6a), 4.34 (dd, $J_{6a,6b}$ = 11.6 Hz, $J_{5,6b}$ = 6.1 Hz, 1H, H-6b), 3.90 (q, $J_{4,5}$ = $J_{5,6a}$ = $J_{5,6b}$ = 5.8 Hz, 1H, H-5), 3.61-3.50 (m, 1H, H-3'), 3.07-2.97 (m, 1H, H-2), 2.39-2.27 (m, 2H, H-4', H-7'), 2.09 (s, 3H, CH_3, Ac), 2.09 (s, 3H, CH_3, Ac), 2.08 (s, 3H, CH_3, Ac), 2.21-1.79 (m, 4H, H-4'', H7'', H-15', H-16'), 1.64-0.80 (m, 22H), 1.01 (s, 3H, CH_3-18'), 0.91 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21'), 0.87 (d, $J_{25,26}$ = 6.6 Hz, 3H, CH_3-26'), 0.86 (d, $J_{25,27}$ = 6.6 Hz, 3H, CH_3-27'), 0.68 (s, 3H, CH_3-19'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -63.1 (d, $J_{CF3,2}$ = 9.6 Hz, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 2927, 2851, 1750, 1456, 1431, 1370, 1230, 1159, 1114, 1047; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{61}F_3NaO_8^+$ (m/z): calc. 749.4211; found 749.4212.

1-*O*-Cholesteryl-3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α/β-D-glucopyranose (4.33)



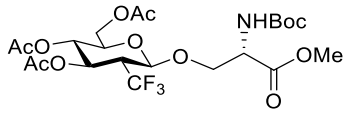
The title compound was prepared following the general procedure **(GP)** using **4.7** (25.7 mg, 0.064 mmol), CH_2Cl_2 (0.35 mL) and 30 % HBr in AcOH (0.35 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, cholesterol (74.3 mg, 0.192 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (60 mg, 0.235 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (21:79) and yield (69%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the remaining cholesterol was submitted to acetylation conditions using Ac_2O (0.1 mL, 1.15 mmol) and pyridine (1 mL, 11.5 mmol) in CH_2Cl_2 (1 mL) for 16 h at room temperature. After standard work-up, the residue was purified by column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.33α** (4 mg, 8% yield) and **4.33β** (26 mg, 56% yield) as white solids.

Data for **4.33α**: White solid. R_f (8:2 hexanes/EtOAc): 0.35; **m.p**: 172-175 °C; **$[\alpha]^D_{25}$** : - 22.1 (0.20, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.67 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.35 (d, $J_{6,7}$ = 4.9 Hz, 1H, H-6'), 5.24 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.00 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.4 Hz, 1H, H-4), 4.27 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a) 4.17 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 4.09 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.46 (m, 1H, H-3'), 2.79 (dqd, $J_{2,3}$ = 11.3 Hz, $J_{2,CF3}$ = 7.7 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.43-2.24 (m, 2H, H-4', H-7'), 2.08 (s, 3H, CH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.12-1.73 (m, 4H, H-4'', H7'', H-15', H-16'), 1.60-0.80 (m, 22H), 1.01 (s, 3H, CH_3-18), 0.91 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21), 0.86 (d, $J_{25,26}$ = 6.6 Hz, 3H, CH_3-26), 0.85 (d, $J_{25,27}$ = 6.6 Hz, 3H, CH_3-27), 0.68 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.00 (d, $J_{CF3,2}$ = 7.4 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.8, 169.6 (3xC=O, Ac), 140.3 (C-5'), 122.2 (C-6'), 93.9 (m, C-1), 79.3 (C-3'), 69.0 (C-4), 67.6 (C-5), 66.9 (q, $J_{C3,F}$ = 2.2 Hz, C-3), 62.3 (C-6), 56.7, 56.1, 50.0, 48.6 (q, $J_{C2,F}$ = 27.2 Hz, C-2), 42.3, 40.0, 39.7, 39.5, 36.9, 36.6, 36.1, 35.8, 31.9, 31.8, 28.2, 28.0, 27.5, 24.3, 23.8, 22.8, 22.6 (C-26, C-27), 21.0, 20.7, 20.63, 20.62 (3xCH_3, Ac), 19.3 (C-18), 18.7 (C-21), 11.8 (C-19); **FT-IR (neat)** ν in cm^{-1}: 2933, 2867, 2850, 1748, 1465, 1456, 1436, 1378, 1365, 1233, 1185, 1159, 1142,

1125, 1087, 1044, 1030; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{40}H_{65}F_3NO_8^+$ (m/z): calc. 744.4657; found 744.4676.

Data for **4.33β:** White solid. R_f (8:2 hexanes/EtOAc): 0.28; **m.p**: 170-172 °C; $[\alpha]^D_{25}$: + 48.1 (0.30, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.43 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.37 (d, $J_{6',7'}$ = 5.2 Hz, 1H, H-6'), 5.01 (t, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.1 Hz, 1H, H-4), 4.76 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 4.28 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6a}$ = 5.1 Hz, 1H, H-6a), 4.09 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-6b), 3.68 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{5,6b}$ = 2.6 Hz, 1H, H-5), 3.54 (m, 1H, H-3'), 2.68 (dp, $J_{2,3}$ = 10.7 Hz, $J_{1,2}$ = $J_{2,CF3}$ = 7.7 Hz, 1H, H-2), 2.34-2.22 (m, 2H, H-4', H-7'), 2.07 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.11-1.76 (m, 4H, H-4'', H7'', H-15', H-16'), 1.60-0.8 (m, 22H), 1.00 (s, 3H, CH_3-18), 0.90 (d, $J_{20,21}$ = 6.5 Hz, 3H, CH_3-21), 0.86 (d, $J_{25,26}$ = 6.6 Hz, 3H, CH_3-26), 0.85 (d, $J_{25,27}$ = 6.6 Hz, 3H, CH_3-27), 0.68 (s, 3H, CH_3-19); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.53 (d, $J_{CF3,2}$ = 7.4 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.7, 169.65 (3xC=O, Ac), 140.1 (C-5'), 122.2 (C-6'), 97.0 (q, $J_{C1,F}$ = 1.8 Hz, C-1), 80.0 (C-3'), 71.2 (C-5), 69.0 (C-4), 68.1 (q, $J_{C3,F}$ = 2.5 Hz, C-3), 62.1 (C-6), 56.7, 56.1, 50.0, 49.7 (m, C-2), 42.3, 39.7, 39.5, 38.2, 37.1, 36.7, 36.1, 35.7, 31.9, 31.8, 29.7, 29.4, 28.2, 28.0, 24.3, 23.8, 22.8, 22.6 (C-26, C-27), 21.0, 20.7 (CH_3, Ac), 20.6 (2xCH_3, Ac), 19.3 (C-18), 18.7 (C-21), 11.8 (C-19); **FT-IR (neat)** ν in cm^{-1}: 2933, 2867, 2852, 1755, 1464, 1436, 1376, 1364, 1294, 1220, 1185, 1132, 1080, 1045, 906; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{40}H_{61}F_3NaO_8^+$ (m/z): calc. 749.4211; found 749.4214.

1-*O*-N-(tert-butoxycarbonyl)-L-serine methyl ester-3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-β-D-glucopyranose (4.34):

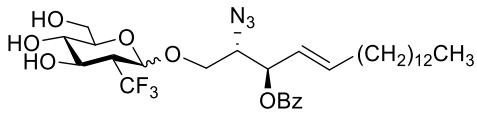


The title compound was prepared following the general procedure **(GP)** using **4.7** (15.6 mg, 0.039 mmol), CH_2Cl_2 (0.3 mL) and 30 % HBr in AcOH (0.3 mL). After standard work-up, glycosylation was carried out in a Schlenk flask

using the crude bromopyranoside, Boc-Serine-OMe (17.1 mg, 0.078 mmol), dry CH_2Cl_2 (0.4 mL), dry toluene (0.4 mL), preactivated 4 Å MS and AgOTf (20 mg, 0.078 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (5:95) and yield (35%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard.). Due to purification issues, the remaining acceptor was submitted to acetylation conditions using Ac_2O (44.5 µL, 0.47 mmol) and pyridine (0.35 mL, 4.67 mmol) in CH_2Cl_2 (0.35 mL) for 16 h at room temperature. After standard work-up, the residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.34β** (5 mg, 24%) as a white solid.

Foamy white solid. $\boldsymbol{R_f}$ (8:2 hexanes/EtOAc): 0.10; **m.p**: 130-132 °C; $\boldsymbol{[\alpha]^{D}_{25}}$: + 74.0 (0.28, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.58 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.45 (d, $J_{NH,2'}$ = 8.0 Hz, 1H, NH-Boc), 5.08 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.02 (dd, $J_{4,5}$ = 10.2 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-4), 4.50 (dt, $J_{NH,2'}$ = 8.0 Hz, $J_{1',2'}$ = 3.1 Hz, 1H, H-2'), 4.29 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 4.07 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 2.2 Hz, 1H, H-6b), 4.03-3.96 (m, 2H, H-1', H-5), 3.93 (dd, $J_{1',1''}$ = 10.1 Hz, $J_{1'',2'}$ = 3.0 Hz, 1H, H-1''), 3.76 (s, 3H, COO-CH_3), 2.81 (dqd, $J_{2,3}$ = 11.3 Hz, $J_{2,CF3}$ = 7.8 Hz, $J_{1,2}$ = 3.6 Hz, 1H, H-2), 2.09 (s, 3H, CH_3, Ac), 2.05 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 1.48 (s, 9H, 3xCH_3, Boc); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.25 (d, $J_{CF3,2}$ = 7.6 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.8, 170.0, 169.7, 169.4 (4xC=O, Ac, COOMe), 95.8 (q, $J_{C1,F}$ = 2.8 Hz, C-1), 69.4 (C-1'), 68.5 (C-4), 67.9 (C-5), 66.7 (C-3), 61.6 (C-6), 53.7 (C-2), 52-7 (CH_3, COOMe), 48.3 (q, $J_{C2,F}$ = 24.1 Hz, C-2), 28.3 (CH_3, Boc), 20.7, 20.6 (3xCH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 2957, 2918, 2850, 1748, 1715, 1516, 1456, 1437, 1367, 1347, 1225, 1180, 1159, 1140, 1040; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{22}H_{32}F_3NNaO_{12}^+$ (m/z): calc. 582.1769; found 582.1767.

(2S,3S,4E)-2-azido-3-*O*-benzoyl-1-*O*-(2-deoxy-2-trifluoromethyl-α/β-D-glucopyranosyl)-4-octadecene-1,3-diol (4.35):



The title compound was prepared following the general procedure **(GP)** using **4.7** (25.7 mg, 0.064 mmol), CH_2Cl_2 (0.35 mL) and 30 % HBr in AcOH (0.35 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, (2S,3S,4E)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (74.3 mg, 0.192 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (32.9 mg, 0.128 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (49:51) and yield (24%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the crude was directly deprotected using MeONa (2 mg, 0.027 mmol) in MeOH (1 mL). After 6h mixing at room temperature, it was neutralized with Dowex 50W-X8 ion exchange resin, the resin was filtered and washed with MeOH twice. The solvent was removed under the reduced pressure and the crude was purified by preparative TLC using a mixture of CH_2Cl_2:MeOH (10:0.5) to afford an anomeric mixture **4.35** (α:β, 1.6:1), (10 mg, 18% yield) as an a colourless syrup.

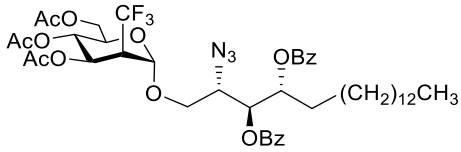
Data for **4.35α/β:** R_f (9:1, CH_2Cl_2:MeOH): 0.39; **FT-IR (neat)** ν in cm^{-1}: 3363, 2924, 2853, 2096, 1724, 1634, 1453, 1316, 1264, 1170, 1122, 1068, 1026, 711; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{32}H_{48}F_3N_3NaO_7^+$ (m/z): calc. 666.3337; found 666.3312.

Data for **4.35α**: Inseparable mixture of α and β. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 8.09-8.02 (m, 2H, ArH, Bz), 7.68-7-62 (m, 1H, ArH, Bz), 7.52-7.42 (m, 2H, ArH, Bz), 6.01-5.88 (m, 1H, H-11'), 5.63-5.51 (m, 2H, H-9', H-10'), 5.09 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.20 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 3.93-3.56 (m, 5H, H-5, H-6a, H-6b, H-7', H-8'), 3.60 (t, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, 1H, H-4), 3.49 (dd, $J_{7',7''}$ = 10.5 Hz, $J_{7'',8'}$ = 7.3 Hz, 1H, H-7''), 2.63-2.46 (m, 1H, H-2), 2.14-2.03 (m, 2H, CH_2-11'), 1.41-1.19 (m, 24H, CH_2-12'-23'), 0.88 (t, J = 6.9 Hz, 3H, CH_3-24'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -64.20 (m, 3F, CF_3); **^{19}F NMR** (MeOD, 376.5 MHz) δ in ppm: -65.50 (d, $J_{CF3,H2}$ = 8,3 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 165.2 (C=O, Bz), 139.3 (C-11'), 133.4, 129.8, 129.75, 128.5 (CH-Ar, Bz), 122.8 (C-10'), 124.5 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 96.3 (q, $J_{C1,F}$ = 3.9 Hz, C-1), 74.6 (C-

9'), 71.6 (C-5), 71.4 (C-4), 68.4 (C-3), 67.3 (C-7'), 63.9 (C-8'), 62.2 (C-6), 50.0 (q, $J_{C2,F}$ = 24.8 Hz, C-2), 32.4 (C-11'), 31.9, 29.69, 29.68, 29.66, 29.6, 29.4, 29.3, 29.2, 29.1, 28.7, 28.68, 22.7 (C-24').

Data for **4.35β**: Inseparable mixture of α and β. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 8.09-8.02 (m, 2H, ArH, Bz), 7.68-7-62 (m, 1H, ArH, Bz), 7.52-7.42 (m, 2H, ArH, Bz), 6.01-5.88 (m, 1H, H-11'), 5.74 (dd, $J_{9',10'}$ = 8.1 Hz, $J_{8',9'}$ = 4.6 Hz, 1H, H-9'), 5.63-5.51 (m, 1H, H-10'), 4.62 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 3.96 (td, $J_{7',8'}$ = $J_{7'',8'}$ = 6.1 Hz, $J_{8',9'}$ = 4.6 Hz, 1H, H-8'), 3.93-3.56 (m, 6H, H-3, H-4, H-6a, H-6b, H-7', H-7''), 3.34 (dt, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 3.7 Hz, 1H, H-5), 2.63-2.46 (m, 1H, H-2), 2.14-2.03 (m, 2H, CH_2-11'), 1.41-1.19 (m, 24H, CH_2-12'-23'), 0.88 (t, J = 6.9 Hz, 3H, CH_3-24'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -64.20 (m, 3F, CF_3); **^{19}F NMR** (MeOD, 376.5 MHz) δ in ppm: -65.29 (d, $J_{CF3,H2}$ = 8,2 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 165.5 (C=O, Bz), 139.4 (C-11'), 133.4, 129.9, 128.5 (CH-Ar, Bz), 122.4 (C-10'), 124.5 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 98.7 (q, $J_{C1,F}$ = 2.9 Hz, C-1), 74.9 (C-5), 74.7 (C-9'), 70.9 (C-3), 70.3 (C-4), 68.6 (C-7'), 63.3 (C-8'), 61.8 (C-6), 51.5 (m, C-2), 32.4 (C-11'), 31.9, 29.69, 29.68, 29.66, 29.6, 29.4, 29.3, 29.2, 29.1, 28.7, 28.68, 22.7 (C-24').

(2S,3S,4R)-2-azido-3,4-di-*O*-benzoyl-1-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α-D-mannopyranosyl)-1,3,4-octadecanetriol (4.36):

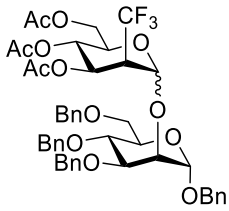


The title compound was prepared following the general procedure **(GP)** using **4.8** (23.2 mg, 0.058 mmol), CH_2Cl_2 (0.4 mL) and 30 % HBr in AcOH (0.4 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, (2S,3S,4R)-2-azido-3,4-di-*O*-benzoyl-1,3,4-octadecanetriol (63.9 mg, 0.116 mmol), dry CH_2Cl_2 (0.6 mL), dry toluene (0.6 mL), preactivated 4 Å MS and AgOTf (29.8 mg, 0.116 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (95:5) and yield (68%) using 1,4-

difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the remaining acceptor was submitted to acetylation conditions using Ac_2O (66.3 µL, 0.69 mmol) and pyridine (0.56 mL, 6.69 mmol) in CH_2Cl_2 (0.56 mL) for 16h at room temperature. After standard work-up, the residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.36α** (33 mg, 64%) as a colourless syrup.

R_f (8:2, hexanes:EtOAc): 0.23; **$[\alpha]^D_{25}$** : + 23.9 (0.35, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 8.05-7.97 (m, 4H, ArH, Bz), 7.66-7.54 (m, 2H, ArH, Bz), 7.52-7.40 (m, 4H, ArH, Bz), 5.53 (t, $J_{8',9'} = J_{9',10'}$ = 5.4 Hz, 1H, H-9'), 5.51-5.44 (m, 1H, H-10'), 5.40-5.29 (m, 2H, H-3, H-4), 5.16 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1), 4.24-4.08 (m, 2H, H-7', H-6a), 4.06 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 4.7 Hz, 1H, H-6b), 3.99 (ddd, $J_{7'',8'}$ = 8.3 Hz, $J_{8',9'}$ = 5.4 Hz, $J_{7',8'}$ = 2.6 Hz, 1H, H-8'), 4.02-3.90 (m, 1H, H-5), 3.71 (dd, $J_{7,7'}$ = 10.6 Hz, $J_{7'',8'}$ = 8.3 Hz, 1H, H-7''), 3.19 (qdd, $J_{2,CF3}$ = 9.6 Hz, $J_{2,3}$ = 4.7 Hz, $J_{1,2}$ = 1.7 Hz, 1H, H-2), 2.06 (s, 3H, CH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 2.00 (s, 3H, CH_3, Ac), 1.93-1.78 (m, 2H, CH_2-11'), 1.46-1.16 (m, 24H, CH_2-12'-23'), 0.87 (t, J = 6.9 Hz, 3H, CH_3-24'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.66 (d, $J_{CF3,2}$ = 9.6 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.9, 169.4 (3xC=O, Ac), 165.8, 165.1 (2xC=O, Bz) 133.7, 133.4, 129.8, 129.7, 129.55, 129.0, 128.7, 128.6 (CH-Ar, Bz), 124.5 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 96.3 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 72.7, 72.6 (C-9', C-10'), 68.8 (C-5), 68.5 (C-7'), 67.4 (C-4), 65.6 (C-3), 62.2 (C-6), 60.9 (C-8'), 45.9 (q, $J_{C2,F}$ = 25.0 Hz, C-2), 31.9, 30.2 (C-11'), 29.68, 29.66, 29.64, 29.58, 29.49, 29.39, 29.35, 29.33, 25.3, 22.7, 20.7, 20.6, 20.5 (3xCH_3, Ac), 14.1 (C-24'); **FT-IR (neat)** ν in cm^{-1}: 2924, 2853, 2099, 1750, 1725, 1452, 1369, 1314, 1264, 1228, 1177, 1159, 1121, 1094, 1068, 1054, 1026, 712; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{45}H_{60}F_3N_3NaO_{12}^+$ (m/z): calc. 914.4021; found 914.4016.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α/β-D-mannosyl-(1→2)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (4.37)



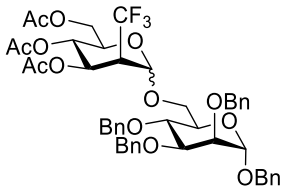
The title compound was prepared following the general procedure **(GP)** using **4.8** (20.5 mg, 0.051 mmol), CH_2Cl_2 (0.36 mL) and 30 % HBr in AcOH (0.36 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, 1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (53.7 mg, 0.102 mmol), dry CH_2Cl_2 (0.5 mL), dry toluene (0.5 mL), preactivated 4 Å MS and AgOTf (26.2 mg, 0.102 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. Reaction was initiated by addition of AgOTf (26,2 mg, 0.102 mmol) solution in dry toluene (0.5 mL) and the reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (86:14) and yield (54%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the remaining acceptor was submitted to acetylation conditions using Ac_2O (58 µL, 0.61 mmol) and pyridine (0.5 mL, 6.12 mmol) in CH_2Cl_2 (0.5 mL) for 16 h at room temperature. After standard work-up, the residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to afford **4.37α** (20 mg, 45%) a **4.37β** (2 mg, 5%).

Data for **4.37α:** Colourless syrup. R_f (9:1 hexanes/EtOAc): 0.10; $[\alpha]^{D}_{25}$: + 48.9 (0.66, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.42-7.24 (m, 18H, ArH), 7.17-7.13 (m 2H, ArH), 5.42 (d, $J_{1,2}$ = 2.3 Hz, 1H, H-1), 5.40 (d, $J_{2,3}$ = 5.6 Hz, 1H, H-3), 5.30 (t, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, 1H, H-4), 4.94 (d, $J_{1',2'}$ = 2.0 Hz, 1H, H-1'), 4.82 (d, *J* = 10.8 Hz, 1H, CH-Ph), 4.74 (d, *J* = 11.6 Hz, 1H, CH-Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH-Ph), 4.65 (d, *J* = 12.2 Hz, 1H, CH-Ph), 4.64 (d, *J* = 11.6 Hz, 1H, CH-Ph), 4.56 (d, *J* = 12.3 Hz, 1H, CH-Ph), 4.50 (d, *J* = 10.8 Hz, 1H, CH-Ph), 4.48 (d, *J* = 11.8 Hz, 1H, CH-Ph), 4.15-4.02 (m, 3H, H-5, H-6a, H-6b), 4.02 (t, $J_{1',2'}$ = $J_{2',3'}$ = 2.0 Hz, 1H, H-2'),

3.97 (dd, $J_{3'4'}$ = 9.1 Hz, $J_{2',3'}$ = 2.4 Hz, 1H, H-3'), 3.92 (t, $J_{3',4'} = J_{4',5'}$ = 9.2 Hz, 1H, H-4'), 3.82 (ddd, $J_{4',5'}$ = 9.2 Hz, $J_{5',6a'}$ = 4.7 Hz, $J_{5',6b'}$ = 1.9 Hz, 1H, H-5'), 3.75 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6a'}$ = 4.7 Hz, 1H, H-6a'), 3.70 (dd, $J_{6a',6b'}$ = 10.6 Hz, $J_{5',6b'}$ = 1.9 Hz, 1H, H-6b'), 3.31 (qdd, $J_{2,CF3}$ = 9.6 Hz, $J_{2,3}$ = 5.6 Hz, $J_{1,2}$ = 2.5 Hz, 1H, H-2), 2.07 (s, 3H, CH_3, Ac), 2.07 (s, 3H, CH_3, Ac), 2.05 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.8 (d, $J_{CF3,2}$ = 9.6 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.6, 169.8, 169.6 (3xC=O, Ac), 138.3, 138.1, 138.0, 137.0, 128.5, 128.45, 128.40, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.65, 127.6, 127.5 (Ar), 124.71 (q, $J_{C,F}$ = 280.8 Hz, CF_3), 97.7 (C-1'), 96.8 (q, $J_{C1,F}$ = 4.1 Hz, C-1), 79.8 (C-3'), 76.0 (C-2'), 75.3 (CH_2Ph), 74.9 (C-4'), 73.3 (CH_2Ph), 73.1 (CH_2Ph), 72.3 (C-5'), 69.1 (CH_2Ph), 69.0 (C-6'), 68.6 (C-5), 67.7 (C-3), 66.0 (C-4), 62.4 (C-6), 46.0 (q, $J_{C2,F}$ = 24.9 Hz, C-2), 20.7, 20.65, 20.6 (3xCH_3, Ac); **FT-IR (neat)** ν in cm^{-1}: 2917, 2867, 1749, 1497, 1455, 1436, 1368, 1306, 1267, 1226, 1178, 1120, 1092, 1051, 978, 737, 699; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{47}H_{51}F_3NaO_{13}^+$ (m/z): calc. 903.3174; found 903.3173.

Data for **4.37β:** Colourless syrup. R_f (9:1 hexanes/EtOAc): 0.08; Selected spectroscopic data: **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.37-7.19 (m, 18H, ArH), 7.15-7.11 (m, 2H, ArH), 5.78 (d, $J_{2,3}$ = 3.4 Hz, 1H, H-3), 5.77 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.14 (ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{5,6a}$ = 2.4 Hz, 1H, H-5), 4.91 (d, $J_{1',2'}$ = 1,8 Hz, 1H, H-1), 4.79 (d, J = 10.8 Hz, 1H, CH-Ph), 4.72-4.68 (m, 3H, 3xCH-Ph), 4.65 (d, J = 12.0 Hz, 1H, CH-Ph), 4.53-4.45 (m, 3H, 3xCH-Ph), 4.43 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 2.5 Hz, 1H, H-6a), 4.30 (dd, $J_{3',4'}$ = 9.8 Hz, $J_{2',3'}$ = 3.1 Hz, 1H, H-3'), 4.06 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-2'), 3.97 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.1 Hz, 1H, H-6b), 3.98-3.93 (m, 1H, H-4), 3.92 (t, $J_{3',4'}$ = 9.5 Hz, 1H, H-4'), 3.80 (ddd, $J_{4',5'}$ = 9.6 Hz, $J_{5',6b'}$ = 4.5 Hz, $J_{5',6a'}$ = 1.9 Hz, 1H, H-5'), 3.73 (dd, $J_{6a',6b'}$ = 10.4 Hz, $J_{5',6a'}$ = 4.6 Hz, 1H, H-6a'), 3.67 (dd, $J_{6a',6b'}$ = 10.4 Hz, $J_{5',6b'}$ = 2.0 Hz, 1H, H-6b'), 2.68 (m, 1H, H-2), 2.04 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.6 (d, $J_{CF3,2}$ = 9.4 Hz, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 2920, 2867, 1747, 1435, 1369, 1306, 1265, 1227, 1120, 1051, 740, 700; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{47}H_{51}F_3NaO_{13}^+$ (m/z): calc. 903.3174; found 903.3167.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α/β-D-mannosyl-(1→2)-1,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose (4.38)



The title compound was prepared following the general procedure **(GP)** using **4.8** (21.5 mg, 0.053 mmol), CH_2Cl_2 (0.38 mL) and 30 % HBr in AcOH (0.38 mL). After standard work-up, glycosylation was carried out in a Schlenk flask using the crude bromopyranoside, 1,2,3,4-tetra-*O*-benzyl-α-D-mannopyranose (55.8 mg, 0.106 mmol), dry CH_2Cl_2 (0.55 mL), dry toluene (0.55 mL), preactivated 4 Å MS and AgOTf (27.2 mg, 0.106 mmol). The reaction mixture was stirred under argon for 2 h at -80 °C. After standard work-up, ^{19}F NMR analysis indicated an α/β ratio (93:7) and yield (60%) using 1,4-difluorobenzene (5 µL, 0.048 mmol) as internal standard. Due to purification issues, the remaining acceptor was submitted to acetylation conditions using Ac_2O (60 µL, 0.64 mmol) and pyridine (0.5 mL, 6.36 mmol) in CH_2Cl_2 (0.5 mL) for 16 h at room temperature. After standard work-up, the residue was purified by flash column chromatography (from hexanes to 1:4 EtOAc/hexanes) to obtain a fraction containing an anomeric mixture of **4.38** (α:β 10:1) (27 mg, 48%) as colourless syrup.

Data for **4.38α/β:** R_f (1:9 EtOAc/hexanes): 0.12; **FT-IR (neat)** ν in cm^{-1}: 3031, 2928, 1749, 1496, 1455, 1435, 1367, 1307, 1267, 1227, 1178, 1154, 1119, 1092, 1051, 1027, 977, 913, 737, 699; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{47}H_{51}F_3NaO_{13}^+$ (m/z): calc. 903.3174; found 903.3170.

Data for **4.38α:** Inseparable mixture of α:β (10:1). Colourless syrup. R_f (9:1 hexanes/EtOAc): 0.12; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.35-7.20 (m, 20H, ArH), 5.39 (dd, $J_{3,4}$ = 9.7 Hz, $J_{2,3}$ = 5.4 Hz, 1H, H-3), 5.42-5.32 (m, 2H, H-1, H-4), 4.97 (d, J = 11.3 Hz, 1H, CH-Ph), 4.83 (d, $J_{1',2'}$ = 1,9 Hz, 1H, H-1'), 4.70-4.55 (m, 6H, 6xCH-Ph), 4.41 (d, J = 12.0 Hz, 1H, CH-Ph), 4.14 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 2.2

Hz, 1H, H-6a), 4.06 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 4.7 Hz, 1H, H-6b), 4.02-3.99 (ddd, $J_{4,5}$ = 9.4 Hz, $J_{5,6b}$ = 4.7 Hz, $J_{5,6a}$ = 2.2 Hz, 1H, H-5), 3.97-3.87 (m, 2H, H-4', H-3'), 3.82 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{5',6a'}$ = 4.8 Hz, 1H, H-6a'), 3.77 (d, $J_{1',2'}$ = 2.4 Hz, 1H, H-2'), 3.83-3.73 (m, 1H, H-5'), 3.72 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{5',6b'}$ = 1.8 Hz, 1H, H-6b'), 3.23 (qdd, $J_{2,CF3}$ = 9.8 Hz, $J_{2,3}$ = 5.6 Hz, $J_{1,2}$ = 1.4 Hz, 1H, H-2), 2.04 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.32 (d, $J_{CF3,2}$ = 9.8 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.8, 169.9, 169.5 (3xC=O, Ac), 138.3, 138.2, 138.0, 137.1, 128.45, 128.4, 128.35, 128.3, 127.8, 127.7, 127,68, 127.65, 127.6, 127.5 (Ar), 124.7 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 97.2 (C-1'), 95.5 (q, $J_{C1,F}$ = 4.9 Hz, C-1), 80.2 (C-3'), 75.1 (CH_2Ph), 74.4 (C-4'), 74.3 (C-2'), 72.7 (CH_2Ph), 72.1 (CH_2Ph), 71.9 (C-5'), 69.0 (CH_2Ph), 68.2 (C-5), 67.7 (C-4), 66.7 (C-6'), 65.7 (C-3), 62.2 (C-6), 46.0 (q, $J_{C2,F}$ = 25.1 Hz, C-2), 20.7 (2xCH_3, Ac), 20.6 (CH_3, Ac).

Data for **4.38β:** Inseparable mixture of α:β (1:6). Colourless syrup. R_f (9:1 hexanes/EtOAc): 0.10; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.38 (dd, J = 7.2 Hz, J = 2.2 Hz, 2H, ArH), 7.35-7.20 (m, 18H, ArH), 5.43-5.31 (m, 2H, H-3, H-4), 5.28 (s, 1H, H-1), 4.99-4.87 (m, 3H, 3xCH-Ph), 4.80 (d, J = 12.6 Hz, 1H, CH-Ph), 4.65-4.43 (m, 3H, 3xCH-Ph), 4.37 (s, 1H, H-1'), 4.36 (d, J = 12.1 Hz, 1H, CH-Ph), 4.18-4.10 (m, 1H, H-6a), 4.10-4.00 (m, 2H, H-5, H-6b), 3.92-3.70 (m, 3H, H-4', H-6a', H-6b'), 3.87 (d, $J_{2',3'}$ = 2.9 Hz, 1H, H-2'), 3.46 (dd, $J_{3',4'}$ = 9.3 Hz, $J_{2',3'}$ = 2.9 Hz, 1H, H-3'), 3.38 (ddd, $J_{4',5'}$ = 9.8 Hz, $J_{5',6a'}$ = 6.0 Hz, $J_{5',6b'}$ = 1.8 Hz, 1H, H-5'), 3.24 (qd, $J_{2,CF3}$ = 9.8 Hz, $J_{2,3}$ = 5.4 Hz, 1H, H-2), 2.01 (s, 3H, CH_3, Ac), 1.97 (s, 3H, CH_3, Ac), 1.87 (s, 3H, CH_3, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -62.22 (d, $J_{CF3,2}$ = 9.8 Hz, 3F, CF_3); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.7, 169.9, 169.5 (3xC=O, Ac), 138.6, 138.2, 137.9, 137.3, 128.5, 128.45, 128.40, 128.3, 128.1, 128.0, 127.95, 127.9, 127.8, 127.7, 127.65, 127.6, 127.4 (Ar), 124.71 (q, $J_{C,F}$ = 281.0 Hz, CF_3), 100.0 (C-1'), 96.7 (q, $J_{C1,F}$ = 4.5 Hz, C-1), 82.3 (C-3'), 75.10 (CH_2Ph, C-5'), 74.3 (C-4'), 73.9 (CH_2Ph), 73.8 (C-2'), 71.3 (CH_2Ph), 70.7 (CH_2Ph), 68.3 (C-5), 67.7 (C-4), 67.2 (C-6'), 65.7 (C-3), 62.1 (C-6), 46.2 (q, $J_{C2,F}$ = 24.7 Hz, C-2), 20.7, 20.6, 20.5 (3xCH_3, Ac).

Chapter IV

CHAPTER V

STUDY AND COMPARISON OF SOME PHYSICOCHEMICAL PROPERTIES OF 2-DEOXY-2-FLUORO AND 2-DEOXY-2-TRIFLUOROMETHYL GLYCOSIDES

Chapter V

5.1. INTRODUCTION

Glycans are well recognized as relevant players in a variety of biological processes, such as cell-adhesion, immune regulation, inflammation, host-pathogen interactions, and cancer metathesis. However, native glycan structures have shown little success due to their poor pharmacokinetic properties, which are mainly the high polarity, low binding affinity, low stability, and rapid excretion (**Figure 5.1**).[1] The great interest of discovering the roles and applications of glycans as therapeutics has led to the successful production of glycomimetic compounds, which imitate the structure and function of native glycans but with improved pharmacokinetic (PK) and pharmacodynamic (PD) features. There are multiple approaches to glycomimetic design, and among them, fluorinated glycomimetics have shown to be a useful tool in the development of new drugs since fluorine substitution has proved to improve binding affinity, bioavailability and glycosyl bond stability, among others.[2]

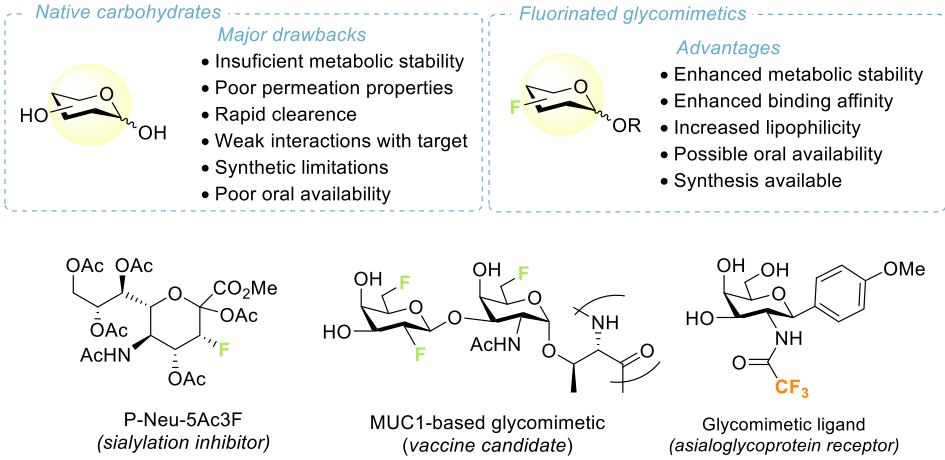


Figure 5.1. Top: Major drawbacks of native carbohydrates as therapeutic drugs and advantages of fluorinated glycomimetics. Bottom: Examples of successful fluorinated glycomimetic compounds.

[1] Hevey, R. *Chem. Eur. J.* **2021**, *27*, 2240-2253.
[2] Hevey, R. *Pharmaceuticals*, **2019**, *12*, 55.

Fluorinated drugs have become increasingly significant over the last decades, and more importantly, the strategic introduction of fluorine motifshas become a common step in the design and optimization of new drugs. Strategic fluorine substitution is normally used to improve physicochemical, adsorption and distribution properties.[3] The bioisosteric replacement of hydroxyl substituents with one or more fluorine atoms has successfully generated glycomimetic compounds with improved properties (**Figure 5.1**).

5.1.1. Effect of fluorine substitution on sugar properties

Orally available drugs must present adequate pharmacokinetic properties for an efficient adsorption, distribution, metabolism, and excretion (ADME) by the organism. Primary goals of clinical pharmacokinetics include enhancing the efficiency and decreasing the toxicity of the drug. Consequently, the bioisosteric replacement of functional groups is commonly used in medicinal chemistry to improve the ADMET properties of a molecule. For instance, deoxyfluorination of sugars has proved to overcome some of the major drawbacks of native carbohydrates as therapeutic drugs, and the most important changes include higher lipophilicity, improved binding affinity, increased bioavailability and enhanced stability and resistance towards hydrolysis, among others (**Figure 5.2**).[4]

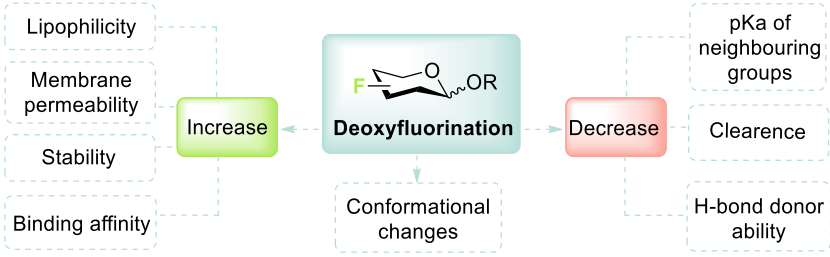


Figure 5.2. Main effects of deoxyfluorination in sugar properties.

[3] Meanwell N. A. *J. Med. Chem*. **2018**, *61*, 5822−5880.
[4] Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. *J. Med. Chem.* **2015**, *58*, 8315-8359.

a. Stability of the glycosidic bond

One of the major drawbacks of native carbohydrates is the lability of the glycosidic bond against glycosidases and acidic media. Thus, improvement of the stability of the glycosidic bond has been a major approach to increase its bioavailability. Two main strategies have proved to substantially increase the stability of the glycosidic bond. First, the substitution of the glycosidic oxygen with a more stable carbon, selenium, or sulphur bond,[5] and second, the introduction of an electron-withdrawing group in the pyranose ring, which destabilize the oxocarbenium intermediate produced after the cleavage of glycosidic bond (see Chapters III, IV). The mechanism of carbohydrate hydrolysis has been the focus of a great deal of research,[6] and the stabilizing effect of fluorine substitution was realised very early in acid-catalysed hydrolysis studies developed by Withers and co-workers.[7] The stronger inductive effect of fluorine compared to hydroxyl groups, hampers the formation of the oxocarbenium ion more difficult, making fluorinated carbohydrates more resistant than the non-fluorinated congeners. This destabilizing effect on the formation of oxocarbenium transition state has also been exploited for mechanism-based enzyme inhibition.[8] Deoxyfluorinated carbohydrates have been largely investigated as enzyme-inhibitors, and its inactivation has usually been achieved using sugars with a fluorine atom adjacent to the anomeric carbon.[9] Therefore, fluorine substitution increases the stability of the glycosidic bond, making it resistant to acid-catalysed hydrolysis and to the enzymatic activity of glycosidases.

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[5] Compañón, I.; Guerreiro, A.; Mangini, V.; Castro-Lopez, J.; Escudero-Casao, M.; Avenoza, A.; Busto, J. H.; Castillón, S.; Jiménez-Barbero, J.; Asensio, J. L.; Jiménez-Osés, G.; Boutureira, O.; Peregrina, J. M.; Hurtado-Guerrero, R.; Fiammengo, R.; Bernardes, G. J. L.; Corzana, F. *J. Ac. Chem. Soc.* **2019**, *141*, 4063-4072.

[6] a) Banait, N. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1991**, *113*, 7951-7958; b) Zhu, J.; Bennet, A. J. *J. Am. Chem. Soc.* **1998**, *120*, 3887-3893.

[7] a) Withers, S. G.; MacLennan, D. J.; Street, I. P. *Carbohydr. Res.* **1986**, *154*, 127-144; b) Withers, S. G.; Percival, M. D.; Street, I. P. *Carbohydr. Res.* **1989**, *187*, 43-66.

[8] a) Street, I. P.; Kempton, J. B.; Withers, S. G. *Biochemistry*, **1992,** *31*, 9970-9978; b) Rempel, B. P.; Withers, S. G. *Glycobiology*, **2008**, *18,* 570-586.

[9] Thanna, S.; Lindenberger, J. J.; Gaitonde, V. V.; Ronning, D. R.; Sucheck, S. J. *Org. Biomol. Chem.* **2015**, *13*, 7542-7550.

b. Lipophilicity

Lipophilicity is defined as the partition coefficient of a given molecule between a nonpolar matrix and an aqueous phase, and it reflects the balance between the hydrophobic and hydrophilic parameters of the molecule. It is determinant of many other drug properties, such as solubility, membrane permeability, bioavailability, drug absorption, distribution, metabolism, and clearance rate.[10] Lipophilicity modulation has become an important tool in the aim of potency optimization in the development of new drugs, thus, new methods for controlling lipophilicity are of great interest.[11] In this context, fluorine substitution has been generally used to decrease the lipophilicity of aliphatic compounds and, in contrast, for aromatic compounds, it normally increases the lipophilicity. In the case of sugars, there is not a direct rule that dictates the relation between deoxyfluorination and lipophilicity values. Carbohydrate's lipophilicity has been difficult to determine due to the troublesome quantification derived from low concentration operating conditions.[12] Recently, Linclau and co-workers presented a valuable ^{19}F NMR protocol for determining log *P* values, an experimental investigation that allowed to stablish correlations between fluorination and log *P* values for fluorinated carbohydrates.[13] They proved that monofluorination has a great impact on lipophilicity leading to an increase in the log *P* value, and exists a clear relation on the effect of fluorination site and the sugar configuration. An example is shown in **Figure 5.3,** in which a great increase of log *P* value is observed from native glucose (-3.24) to 6F-glucose (-2.36), and it also depends on the fluorination position, since 2F-glucose shows higher log *P* value (-2.21) compared to the 6F-analogue. Lipophilicity rises along with the increasing number of fluorine atoms and the highest log *P* value is obtained when substituting 3 positions of glucose (log *P (***2F,3F,4F-*Glc*** = -0.17).

[10] Arnott, J. A.; Planey, S. L. *Expert Opin. Drug Discov.* **2012**, *7*, 863-875.
[11] Di, L. Kerns, E. Drug-like properties: concepts, structure design and methods from ADME to toxicity optimization. Academic press **2015.**
[12] Mazzobre, M. F.; Román, M. V.; Mourelle, A. F.; Corti, H. R. *Carbohydr. Res.* ***2005***, *340,* 1207.
[13] Linclau, B.; Wang, Z.; Compain, G.; Paumelle, V.; Fontenelle, C. Q.; Wells, N.; Weymouth-Wilson, A. *Angew. Chem. Int. Ed.* **2016**, *55*, 674–678.

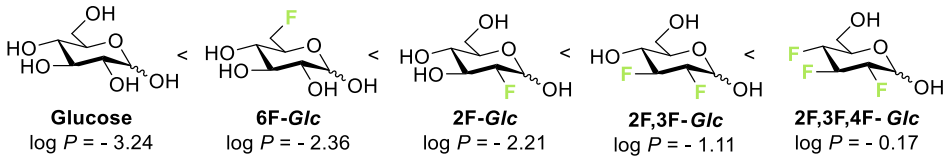

Figure 5.3. Examples of lipophilicities of fluorinated carbohydrates.[14]

c. Binding affinity

Binding affinity and selectivity are key factors for drug efficiency, however, sugars normally present weak interactions due to their high hydrophilicity. Polar carbohydrates are highly solvated, and the low binding affinities have been attributed to the high energetic cost for desolvation prior to protein binding. Polar interactions, such as, hydrogen bonding, metal chelation, and salt bridging are determinant for the binding specificity, and hydrophobic interactions are considered to be the major contributors to binding affinity.[7] Combined, all the sugar-protein interactions are normally insufficient to compensate the enthalpic desolvation costs associated. Efforts to overcome the poor binding affinity include deoxyfluorination, which reduces the polar surface area making it less solvated while still maintaining the necessary interactions with the binding site. Deoxyfluorination has a direct impact in the pK_a, conformation, hydrophobic interactions, and lipophilicity, so it can also affect to the binding selectivity. Considering molecular interactions, hydrogen bonds and CH-π interactions are the two key interactions involved in the binding event, and both are affected by fluorination. Deoxyfluorination of hydroxyl groups which are non-essential for binding has proved to improve the binding affinity and it is explained by the favoured hydrophobic desolvation and electrostatic interactions. Elimination of hydroxyl groups leads to a reduction of the polar surface area, thereby, the desolvation energetic costs associated with binding are also reduced. In addition, it can potentially generate new hydrophobic interactions with the protein surface.[15] Since it involves the loss of

[14] St-Gelais, J.; Bouchard, M.; Denavit, V.; Giguère, D. *J. Org. Chem.* **2019**, *84*, 8509−8522.
[15] Hevey, R. *Biomimetics*, **2019**, *4*, 53.

hydrogen bond donating ability at this position (maintaining a weak hydrogen bond acceptor capacity), this can be detrimental for the affinity and selectivity of proteins when the hydroxyl group is involved in the binding event.[16] Considering CH-π interactions, they are usually described as weak polar interactions in which the hydrogen atom of the polarized C-H bond acts as donor and the delocalized electron density of the sp^2-aromatic amino acids acts as acceptor. Deoxyfluorination involves the replacement of the hydroxyl group by a highly electronegative F atom, which effectively polarises C-H bonds, enabling them to interact with electron rich amino acids.[17]

d. Oral bioavailability

High oral bioavailability is an important parameter in the development of therapeutic agents. Native sugars have a limited use as orally administered drugs due to their high hydrophilicity that makes them unable to passively cross the intestinal enterocyte layer. Lipinski and Veber rules determined that the passive permeation normally requires molecules to have reduced molecular flexibility, low molecular weight, limited polar surface area and a low number of hydrogen-bond donors and acceptors.[18] Deoxyfluorination, as mentioned before, involves the substitution of a hydroxyl group by a fluorine atom which reduces the polar surface area and the number of hydrogen bond donors, fitting the sugar within the previous rules. In addition, the longer hydrolytic stability and enhanced lipophilicity significantly contribute to enhance the oral bioavailability by improving the passive permeation through the intestinal membrane.[1]

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[16] Van Straaten, K. E.; Kuttiyatveetil, J. R.; Sevrain, C. M.; Villaume, S. A.; Jimenez-Barbero, J.; Linclau, B.; Vincent, S. P.; Sanders, D. A. *J. Am. Chem. Soc.* **2015**, *137*, 1230-1244.

[17] Jiménez-Moreno, E.; Jiménez-Osés, G.; Gómez, A. M.; Santana, A. G.; Corzana, F.; Bastida, A. J.; Jiménez-Barbero, J.; Asensio, J. L. *Chem. Sci.* **2015**, *6*, 6076-6085; b) Fernández-Alonso, M. C.; Cañada, F. J.; Jimenez-Barbero J.; Cuevas, G. *J. Am. Chem. Soc.* **2005**, *127*, 7379-7386.

[18] a) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3-25; b) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45,* 2615-2623.

5.1.2. Comparison between F and CF_3

Although fluorine and trifluoromethyl groups have similar Pauling electronegativities (4.0 for fluorine and 3.5 for CF_3), the trifluoromethyl group has higher hydrophobicity (0.88 for CF_3 and 0.14 for F) and higher steric hindrance, which is comparable to the isopropyl substituent (**Figure 5.4**).[3]

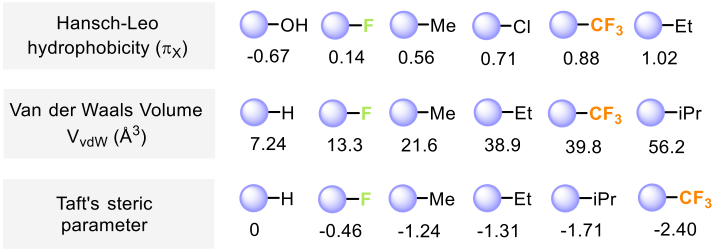


Figure 5.4. Properties of fluorine and trifluoromethyl groups.

Moreover, F and CF_3 groups present different effects when attached to π-systems. For instance, carbocations are partially stabilized by the donation of a non-bonding electron pair from fluorine, whereas α-trifluoromethyl cations are destabilized by inductive effect. α-Fluorine-carbanions are destabilized by electronic repulsion whereas CF_3 stabilizes the negative charge by hyperconjugation. In vinyl and aromatic systems, fluorine can act as an electron-donating group by resonance conjugation. Trifluoromethyl analogues show pure inductive effects, lowering the electron density on the π-system.[19]

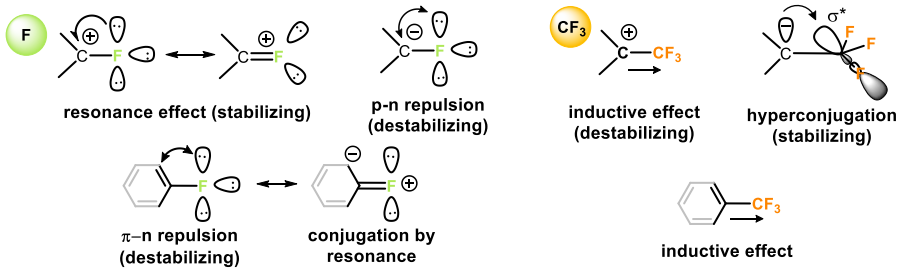


Figure 5.5. Fluorine and trifluoromethyl effects on π-systems.

[19] Hiyama, T. Organofluorine compounds: chemistry and applications. Springer Science & Business. **2000.**

We hypothesised that the introduction of a fluorinated group on the C-2 position of glycosides would induce several changes in the electronic structure and in the stability of the modified glycoside. By studying the effect of the introduction of different fluorinated groups, fluorine and trifluoromethyl could be used to modulate the physicochemical and pharmacokinetic properties of 2-deoxy- and 2-OH-glycosides.

5.2. OBJECTIVES

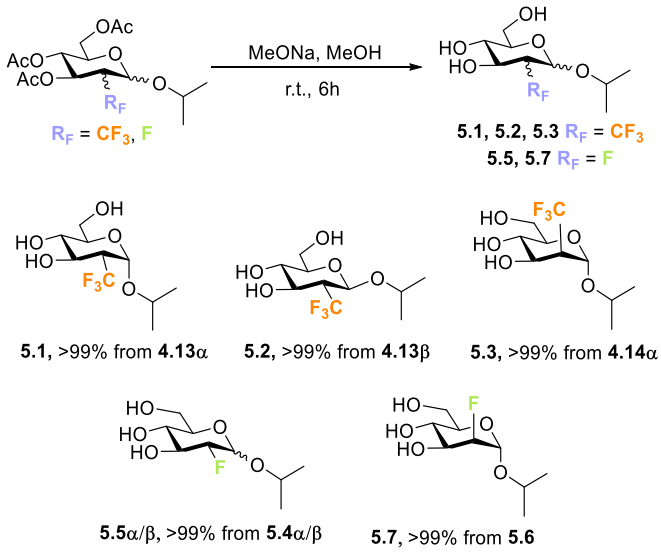
The general aim of this chapter is to study and compare different physicochemical properties of 2-deoxy-2-fluoro and 2-deoxy-2-trifluoromethyl glycosides to gain insight into the changes induced by both motifs. In this context, the objectives of this chapter are:

1. To synthetize deprotected 2-deoxy-2-fluoro and 2-deoxy-2-trfluoromethyl glycosides with different sugar configurations (*gluco* and *manno*) and both anomeric dispositions (α and β).
2. To address computational and experimental (NMR analysis) evaluation of glycosides conformation, potential energy surface, dipole moments and Van der Waals volume calculation.
3. Determination of the lipophilicity of the fluorinated and trifluoromethylated derivatives.
4. Probe the stability of the fluorinated and trifluoromethylated derivatives under intracellular physiological standard conditions, considering two principal media: cytosol mimic and lysosome mimic media.
5. Determination of the acid-catalysed hydrolysis rate and the half-life times of the deprotected 2-fluoro and 2-trifluoromethylpyranoses and study the influence of the fluorinated motif in the pyranose stability.

5.3. RESULTS AND DISCUSSION

5.3.1. Synthesis of deprotected F- and CF_3-glycosides

Considering the inherent properties of the fluorine atom and the effects that deoxyfluorination imparts to sugars, we expected that monofluorinated and trifluoromethylated glycosides would show increased lipophilicity, enhanced hydrolytic stability and higher stability under standard physiological conditions. To this end, different deprotected fluorinated and trifluoromethylated sugars were synthetised. *Gluco* and *manno* α-glycosides were selected to study the effect of the sugar configuration and α and β glucosides were used as model substrates to study the effect of the anomeric configuration. Acetyl-protected glycosides were subjected to deprotection conditions to obtain 2-deoxy-2-trifluoromethyl glycosides **5.1-5.3** and 2-deoxy-2-fluoro glycosides **5.5α/β** and **5.7** in quantitative yields (**Scheme 5.1**). Peracetylated 1-*O*-isopropyl-2-deoxy-2-fluoro-pyranosides **5.4** (*Glc*) and **5.6** (*Man)* were obtained subjecting the corresponding bromide to glycosylation conditions in the presence of 2-propanol, obtaining the desired products **5.4** and **5.6** in excellent yields (85% for **5.4**, and 82% for **5.6**).



Scheme 5.1. Deprotection of selected trifluoromethylated and fluorinated glycosides.

The configuration and conformation of the products was confirmed by ^{1}H, ^{13}C, ^{19}F, HSQC, HMBC, NOESY and COSY. $^{1}J_{C1,H1}$ values of 160–170 Hz are diagnostic of an axial anomeric proton that is associated with a β-glycoside adopting a $^{4}C_{1}$ conformation, whereas $^{1}J_{C1,H1}$ > 170 Hz indicates an α-glycoside for the same conformation. The vicinal coupling constants and NOE contacts suggested that all the products display the $^{4}C_{1}$ conformation (**Figure 5.6**).

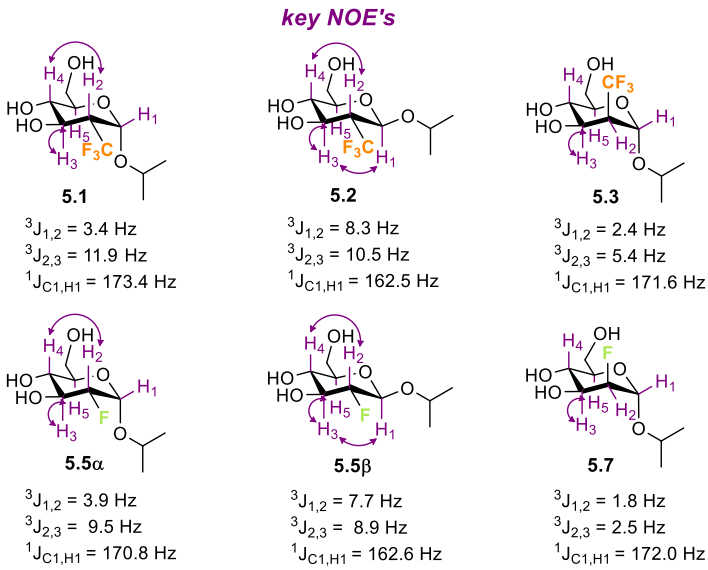


Figure 5.6. Conformational analysis of the deprotected 2-deoxy-2-fluoro- and 2-trifluoromethyl-glycosides in solution.

5.3.2. Electrostatic potential surface calculation

In order to evaluate the electronic and steric differences of the synthetised fluorinated compounds, computational calculations were carried out to determine optimized conformations, dihedral angles, potential energy surface and dipole moment magnitude and direction. In addition, Van der Waals volumes were calculated for the synthetised glycosides (**Figure 5.7**),[20] yielding a value of 231.3 Å^{3} for 2-deoxy-2-trifluoromethyl glycosides (**5.1, 5.2** and **5.3**) and a value of 201.9 Å^{3} for 2-deoxy-2-fluoro glycosides (**5.5α, 5.5β** and

[20] Zhao, Y. H.; Abraham, M. H.; Zissimos, A. M. *J. Org. Chem.* **2003**, *68*, 7368−7373.

5.6). As expected, the trifluoromethyl group increases the Van der Waals volume of the molecule in almost 30 Å^3. Same results were obtained in the case of 2-deoxy-2-fluoro and 2-trifluoromethyl glycosides in Chapter IV.

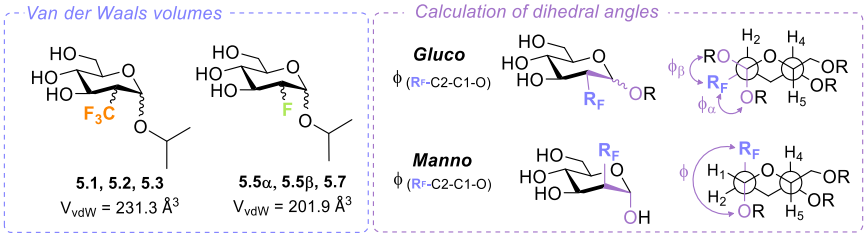


Figure 5.7. Calculated Van der Waals volumes and calculation of dihedral angles.

Optimized conformations, dihedral angles, dipolar moments, and potential energy surfaces were calculated using a Gaussian 9 software, and computational parameters are reported in the experimental section. Selected computational results for 2-deoxy-2-trifluoromethyl glycosides are shown in **Figure 5.8** and results obtained for 2-deoxy-2-fluoro-glycosides are shown in **Figure 5.9.** Full computational results are reported in the experimental section **5.5.4**. Optimized conformations were obtained for all the sugars and dihedral angles were measured as indicated in **Figure 5.7**. Results obtained for both optimal conformations and dihedral angles suggest that the introduction of a trifluoromethyl motif in the C-2 position slightly distorts the conformation of the sugar, particularly in *manno* glycosides, as previously demonstrated in Chapter IV. Differences in dihedral angles (CF_3-C2-C1-O) between 2-CF_3-*manno* glycoside **5.3** (ϕ = 158.5°) and 2-F-*manno* glycoside **5.7** (ϕ = 168.6°) of 10° were higher than in 2-CF_3- and 2-F-*gluco* glycosides (ϕ **5.1/5.5a** = 58.2°/54.8° and ϕ **5.2/5.5a** = -63.8°/-66.4°), where there was just up to 3.4°. As demonstrated in the previous section, all the sugars maintain a $^4C_1$ chair conformation in solution. Therefore, it can be concluded that the trifluoromethyl group has little impact on the conformation of the sugar when located in axial position at C-2, in *manno* glycosides, suggesting that repulsive interactions between the trifluoromethyl group and the hydroxyl group in C-6 are limited.

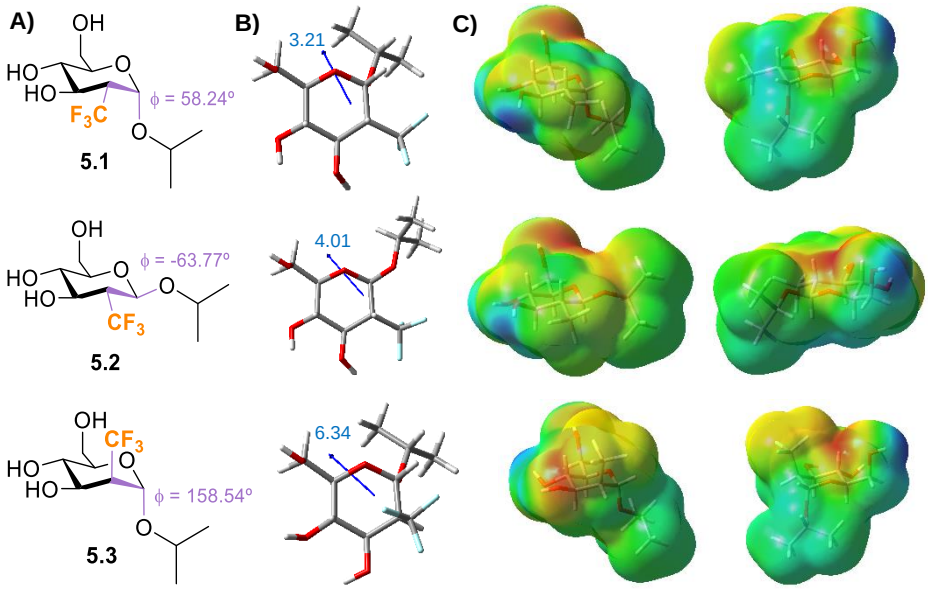


Figure 5.8. Computational results obtained for 2-CF_3-glycosides **5.1, 5.2** and **5.3. A)** Optimized conformation and dihedral angle. **B)** Dipolar moment direction and magnitude. **C)** Potential energy surface.

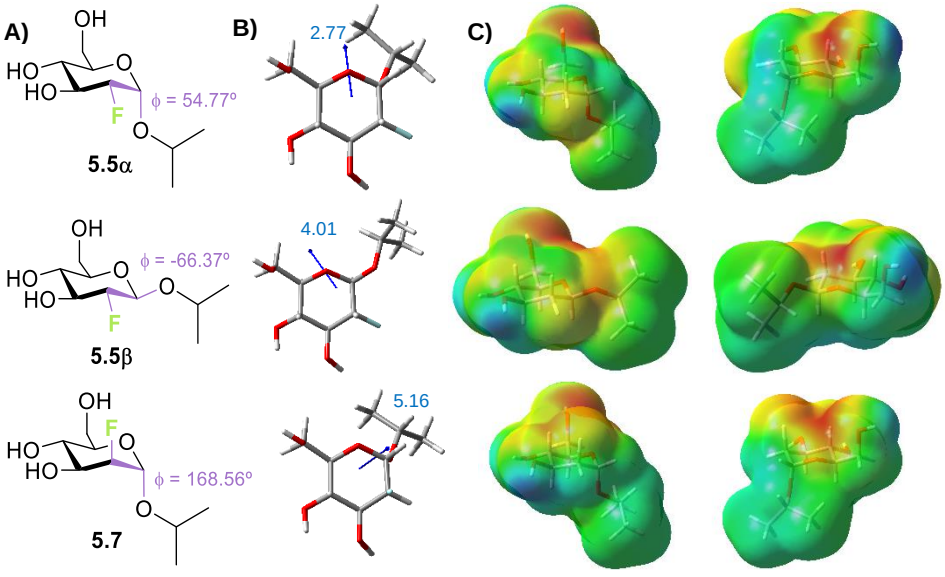


Figure 5.9. Results obtained for 2-F-glycosides **5.5α, 5.5β** and **5.7. A)** Optimized conformation and dihedral angle. **B)** Dipolar moment. **C)** Potential energy surfaces.

Regarding the dipolar moments, directions and magnitudes were obtained for all the sugars and selected results are reported in **Figure 5.8** and **5.9**. Looking into 2-CF_3-glycosides, dipolar moment directions seemed similar for all the sugars, however, big differences in the magnitudes were observed for *gluco* and *manno* glycosides (**Figure 5.8**). Similar values were obtained for α and β glucosides (μ (**5.1**) = 3.21 and μ (**5.2**) = 4.01), however, mannoside **5.3** presented a much higher dipolar moment (μ = 6.34). These differences can be explained by observing the sugar configurations and potential energy surfaces in section **5.5.4**. It can be noted that for all configurations, an electropositive region is located in the bottom face, while an electronegative region is placed in the top face of the sugar. In the case of 2-CF_3-*manno* glycoside **5.3,** this region is much higher than the ones of the corresponding *gluco* derivatives, therefore, the position of the trifluoromethyl group in the top face of the sugar highly contributes to the magnitude of the dipole moment. Similar results were obtained in the calculation of dipole moments of 2-fluoro-glycosides. For these sugars, the direction of the dipolar moment slightly changes in the case of 2-F-*manno* glycoside **5.7** in comparison with *gluco* analogues. Magnitudes of the dipole moments followed the same trend than 2-CF_3-glycosides, in contrast, 2-F-*manno* glycoside **5.7** presents much higher magnitude (μ (**5.7**) = 5.16) than the corresponding 2-F-*gluco* derivatives (μ (**5.5α**) = 2.77 and μ (**5.5β**) = 4.01). However, for 2-F-*gluco* sugars, higher differences in magnitude between α and β glycosides were observed. Interestingly, same magnitude and direction of dipole moments were obtained for trifluoromethylated **5.2** and fluorinated **5.5β** β-glucopyranosides, moreover, no big differences were observed in the dihedral angles. It can be concluded that for *gluco* configuration, the introduction of a trifluoromethyl group does not have a great influence in the conformation and the electronics of the molecule in comparison with fluorine. Overall, dipole moments followed the same trend for 2-F and 2-CF_3- glycosides with slightly variations in magnitude and direction for *manno* glycosides.

Finally, potential energy surfaces for all the sugars were calculated using Gaussian, and selected images are presented in **Figure 5.8** and **Figure 5.9.** It can be concluded that the trifluoromethyl group highly contributes to the

overall volume of the molecule, however, it does not have a great influence in the electronic charge distribution, and similar potential energy surfaces can be observed for all the pairs of 2-F- and 2-CF_3-glycosides.

Considering all the computational results obtained, it can be concluded that the trifluoromethyl group has a minor effect in the conformation of the sugar. This effect is stronger when located in axial position, in which the CF_3 slightly distorts the CF_3-C2-C1-O angle by repulsions with hydroxyl group in C-6. Considering the electronics of the sugar, the trifluoromethyl group does not have a great influence in the magnitude and distribution of the charge, and similar dipolar moments and potential energy surfaces were obtained for the pairs of 2-F- and 2-CF_3-glycosides. Overall, the CF_3 does not have a great influence in the conformation and the electronics of the sugar in comparison with fluorine.

5.3.3. Physicochemical properties of F- and CF_3-glycosides

Once the final products were obtained and the conformation was confirmed by NMR, physicochemical properties of these products were evaluated. We decided to study their lipophilicity, their resistance to acid-catalysed hydrolysis and their stability under physiological standard conditions. Higher lipophilicity is expected because of the increase in fluorination degree, although subtle differences in polarity may arise from the different configuration of the fluorinated motif or the configuration of the anomeric carbon. To this aim, different fluorinated motifs (CF_3 and F), sugars configurations (*manno* and *gluco*) and anomeric pairs (**5.1/5.2** and **5.5α/β**) were selected to study the influence of these structural changes and the fluorinated motif on some physicochemical properties. In addition, we expect that the introduction of a polyfluorinated group would increase, or maintain, the metabolic and hydrolytic stability of the glycosidic bond in comparison with fluorine.

5.3.3.1. Lipophilicity measurement

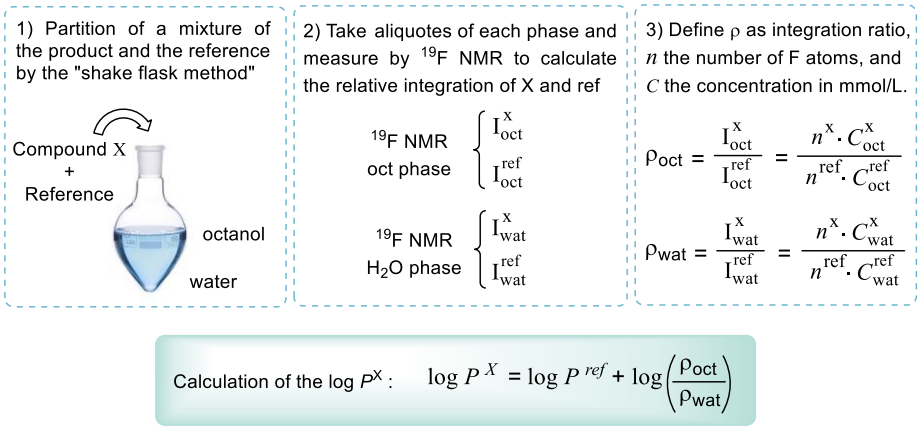
The lipophilicity of a compound can be measured experimentally by determining its partition coefficient P, between 1-octanol and water and it is expressed as *log* P. It is a rapid and effective tool for initial property assessment, as indicated by Lipinski in the rule of 5.[18] Moreover, this value has been widely used in medicinal chemistry as a measure of the membrane permeability, and optimum values (dictated by the CNS MPO (central nervous system multiparameter optimization)) are between +1 to +3 for orally available drugs.[11]

$$\log P = \log\left(\frac{[F-sugar]_{octanol}}{[F-sugar]_{water}}\right)$$

As mentioned before, lipophilicity affects to the physicochemical, metabolic, permeability and solubility properties of a molecule, and it has been correlated to drug activity and ADMET properties. The relative lipophilicity of sugars has a great interest because of their role in biological systems, and many different methods have been used to determine their log *P* values, though sometimes, great differences on the values had been obtained for native sugars. Reported lipophilicities for D-glucose are in good agreement, and log *P* values between -3 and -3.3 have been obtained.[12] However, these values are quite far from the optimal values required for membrane permeability. Deoxyfluorination has proved to highly increase the lipophilicity, and moreover, introduction of more than one fluorine atom has proved to increase lipophilicity close to optimal values. However, deoxyfluorination of these positions could have a negative effect on the binding affinity and selectivity since the ability of hydrogen-bond donor is lost at this position. Therefore, it is of vital importance to improve lipophilicity values of sugars but without compromising the binding selectivity and affinity to the protein. Considering this, we expected that the introduction of a trifluoromethyl moiety adjacent to the anomeric carbon would cause a great increase in lipophilicity, and since only one position is substituted, it may not have a negative effect on its binding affinity and selectivity. We also expected that structural variations would have

a moderate effect on its value. Our aim is to compare and evaluate the values obtained for different fluorinated and trifluoromethylated sugars, in order to understand how the lipophilicity is affected by the fluorinated motif and sugar configuration, and how they can be directly used to modulate this property.

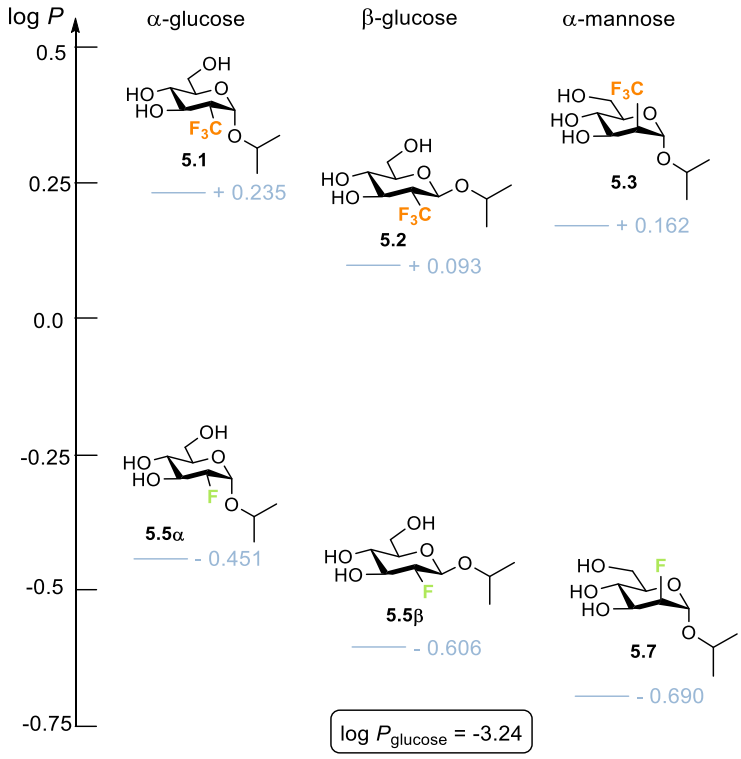
The water/1-octanol partition coefficients were determined employing the "shake flask method" developed by Linclau and co-workers.[21] The method is based on the relative integration of the ^{19}F NMR signals of a product and an internal reference in each phase. A mixture of the reference (2,2,2-trifluoroethanol) and the product was partitioned between 1-octanol and MilliQ® water (**Scheme 5.2**, **1**). An aliquot of each phase was transferred to an NMR tube and analysed by ^{19}F NMR. The FIDs obtained were reprocessed to obtain the integration ratios of the compound X and the reference; I_{oct}^{X}, I_{oct}^{ref}, I_{wat}^{X} and I_{wat}^{ref} (**Scheme 5.2**, **2**). The ratios of these values were defined as ρ_{oct} for the organic phase and ρ_{wat} for the aqueous phase (**Scheme 5.2, 3**), which correspond to the ratio of respective concentrations. The equation of the log P^X calculation shows that it depends on the log *P* of the reference and the logarithm of the ratio of the measured ρ values.



Scheme 5.2. Procedure for the determination of the log *P* value.

[21] Linclau, B.; Wang, Z.; Compain, G.; Paumelle, V.; Fontenelle, C. Q.; Wells, N.; Weymouth-Wilson, A. *Angew. Chem. Int. Ed.* **2016**, *55*, 674–678.

Results obtained for 2-F- and 2-CF_3- glycosides are presented in **Scheme 5.3**. As we envisioned, 2-CF_3-sugars presented higher lipophilicity compared to the corresponding 2-fluoro analogues and values differ from 0.65 to 0.85 log *P* units. Promising results were obtained for trifluoromethyl glycosides since positives values were obtained for all the products, which is of great importance considering the huge increment of lipophilicity when introducing the trifluoromethyl motif (log $P_{glucose}$ = -3.24). Interestingly, results obtained show that lipophilicity is also dependant on the anomeric configuration and 1,2-*cis*-glycosides (**5.1** and **5.5α**) present higher lipophilicity than the corresponding 1,2-*trans*-glycosides (**5.2** and **5.5β**). For instance, 2-F-α-glucose **5.5α** is 0.15 log *P* units more lipophilic than 2-F-β-glucose **5.5β**, and 2-CF_3-α-glucose **5.1** is 0.14 log *P* units more lipophilic than 2-CF_3-β-glucose **5.2**.



Scheme 5.3. Results obtained in the determination of the *log* P following the "shake flask method" by ^{19}F NMR analysis.

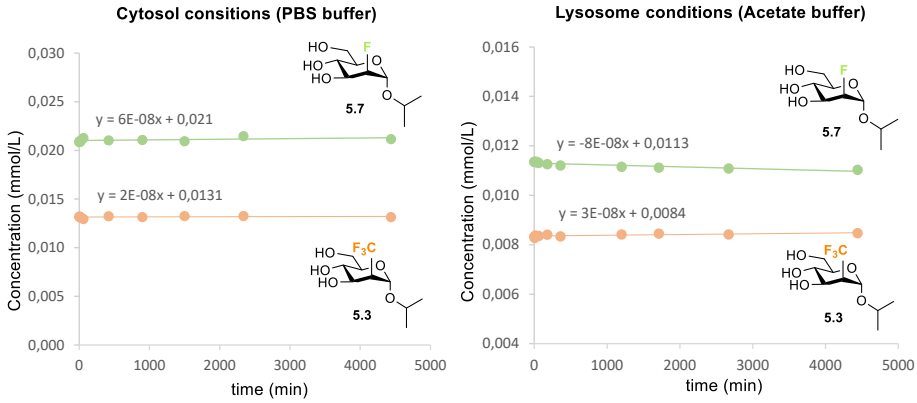
Moreover, lipophilicity showed to be dependent on the sugar configuration and differences were obtained for *gluco* and *manno* glycosides. Considering trifluoromethylated sugars, differences between $2CF_3$-α-glucose **5.1** and $2CF_3$-α-mannose **5.3** are only of 0.07 log *P* units. In contrast, for the fluorinated analogues, differences between 2F-α-glucose **5.5α** and 2F-α-mannose **5.7** are of 0.24 log *P* units, which is more significant.

5.3.3.2. Stability in physiological conditions

Despite being administrated by different routes, most drugs are distributed to the target tissue via the circulatory systems. Thus, to prove that our 2-deoxy-2-fluoro and 2-deoxy-2-trifluoromethyl carbohydrates are suitable structures for drug development we decided to evaluate their stability under standard physiological conditions. Pharmacokinetics study the different processes that a drug suffers when is administrated to a living organism. Considering oral-available drugs, the drug must pass or permeate though the membrane of the epithelial cells and it must be stable under the different intracellular conditions. Once the drug has entered the cytosol, where there is a neutral pH of 7.4, it could be taken by the lysosomes, which have an acid pH (between 4.5 and 5), due to the presence of different hydrolases that work in acid medium.[1,11] Considering this background we decided to study the stability of our glycosides under two different media, a neutral medium (mimicking cytosol) and an acidic medium (mimicking lysosomes). To mimic the cytosol medium, Phosphate Buffered Saline (PBS) with pH of 7.4 was used, which is an isotonic buffer frequently used in biological applications, such as washing cells, transportation of tissues, and dilutions.[22] PBS buffer closely mimics the pH, osmolarity, and ion concentrations of the human body. To mimic the lysosomes acid medium, acetate buffer with pH of 4.5 was used.

[22] a) Schuster, J.; Mahler, H. C.; Koulov, A.; Joerg, S.; Racher, A.; Huwyler, J.; Detampel, P.; Mathaes, R. *Eur. J. Pharm. Biopharm.* **2020**, *152*, 193-201; b) Michnik, A.; Kiełboń, A.; Duch, K.; Sadowska-Krępa, E.; Pokora, I. *J. Therm. Anal. Calor.* **2022**, *147*, 6739-6743.

The methodology consisted in the incubation of the fluorinated sugars in the buffered media and the monitoring of the chemical shift by ^{19}F NMR. For this evaluation, fluorinated and trifluoromethylated mannoglycosides (**5.3** and **5.7**) were selected as probes. Each compound was dissolved in the buffered medium (PBS or acetate buffer) in the presence of an internal standard (TFE), sealed using a rubber septum and successively mixed to obtain a homogeneous solution. The initial concentration of the fluorinated sugar was determined by ^{19}F NMR and then the tube was incubated at the required temperature (37 ± 0.02 °C). At appropriate intervals, the tube was cooled at 0°C and analysed for the fluorinated carbohydrate decomposition by ^{19}F NMR until reaching to a total of 3 days. Results obtained for both glycosides in the corresponding buffers are presented in **Scheme 5.4**. As we envisioned under neutral pH conditions mimicking cytosol (**Scheme 5.4**, **left**), both fluorinated glycosides proved to be stable, and no decomposition was observed after 74 h at 37 °C. In contrast, under acidic conditions mimicking lysosomes (**Scheme 5.4**, **right**), the fluorinated mannopyranoside **5.3** proved to be highly stable and only a slightly decomposition was observed (3% of hydrolysis product), whereas the trifluoromethyl mannopyranoside **5.7** proved to be totally stable under these conditions after 74h.



Scheme 5.4. Study of the stability of 2-deoxy-2-fluoro- and trifluoromethyl-mannopyranosides **5.3** and **5.7** under intracellular physiological conditions.

5.3.3.3. Acid hydrolysis resistance

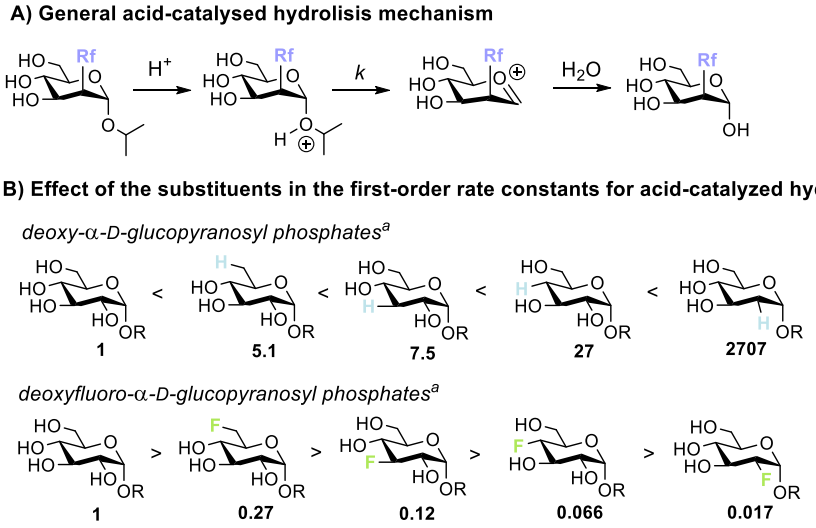
As mentioned in the introduction, one of the major drawbacks of carbohydrate-derived drugs is the poor resistance of the glycosidic bond towards glycosidases and acidic media. Deoxyfluorination has been widely used to increase the stability of the glycosidic linkage, and many examples proved that 2-deoxy-2-fluoro-glycosides are resistant to glycosidases and acid-media, due to the destabilization of the oxocarbenium intermediate state. With this in mind, our aim is to evaluate the effect of the trifluoromethyl moiety in the stability of the glycosidic bond and compare its acid-resistance with a fluorinated analogue.

The currently accepted mechanism for the acid-catalysed hydrolysis of glycosidic bonds involves a rapid, equilibrium-controlled protonation of the glycosidic oxygen atom to form a conjugate acid, which in a slow, rate-determining heterolysis, decomposes to form the oxocarbenium ion. Finally, water is readily added giving the hydrolysed glycoside (**Scheme 5.5, A)**). Several studies have indicated that polar effects contribute strongly to the relative rates of glycoside hydrolysis. Moreover, our results indicate that sugar configuration influences the hydrolysis rate of the compounds.[23] Generally, the rate of hydrolysis of a glycoside increases because of deoxygenation,[24] presumably because replacement of an electron-withdrawing hydroxyl group with a hydrogen stabilizes the electron-deficient transition state. However, the increase in the rate of hydrolysis of the deoxy glycosides is not simply a function of the distance of the substitution from the anomeric centre. In the studies carried out to date, the relative rates of hydrolysis of monodeoxygenated glycosides and glucosyl phosphates are: 2-deoxy > 4-deoxy > 3-deoxy > 6-deoxy > parent (**Scheme 5.5, B)**). Conversely, when a hydroxyl is replaced by a more electronegative fluorine atom, the rate of glycoside

[23] a) Timell, T. E. *Can. J. Chem.* **1964,** *42*, 1456-1472; b) O'Connor, J. V.; Barker, R. *Carbohydr. Res*. **1979**, *73*, 227-234.

[24] Franks, F.; Ravenhill, *J.* R.; Reid, D. S. *J. Solution Chem*. **1972**, *1*, 3-9; b) Mega, T.; Matsushima, Y. *J. Biochem.* **1983**, *94*, 1637-1647; c) Overend, W. G.; Rees, C. W.; Sequeria, J. S. *J. Am. Chem. Soc.* **1962**, *84*, 3429-3440.

hydrolysis decreases. Indeed, a study of the rates of hydrolysis of a series of deoxyfluoro glucosyl phosphates showed the exact inverse order, parent > 6-deoxyfluoro > 3-deoxyfluoro > 4-deoxyfluoro > 2-deoxyfluoro (**Scheme 5.5, B)**).[7]

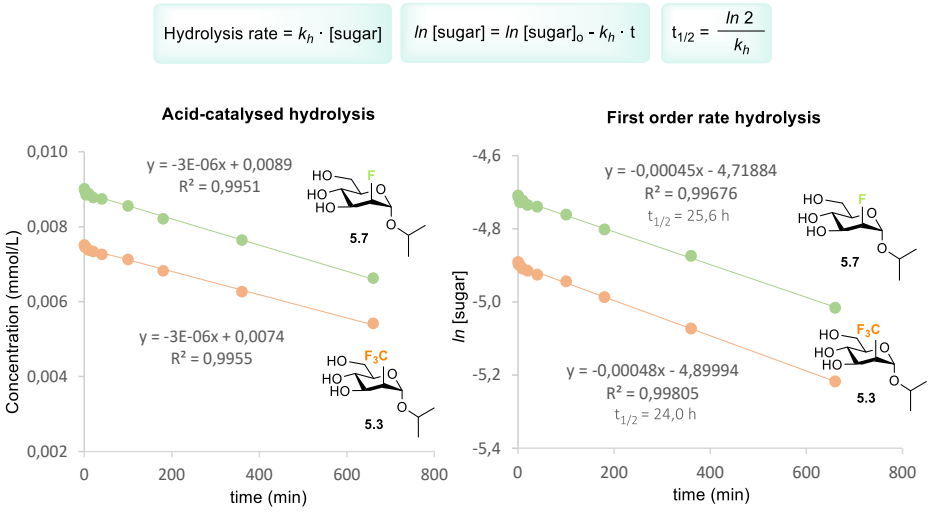


Scheme 5.5: A) General acid-catalysed hydrolysis mechanism for 2-substituted glycosides. B) Influence of the C-2 substituents on the hydrolysis rates.[7]

To further investigate the effect of the trifluoromethyl moiety in the relative rate of the glycoside hydrolysis we developed a new method for the determination of hydrolysis rates based on previous studies. The outcome of the hydrolysis reaction of fluorinated compounds could be easily followed by ^{19}F NMR and quantified by using an internal standard. Hydrolysis conditions employed were identical to those described previously.[25] Acid-catalysed hydrolysis was monitored by incubating a solution of the sugar [0.025 M] in 0.5 M of sulfuric acid at the required temperature (± 0.02 °C) in a sealed NMR tube and using trifluoroethanol (5 µL) as internal standard. At appropriate intervals, the tube was cooled at 0 °C and analysed for sugar hydrolysis by ^{19}F NMR.

[25] a) Timell, T. E. *Can. J. Chem.* **1964**, *42*, 1456-1472; b) Timell, T. E., Enterman, W., Spencer, F., Soltes, E. J. *Can. J. Chem.* **1965**, *43*, 2296-2305.

Following previous reports,[25] different temperatures were tested for the acid-catalysed hydrolysis of the corresponding fluorinated glycosides, however, these compounds probe to be stable under most acid media and no hydrolysed product was observed (sugar [0.025 M], H_2SO_4 [0.5 M] at 25 °C, 37 °C and 60 °C). Finally, hydrolysis product was observed subjecting the sugar [0.025 M] to H_2SO_4 [0.5 M] at 70 °C. Rate constants were determined from plots of $\ln[C_t]$ versus time, where C_t is the concentration of the starting material in mol/L at time t. All plots were clearly linear for each compound with excellent correlation coefficients ranging from 0.995 to 0.998 and comprising more than 10 data points in each case. Hydrolysis rates are generally first order or pseudo first order under most environmental conditions (where the pH is generally buffered) with an overall observed hydrolysis rate constant k_h. First-order rate-constants and half-life times for the acid-catalysed hydrolysis of trifluoromethyl pyranoside **5.3** and fluoropyranoside **5.7** are presented in **Scheme 5.6**. Both compounds presented similar hydrolysis rates, with a value of $4.8 \cdot 10^4$ min^{-1} for the trifluoromethyl glycoside **5.3** and $4.5 \cdot 10^4$ min^{-1} for the fluorinated compound **5.7**. Therefore, no big differences were either observed for the half-life times, which correspond to 24 h for compound **5.3** and 25 h for compound **5.7.**



Scheme 5.6. Hydrolysis rate determination under H_2SO_4 [0.5 M] at 70 °C.

5.4. CONCLUSIONS

In summary, we efficiently synthesised deprotected 2-deoxy-2-fluoro and 2-trifluoromethyl glycosides as probes to study and compare some important physicochemical parameters induced by the fluorinated motif. The conclusions that arise from these studies are:

The computational evaluation of 2-F and 2-CF_3 glycosides demonstrated that they maintain the $^4C_1$ chair conformation, and minimal distortion is observed for the sterically demanding CF_3 moiety. Differences between F and CF_3 were higher for *manno* glycosides, and dipole moments and dihedral angles presented higher variations. In contrast, *gluco* derivatives presented similar electronic and steric properties.

Results obtained for the lipophilicity determination showed that the trifluoromethyl moiety increases the lipophilicity more than the fluorine atom, and values differ from 0.65 to 0.85 log *P* units in *gluco/manno* derivates. The anomeric configuration had a slightly effect in lipophilicity, and 1,2-*cis* glycosides presented higher lipophilicities than the 1,2-*trans*-analogues. 2CF_3-glycosides are promising compounds for biological studies since they present positive log *P* values, which are close to optimal values for membrane permeability (log *P* = 1-3) and it supposes a great increment from the native sugar (log P_{glc} = -3.24).

Fluorinated and trifluoromethylated analogues proved to be stable under cytosol mimic conditions (PBS buffer pH 7.4) and lysosome mimic conditions (Acetate buffer pH 4.5). Moreover, acid-hydrolysis resistance was demonstrated by subjecting the fluorinated compounds to extreme acid conditions and following the progress by ^{19}F NMR. Sugars proved to be stable under H_2SO_4 [0.5 M] at 25 °C, 37 °C, and 60 °C, and finally, hydrolysis product was observed at 70 °C. Determined first-order hydrolysis rate-constants and half-life times for 2F- and 2-CF_3-glycosides were similar, indicating that F and CF_3 have similar stabilizing effect, and both improve the resistance to acid-catalysed hydrolysis.

5.5. EXPERIMENTAL SECTION

5.5.1. General considerations

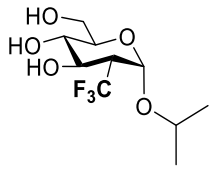
Proton (^{1}H NMR), carbon (^{13}C NMR) and fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (400 MHz for ^{1}H, 100.6 MHz for ^{13}C and 376.5 MHz for ^{19}F). Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^{1}H NMR: $CDCl_3$ = 7.26 and ^{13}C NMR: $CDCl_3$ = 77.00) and $CFCl_3$. Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent. Infrared (IR) spectra were recorded on a Jasco FT/IR-600 Plus ATR Specac Golden Gate spectrophotometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra (HRMS) were recorded on an Agilent 1100 Series LC/MSD mass spectrometer with electrospray ionization (ESI) by the Servei de Recursos Científics (URV). Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using commercial aluminum backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (max = 254 nm) and 6% H_2SO_4 in EtOH. Flash column chromatography was carried out using silica gel 60 A CC (230-400 mesh). Mobile phases are reported in relative composition (e.g. 1:1 EtOAc/hexane v/v). All solvents were used as supplied (Analytical, synthesis or HPLC grade), without prior purification. All reagents were used as received from commercial suppliers. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium sulphate (Na_2SO_4) were used as drying agents after reaction work-up, as indicated.

5.5.2. General deprotection procedure

To a solution of the peracetylated sugar (1 mmol) in MeOH (20 mL/mmol) was added MeONa (0.6 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The mixture was filtered to separate the Dowex® resin, washed with MeOH and then concentrated under vacuum. The crude was purified by flash column chromatography to give the desired deprotected product.

5.5.3. Synthesis of deprotected fluoro and trifluoromethyl glycosides

1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-α-D-glucopyranose (5.1)

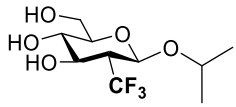


To a solution of **4.13α** (4.5 mg, 0.011 mmol) in MeOH (0.3 mL) was added MeONa (0.35 mg, 0.006 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The crude was filtered to separate the Dowex® resin, washed with MeOH and then concentrated under vacuum. The crude was purified by flash column chromatography (from CH_2Cl_2 to CH_2Cl_2:MeOH 9:1) to give the desired product **5.1** (3.0 mg) in quantitative yield.

Colourless needles. R_f (9:1, CH_2Cl_2:MeOH): 0.19; $[\alpha]^{D}_{25}$: + 80.5 (0.29, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.13 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 3.95 (dd, $J_{2,3}$ = 11.9 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 3.90 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.77 (ddd, $J_{6a,6b}$ = 11.6 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{6a,OH}$ = 2.1 Hz, 1H, H-6a), 3.67 (dd, $J_{6a,6b}$ = 11.6 Hz, $J_{5,6b}$ = 5.3 Hz, Hz, 1H, H-6b), 3.67-3.60 (m, 1H, H-5), 3.32-3.21 (m, 1H, H-4), 2.48 (dqd, $J_{2,3}$ = 11.8 Hz, $J_{2,CF3}$ = 8.4 Hz, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 1.19 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.12 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in

ppm: -65.48 (d, $J_{CF3,2}$ = 8.4 Hz, 3F, CF_3-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 125.2 (q, $J_{C1,F}$ = 279.8 Hz, CF_3), 93.4 (q, $J_{1,F}$ = 4.6 Hz, C-1), 72.5 (C-5), 71.0 (C-4), 69.9 (C-7), 68.0 (q, $J_{1,F}$ = 2.2 Hz, C-3), 61.2 (C-6), 52.2 (q, $J_{C2,F}$ = 23.9 Hz, C-2), 22.2 (C-8), 20.0 (C-8'); **FT–IR (neat)** ν in cm^{-1}: 3428, 3371, 3303, 2975, 2925, 1465, 1384, 1273, 1260, 1181, 1083, 1058, 1023, 982; **HRMS (TOF ES$^+$)** for $(2M+Na)^+$ $C_{20}H_{34}F_6NaO_{10}^+$ (m/z): calc. 571.1948; found 571.1947.

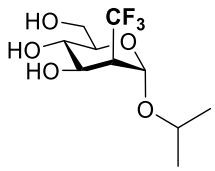
1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-β-D-glucopyranose (5.2)



To a solution of **4.13β** (18 mg, 0.045 mmol) in MeOH (0.9 mL) was added MeONa (1.5 mg, 0.027 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The crude was filtered to separate the Dowex® resin, washed with MeOH and then concentrated under vacuum. The crude was purified by flash column chromatography (from CH_2Cl_2 to CH_2Cl_2:MeOH 9:1) to give the desired product **5.2** (12 mg) in quantitative yield.

White powder. ***R$_f$*** (9:1, CH_2Cl_2:MeOH): 0.19; **[α]$^D_{25}$**: - 32.4 (0.57, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 4.69 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1), 3.95 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.84 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6a}$ = 2.0 Hz, 1H, H-6a), 3.69-3.60 (m, 2H, H-3, H-6b), 3.32-3.21 (m, 2H, H-4, H-5), 2.27 (dp, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = $J_{2,CF3}$ = 8.2 Hz, 1H, H-2), 1.19 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.12 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -65.16 (d, $J_{CF3,2}$ = 8.2 Hz, 3F, CF_3-β-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 125.6 (q, $J_{C1,F}$ = 281.6 Hz, CF_3), 93.4 (q, $J_{1,F}$ = 2.6 Hz, C-1), 76.0 (C-5), 71.2 (C-7), 70.75 (C-4), 70.7 (C-3), 61.3 (C-6), 52.2 (q, $J_{C2,F}$ = 22.2 Hz, C-2), 22.4 (C-8), 20.3 (C-8'); **FT–IR (neat)** ν in cm^{-1}: 3551, 3402, 3236, 2976, 2922, 1616, 1467, 1451, 1383, 1270, 1239, 1186, 1077, 1032, 1101, 930; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{10}H_{17}F_3NaO_5^+$ (m/z): calc. 297.0920; found 297.0916.

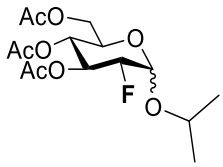
1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-α-D-mannopyranose (5.3)



To a solution of **4.14** (14.5 mg, 0.036 mmol) in MeOH (0.8 mL) was added MeONa (1.2 mg, 0.021 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The crude was filtered to separate the Dowex® resin, washed with MeOH and then concentrated under vacuum. The crude was purified by flash column chromatography (from CH_2Cl_2 to CH_2Cl_2:MeOH 9:1) to give the desired product **5.3** (9.5 mg) in quantitative yield.

Colourless syrup. R_f (9:1, CH_2Cl_2:MeOH): 0.25; $[\alpha]^D_{25}$: + 78.7 (0.72, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.25 (d, $J_{1,2}$ = 2.4 Hz, 1H, H-1), 4.10 (t, $J_{2,3}$ = $J_{3,4}$ = 5.6 Hz, 1H, H-3), 4.01 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.88-3.79 (m, 1H, H-6a), 3.72-3.62 (m, 3H, H-4, H-5, H-6b), 2.78 (qdd, $J_{2,CF3}$ = 10.4 Hz, $J_{2,3}$ = 5.4 Hz, $J_{1,2}$ = 2.4 Hz, 1H, H-2), 1.25 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.19 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -63.17 (d, $J_{CF3,2}$ = 10.3 Hz, 3F, CF_3-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 126.0 (q, $J_{C1,F}$ = 280.3 Hz, CF_3), 93.3 (q, $J_{1,F}$ = 13.1 Hz, C-1), 73.5 (C-5), 68.9 (C-7), 68.5 (C-3), 68.0 (C-4), 61.8 (C-6), 52.2 (q, $J_{C2,F}$ = 23.4 Hz, C-2), 22.1 (C-8), 20.0 (C-8'); **FT–IR (neat)** ν in cm^{-1}: 3363, 2975, 2933, 1456, 1384, 1332, 1267, 1169, 1123, 1103, 1077, 1040, 975; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{10}H_{17}F_3NaO_5^+$ (m/z): calc. 297.0920; found 297.0909.

1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-glucopyranose (5.4)



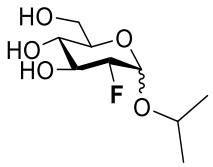
To a solution of 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **3.11-Br** (96.0 mg, 0.26 mmol) and 2-propanol (59.4 µL, 0.776 mmol) in dry toluene (2.6 mL) under an Argon atmosphere, were added 4 Å molecular sieves, allyl tributyltin (104.8 µL, 0.338 mmol) and TfOH (20 µL, 0.2 mmol). The mixture was stirred at 80°C for 2h. When the reaction has finished, the crude was diluted with EtOAc and washed with sat. aq. solution of $NaHCO_3$ and KF. The organic combined layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude was purified by flash column chromatography (from hexanes to 8:2 hexanes/EtOAc) to afford the product **5.4α/β** (77 mg, 85%) as an inseparable mixture of the α/β anomers (ratio α:β = 1:3.5).

Data for **5.4α:** Colourless syrup. R_f (8:2, hexanes:EtOAc): 0.16; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.49 (ddt, $J_{3,F}$ = 11.7 Hz, $J_{3,4}$ = $J_{2,3}$ = 9.5 Hz, 1H, H-3), 5.08 (dd, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.95 (t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.43 (ddd, $J_{2,F}$ = 49.7 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.10-3.95 (m, 3H, H-5, H-6a, H-6b), 3.90 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 2.04 (s, 3H, CH_3, Ac), 2.02 (s, 3H, CH_3, Ac), 2.00 (s, 3H, CH_3, Ac), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.18 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -200.87 (dd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 11.8 Hz, 1F, 2F-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.5, 170.0, 169.4 (3C=O, Ac), 94.6 (d, $J_{C1,F}$ = 20.5 Hz, C-1), 87.0 (d, $J_{C2,F}$ = 194.8 Hz, C-2), 71.9 (C-7), 70.8 (d, $J_{C3,F}$ = 19.4 Hz, C-3), 67.1 (C-4), 61.9 (C-6), 23.0 (C-8), 21.6 (C-8'), 20.7 (OAc), 20.6 (OAc), 20.5 (OAc).

Data for **5.4β:** Colourless syrup. R_f (8:2, hexanes:EtOAc): 0.16; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.27 (ddt, $J_{3,F}$ = 14.5 Hz, $J_{3,4}$ = $J_{2,3}$ = 9.3 Hz, 1H, H-3), 4.97 (t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.60 (dd, $J_{1,2}$ = 7.7 Hz, $J_{1,F}$ = 2.6 Hz, 1H, H-1), 4.67 (ddd, $J_{2,F}$ = 50.3 Hz, $J_{2,3}$ = 9.4 Hz, $J_{1,2}$ = 7.9 Hz, 1H, H-2), 4.22 (dd, $J_{6a,6b}$ = 12.2

Hz, $J_{5,6a}$ = 5.0 Hz, 1H, H-6a), 4.07 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-6b), 3.98 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.67 (ddd, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-5), 2.04 (s, 3H, CH_3, Ac), 2.03 (s, 3H, CH_3, Ac), 1.99 (s, 3H, $3CH_3$, Ac), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.18 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -199.42 (ddd, $J_{F,2}$ = 50.6 Hz, $J_{F,3}$ = 14.5 Hz, $J_{F,1}$ = 2.6 Hz, 1F, 2F-β-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.5, 170.0, 169.6 (3C=O, Ac), 98.9 (d, $J_{C1,F}$ = 22.5 Hz, C-1), 89.3 (d, $J_{C2,F}$ = 190.6 Hz, C-2), 72.9 (d, $J_{C3,F}$ = 19.9 Hz, C-3), 72.8 (C-7), 71.6 (C-5), 68.3 (d, $J_{C3,F}$ = 7.4 Hz, C-4), 62.0 (C-6), 23.2 (C-8), 21.8 (C-8'), 20.64 (OAc), 20.62 (OAc), 20.5 (OAc); spectroscopic data were identical to the previously reported.[26]

1-*O*-Isopropyl-2-deoxy-2-fluoro-α/β-D-glucopyranose (5.5)



To a solution of **5.4** (65.5 mg, 0.187 mmol) in MeOH (3.8 mL) was added MeONa (6.0 mg, 0.112 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The crude was filtered to separate the Dowex® resin, washed with MeOH and then concentrated under vacuum. The crude was purified by flash column chromatography (from CH_2Cl_2 to CH_2Cl_2:MeOH 9:1) to give the desired product **5.5** (50 mg) as an inseparable mixture of the α/β anomers (ratio α:β = 1:3.6) in quantitative yield.

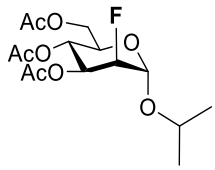
Data for **5.5α/β: FT–IR (neat)** ν in cm^{-1}: 3367, 2974, 2930, 1456, 1418, 1384, 1373, 1339, 1165, 1125, 1076, 1027; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_9H_{17}FNaO_5^+$ (m/z): calc. 247.0952; found 247.0946.

[26] Bucher, C.; Gilmour, R. *Angew. Chem. Int. Ed.* **2010,** *49*, 8724-8728.

Data for **5.5α:** Colourless syrup. R_f (9:1, CH_2Cl_2:MeOH): 0.18; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.12 (dd, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.21 (ddd, $J_{2,F}$ = 50.0 Hz, $J_{2,3}$ = 9.5 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 3.98 (m, 1H, H-7), 3.91-3.76 (m, 3H, H-3, H-6a), 3.73-3.51 (m, 1H, H-5, H-6b), 3.38-3.29 (m, 1H, H-4), 1.27 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.20 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -202.35 (dd, $J_{F,2}$ = 50.0 Hz, $J_{F,3}$ = 12.8 Hz, 1F, 2F-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 94.6 (d, $J_{C1,F}$ = 21.0 Hz, C-1), 89.9 (d, $J_{C2,F}$ = 189.4 Hz, C-2), 71.9 (C-5), 71.9 (d, $J_{C3,F}$ = 17.2 Hz, C-3), 70.1 (C-7), 70.0 (d, $J_{C3,F}$ = 7.4 Hz, C-4), 61.0 (C-6), 22.2 (C-8), 20.4 (C-8').

Data for **5.5β:** Colourless syrup. R_f (9:1, CH_2Cl_2:MeOH): 0.16; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 4.63 (dd, $J_{1,2}$ = 7.7 Hz, $J_{1,F}$ = 2.5 Hz, 1H, H-1), 4.08 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.91 (ddd, $J_{2,F}$ = 51.3 Hz, $J_{2,3}$ = 8.9 Hz, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 3.91-3.87 (m, 1H, H-6a), 3.69 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 5.3 Hz, 1H, H-6b), 3.62 (dt, $J_{3,F}$ = 15.9 Hz, $J_{2,3}$ = $J_{2,3}$ = 8.9 Hz, 1H, H-3), 3.38-3.29 (m, 2H, H-4, H-5), 1.26 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.21 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -200.41 (ddd, $J_{F,2}$ = 51.3 Hz, $J_{F,3}$ = 15.9 Hz, $J_{F,1}$ = 2.5 Hz, 1F, 2F-β-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 98.5 (d, $J_{C1,F}$ = 22.7 Hz, C-1), 92.1 (d, $J_{C2,F}$ = 185.5 Hz, C-2), 72.8 (C-5), 75.1 (d, $J_{C3,F}$ = 17.5 Hz, C-3), 71.6 (C-7), 69.9 (d, $J_{C3,F}$ = 7.8 Hz, C-4), 61.1 (C-6), 22.3 (C-8), 20.7 (C-8').

1-*O*-Isopropyl-2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-mannopyranose (5.6)

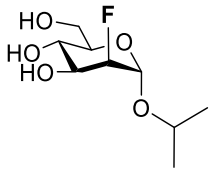


To a solution of 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α-D-manno-pyranosyl bromide **3.12-Br** (33.6 mg, 0.09 mmol) and 2-propanol (13.8 µL, 0.18 mmol) in dry toluene (1.4 mL) under an Argon atmosphere, were added 4 Å molecular sieves, allyl tributyltin (36.6 µL, 0.118 mmol) and TfOH (8.1 µL, 0.09 mmol). The mixture was stirred at 80°C for 2h. When the reaction has finished, the crude was diluted with EtOAc and washed with sat. aq. solution of $NaHCO_3$

and KF. The organic combined layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude was purified by flash column chromatography (from hexanes to 8:2 hexanes/EtOAc) to afford **5.6** (25.8 mg, 82%) as colourless syrup.

Colourless syrup. R_f (8:2, hexanes:EtOAc): 0.29; $[\alpha]^{D}_{25}$: + 73.0 (1.29, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.35-5.28 (m, 1H, H-4), 5.23 (ddd, $J_{3,F}$ = 29.6 Hz, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 2.5 Hz, 1H, H-3), 5.08 (dd, $J_{1,F}$ = 7.4 Hz, $J_{1,2}$ = 1.8 Hz, 1H, H-1), 4.67 (dt, $J_{2,F}$ = 50.3 Hz, $J_{1,2}$ = $J_{2,3}$ = 2.1 Hz, 1H, H-2), 4.26 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 4.9 Hz, 1H, H-6a), 4.10 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-6b), 4.04 (ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-5), 3.95 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 2.08 (s, 6H, 2xCH_3, Ac), 2.04 (s, 3H, CH_3, Ac), 1.24 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.18 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -203.29 (ddd, $J_{F,2}$ = 50.2 Hz, $J_{F,3}$ = 29.6 Hz, $J_{F,1}$ = 7.3 Hz, 1F, F-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 170.8, 170.2, 169.5 (3C=O, Ac), 95.5 (d, $J_{C1,F}$ = 29.0 Hz, C-1), 87.5 (d, $J_{C2,F}$ = 179.7 Hz, C-2), 71.0 (C-7), 70.0 (d, $J_{C3,F}$ = 29.0 Hz, C-3), 68.4 (C-5), 66.0 (C-4), 62.2 (C-6), 23-0 (C-8), 21.4 (C-8'), 20.8 (OAc), 20.7 (OAc), 20.6 (OAc); **FT–IR (neat)** ν in cm^{-1}: 2974, 2932, 1744, 1455, 1435, 1370, 1224, 1163, 1133, 1089, 1048, 979; **HRMS (TOF ES$^+$)** for $(M+NH_4)^+$ $C_{15}H_{27}FNO_8^+$ (m/z): calc. 368.1715; found 368.1723. Spectroscopic data were identical to the previously reported.[26]

1-*O*-Isopropyl-2-deoxy-2-fluoro-α-D-mannopyranose (5.7)



To a solution of **5.6** (25.8 mg, 0.073 mmol) in MeOH (1.5 mL) was added MeONa (1.2 mg, 0.021 mmol) and the reaction was stirred at room temperature for 6 hours. After complete consumption of the starting material, Dowex® 50WX2 50-100 (H^+) was added and the mixture stirred for 30 minutes. The crude was filtered to separate the Dowex® resin, washed with MeOH and

then concentrated under vacuum. The crude was purified by flash column chromatography (from CH_2Cl_2 to CH_2Cl_2:MeOH 9:1) to give the desired product **5.7** (19.5 mg) in quantitative yield.

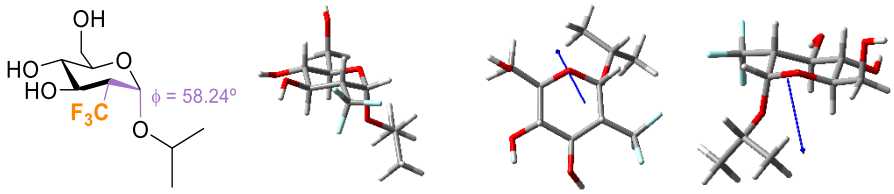
Colourless syrup. R_f (9:1, CH_2Cl_2:MeOH): 0.21; $[\alpha]^D_{25}$: + 87.1 (0.80, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.04 (dd, $J_{1,F}$ = 7.8 Hz, $J_{1,2}$ = 1.9 Hz, 1H, H-1), 4.49 (ddd, $J_{2,F}$ = 50.1 Hz, $J_{2,3}$ = 2.6 Hz, $J_{1,2}$ = 2.0 Hz, 1H, H-2), 3.99 (hept., $J_{7,8}$ = 6.2 Hz, 1H, H-7), 3.82 (dd, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 1.6 Hz, 1H, H-6a), 3.71 (ddd, $J_{3,F}$ = 30.8 Hz, $J_{3,4}$ = 9.4 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-3), 3.68 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 5.4 Hz, 1H, H-6b), 3.63-3.55 (m, 2H, H-4, H-5), 1.21 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8), 1.15 (d, $J_{7,8}$ = 6.2 Hz, 3H, CH_3-8'); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -205.84 (ddd, $J_{F,2}$ = 50.1 Hz, $J_{F,3}$ = 30.9 Hz, $J_{F,1}$ = 7.8 Hz, 1F, F-α-anomer); **^{13}C NMR** (100.6 MHz, $CDCl_3$) δ in ppm: 95.4 (d, $J_{C1,F}$ = 29.5 Hz, C-1), 87.5 (d, $J_{C2,F}$ = 175.6 Hz, C-2), 73.3 (C-5), 70.3 (d, $J_{C3,F}$ = 17.4 Hz, C-3), 69.3 (C-7), 67.4 (C-4), 61.3 (C-6), 22.2 (C-8), 20.1 (C-8'); **FT–IR (neat)** ν in cm^{-1}: 3378, 2972, 2928, 1456, 1381, 1312, 1114, 1064, 972; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_9H_{17}FNaO_5^+$ (m/z): calc. 247.0952; found 247.0948.

5.5.4. Electrostatic potential surface calculation

DFT calculations were performed using Gaussian 09 software.[27] Geometry optimization was conducted at the CPCM (water) B3LYP/6-311+G(d,p) level of theory. Frequencies were calculated at the same level of theory and used to verify the nature of all stationary points as minima.

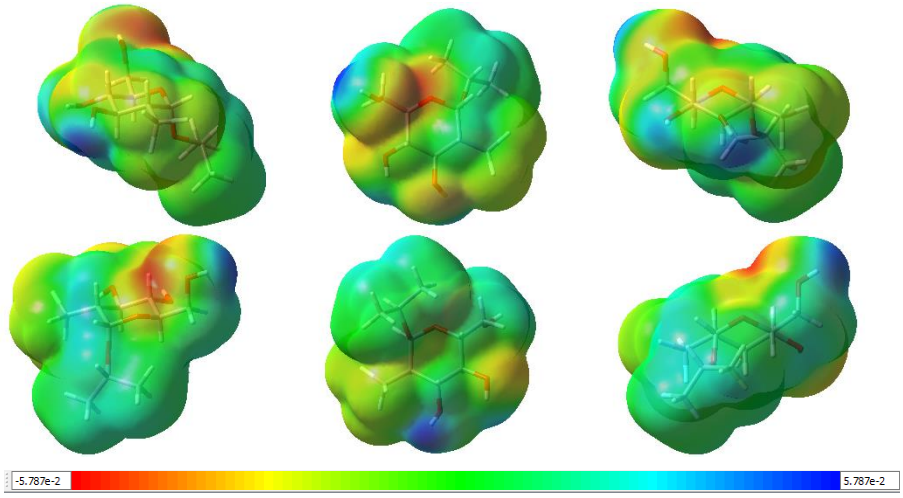
1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-α-D-glucopyranose (5.1)

A) Optimized conformation: **B) Dipole moment calculation:**



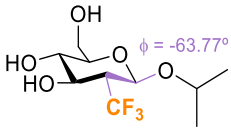
[27] Chalmers, A. A.; Hall, R. H. *J. Chem. Soc. Perkin Trans. 2*, **1974**, 728-732.

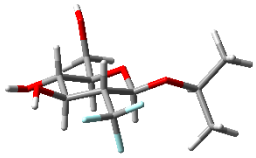
C) Potential energy surfaces:



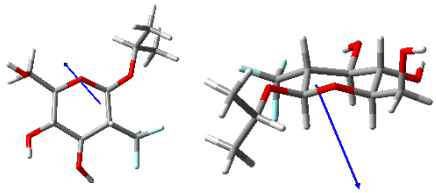
1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-β-D-glucopyranose (5.2)

A) Optimized conformation:

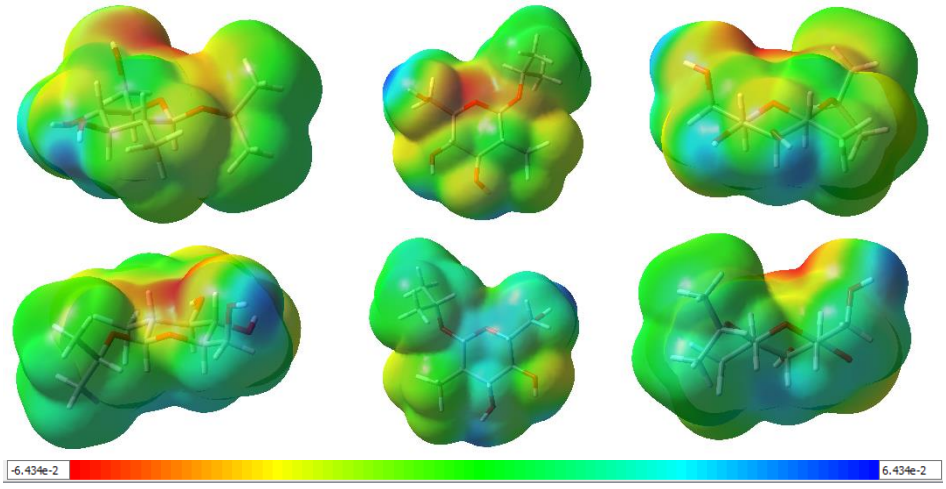




B) Dipole moment calculation:

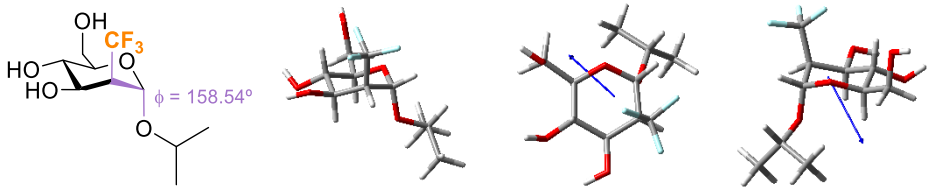


C) Potential energy surfaces:

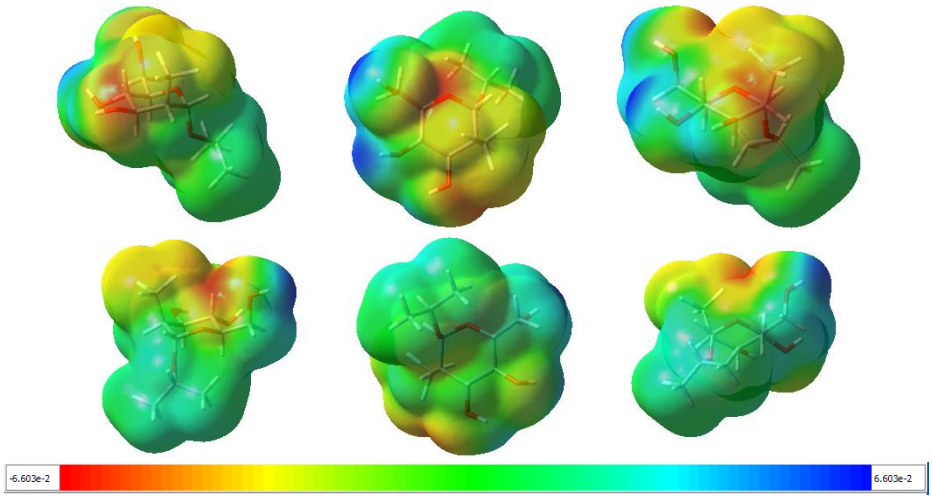


1-*O*-Isopropyl-2-deoxy-2-trifluoromethyl-α-D-mannopyranose (5.3)

A) Optimized conformation: **B) Dipole moment calculation:**

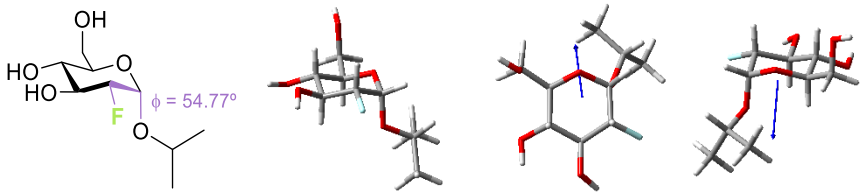


C) Potential energy surfaces:

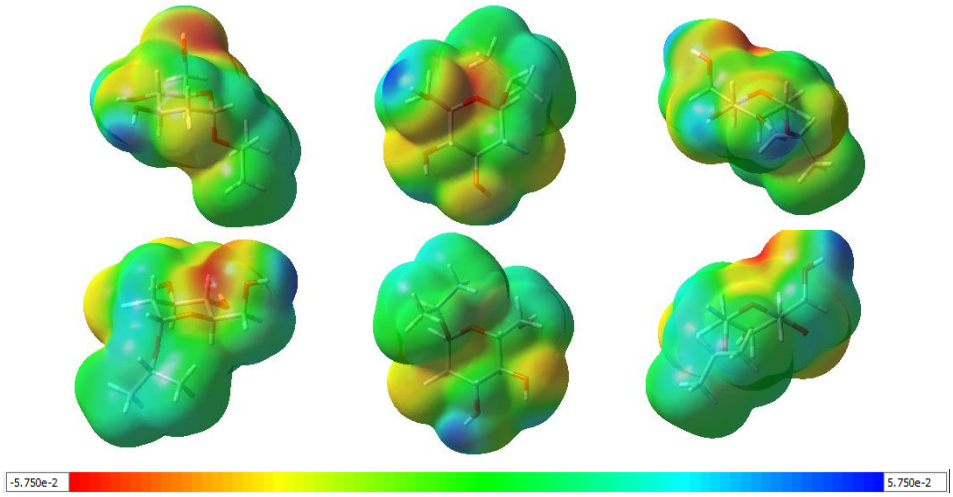


1-*O*-Isopropyl-2-deoxy-2-fluoro-α-D-glucopyranose (5.5α)

A) Optimized conformation: **B) Dipole moment calculation:**

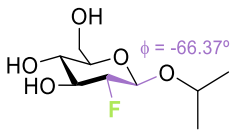


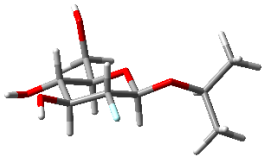
C) Potential energy surfaces:



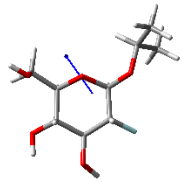
1-*O*-Isopropyl-2-deoxy-2-fluoro-β-D-glucopyranose (5.5β)

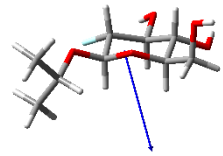
A) Optimized conformation:



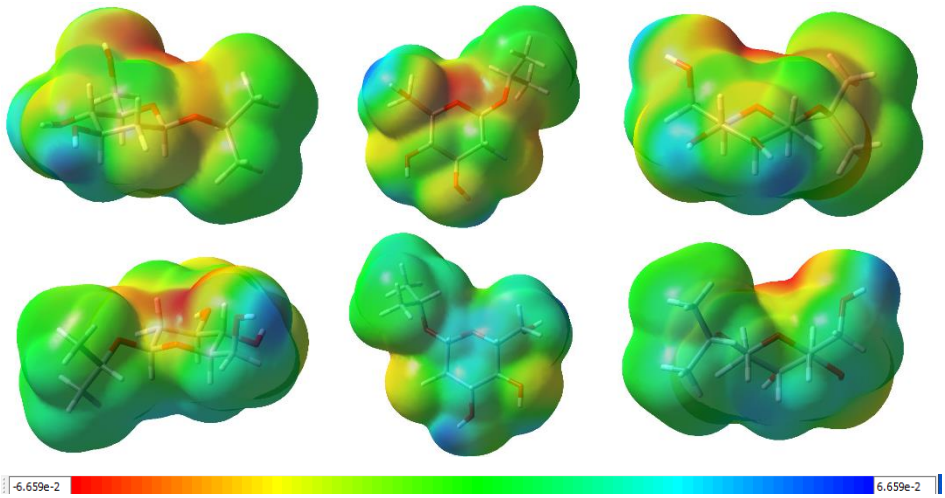


B) Dipole moment calculation:



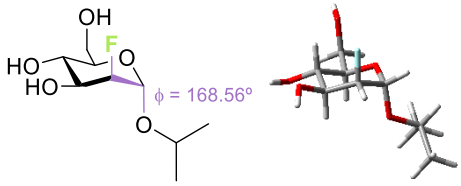


C) Potential energy surfaces:

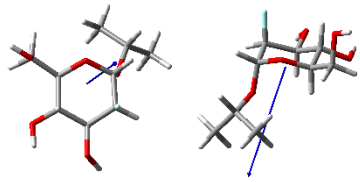


1-*O*-Isopropyl-2-deoxy-2-fluoro-α-D-mannopyranose (5.7)

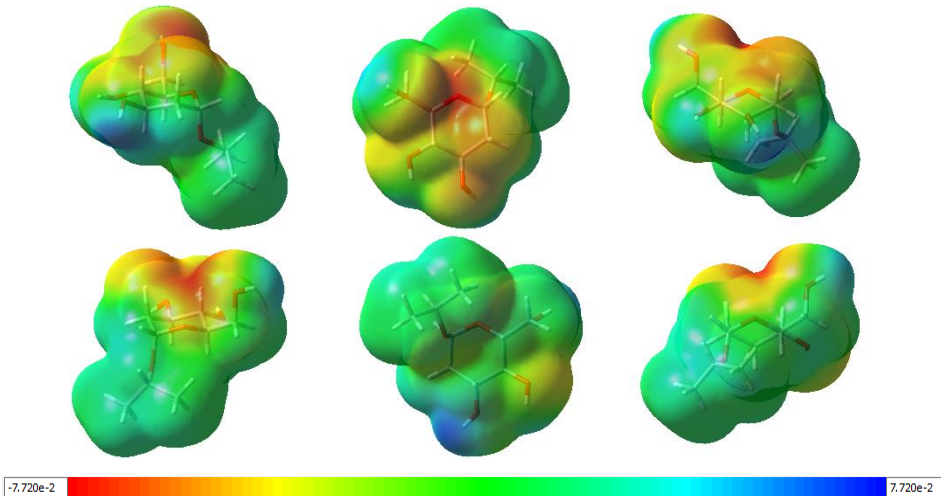
A) Optimized conformation:



B) Dipole moment calculation:



C) Potential energy surfaces:



5.5.5. Lipophilicity measurement protocol

The detailed protocol was obtained from the new "shake flask method" developed by the group of Linclau.[21] The process includes 4 steps, and it was repeated duplicate for each compound X:

a. Partitioning:

To a 10 mL pear-shaped flask was added 2 mL of freshly distilled 1-octanol, compound X (1-10 mg), the reference compound (TFE) and 2 mL of Milli-Q® water. The resulting mixture was stirred at 25 °C for 2 hours controlling the temperature by an immersion cooler, then it was left to stand at 25 °C overnight to enable complete phase separation.

b. Sample preparation:

Using 1 mL disposable syringe an aliquot of 0.5-0.6 mL was carefully taken from the upper 1-octanol phase. When taking an aliquot of the lower water phase, to avoid the contamination of the syringe with the upper octanol phase, 0.05 mL of air was taken into syringe before putting the needle into the solution, and while moving through the upper octanol layer, the air was gently pushed out. Upon reaching the water phase, all air bubbles are pushed out of the syringe, the aliquot takes, and the needle quickly removed from the solution. Then a small amount of water phase was discarded (to ensure all traces of octanol are out of the needle, leaving 0.6 mL sample in the syringe. For each aliquot the needle was carefully wiped with dry tissue and then an aliquot of 0.5 mL of sample was injected into the new NMR tube, followed by addition of 0.1 mL acetone-d^6. The NMR tubes were sealed using a rubber septum stopper and inverted carefully for 20 times to obtain a homogenous solution for NMR measurement.

c. NMR measurement

Fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (376.5 MHz for ^{19}F NMR). Parameters used in the determination of lipophilicities were obtained from the experiments carried out by Linclau and co-workers. Using our robot, it was not possible to carried out ^{19}F with proton decoupling (^{19}F {^{1}H} NMR), so the spectra was recorded using ^{19}F NMR with proton coupling. Firstly, the sealed tube was inserted to the NMR spectrometer and following automatic locking and gradient shimming, a simple ^{19}F spectrum was recorded on the non-spinning sample to assess the required spectral width (SW) and frequency offset point (O1P). The O1P is centered between the two diagnostic F signals and the spectral width (SW) is left at 200 ppm (it can be reduced if better S/N ratio is required (should be >250)). Then, the 90° pulse was measured with the automated pulsecal routine obtaining a power (PW) of 16 µs. The measured 90° pulse, SW and O1P were transferred into the inversion-recovery experiment. For practical purposes they recommended to use a D1 of

30 sec for the octanol sample and of 60 sec for the water sample as pulse delay, given the D1 value should be greater than 5*T1 for quantitative integration. The number of transients (NS) is selected or each sample to afford a suitable signal:noise for the calculation (the SNR was measured in the robot while acquiring the ^{19}F NMR spectra and stopped when obtaining SNR > 250).

d. Data processing:

Data were processed using a MestRe Nova NMR software. The obtained FID file was reprocessed using following conditions: WFunction (LB = 2, Exponential), Zero Filling (increasing points from 65536 to 262144) and then Fourier transform, followed by phasing with mouse and auto baseline correction. The integration ratio was obtained by manual integration (bias correction can be applied via adjusting tilt and slope if integral curve is not parallel to the baseline).

5.5.6. Preparation of buffered solutions

PBS buffer (pH 7.4): To prepare a PBS buffer solution [0.15M] with pH = 7.4, 200 mg of NaCl, 5 mg of KCl, 36 mg of Na_2HPO_4 and 6 mg of KH_2PO_4 were added to a flask containing 25 mL of Milli-Q® water. The mixture was stirred for 15 min and then the pH was checked using pH-metro. If needed, HCl 1M and NaOH 1M were used to adjust the pH to 7.4.

Acetate buffer (pH 4.5): To prepare an Acetate buffer solution [0.1M] with pH = 4.5, 74 mg of NaOAc and 61.8 µL of AcOH were added to a flask containing 20 mL of Milli-Q® water. The mixture was stirred for 15 min and then the pH was checked using pH-metro. If needed, HCl 1M and NaOH 1M were used to adjust the pH to 4.5.

5.5.7. Procedure for study the stability under intracellular physiological conditions

To study the stability of the compounds under the intracellular mimicked conditions, fluorinated sugars were incubated at 37 °C in the corresponding buffers and monitored by ^{19}F NMR. To prepare the samples, the

sugar (1-5 mg) was dissolved in 0.6 mL of aqueous buffer and transferred to a NMR tube, then 5 µL of the standard pattern (TFE) were added, the NMR tube was closed using a rubber septum and mixed vigorously. The sample was incubated at 37 °C and monitored by ^{19}F NMR at determined times.

5.5.8. Hydrolysis rate determination

Samples for the acid-catalysed hydrolysis rate determination were prepared by adding the sugar (1-5 mg, 0.025 M), trifluoroethanol (5 µL) as standard pattern and 0.5 M of sulfuric acid into a sealed NMR tube at 0°C. The mixture was vigorously mixed, and the initial concentration of the sugar was determined by ^{19}F NMR. Then, NMR tubes were heated at 70 °C (±0.02) and at appropriate intervals, the tubes were cooled at 0 °C and analysed for fluorinated sugar hydrolysis by ^{19}F NMR. To determine the acid-catalysed hydrolysis rate of the compounds the results were analysed and adjusted to a first-order plot. Rate constants k_h were obtained from plots of $\ln[C_t]$ versus time, where C_t is the concentration of the starting material in mol/L at time t. Half-life $t_{1/2}$ times were determined as $t_{1/2} = \ln 2/k_h$.

Chapter V

CHAPTER VI

STEREOSELECTIVE SYNTHESIS OF FLUORINATED AND TRIFLUOROMETHYLATED TN-ANTIGEN ANALOGUES

Chapter VI

6.1. INTRODUCTION

For many years biological information storage and transfer seemed to only depend on nucleic acids and proteins. In contrast, sugar molecules have been exclusively related to building blocks of protective cell wall substituents or as biological fuel in metabolism. This idea has been questioned over the years and the developments in carbohydrate synthesis and understanding of glycan-protein interactions have led to consider carbohydrates as important and versatile molecules in biorecognition and signalization processes. Their natural occurrence, abundant presence at cell surface and extracellular matrix and structural complexity, makes carbohydrates a versatile platform for bioinformation encoding, described by the term "sugar code".[1] The human glycome is composed primarily by only 10 building blocks (**Figure 6.1**) that can adopt different molecular forms, such as, glycoproteins, glycolipids, or proteoglycans.

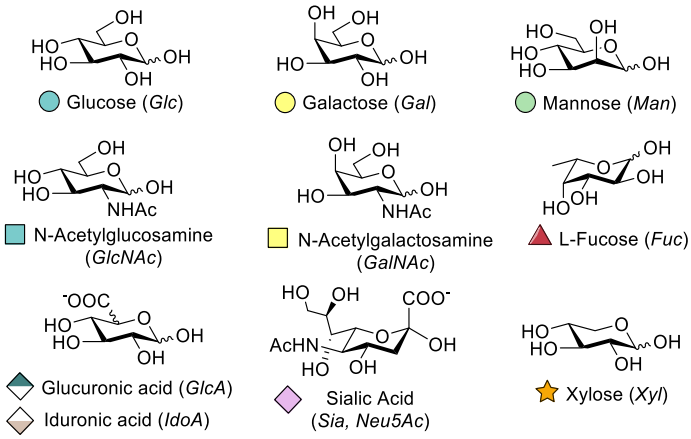


Figure 6.1. Major sugars comprising the human glycome and symbol representations. A colour-symbol nomenclature has been agreed in the field.

[1] a) Gabius, H. J.; Siebert, H. C.; André, S.; Jiménez-Barbero, J.; Rüdiger, H. *ChemBioChem* **2004**, *5*, 740-764; b) The Sugar Code. Fundamentals of glycosciences, H. J. Gabius, ed.: Wiley-VCH, Weinheim, Gemany, **2009**; c) Gabius, H. J. *Biosystems* **2018**, *164*, 102-111; d) Kaltner, H.; Abad-Rodríguez, J.; Corfield, A. P.; Kopitz, J.; Gabius, H. J. *Biochem. J.* **2019**, *476*, 2623-2655; e) Bernardi, A.; Sattin, S. *Eur. J. Org. Chem.* **2020**, *2020*, 4652-4663.

Their wide diversity is explained by the many assembly possibilities of monosaccharide building blocks, which have various positions on the pyranose or furanose rings. Additionally, the structural complexity is increased by the two possible configurations of the anomeric centre, α and β isomers, by the linear or branched linkages or the presence of other functional groups (such as ethers, sulphates or phosphates).[2] The high-density encoding system is essential to allow cells to communicate efficiently and assure high selectivity in protein-ligand interactions. Many fundamental biological processes are controlled by sugar-mediated information, such as, modulation of cell adhesion, protein folding control, intra and extracellular trafficking of glycoconjugates, cell-signalling, host defence pathways,[3] inflammation,[4] immune regulation,[5] and tumour progression.[6] The complexity of glycans, their enormous potential in information coding and their involvement in important biological processes has led to consider oligosaccharides as functional active units for the rational design of therapeutics and the identification of new targets in drug design.

Glycosylation is one the most diverse and major posttranslational modifications of glycoproteins. Focusing on the sugar-amino acid linkage, it could be classified in two major types: *N*-glycosylation (Asn-linked) and *O*-glycosylation (Ser/Thr-linked). *N*-linked glycans are attached to protein asparagine residues and are invariably branched structures that display varied termini. *O*-linked glycans are attached to protein serine or threonine residues and typically consist of shorter branched or unbranched structures (**Figure 6.2, A**).

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[2] Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C. W.; Seeberger, P. H. *ACS Chem. Biol*. **2007**, *2*, 685-691.

[3] Imberty, A.; Chabre, Y. M.; Roy, R. *Chem. Eur. J.* **2008**, *14*, 7490-7499.

[4] Schnaar, R. L. *J. Leukoc. Biol.* **2016**, *99*, 825-838.

[5] Meiers, *J*.; Siebs, E.; Zahorska, E.; Titz, A. *Curr. Opin. Chem. Biol.* **2019**, *53*, 51-67.

[6] a) Gorelik, E.; Galili, U.; Raz, A. *Cancer Metastasis Rev.* **2001**, *20,* 245-277; b) Ghazarian, H.; Idoni, B.; Oppenheimer, S. B. *Acta Histochem.* **2011**, *113*, 236-247; c) Scott, E.; Elliott, D. *J*.; Munkley, J. *Clin. Chim. Acta* **2020**, *502*, 167-173; d) Beckwith, D. M.; Cudic, M. *Semin. Immunol.* **2020**, *47*, 101389.

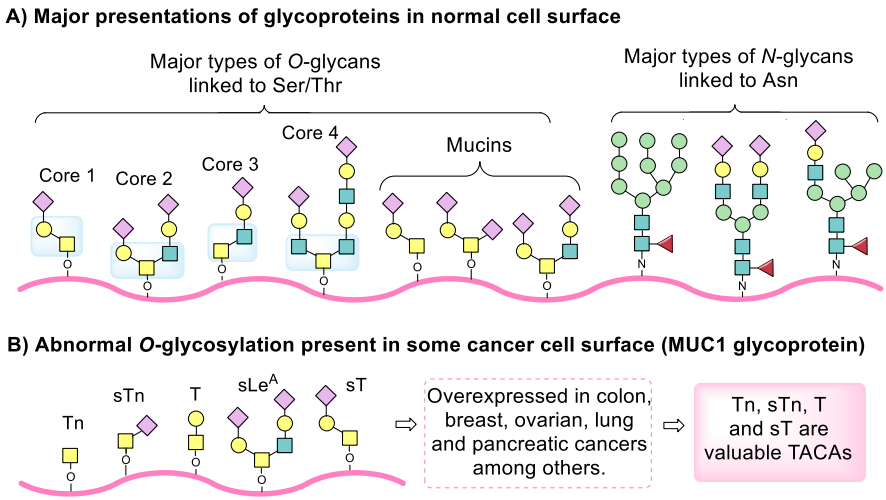

Figure 6.2. A) Representation of the major types of glycoproteins in normal cell surface, including *O*-linked glycans (left) and *N*-linked glycans (right). B) Representation of abnormal *O*-glycosylation occurring on Mucin 1 glycoproteins on some cancer cell surface. TACAs: tumour-associated carbohydrate antigens.

Aberrant glycosylation is one of the main factors involved in carcinogenesis, and abnormal glycosidic patterns are displayed on the surface of some cancer cells. Common anomalies include glycan truncation and density changes of glycan epitopes. A common irregularity occurring in adenocarcinomas is the over-simplification of the glycosylation pattern of mucins. The overexpression of mucins and truncation of their glycosylated branches are associated with malignant diseases.[7] Among the 21 human mucins, MUC1 is the most studied and most widely used as a tumoral marker.[8] The tumour-associated MUC1 exposes specific and structurally simple mono- and disaccharide antigens (Tn, sTn, T, sLe^A, sT) to the immune system (**Figure 6.2, B**). These antigens are expressed in aggressive cancers like breast, pancreas, and colon cancers, but are

[7] Hollingsworth, M. A.; Swanson, B. J. *Nat. Rev. Cancer* **2004**, *4*, 45-60.

[8] a) Tarp, M. A.; Clausen, H. *Biochim. Biophys. Acta* **2008**, *1780*, 546-563; b) Taylor-Papadimitriou, J.; Burchell, J.; Miles, D. W.; Dalziel, M. *Biochim. Biophys. Acta* **1999**, *1455*, 301-313; c) Guillen-Poza, P.A.; Sánchez-Fernández, E. M.; Artigas, G.; García Fernández, J. M.; Hinou, H.; Ortiz Mellet, C.; Nishimura, S. I.; Garcia-Martin, F. *J. Med. Chem.* **2020**, *63*, 8524-8533.

almost absent in normal cells.[9] These considerations make these structures valuable Tumour-Associated Carbohydrate Antigens (TACAs).[10] TACAs are promising candidates for the design of anticancer vaccines.[11] Moreover, many TACAs are secreted into the serum by tumoral cells, making them suitable targets for both early diagnostic and cancer vaccines. The great advantage offered by cancer immunotherapy over other classical therapies (i.e., surgery, chemotherapy, and radiation) has promoted the design of tumour-associated antigen molecules that generate a specific response against cancer.[12]

The Tn antigen (α-*O*-*Gal*NAc-Ser/Thr) is one of the most specific human tumour-associated structures,[13] and it is expressed in approximately 90% of carcinomas, showing a direct correlation between the aggressiveness of the cancer and the occurrence of the Tn antigen.[14] The premature detection of the Tn antigen is essential to treat and eradicate tumours. In this regard, it has been largely used as cancer biomarker and it has been recognized by antibodies and lectins.[15] It is also considered as an ideal immunotherapy target because it is masked on normal cells and overexpressed on cancer cells. However, it presents some disadvantages, mainly related to the pharmacokinetic drawbacks associated with carbohydrates, which are the low immunogenicity and reduced metabolic stability. Carbohydrates alone can only induce poor immunogenicity because they are unable to produce powerful T-cell-dependent immune responses, which are critical for cancer immunotherapy. Moreover, due to their low metabolic stability, low solubility, and fast excretion, it is difficult to elicit an effective and long-lasting response (**Figure 6.3**).[16]

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[9] Jin, K. T.; Lan, H. R.; Chen, X. Y.; Wang, S. B.; Ying, X. J.; Lin, Y.; Mou, X. Z. *Biotechnol. Lett.* **2019**, *41*, 641-650.
[10] Beatson, R. E.; Taylor-Papadimitriou, J.; Burchell, J. M. *Immunotherapy* **2010**, *2*, 305-327.
[11] Morelli, L.; Poletti, L.; Lay, L. *J. Org. Chem.* **2011**, *2011*, 5723-5777.
[12] Buskas, T.; Thompson, P.; Boons, G. J. *Chem. Commun.* **2009**, 5335-5349.
[13] Ju, T.; Otto, V. I.; Cummings, R. D. *Angew. Chem., Int. Ed.* **2011**, *50*, 1770-1791.
[14] Springer, G. F. *J. Mol. Med.* **1997**, *75*, 594-602.
[15] Madariaga, D.; Martinez-Saez, N.; Somovilla, V. J.; Coelho, H.; Valero-González, J.; Castro-López, J.; Asensio, J. L.; Jiménez-Barbero, J.; Busco, J. H.; Avenoza, A.; Marcelo, F.; Hurtado-Guerrero, R.; Corzana, F.; Peregrina, J. M. *ACS Chem. Biol.* **2015**, *10*, 747-756.
[16] Nativi, C.; Papi, F.; Roelens, S. *Chem. Commun.* **2019**, *55*, 7729-7736

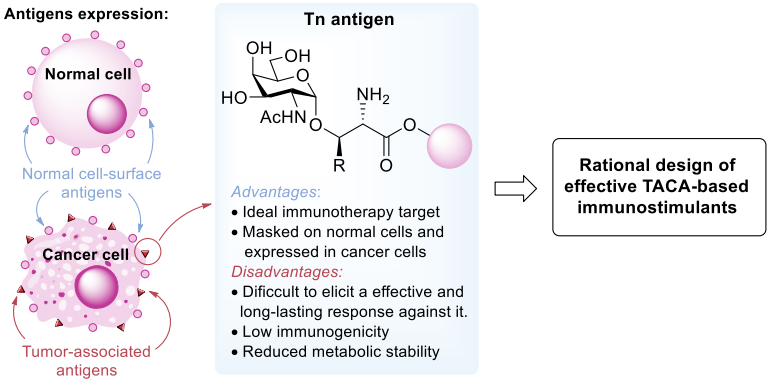

Figure 6.3. Representation of the antigens present in the surface of normal and cancer cells. Among the TACAs, the Tn antigen present some disadvantages that could be overcame with a rational design of effective analogues.

In this context, three main strategies have been used to overcome the drawbacks of the Tn antigen and other TACAs: 1) couple TACAs to immunogenic carriers to improve its immunogenicity (clustered or multivalent conjugate vaccines), 2) couple TACAs to T-cell peptide epitopes or adjuvants (multicomponent glycoconjugate vaccines),[12] and 3) develop synthetic vaccines based on chemically modified TACAs to improve their pharmacokinetic properties.[17] Focusing on the last strategy, the low metabolic stability of TACA-based vaccines is attributed to the glycosidic linkage, which is cleaved *in vivo* by glycosidases or acid media, reducing its bioavailability and, therefore, its immune response. To overcome this problem, recent studies proposed the design of hydrolytic-resistant TACA analogues or mimetics. Moreover, natural TACAs are innate in the human body, which could cause immunotolerance and immunosuppression, reducing the immunogenic response. In contrast, unnatural TACA mimics may be more immunogenic and could generate a robust antibody cross-reactive response to the natural TACA antigens. The potential of the Tn antigen as a therapeutic target has been widely investigated, either in the native form or in related structures (**Figure 6.4**).[16]

[17] Feng, D.; Shaikh, A. S.; Wang, F. *ACS Chem. Biol.* **2016**, *11*, 850-863.

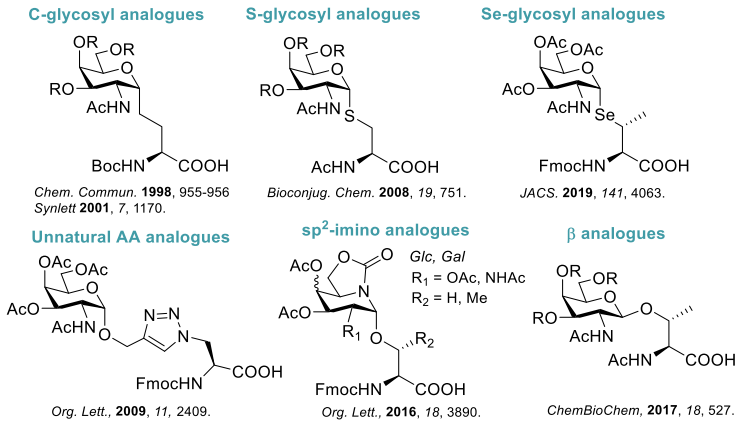

Figure 6.4. Examples of synthetic Tn antigen analogues.

Among them, fluorinated Tn antigen analogues have been widely investigated, since deoxyfluorination increases the metabolic stability and the lipophilicity and has proved to improve the immunogenicity of sTn antigen analogues.[18] Of great importance is the work developed by Hoffman-Röder and co-workers, who studied the effect of fluorine introduction in the immunological and metabolic properties of mucin glycopeptides (**Figure 6.5**). These building blocks were easily applied to the synthesis of F-analogues of MUC1 tandem repeat-glycopeptide antigens via SPPS (solid-phase peptide synthesis).

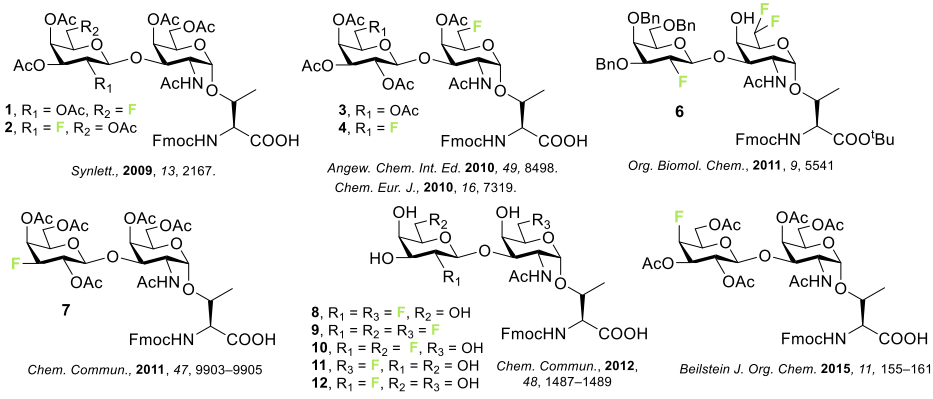

Figure 6.5. Examples of fluorinated Tn antigen analogues.

[18] Yang, F.; Zheng, X. J.; Huo, C. X.; Wang, Y.; Zhang, Y.; Ye, X. S. *ACS Chem. Biol.* **2011**, *6*, 252-259.

In addition to the poor stability and immunogenicity of the Tn antigen, another drawback is its difficult obtention in high amounts, which limits its use as therapeutic target. With these considerations, we hypothesise that the introduction of a fluorinated motif in the C-2 of the sugar moiety of the Tn-antigen will affect its pharmacokinetic properties, by increasing the metabolic stability and lipophilicity, and could have an important effect in immunogenicity. The resulting glycopeptides could be easily employed in the synthesis of F-analogues of MUC1 tandem-repeat glycopeptide antigens via SPPS and could be used to elucidate the effects of the fluorinated motif on metabolic and immunological properties.

6.2. OBJECTIVES

The general aim in this chapter is to synthetize fluorinated and trifluoromethylated analogues of the Tn antigen. To this end, two objectives are addressed: 1) The stereoselective synthesis of 2-deoxy-2-fluoro-α-D-glycopeptides analogues of the Tn antigen applying the method described in **Chapter III** and; 2) the stereoselective synthesis of 2-deoxy-2-CF_3-α-D-glycopeptides analogues of the Tn antigen applying the method described in **Chapter IV** (**Figure 6.6**).

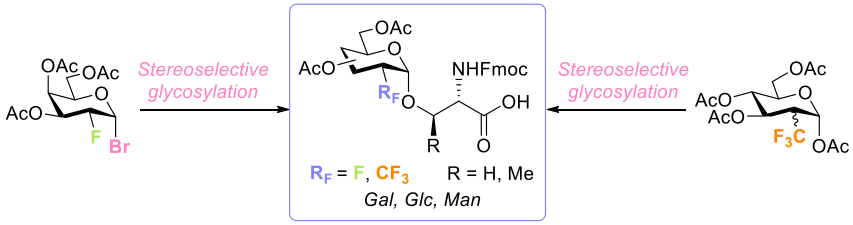

Figure 6.6. Proposed synthesis of the fluorinated and trifluoromethylated analogues of the Tn antigen.

6.3. RESULTS AND DISCUSSION

The obtention of glycopeptides by enzymatic synthesis has been tough due to the problematic control of the enzymatic activities of different glycosyltransferases that lead to a heterogeneous mixture of glycoforms, which are generally inseparable by available chromatographic methods. Chemical synthesis has appeared as a valuable strategy to solve this problem, and many different methods that gave access to a variety of glycopeptides and glycoproteins have been described.[19] Moreover, the introduction of solid-phase peptide synthesis (SPPS) has led to remarkable developments in peptide/protein chemistry and their related fields. It allowed the preparation of diverse peptide libraries on solid-phase supports in a combinatorial manner, and these have been used as tools in many areas of biomedicine.[20]

Nowadays, the assembly of simple peptides containing α-*Gal*NAc is relatively straightforward. However, the main obstacle in the synthesis of complex *O*-glycosyl amino acids is obtaining high α-selectivity in the formation of the *O*-Ser/Thr mucin-type linkage. In addition to the difficult synthesis of glycopeptides, the complexity of the problem is increased due to the simultaneous occurrence of different functional groups in a single molecule. Most used protecting groups included, acetyl protecting groups for the free hydroxyl groups of the sugar, Fmoc and Boc protecting groups for the amine moiety of the amino acid, and methyl, allyl, *tert*-butyl, benzoyl, or benzyl protecting groups for the acid moiety.[19]

6.3.1. Stereoselective synthesis of 2-deoxy-2-fluoro glycopeptides

The first objective is to obtain 2-deoxy-2-fluoro analogues of the Tn antigen. For this purpose, the stereoselective microwave-assisted synthesis developed in Chapter III was applied to this synthesis. First, different acceptors and deprotection conditions were evaluated to select the best performer.

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[19] Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495-4537.

[20] a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149; b) Pellois, J. P.; Zhou, X.; Srivannavit, O.; Zhou, T.; Gulari, E.; Gao, X. *Nat. Biotechnol.* **2002**, *20*, 922-926.

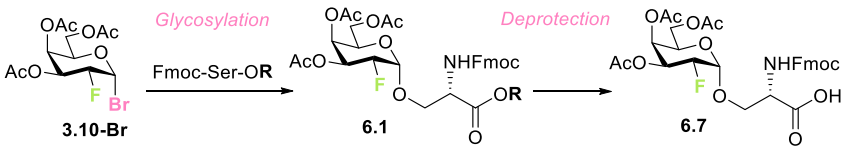
2-Deoxy-2-fluoro-galactoysl bromide **3.10-Br** and Fmoc-protected serine amino acids were chosen as starting materials to find the optimal glycosylation and deprotection conditions and the best acceptor. First, microwave-assisted glycosylation method developed in Chapter III was applied to the fluorinated sugar **3.10-Br** and Fmoc-Ser-OMe (**Table 6.1**, entry 1), obtaining **6.1** in a 70% glycosylation yield (determined by ^{19}F NMR using 1,4-difluorobenzene as internal standard) and good α-selectivity (3:1 α:β ratio). Purification by flash column chromatography allowed the separation of both isomers. The resulting α-glycopeptide **6.1α** was submitted to the deprotection of the methyl ester following a protocol that involves the use of a LiI in ethyl acetate under reflux.[21] After two cycles, only 37% of the deprotected-α-glycopeptide **6.7** was obtained (**Table 6.1**, entry 6). Other lithium salts (LiBr and LiCl in THF)[22] were used, however, lower yields were obtained. Due to the unsatisfactory results, other protected amino acids were studied. Next, microwave-assisted glycosylation method was applied to the fluorinated sugar **3.10-Br** and Fmoc-Ser-OAllyl (**Table 6.1**, entry 2), obtaining **6.1** in 57% yield (determined by ^{19}F NMR) and good α-selectivity (3:1 α:β ratio). Again, the α-glycopeptide was submitted to the deprotection of the allyl ester, following a protocol that involves the use of $Pd(PPh_3)_4$ and *N*-methyl morpholine (NMM) in a mixture of chloroform and acetic acid (20:1, entry 7).[23] However, after 3 days, the reaction was incomplete and analysis of the crude showed the presence of multiple by-products. TLC analysis showed the decomposition of Fmoc, probably due to the presence of NMM. Therefore, allyl protected amino acids did not exhibit good results in the deprotection reaction and other protected amino acids were tested.

When using *tert*-butyl protected amino acid (**Table 6.1**, entry 3), a mixture of more than 7 products were observed in the reaction crude, probably due to the incompatibility of *tert*-butyl ester protecting groups and the acid medium at high temperatures. To prove the applicability of the method, promoters (triflic acid and allyltributyltin) were substituted by tributyltin triflate

[21] Mayato, C.; Dorta, R. L.; Vázquez, J. T. *Tetrahedron Lett.* **2008**, *49*, 1396-1398.
[22] Mattsson, S.; Dahlström, M.; Karlsson, S. *Tetrahedron Lett.* ***2007***, *48*, 2497-2499.
[23] Ohta, Y.; Itoh, S.; Shigenaga, A.; Shintaku, S.; Fujii, N.; Otaka, A. *Org. Lett.* **2006**, *8*, 467-470.

(entry 4), which under the same conditions gave the desired glycopeptide **6.1** in 57% glycosylation yield and with good α:β ratio (3.5:1). Delightfully, when the isolated α-glycopeptide **6.1α** was submitted to deprotection conditions using a mixture of trifluoroacetic acid and water (TFA:H_2O 9:1) the corresponding acid **6.7** was isolated with excellent yields (87%, entry 8) and no epimerization was observed. Considering the difficulties observed in the deprotection of other ester-protected amino acids (OMe and OAllyl), *tert*-butyl protected amino acids were selected as the reagents of choice. Due to the incompatibility of *tert*-butyl protected amino acids and our microwave-assisted glycosylation method, other glycosylation conditions to improve yields and stereoselectivities were studied.

Table 6.1. Optimization of the glycosylation reaction between 2-F-galactosyl bromide and different serine acceptors, followed by optimization of the deprotection reaction.



entry	R	Glycosylation conditions[a,b]	Yield[c] (%)[d]	α:β ratio[d]
1	Me	acceptor (2 eq.), allyltributyltin (1.2 eq.), 4 Å MS, TfOH (0.2 eq.), μw heating, 90 ºC, 2 h, toluene	45 (70)	3:1
2	Allyl	acceptor (2 eq.), allyltributyltin (1.2 eq.),4 Å MS, TfOH (0.2 eq.), μw heating, 90 ºC, 4 h, toluene	26 (57)	3:1
3	^{t}Bu	acceptor (2 eq.), allyltributyltin (1.2 eq.), 4 Å MS, TfOH (0.2 eq.), μw heating, 90 ºC, 2 h, toluene	ND[e]	ND
4	^{t}Bu	acceptor (2 eq.), tributyltintriflate (1.2 eq.), 4 Å MS, μw heating, 90 ºC, 1 h, toluene	34 (57)	3.5:1
5	^{t}Bu	acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq.), 4 Å MS, 3 h, 0 ºC to r.t., toluene	54 (72)	3:1

entry	R	Deprotection conditions	Yield (%)[c]
6	Me	LiI (6 eq.), EtOAc, reflux, 20 h	37
7	Allyl	$Pd(PPh_3)_4$ (0.2 eq.), NMM (1 eq.), $CHCl_3$:AcOH (20:1), r.t.	ND[e]
8	^{t}Bu	TFA:H_2O (9:1), r.t., 3 h	87

[a]General conditions for MW-assisted glycosylation: donor (1 eq.) acceptor (2 eq.), allyltributyltin (1-2 eq), TfOH (0.2 eq.), 4 Å MS, toluene [0.02 M]. μw heating: 90 ºC, 300 W, 250 psi.[b]General conditions for glycosylation with Ag_2O: donor (1 eq.), acceptor (1.2 eq.), Ag_2O (2 eq.), TfOH (0.2 eq), 4 Å MS, toluene [0.02 M]. [c]Isolated yields of the α-isomer. [d]In parentesis, yields and α:β ratios determined by ^{19}F NMR using 1,4-difluorobenzene as internal pattern. [e]Fmoc decomposition was observed.

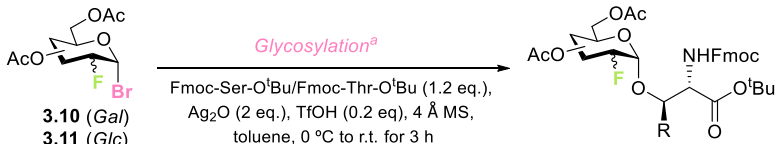
Of great interest is the recent work presented by Demchenko and co-workers in the stereocontrolled 1,2-*cis* glycosylation using silver promoters.[24] Following the seminal report of Demchemko,[24b] the starting 2F-galactosyl bromide **3.10-Br** and Fmoc-Ser-O^{t}Bu were treated with silver oxide (1 equivalents) and triflic acid (0.2 equivalents) to afford glycopeptide **6.1** (**Table 6.1**, entry 5) in 54% isolated yield (72% ^{19}F NMR yield). Results were better than in the conditions previously tested (entries 1 to 4), and almost the same stereoselectivity (3:1, α:β ratio) was obtained in all the cases. In addition, milder conditions and same reaction times were used in comparison with previous conditions, therefore, these were selected as the optimal glycosylation conditions.

Finally, glycosylation and deprotection conditions were applied to the stereoselective synthesis of fluorinated analogues of the Tn antigen, including different sugar configurations (*Gal, Glc, Man*) and serine and threonine amino acids (**Table 6.2**). Glycosylation reaction between 2-F-glycosyl bromides **3.10-Br**, **3.11-Br** and **3.12-Br** and Fmoc-Ser-O^{t}Bu amino acids gave the desired glycopeptides **6.1**, **6.2** and **6.3** in 39-54% isolation yields (60-72% ^{19}F NMR yields, **Table 6.2**, entries 1-3). Similar α-selectivities were obtained for *galacto* and *gluco* configurations (α:β ratio, 3:1 for **6.1** (*Gal*) entry 1, and 2.5:1 for **6.2** (*Glc*) entry 2). As expected, for *manno* glycosyl donors only the α-glycopeptide **6.3** was obtained. Glycosylation reaction between 2-F-glycosyl bromides **3.10-Br**, **3.11-Br**, and **3.12-Br** and Fmoc-Thr-O^{t}Bu amino acids gave the corresponding glycopeptides **6.4**, **6.5** and **6.6** in better isolated yields (43-64%, **Table 6.2,** entries 4-6) and better crude ^{19}F NMR yields (72-82%) compared to serine analogues (**Table 6.2,** entries 1 to 3). However, α-selectivities obtained for *galacto* (1.5:1 α:β ratio of **6.4**, entry 4) and *gluco* (2:1 α:β ratio of **6.5,** entry 5) glycopeptides were lower compared to the serine analogues (3:1 α:β ratio of **6.1** (*Gal*), entry 1) (2.5:1 α:β ratio of **6.2** (*Glc*), entry 2). Smaller differences were obtained for *gluco* derivates, however, in the case of *galacto* glycopeptides the α-selectivity is

[24] a) Shadrick, M.; Singh, Y.; Demchenko, A. V. *J. Org. Chem.* **2020***, 85,* 15936-15944; b) Singh, Y.; Demchenko, A. V. *Chem. Eur. J.* **2020**, *26*, 1042-1051; c) Geringer, S. A.; Singh, Y.; Hoard, D. J.; Demchenko, A. V. *Chem. Eur. J.* **2020***, 26,* 8053-8063.

reduced by half when using threonine. The lower α-selectivities obtained for threonine glycopeptides could be explained by the higher steric hindrance of the acceptor, since serine is a primary hydroxyl acceptor and threonine is a secondary hydroxyl acceptor. As expected, *manno* glycopeptide **6.6** was again obtained again as a sole α-isomer. Final products were purified by flash column chromatography obtaining two separated fractions of pure α and β isomers, which were fully characterized by NMR (^{1}H, ^{19}F, ^{13}C, HSQC, HMBC, NOESY) and results were consistent with a $^{4}C_{1}$ chair conformation.

Table 6.2. Scope of the glycosylation reaction between 2-F-α-glycosyl bromides and Fmoc-Ser/Thr-O^{t}Bu amino acids.



3.10 (*Gal*)
3.11 (*Glc*)
3.12 (*Man*)

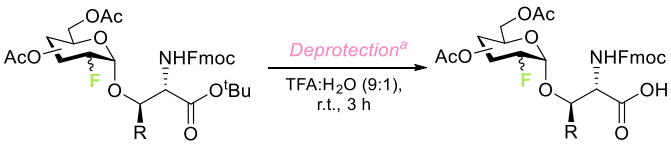
6.1-6.6

entry	donor	R	Glycosylation product	Isolated yield[b] (%)	^{19}F NMR yield[c] (%)	α:β ratio[c]
1	**3.10** (*Gal*)	H	**6.1**	54	72	3:1
2	**3.11** (*Glc*)	H	**6.2**	39	69	2.5:1
3	**3.12** (*Man*)	H	**6.3**	40	60	1:0
4	**3.10** (*Gal*)	Me	**6.4**	64	82	1.5:1
5	**3.11** (*Glc*)	Me	**6.5**	57	75	2:1
6	**3.12** (*Man*)	Me	**6.6**	43	72	1:0

[a]General conditions for glycosylation: donor (1 eq.), Fmoc-Ser-O^{t}Bu or Fmoc-Thr-O^{t}Bu (1.2 eq.), Ag_2O (2 eq.), TfOH (0.2 eq), 4 Å MS, toluene [0.02 M], 0 ºC to r.t. for 3 h. [b]Isolated yields. [c]Yields and α:β ratios determined by ^{19}F NMR using 1,4-difluorobenzene as internal standard.

Once *tert*-butyl protected glycopeptides were obtained, pure α-isomers were submitted to optimized deprotection conditions (**Table 6.3**). Deprotected serine glycopeptides **6.7**, **6.8** and **6.9** were obtained in excellent yields (84-89%, entries 1-3). However, threonine glycopeptides **6.10**, **6.11** and **6.12** were obtained in lower yields (60-68%, entries 4-6). Final products were fully characterized by NMR (^{1}H, ^{19}F, ^{13}C, HSQC, HMBC, NOESY) and results obtained are consistent with a $^{4}C_{1}$ chair conformation.

Table 6.3. Deprotection of the *tert*-butyl ester of 2-deoxy-2-fluoro glycopeptides.



6.1-6.6 **6.7-6.12**

entry	SM	R	Product	Deprotection yield[b] (%)
1	**6.1** (*Gal*)	H	**6.7**	87
2	**6.2** (*Glc*)	H	**6.8**	84
3	**6.3** (*Man*)	H	**6.9**	89
4	**6.4** (*Gal*)	Me	**6.10**	68
5	**6.5** (*Glc*)	Me	**6.11**	60
6	**6.6** (*Man*)	Me	**6.12**	65

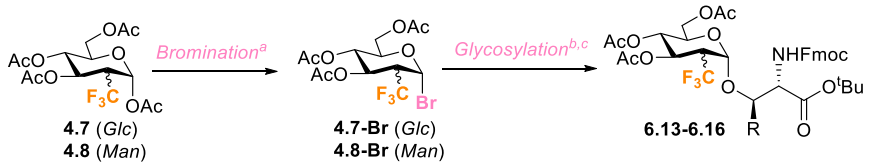
[a]General conditions for deprotection of the *tert*-butyl ester: *tert*-butyl protected glycoamino acid (1 equiv.), TFA:H$_2$O (9:1), r.t. for 3 h. [b]Isolated yields.

6.3.2. Stereoselective synthesis of 2-deoxy-2-trifluoromethyl glycopeptides

The second objective on this chapter is to obtain 2-deoxy-2-trifluoromethylated analogues of the Tn antigen. Results obtained for 2-F-glycosides showed that best results were obtained for *tert*-butyl protected amino acids and deprotection conditions using TFA and water. In consequence, Fmoc-Ser/Thr-O^{t}Bu amino acids were chosen as acceptors.

Initiallly, the stereoselective glycosylation method developed in Chapter IV was initially tested for trifluoromethyl glycosides **4.7** and **4.8** and serine and threonine amino acids (**Table 6.4**, entries 1 to 4). The required glycosyl bromides **4.7-Br** and **4.8-Br** were freshly prepared and directly submitted to glycosylation reaction to avoid their decomposition. Therefore, glycosylation yields correspond to a two-step bromination-glycosylation sequence.

Table 6.4. Optimization of the glycosylation reaction between 2-deoxy-2-trifluoromethyl glycosides and Fmoc-Ser/Thr-O^{t}Bu.



entry	donor	R	Glycosylation conditions[b,c]	Product	yield (%)[d]	α:β ratio[d]
1	**4.7** (*Glc*)	H	acceptor (1.2 eq.), AgOTf (2 eq.), TfOH (0.2 eq.), 4 Å MS, -80 ºC, 2 h, toluene:CH_2Cl_2 (1:1)	**6.13**	33	1.5:1
2	**4.8** (*Man*)	H	acceptor (1.2 eq.), AgOTf (2 eq.), TfOH (0.2 eq.), 4 Å MS, -80 ºC, 2 h, toluene:CH_2Cl_2 (1:1)	**6.14**	67	1:0
3	**4.7** (*Glc*)	Me	acceptor (1.2 eq.), AgOTf (2 eq.), TfOH (0.2 eq.), 4 Å MS, -80 ºC, 2 h, toluene:CH_2Cl_2 (1:1)	**6.15**	0	ND
4	**4.8** (*Man*)	Me	acceptor (1.2 eq.), AgOTf (2 eq.), TfOH (0.2 eq.), 4 Å MS, -80 ºC, 2 h, toluene:CH_2Cl_2 (1:1)	**6.16**	10	1:0
5	**4.7** (*Glc*)	H	acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq.), 4 Å MS, 3 h, -80 ºC, toluene	**6.13**	0	ND
6	**4.7** (*Glc*)	H	acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq.), 4 Å MS, 3 h, -20 ºC, toluene	**6.13**	25	1.6:1
7	**4.7** (*Glc*)	H	acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq.), 4 Å MS, 3 h, -10 ºC, toluene	**6.13**	42	1.5:1
8	**4.7** (*Glc*)	H	acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq.), 4 Å MS, 3 h, 0 ºC, toluene	**6.13**	35	1.5:1

[a]General conditions for bromination: 1-OAc trifluoromethyl glycoside (1 eq.) 33% HBr/AcOH, CH_2Cl_2.[b]General conditions for glycosylation with AgOTf: donor (1 eq.), acceptor (1.2 eq.), AgOTf (2 eq.), TfOH (0.2 eq), 4 Å MS, toluene:CH_2Cl_2 (1:1) [0.02 M]. [c]General conditions for glycosylation with Ag_2O: donor (1 eq.), acceptor (1.2 eq.), Ag_2O (1 eq.), TfOH (0.2 eq), 4 Å MS, toluene [0.02 M]. [d]In parentesis, yields and α:β ratios determined by ^{19}F NMR using 1,4-difluorobenzene as internal standard. [e]Only trifluoromethyl-D-glucal was obtained. ND = not determined.

In a first attempt, freshly prepared 2-trifluoromehtyl glucosyl bromide **4.7-Br** and Fmoc-Ser-O^{t}Bu were submitted to glycosylation conditions (**Table 6.4**, entry 1), using silver triflate and triflic acids as promoters, in the presence of 4 Å MS, and in a mixture 1:1 of dichloromethane:toluene. Reaction was stirred for 2 h at -80 °C and then the reaction crude was analysed by ^{19}F NMR using 1,4-difluorobenzene as internal standard. In order to identify all the products, reaction crudes were carefully purified by flash column chromatography. ^{19}F NMR spectrum of the reaction crude showed that compound **6.13** was obtained in 33% with a α:β ratio of 1.5:1 (**Table 6.4**, entry 1). However, 61% of elimination

product **4.4** (2-trifluoromethyl-D-glucal) was also obtained. The same conditions were then applied to 2-trifluoromethyl mannosyl bromide **4.8-Br** and Fmoc-Ser-O^{t}Bu (**Table 6.4**, entry 2). ^{19}F NMR spectrum of the reaction crude showed that glycopeptide **6.14** was obtained in 67% yield getting only the α-isomer and yielding only 5% of elimination product. When same conditions were applied to trifluoromethyl glycosides **4.7** and **4.8** and threonine amino acids, results were poor (entries 3 and 4), and the elimination product **4.4** was mainly obtained. In the case of 2-trifluoromehtyl glucosyl bromide **4.7-Br** and Fmoc-Thr-O^{t}Bu (**Table 6.4**, entry 3), no glycosylation product **6.15** was observed, and elimination product (89%), acetylated 2-trifluoromethyl glucoside **4.7** (8%) and other side products were identified in the ^{19}F NMR spectrum of the reaction crude. Same conditions were applied for 2-trifluoromethyl mannosyl bromide **4.8-Br** and Fmoc-Thr-O^{t}Bu (**Table 6.4**, entry 4), and glycosylation product **6.15** was identified in the ^{19}F NMR spectrum of the reaction crude but in just 10% of yield. Considering the poor results obtained when using Fmoc-Thr-O^{t}Bu amino acids as acceptors, other glycosylation conditions were evaluated to improve the yields.

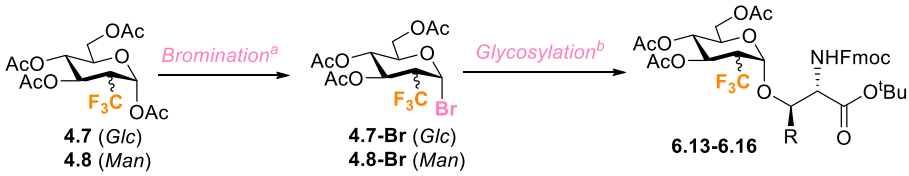
Considering the good results obtained for 2-deoxy-2-fluoro glycosyl donors when submitted to Demchenko glycosylation conditions,[24] these, were also applied to 2-deoxy-2-trifluoromethyl glycosides. In a first attempt, 2-CF_3-glucosyl bromide **4.7-Br** and Fmoc-Ser-O^{t}Bu were submitted to the glycosylation protocol using 1 equivalent of silver oxide and 0.2 equivalents of triflic acid, in the presence of 4 Å MS and using toluene as solvent. Reaction temperature was set to -80 °C to avoid the formation of the elimination product **4.4** and the reaction was stirred for 3 h (**Table 6.4**, entry 5). However, only starting trifluoromethyl glucosyl bromide **4.7-Br** was observed in the ^{19}F NMR spectrum. It is important to notice that silver equivalents are maintained in both glycosylation conditions (entries 1-4 *vs* entries 5-8), however, it seems that silver oxide needs higher temperatures to activate the glycosyl donor in comparison with silver triflate, that activates the glycosyl donor even at -80 °C. Thus, increasing glycosylation temperature to -20 °C (**Table 6.4**, entry 6) allowed the activation of the glycosyl donor and almost full conversion was achieved in 3 h. ^{19}F NMR analysis of the reaction crude showed the formation of product **6.13** in

a 25% yield with an α:β ratio of 1.6:1 (**Table 6.4**, entry 6). Elimination product was obtained in 32% yield and acetylated 2-CF_3-glucoside **4.7** was obtained in 25% yield, and other rearranged by-products were observed in the reaction crude. In view of the low yields, glycosylation reaction was evaluated at -10 °C under similar conditions (**Table 6.4**, entry 7). Compound **6.13** was obtained in higher yield (42%, entry 7) and practically same stereoselectivity (1.5:1, α:β ratio). Elimination product was obtained in slightly higher yield (35% yield, entry 7) compared to reaction at -20 °C (32%, entry 6). Again, 20% of acetylated 2-CF_3-glucoside **4.7** and other transposition products were obtained. These glycosylation conditions (3 h at -10 °C, entry 7) allowed to obtain the desired glycopeptide **6.13** in good yield without favouring the side elimination reaction. Lastly, glycosylation reaction was repeated at 0 °C, however, compound **6.13** was obtained in lower yields (35%, entry 8) and the formation of elimination product **4.4** was increased (40%). Therefore, optimal conditions involved the use of 1 eq. of silver oxide, 0.2 eq. of triflic acid, preactivated 4 Å MS and toluene as solvent at -10 °C for 3 h.

Optimal conditions were then applied to both trifluoromethyl glycosides **4.7** and **4.8** and serine and threonine amino acids (**Table 6.5**). Good yields were achieved for *gluco* glycopeptides **6.13** and **6.15**, although, α-selectivity was low. Glycopeptide **6.13** was isolated in 31% yield (42% ^{19}F NMR yield, entry 1) as a mixture 1.5:1 of α:β isomers. Lower α-selectivity was obtained for the threonine glycopeptide **6.15** (1:1, α:β ratio, entry 2), that was obtained in 29% isolated yield (38% ^{19}F NMR yield). Higher yields and exclusive α-selectivities were obtained for *manno* glycopeptides **6.14** and **6.16**. Under the optimized glycosylation conditions, glycopeptide **6.14** was obtained in a ^{19}F NMR yield (65%, **Table 6.5**, entry 2) similar to that obtain under the former conditions (67%, **Table 6.4**, entry 2). In both cases the α-isomer was obtained exclusively, therefore, for this glycopeptide both conditions are applicable. The crude was purified by flash column chromatography to obtain the desired glycopeptide **6.14** in 34% isolated yield as sole α-isomer. In contrast, glycopeptide **6.16** was obtained in much better ^{19}F NMR yields (50%, **Table 6.5**, entry 4) when compared to previous conditions (10%, **Table 6.4**, entry 4) and pure α-glycopeptide **6.16** was obtained

in 32% isolated yield. *Gluco* glycopeptides were purified by flash column chromatography obtaining two separated fractions of pure α and β isomers. Pure final products were fully characterized by NMR (^{1}H, ^{19}F, ^{13}C, HSQC, HMBC, NOESY) and results obtained were consistent with a $^{4}C_{1}$ chair conformation.

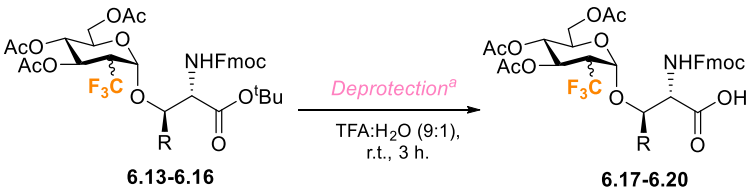
Table 6.5. Scope of the glycosylation reaction between 2-CF_3-glycosides and Fmoc-Ser/Thr-O^{t}Bu amino acids.



entry	donor	R	Product	Isolated yield[c] (%)	^{19}F NMR yield[d] (%)	α:β ratio[d]
1	**4.7** (*Glc*)	H	**6.13**	31	42	1.5:1
2	**4.8** (*Man*)	H	**6.14**	34	65	1:0
3	**4.7** (*Glc*)	Me	**6.15**	29	38	1:1
4	**4.8** (*Man*)	Me	**6.16**	32	50	1:0

[a]General conditions for bromination: donor (1 eq.), 33% HBr/AcOH, 0 ºC to r.t., 3 h. [b]General conditions for glycosylation: donor (1 eq.), Fmoc-Ser-O^{t}Bu or Fmoc-Thr-O^{t}Bu (1.2 eq.), Ag_2O (2 eq.), TfOH (0.2 eq), 4 Å MS, toluene [0.02 M], 0 ºC to r.t. for 3 h. [c]Isolated yields. [d]Yields and α:β ratios determined by ^{19}F NMR using 1,4-difluorobenzene as internal pattern.

Once *tert*-butyl protected glycopeptides were obtained, pure α-isomers were submitted to optimized deprotection conditions (**Scheme 6.1**). The obtention of the final products (**6.17-6.20**) was confirmed by HRMS.



Scheme 6.1. Deprotection of the *tert*-butyl ester of 2-deoxy-2-trifluoromethyl glycopeptides.

6.4. CONCLUSIONS

The stereoselective synthesis of fluorinated and trifluoromethylated analogues of the Tn-antigen was achieved in good to excellent yields and pure α-isomers of all the products were isolated. Glycosylation methods were optimized and those developed in Chapter III and Chapter IV could be applied in some cases. Evaluation of glycosylation and deprotection conditions revealed that the best results were obtained with *tert*-butyl protected amino acids.

In the case of fluorinated glycopeptides, glycosylation method described in Chapter III for the stereoselective synthesis of 2-deoxy-2-fluoro-α-glycosides could not be applied due to the incompatibility of *tert*-butyl protecting groups in acid media at high temperatures. Under these conditions the *tert*-butyl group is hydrolysed to give the corresponding acid, that can act as an acceptor in the glycosylation step yielding a mixture of by-products. In order to increase yields and avoid the formation of by-products, a glycosylation protocol developed by Demchenko[24] was applied to the synthesis of fluorinated Tn antigen analogues. Good to excellent yields (60-82%) and high α-selectivity was achieved in all cases. Higher yields were obtained in the synthesis of threonine glycopeptides **6.4**, **6.5** and **6.6** (72-82%) in comparison with serine analogues **6.1**, **6.2** and **6.3** (60-72%). Fluorinated *manno* glycopeptides **6.3** and **6.6** were obtained with exclusive α-selectivity and good yields (60% for **6.3** and 72% for **6.6**). Glucopeptides **6.2** and **6.5** were obtained in good yields (69 % and 75% respectively) and good α-selectivity (up to 2.5:1 α:β ratio), and galactopeptides **6.1** and **6.4** were obtained in excellent yields (72-82%) and good α-selectivity (up to 3:1 α:β ratio). Isolated α-glycopeptides were submitted to deprotection conditions giving the desired acids in good to excellent yields as pure α isomers. Deprotected serine glycopeptides **6.7**, **6.8** and **6.9** were obtained in higher yields (84-89%) than threonine analogues **6.10**, **6.11** and **6.12** (60-68%).

Regarding trifluoromethylated glycopeptides, glycosylation method described in Chapter IV was effective for the synthesis of trifluoromethylated glycopeptides when using serine amino acids, however, threonine amino acids gave poor results. Demchenko protocol was also applied for 2-trifluoromethyl

glycosides and serine and threonine amino acids, obtaining good overall yields (38-66%, bromination and glycosylation yield) in all the cases. Interestingly, for mannopeptide **6.14**, slightly higher yields were obtaining applying the method described in Chapter IV. However, the protocol developed by Demchenko and co-workers was of wider applicability. Slightly higher ^{19}F NMR yields (65% for **6.14** and 50% for **6.16**) and much better α-selectivity (exclusive α-isomer) were obtained for *manno* trifluoromethyl glycopeptides in comparison with *gluco* analogues, obtained in good yields (42% for **6.13** and 38% for **6.15**) and α-selectivities up to 1.5:1 (α:β ratio).

6.5. EXPERIMENTAL SECTION

6.5.1. General considerations

Proton (^{1}H NMR), carbon (^{13}C NMR) and fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (400 MHz for ^{1}H, 100.6 MHz for ^{13}C and 376.5 MHz for ^{19}F). Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^{1}H NMR: $CDCl_3$ = 7.26 and ^{13}C NMR: $CDCl_3$ = 77.00) and $CFCl_3$. Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent. Infrared (IR) spectra were recorded on a Jasco FT/IR-600 Plus ATR Specac Golden Gate spectrophotometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. High resolution mass spectra (HRMS) were recorded on an Agilent 1100 Series LC/MSD mass spectrometer with electrospray ionization (ESI) by the Servei de Recursos Científics (URV). Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using commercial aluminium backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (max = 254 nm) and 6% H_2SO_4 in EtOH. Flash column chromatography was carried out using silica gel 60 A CC (230–400 mesh). Mobile phases are reported in relative composition (e.g. 1:1 EtOAc/hexane v/v). All solvents were used as supplied (Analytical, synthesis or HPLC grade), without prior purification. All reagents were used as received from commercial suppliers. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulphate ($MgSO_4$) and anhydrous sodium sulphate (Na_2SO_4) were used as drying agents after reaction work-up, as indicated.

6.5.2. General glycosylation protocol for fluorinated glycopeptides

To a solution of the **glycosyl donor** (1 mmol), the **glycosyl acceptor** (1.2 mmol) and preactivated 4Å MS in dried toluene (20 mL/mmol) was added silver oxide (1 mmol) under argon, and the solution was stirred at room temperature for 15 min under darkness. Then, trifluoromethanesulfonic acid (0.2 mmol) was added under argon at 0 °C, and the reaction is let to warm up until room temperature. The reaction was followed by TLC until total consumption of the starting material. Once the reaction had finished, the crude was filtered through a short path of celite and washed with CH_2Cl_2. The filtrate was evaporated under reduced pressure and the crude obtained was analysed by NMR using an internal standard (TFE) to determine the yield and α:β ratio. Then the crude was purified by flash column chromatography.

6.5.3. General bromination and glycosylation protocols for trifluoromethylated glycopeptides

Bromination: To a solution of the **glycosyl acetate** (1 mmol) in CH_2Cl_2 (5 mL/mmol) in an ice-bath was added 33% HBr/AcOH (5 mL/mmol), and the solution was stirred and let to warm up until room temperature. The reaction was followed by TLC. Once total consumption of the starting material was observed, the crude was cool down using an ice-bath, diluted with CH_2Cl_2 and $NaHCO_3$ was added dropwise. The organic layer was then washed with $NaHCO_3$ (x3), brine (x3), dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue obtained correspond to the **glycosyl donor**, which was azeotropically dried twice with toluene and let under reduced pressure for 2 h.

Glycosylation with AgOTf: To the flask containing the **glycosyl donor** were added the **glycosyl acceptor** (1.2 mmol) and preactivated 4 Å MS, and the mixture was dissolved in dried CH_2Cl_2 (10 mL/mmol) and maintained under argon. Then, it was cool down to -80 °C and azeotropically dried silver trifluoromethanesulfonate (2 mmol) dissolved in dried toluene (10 mL/mmol) was transferred to the mixture under argon. The solution was stirred at -80 °C and the reaction followed by TLC. Once the reaction had finished, the crude was

filtered through a short path of celite and washed with CH_2Cl_2. The filtrate was evaporated under reduced pressure and the crude obtained was analysed by NMR using an internal pattern (TFE) to determine the yield and α:β ratio. Then the crude was purified by flash column chromatography.

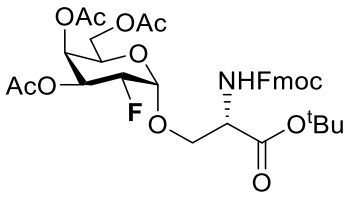
Glycosylation with Ag_2O: To the flask containing the **glycosyl donor** were added the **glycosyl acceptor** (1.2 mmol), preactivated 4 Å MS and silver oxide (1 mmol) and they were dried under reduced pressure for 1 h. Then, the mixture was dissolved in dried toluene (20 mL/mmol) under argon and stirred at room temperature for 15 min under darkness. Then, the reaction was cool down to -10 °C and trifluoromethanesulfonic acid (0.2 mmol) was added under argon, and the reaction is let stirring at the same temperature. The reaction was followed by TLC until total consumption of the starting material. Once the reaction had finished, the crude was filtered through a short path of celite and washed with CH_2Cl_2. The filtrate was evaporated under reduced pressure and the crude obtained was analysed by NMR using an internal pattern (TFE) to determine the yield and α:β ratio. Then the crude was purified by flash column chromatography.

6.5.4. General procedure for the deprotection of the tert-butyl ester group

To a flask containing the *tert*-butyl ester glycopeptide (1 mmol) was added trifluoroacetic acid (18 mL/mmol) and distilled water (2 mL/mmol). The reaction was let stirring at room temperature until total consumption of the starting material. Once the reaction had finished, the crude was evaporated under reduced pressure and purified by flash column chromatography.

6.5.5. Synthesis of fluorinated analogues of the Tn antigen

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-galactopyranosyl)-L-serine *tert*-butyl ester (6.1)



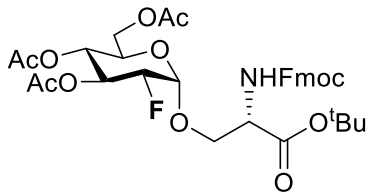
The general procedure was applied using galactosyl bromide **3.10-Br** (60.4 mg, 0.173 mmol), Fmoc-Ser-O^{t}Bu (79.6 mg, 0.207 mmol), preactivated 4 Å MS, silver oxide (40.0 mg, 0.173 mmol) and TfOH (3 µL, 0.035 mmol) in 3.5 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 72% yield of **6.1** obtaining an α:β ratio of 3:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.1α** as a white foam (32.8 mg, 28.2%), **6.1β** as a colourless syrup (15.3 mg, 13.1%) and an inseparable mixture of **6.1α/β** (14.3 mg, 12.2%).

Data for **6.1α:** White foam. R_f (3:7 EtOAc/hexane): 0.24; **[α]$^{D}_{25}$** : + 86.3 (1.64, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.76 (dd, J = 7.5 Hz, J = 1.0 Hz, 2H, CH-Ar), 7.64 (d, J = 7.4 Hz, 2H, CH-Ar), 7.40 (t, J = 7.5 Hz, 2H, CH-Ar), 7.33 (tdd, J = 7.5 Hz, J = 2.0 Hz, J = 1.3 Hz, 2H, CH-Ar), 5.89 (d, $J_{2',NH'}$ = 7.7 Hz, 1H, NH'), 5.47 (s, 1H, H-4), 5.34 (td, $J_{3,F}$ = $J_{3,4}$ = 10.5 Hz, $J_{2,3}$ = 3.4 Hz, 1H, H-3), 5.06 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.75 (ddd, $J_{2,F}$ = 49.7 Hz, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.8 Hz, 1H, H-2), 4.45 (dt, $J_{2',NH'}$ = 7.5 Hz, $J_{2',1'}$ = $J_{2',1''}$ = 2.8 Hz, 1H, H-2'), 4.42-4.35 (m, 2H, CH_2-O-Fmoc), 4.25 (t, J = 7.2 Hz, 1H, CH-Fmoc), 4.22 (t, $J_{5,6a}$ = $J_{5,6b}$ = 6.4 Hz, 1H, H-5), 4.29-4.19 (m, 2H, H-1', H-1'', H-6a, H-6b), 2.14, 2.04, 1.97 (s, 9H, $3CH_3$, Ac), 1.49 (s, 9H, CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –208.1 (ddd, $J_{F,2}$ = 49.8 Hz, $J_{F,3}$ = 10.8 Hz, $J_{F,1}$ = 3.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.0, 169.9, 168.4 (C=O, Ac, Fmoc), 155.8 (C=O, Ser), 143.87, 143.81, 141.3, (C-Ar, Fmoc), 127.78, 127.76, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 97.5 (d, J = 20.9 Hz, C-1), 85.0 (d, J = 191.6 Hz, C-2), 83.2 (O-C-$(CH_3)_3$, O^{t}Bu), 70.3 (C-1'), 68.6 (d, J = 7.6 Hz, C-4),

68.0 (d, *J* = 18.7 Hz, C-3), 67.3 (C-5), 67.0 (CH_2-Fmoc), 61.5 (C-6), 54.9 (C-2'), 47.1 (CH-Fmoc), 28.0 ((CH_3)$_3$, O^{t}Bu), 20.69, 20.61, 20.59 (CH_3, OAc); **FT-IR (neat)** *v* in cm^{-1}: 2360, 2341, 1750, 1718, 1370, 1227, 1155, 1076, 1053; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{34}H_{40}FNNaO_{12}^+$ (m/z): calc. 696.2427; found 696.2425.

Data for **6.1β:** Uncoloured syrup. R_f (3:7 EtOAc/hexane): 0.16; **[α]$^D_{25}$** : + 21.7 (1.53, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.61 (d, *J* = 7.1 Hz, *J* = 4.3 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.5 Hz, 2H, CH-Ar), 7.31 (td, *J* = 7.5 Hz, 2H, CH-Ar), 5.72 (d, $J_{2',NH'}$ = 8.1 Hz, 1H, NH'), 5.40 (t, *J* = 2.5 Hz, 1H, H-4), 5.10 (ddd, $J_{3,F}$ = 13.4 Hz, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3), 4.58-4.42 (m, 2H, H-1, H-2), 4.45 (dt, $J_{2',NH'}$ = 8.1 Hz, $J_{1',2'}$ = $J_{1',2''}$ = 3.1 Hz, 1H, H-2'), 4.39 (dd, *J* = 7.2 Hz, *J* = 4.3 Hz, 2H, CH_2-Fmoc), 4.33 (dd, $J_{1',1''}$ = 9.7 Hz, $J_{1',2'}$ = 2.8 Hz, 1H, H-1'), 4.25 (t, *J* = 7.1 Hz, 1H, CH-Fmoc), 4.17-4-05 (m, 2H, H-6a, H-6b), 4.91-3.84 (m, 2H, H-5, H-1''), 2.12, 2.06, 2.04 (s, 9H, 3CH_3, Ac), 1.49 (s, 9H, CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –206.4 (ddd, $J_{F,2}$ = 50.1 Hz, $J_{F,3}$ = 13.4 Hz, $J_{F,1}$ = 7.0 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.0, 169.96, 168.6 (C=O, Ac, Fmoc), 155.9 (C=O, Ser), 143.9, 143.8, 141.3 (C-Ar, Fmoc), 127.8, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 100.6 (d, *J* = 22.9 Hz, C-1), 87.8 (d, *J* = 187.3 Hz, C-2), 82.7 (O-**C**-(CH_3)$_3$, O^{t}Bu), 71.0 (d, *J* = 19.0 Hz, C-3), 70.7 (C-5), 69.8 (C-1'), 67.5 (d, *J* = 8.3 Hz, C-4), 67.2 (CH_2-Fmoc), 61.1 (C-6), 54.7 (C-2'), 47.1 (CH-Fmoc), 27.9 ((CH_3)$_3$, O^{t}Bu), 20.66, 20.62, 20.51 (CH_3, OAc); **FT-IR (neat)** *v* in cm^{-1}: 2360, 2341, 1748, 1519, 1507, 1369, 1226, 1156, 1076, 1057; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{34}H_{40}FNNaO_{12}^+$ (m/z): calc. 696.2427; found 696.2425.

N^α-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-serine *tert*-butyl ester (6.2)



The general procedure was applied using glucosyl bromide **3.11-Br** (84.0 mg, 0.226 mmol), Fmoc-Ser-O^{t}Bu (104.3 mg, 0.272 mmol), preactivated 4 Å MS,

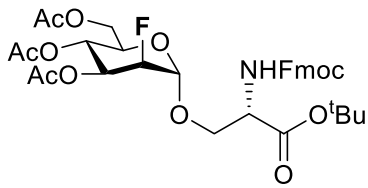
silver oxide (52.5 mg, 0.226 mmol) and TfOH (4 µL, 0.045 mmol) in 4.5 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 68.6% yield of **6.2** obtaining an α:β ratio of 2.5:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.2α** as a white foam (37.5 mg, 24.6%), **6.2β** as a colourless syrup (13.9 mg, 9.1%) and an inseparable mixture of **6.2α/β** (7.1 mg, 4.7%).

Data for **6.2α:** White foam. R_f (3:7 EtOAc/hexane): 0.36; $[\alpha]^D_{25}$: + 87.3 (0.42, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (dd, *J* = 7.6 Hz, *J* = 1.0 Hz, 2H, CH-Ar), 7.63 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.41 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.32 (td, *J* = 7.4 Hz, *J* = 1.0 Hz, 2H, CH-Ar), 5.85 (d, $J_{2',NH'}$ = 7.7 Hz, 1H, NH'), 5.48 (dt, $J_{3,F}$ = 11.9 Hz, $J_{2,3} = J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.02 (d, $J_{1,2}$ = 3.8 Hz, 1H, H-1), 4.98 (t, $J_{3,4} = J_{4,5}$ = 9.9 Hz, 1H, H-4), 4.49 (ddd, $J_{2,F}$ = 49.1 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.48-4.38 (m, 3H, CH_2-O-Fmoc, H-2'), 4.25 (t, *J* = 7.1 Hz, 1H, CH-Fmoc), 4.21 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a), 4.17-3.96 (m, 4H, H-1', H-1'', H-5, H-6b), 2.07, 2.05, 2.02 (s, 9H, $3CH_3$, Ac), 1.50 (s, 9H, CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –201.07 (dd, $J_{F,2}$ = 49.1 Hz, $J_{F,3}$ = 11.9 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 170.0, 169.7, 169.4 (C=O, Ac, Fmoc), 155.9 (C=O, Ser), 143.8, 141.3, (C-Ar, Fmoc), 127.78, 127.75, 127.1, 125.2, 125.1, 120.0, (CH-Ar, Fmoc), 97.0 (d, $J_{C1,F}$ = 20.7 Hz, C-1), 87.1 (d, $J_{C2,F}$ = 195.4 Hz, C-2), 83.3 (O-C-$(CH_3)_3$, O^{t}Bu), 70.5 (d, $J_{C3,F}$ = 19.8 Hz, C-3), 70.5 (C-1'), 68.0 (d, $J_{C4,F}$ = 7.3 Hz, C-4), 67.8 (C-5), 67.2 (CH_2-Fmoc), 61.7 (C-6), 54.9 (C-2'), 47.1 (CH-Fmoc), 27.9 ($(CH_3)_3$, O^{t}Bu), 20.75, 20.69, 20.62 (CH_3, OAc); **FT-IR (neat)** *v* in cm^{-1}: 1748, 1518, 1450, 1368, 1223, 1158, 1077, 1034; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{34}H_{40}FNNaO_{12}^+$ (m/z): calc. 696.2427; found 696.2418.

Data for **6.2β:** Uncoloured syrup. R_f (3:7 EtOAc/hexane): 0.32; $[\alpha]^D_{25}$: + 28.1 (0.68, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.61 (dd, *J* = 7.2 Hz, *J* = 4.3 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.2 Hz, 2H, CH-Ar), 7.32 (tdd, *J* = 7.4 Hz, *J* = 2.0 Hz , *J* = 1.2 Hz, 2H, CH-Ar), 5.71 (d, $J_{2',NH'}$ = 8.1 Hz, 1H, NH'), 5.32 (dt, $J_{3,F}$ = 14.6 Hz, $J_{2,3} = J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.01 (t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.56 (dd, $J_{1,2}$ = 7.6 Hz, $J_{1,F}$ = 2.7 Hz, 1H, H-1), 4.45 (dt, $J_{2',NH'}$ = 8.1 Hz, $J_{1',2'}$ =

$J_{1'',2'}$ = 3.0 Hz, 1H, H-2'), 4.41-4.20 (m, 3H, CH_2-O-Fmoc, H-2), 4.33 (dd, $J_{1',1''}$ = 9.7 Hz, $J_{1',2'}$ = 2.9 Hz, 1H, H-1'), 4.27-4.10 (m, 2H, H-6a, CH-Fmoc), 4.12 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 3.86 (dd, $J_{1',1''}$ = 9.7 Hz, $J_{1'',2'}$ = 3.0 Hz, 1H, H-1''), 3.69 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 2.09, 2.07, 2.04 (s, 9H, $3CH_3$, Ac), 1.48 (s, 9H, CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –199.3 (dd, $J_{F,2}$ = 50.6 Hz, $J_{F,3}$ = 14.6 Hz, $J_{F,1}$ = 2.7 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 170.0, 169.6, 168.5 (C=O, Ac, Fmoc), 155.9 (C=O, Ser), 143.9, 143.8, 141.3, (C-Ar, Fmoc), 127.7, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 100.3 (d, $J_{C1,F}$ = 23.1 Hz, C-1), 89.3 (d, $J_{C2,F}$ = 191.4 Hz, C-2), 82.8 (O-C-$(CH_3)_3$, O^{t}Bu), 72.7 (d, $J_{C3,F}$ = 20.0 Hz, C-3), 71.8 (C-1'), 70.0 (C-5), 68.0 (d, $J_{C4,F}$ = 7.3 Hz, C-4), 67.3 (CH_2-Fmoc), 61.7 (C-6), 54.6 (C-2'), 47.1 (CH-Fmoc), 27.9 ($(CH_3)_3$, O^{t}Bu), 20.69, 20.68, 20.60 (CH_3, OAc); **FT-IR (neat)** *ν* in cm^{-1}: 1747, 1508, 1450, 1368, 1343, 1225, 1158, 1036; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{34}H_{40}FNNaO_{12}^+$ (m/z): calc. 696.2427; found 696.2429.

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-mannopyranosyl)-L-serine *tert*-butyl ester (6.3)

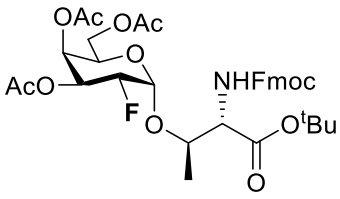


The general procedure was applied using mannosyl bromide **3.12-Br** (70.6 mg, 0.19 mmol), Fmoc-Ser-O^{t}Bu (87.7 mg, 0.229 mmol), preactivated 4 Å MS, silver oxide (44 mg, 0.19 mmol) and TfOH (3.4 µL, 0.038 mmol) in 3.8 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 63.2% yield of **6.3** obtaining an α:β ratio of 1:0. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.3α** as a white foam (51.3 mg, 40%).

R$_f$ (3:7 EtOAc/hexane): 0.23; **[α]$^D_{25}$**: + 49.0 (0.33, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (dd, *J* = 7.5 Hz, *J* = 1.0 Hz, 2H, CH-Ar), 7.63 (d, *J* = 7.3 Hz,

2H, CH-Ar), 7.40 (t, J = 7.5 Hz, 2H, CH-Ar), 7.32 (tdd, J = 7.5 Hz, J = 2.0 Hz, J = 1.3 Hz, 2H, CH-Ar), 5.79 (d, $J_{2',NH'}$ = 7.8 Hz, 1H, NH'), 5.32 (t, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.18 (ddd, $J_{3,F}$ = 28.4 Hz, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 2.2 Hz, 1H, H-3), 4.97 (d, $J_{1,F}$ = 6.1 Hz, 1H, H-1), 4.71 (d, $J_{2,F}$ = 49.8 Hz, 1H, H-2), 4.53-4.36 (m, 3H, CH_2-O-Fmoc, H-2'), 4.29-4.19 (m, 2H, CH-Fmoc, H-6a), 4.14 (dd, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 2.4 Hz, 1H, H-6b), 4.07-3.95 (m, 3H, H-1', H-1'', H-5), 2.09, 2.07, 2.03 (s, 9H, $3CH_3$, Ac), 1.50 (s, 9H, CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –203.9 (ddd, $J_{F,2}$ = 49.8 Hz, $J_{F,3}$ = 28.4 Hz, $J_{F,1}$ = 7.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.7, 170.1, 169.5, 168.5 (C=O, Ac, Fmoc), 155.8 (C=O, Ser), 143.8, 141.3, (C-Ar, Fmoc), 127.8, 127.1, 125.15, 125.1, 120.0, (CH-Ar, Fmoc), 98.1 (d, $J_{C1,F}$ = 29.5 Hz, C-1), 86.6 (d, J = 180.0 Hz, C-2), 83.2 (O-C-$(CH_3)_3$, O^{t}Bu), 70.2 (C-1'), 69.7 (d, J = 16.8 Hz, C-3), 69.1 (C-5), 67.2(CH_2-Fmoc), 65.5 (C-4), 62.0 (C-6), 54.8 (C-2'), 47.1 (CH-Fmoc), 28.0 ($(CH_3)_3$, O^{t}Bu), 20.76, 20.73, 20.66 (CH_3, OAc); **FT-IR (neat)** ν in cm^{-1}: 2360, 2341, 1748, 1717, 1699, 1507, 1226, 1156, 1088, 1049; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{34}H_{40}FNNaO_{12}^+$ (m/z): calc. 696.2427; found 696.2418.

N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-galactopyranosyl)-L-threonine *tert*-butyl ester (6.4)



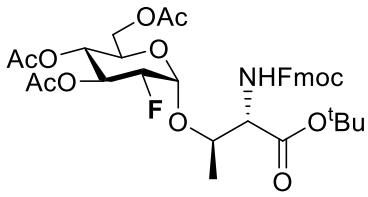
The general procedure was applied using galactosyl bromide **3.10-Br** (50.5 mg, 0.136 mmol), Fmoc-Thr-O^{t}Bu (65 mg, 0.164 mmol), preactivated 4 Å MS, silver oxide (31.6 mg, 0.136 mmol) and TfOH (1.2 µL, 0.013 mmol) in 2.8 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 82% yield of **6.4** obtaining an α:β ratio of 1.5:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.4α** as a white foam (30.1 mg, 32%), **6.4β** as a colourless syrup (17 mg, 18.1%) and an inseparable mixture of **6.4α/β** (12.6 mg, 13.5%).

Data for **6.4α:** R_f (3:7 EtOAc/hexane): 0.30; **[α]$^{D}_{25}$**: + 63.5 (1.41, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (dd, *J* = 7.5 Hz, 2H, CH-Ar), 7.65 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.32 (m, 2H, CH-Ar), 5.53-5.48 (m, 2H, NH', H-4), 5.35 (td, $J_{3,F} = J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3), 5.22 (d, $J_{1,2}$ = 3.9 Hz, 1H, H-1), 4.73 (ddd, $J_{2,F}$ = 49.9 Hz, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.9 Hz, 1H, H-2), 4.47-4.35 (m, 3H, H-1', CH_2-O-Fmoc), 4.35-4.23 (m, 3H, H-2', H-5, CH-Fmoc), 4.12-4.06 (m, 2H, H-6a, H-6b), 2.14, 2.05, 2.04 (s, 9H, $3CH_3$, Ac), 1.47 (s, 9H, CH_3, O^{t}Bu), 1.36 (d, $J_{1',CH3}$ = 6.3 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –206.4 (ddd, $J_{F,2}$ = 49.9 Hz, $J_{F,3}$ = 10.7 Hz, $J_{F,4}$ = 3.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.07, 170.02, 169.9, (C=O, Ac, Fmoc), 156.8 (C=O, Thr), 143.9, 141.3, (C-Ar, Fmoc), 127.7, 127.1, 125.3, 125.28, 120.0, 119.98 (CH-Ar, Fmoc), 97.8 (d, *J* = 20.7 Hz, C-1), 84.9 (d, *J* = 191.8 Hz, C-2), 82.9 (O-C-$(CH_3)_3$, O^{t}Bu), 77.3 (C-1'), 68.6 (d, *J* = 7.4 Hz, C-4), 68.2 (d, *J* = 18.9 Hz, C-3), 67.5 (C-5), 66.9 (CH_2-Fmoc), 61.7 (C-6), 59.2 (C-2'), 47.2 (CH-Fmoc), 28.0 ($(CH_3)_3$, O^{t}Bu), 20.69, 20.67, 20.58 (CH_3, OAc), 18.6 (CH_3, Thr); **FT-IR (neat)** *ν* in cm^{-1}: 2360, 2341, 1750, 1521, 1507, 1370, 1229, 1158, 1076, 1037; **HRMS (TOF ES^{+})** for $(M+Na)^{+}$ $C_{35}H_{42}FNNaO_{12}^{+}$ (m/z): calc. 710.2583; found 710.2568.

Data for **6.4β:** R_f (3:7 EtOAc/hexane): 0.27; **[α]$^{D}_{25}$**: + 12.3 (1.66, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (dd, *J* = 7.6 Hz, 2H, CH-Ar), 7.63 (d, *J* = 7.0 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.32 (tt, *J* = 7.7 Hz, *J* = 1.5 Hz, 2H, CH-Ar), 5.58 (d, $J_{2',NH'}$ = 9.4 Hz, 1H, NH'), 5.40 (s, 1H, H-4), 5.10 (ddd, $J_{3,F}$ = 13.3 Hz, $J_{3,4}$ = 9.8 Hz, $J_{2,3}$ = 3.5 Hz, 1H, H-3), 4.61 (dd, $J_{1,2}$ = 7.5 Hz, $J_{1,F}$ = 3.7 Hz, 1H, H-1), 4.58-4.32 (m, 2H, H-2, H-1'), 4.40 (dd, *J* = 7.3 Hz, *J* = 2.8 Hz, 2H, CH_2-O-Fmoc), 4.35 (dd, $J_{2',NH'}$ = 9.5 Hz, $J_{2',1'}$ = 2.6 Hz, 1H, H-2'), 4.26 (t, *J* = 7.3 Hz, 1H, CH-Fmoc), 4.11 (d, $J_{5,6a,b}$ = 6.7 Hz, 2H, H-6a, H-6b), 3.91 (t, $J_{5,6a} = J_{5,6b}$ = 6.6 Hz, 1H, H-5), 2.09, 2.06, 2.05 (s, 9H, $3CH_3$, Ac), 1.49 (s, 9H, CH_3, O^{t}Bu), 1.28 (d, $J_{1',CH3}$ = 6.1 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –206.8 (ddt, $J_{F,2}$ = 54.0 Hz, $J_{F,3}$ = 13.3 Hz, $J_{F,1}$ = 3.2 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.0, 169.9, 169.0 (C=O, Ac, Fmoc), 156.8 (C=O, Thr), 143.9, 143.8, 141.3, (C-Ar, Fmoc), 127.7, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 97.2 (d, *J* = 23.0 Hz, C-1), 87.7 (d, *J* = 187.3 Hz, C-2), 82.2 (O-C-$(CH_3)_3$, O^{t}Bu), 73.5 (C-1'), 71.1 (d, *J* = 19.2 Hz, C-3), 70.6 (C-5), 67.5 (d, *J* = 8.3 Hz, C-4), 67.3 (CH_2-Fmoc), 61.1 (C-6), 58.8 (C-2'), 47.2 (CH-Fmoc), 27.9

($(CH_3)_3$, O^{t}Bu), 20.69, 20.64, 20.48 (CH_3, OAc), 15.8 (CH_3, Thr); **FT-IR (neat)** *ν* in cm^{-1}: 2360, 2341, 1749, 1508, 1450, 1369, 1322, 1224, 1159, 1061; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{42}FNNaO_{12}^+$ (m/z): calc. 710.2583; found 710.2583.

N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-threonine *tert*-butyl ester (6.5)



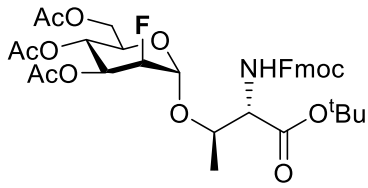
The general procedure was applied using glucosyl bromide **3.11-Br** (50.1 mg, 0.135 mmol), Fmoc-Thr-O^{t}Bu (64.6 mg, 0.162 mmol), preactivated 4 Å MS, silver oxide (31.4 mg, 0.135 mmol) and TfOH (2.4 µL, 0.027 mmol) in 2.7 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 75% yield of **6.4** obtaining an α:β ratio of 2:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.4α** as a white foam (34.5 mg, 37%), **6.5β** as a colourless syrup (10.7 mg, 13.5%) and an inseparable mixture of **6.5α/β** (6.2 mg, 6.7%).

Data for **6.5α:** ***R$_f$*** (3:7 EtOAc/hexane): 0.32; **[α]$^D_{25}$**: + 65.9 (1.22, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.78 (dd, *J* = 7.6 Hz, 2H, CH-Ar), 7.66 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.41 (td, *J* = 7.4 Hz, *J* = 2.9 Hz, 2H, CH-Ar), 7.37-7.29 (m, 2H, CH-Ar), 5.57 (d, $J_{2',NH'}$ = 9.5 Hz, 1H, NH'), 5.50 (dt, $J_{3,F}$ = 11.8 Hz, $J_{3,4}$ = $J_{2,3}$ = 9.6 Hz, 1H, H-3), 5.19 (d, $J_{1,2}$ = 4.0 Hz, 1H, H-1), 4.99 (t, $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, 1H, H-4), 4.52 (ddd, $J_{2,F}$ = 49.2 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 4.0 Hz, 1H, H-2), 4.46-4.21 (m, 7H, H-1', H-2', CH_2-O-Fmoc, CH-Fmoc, H-6a), 4.13-4.05 (m, 2H, H-6b, H-5), 2.08, 2.06, (s, 9H, $3CH_3$, Ac), 1.47 (s, 9H, CH_3, O^{t}Bu), 1.36 (d, $J_{1',CH3}$ = 6.5 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –199.3 (dd, $J_{F,2}$ = 49.2 Hz, $J_{F,3}$ = 11.9 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 170.1, 169.7, 169.8 (C=O, Ac, Fmoc), 156.8 (C=O, Thr), 143.9, 143.8, 141.3, (C-Ar, Fmoc), 127.7, 127.1, 125.3, 120.0, (CH-Ar, Fmoc), 97.2 (d, *J* = 20.4 Hz, C-1), 86.8 (d, *J* = 195.9 Hz, C-2), 82.2 (O-C-$(CH_3)_3$, O^{t}Bu), 77.6

(C-1'), 70.6 (d, *J* = 19.4 Hz, C-3), 68.2 (d, *J* = 6.5 Hz, C-4), 67.7 (C-5), 67.5 (CH_2-Fmoc), 61.8 (C-6), 59.2 (C-2'), 47.2 (CH-Fmoc), 28.0 ($(CH_3)_3$, O^{t}Bu), 20.77, 20.70, 20.65 (CH_3, OAc), 18.6 (CH_3, Thr); **FT-IR (neat)** *ν* in cm^{-1}: 1748, 1508, 1368, 1225, 1159, 1078, 1034; **HRMS (TOF ES$^+$)** for $(M+Na)^+C_{35}H_{42}FNNaO_{12}^+$ (m/z): calc. 710.2583; found 710.2579.

Data for **6.5β:** ***R*$_f$** (3:7 EtOAc/hexane): 0.25; **[α]$^D_{25}$**: + 11.5 (0.71, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.63 (dd, *J* = 7.3 Hz, *J* = 2.2 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.5 Hz, 2H, CH-Ar), 7.31 (t, *J* = 7.5 Hz, 2H, CH-Ar), 5.57 (d, $J_{NH,2'}$ = 9.4 Hz, 1H, NH'), 5.32 (dt, $J_{3,F}$ = 14.5 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, 1H, H-3), 5.01 (t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1H, H-4), 4.62 (dd, $J_{1,2}$ = 7.7 Hz, $J_{1,2}$ = 2.7 Hz, 1H, H-1), 4.51 (qd, $J_{1',CH3}$ = 6.3 Hz, $J_{1',2'}$ = 2.5 Hz, 1H, H-1'), 4.41-4.06 (m, 7H, H-2, CH_2-O-Fmoc, H-2', CH-Fmoc, H-6a, H-6b), 3.70 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 2.1 Hz, 1H, H-5), 2.09, 2.05, 2.04 (s, 9H, $3CH_3$, Ac), 1.47 (s, 9H, CH_3, O^{t}Bu), 1.28 (d, $J_{1',CH3}$ = 6.3 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –199.7 (ddd, $J_{F,2}$ = 50.5 Hz, $J_{F,3}$ = 14.5 Hz, $J_{F,1}$ = 2.6 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 170.1, 169.6, 168.9, (C=O, Ac, Fmoc), 156.8 (C=O, Thr), 143.9, 143.8, 141.3, (C-Ar, Fmoc), 127.7, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 97.0 (d, *J* = 22.7 Hz, C-1), 89.1 (d, *J* = 191.5 Hz, C-2), 82.3 (O-C-$(CH_3)_3$, O^{t}Bu), 73.8 (C-1'), 72.8 (d, *J* = 19.8 Hz, C-3), 71.8 (C-5), 67.9 (d, *J* = 7.6 Hz, C-4), 67.3 (CH_2-Fmoc), 61.7 (C-6), 58.7 (C-2'), 47.2 (CH-Fmoc), 27.9 ($(CH_3)_3$, O^{t}Bu), 20.71, 20.67, 20.60 (CH_3, OAc), 15.9 (CH_3, Thr); **FT-IR (neat)** *ν* in cm^{-1}: 2360, 2339, 1748, 1733, 1520, 1507, 1368, 1226, 1162, 1065, 1034; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{42}FNNaO_{12}^+$ (m/z): calc. 710.2583; found 710.2582.

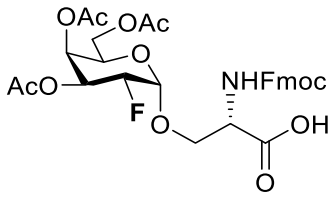
N^α-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-mannopyranosyl)-L-threonine *tert*-butyl ester (6.6)



The general procedure was applied using mannosyl bromide **3.12-Br** (86.0 mg, 0.232 mmol), Fmoc-Thr-O^{t}Bu (110.8 mg, 0.278 mmol), preactivated 4 Å MS, silver oxide (53.8 mg, 0.232 mmol) and TfOH (4.1 µL, 0.046 mmol) in 4.6 mL of toluene. After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 72% yield of **6.6** obtaining an α:β ratio of 1:0. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.6α** as a white foam (67.8 mg, 42.5%).

***R**$_f$* (3:7 EtOAc/hexane): 0.23; **[α]$^D_{25}$**: + 39.1 (0.77, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.78 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.65 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.41 (td, *J* = 7.5 Hz, *J* = 2.4 Hz, 2H, CH-Ar), 7.37-7.30 (m, 2H, CH-Ar), 5.47 (d, $J_{2',NH'}$ = 9.4 Hz, 1H, NH'), 5.35-5.26 (m, 1H, H-4), 5.18 (ddd, $J_{3,F}$ = 28.1 Hz, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 2.4 Hz, 1H, H-3), 5.08 (d, $J_{1,F}$ = 6.3 Hz, 1H, H-1), 4.63 (d, $J_{2,F}$ = 49.8 Hz, 1H, H-2), 4.45-4.32 (m, 4H, CH_2-O-Fmoc, H-2', H-1'), 4.30-4.22 (m, 2H, CH-Fmoc, H-6a), 4.14 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.0 Hz, 1H, H-6b), 4.06 (ddd, $J_{4,5}$ = 8.3 Hz, $J_{5,6a}$ = 5.9 Hz, $J_{5,6b}$ = 2.0 Hz, 1H, H-5), 2.11, 2.09, 2.06, (s, 9H, 3CH_3, Ac), 1.49 (s, 9H, CH_3, O^{t}Bu), 1.33 (d, $J_{1',CH3}$ = 6.3 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –202.9 (ddd, $J_{F,2}$ = 50.3 Hz, $J_{F,3}$ = 27.6 Hz, $J_{F,1}$ = 7.5 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.7, 170.1, 169.5, 169.0 (C=O, Ac, Fmoc), 155.6 (C=O, Thr), 143.9, 143.8, 141.3, (C-Ar, Fmoc), 127.8, 127.1, 125.2, 125.1, 120.04, 120.01, (CH-Ar, Fmoc), 98.7 (d, *J* = 29.8 Hz, C-1), 86.9 (d, *J* = 180.4 Hz, C-2), 83.0 (O-C-(CH_3)$_3$, O^{t}Bu), 77.9 (C-1'), 69.6 (d, *J* = 16.7 Hz, C-3), 69.1 (C-5), 67.5 (CH_2-Fmoc), 66.0 (C-4), 62.2 (C-6), 59.0 (C-2'), 47.2 (CH-Fmoc), 28.0 ((CH_3)$_3$, O^{t}Bu), 20.77, 20.73, 20.66 (CH_3, OAc), 18.3 (CH_3, Thr); **FT-IR (neat)** *ν* in cm^{-1}: 2925, 1748, 1718, 1507, 1369, 1226, 1157, 1048; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{42}FNNaO_{12}^+$ (m/z): calc. 710.2583; found 710.2583.

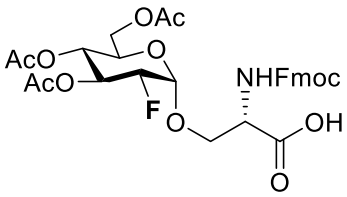
N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-galactopyranosyl)-L-serine (6.7)



The general procedure was applied to **6.1α** (19.0 mg, 0.028 mmol) using trifluoroacetic acid (0.5 mL) and distilled water (56.5 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.7α** as a white solid (15.1 mg, 87%).

R_f (CH_2Cl_2): 0.29; **[α]$^{D}_{25}$**: + 52.5 (0.65, MeOH); **^{1}H NMR** (MeOD, 400 MHz) δ in ppm: 7.83 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.67-7.60 (m, 2H, CH-Ar), 7.42 (t, *J* = 7.2 Hz, 2H, CH-Ar), 7.33 (tdd, *J* = 7.4 Hz, *J* = 2.4 Hz, *J* = 1.2 Hz, 2H, CH-Ar), 5.46 (m, 1H, H-4), 5.41 (td, $J_{2,3} = J_{3,F}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 5.19 (d, $J_{1,2}$ = 3.3 Hz, 1H, H-1), 4.73 (ddd, $J_{F,2}$ = 49.8 Hz, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 4.52 (bs, 1H, H--2'), 4.44 (dd, *J* = 10.3 Hz, *J* = 7.1 Hz, 1H, H-CH-Fmoc), 4.39-4.31 (m, 1H, H-5), 4.31 (m, 1H, H-CH-Fmoc), 4.29 (t, *J* = 7.0 Hz, 1H, CH-Fmoc), 4.14-3.96 (m, 4H, H-6a, H-6b, H-1', H-1'), 2.14, 2.03, 1.90 (s, 9H, 3CH_3, Ac); **^{19}F NMR** (MeOD, 376.5 MHz) δ in ppm: –205.7 (ddd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 10.7 Hz, $J_{F,4}$ = 2.9 Hz, F-2); **^{13}C NMR** (MeOD, 100.6 MHz) δ in ppm: 170.6, 170.5, 170.2 (C=O, Ac), 144.0, 143.9, 141.2 (C=O, C-Ar, Fmoc), 127.42, 127.4, 126.8, 124.9, 119.54 (CH-Ar, Fmoc), 97.1 (d, $J_{1,F}$ = 20.9 Hz, C-1), 85.1 (d, $J_{2,F}$ = 190.8 Hz, C-2), 68.7 (C-1'), 68.6 (d, $J_{4,F}$ = 8.0 Hz, C-4), 68.3 (d, $J_{3,F}$ = 18.9 Hz, C-3), 66.8 (CH_2-Fmoc), 66.6 (C-5), 61.3 (C-6), 47.8 (C-2') 46.9 (CH-Fmoc), 19.2, 19.1, 19.0 (3xCH_3CO); **FT-IR (neat)** *ν* in cm^{-1}: 2956, 2923, 1748, 1717, 1521, 1450, 1373, 1230, 1153, 1075, 1049; **HRMS (TOF ES^{+})** for $[M+NH_4]^+$ for $C_{30}H_{36}FN_2O_{12}^+$ (m/z): 635.2247; calculated: 635.2253.

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-serine (6.8)

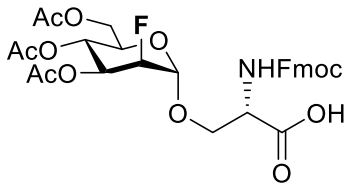


The general procedure was applied to **6.2α** (22.2 mg, 0.033 mmol) using trifluoroacetic acid (0.6 mL) and distilled water (66.0 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.8α** as a white solid (17.0 mg, 84%).

***R**$_f$*(CH_2Cl_2): 0.32; **^{1}H NMR** (MeOD, 400 MHz) δ in ppm: 7.83 (dd, *J* = 7.5 Hz, *J* = 1.0 Hz, 2H, CH-Ar), 7.72 (m, 2H, CH-Ar), 7.42 (t, *J* = 7.5 Hz, 2H, CH-Ar), 7.34 (t, *J* = 7.4 Hz, 2H, CH-Ar), 5.52 (dt, $J_{3,F}$ = 11.8 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.9 Hz, 1H, H-3), 5.14 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.99 (t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 4.64 (ddd, $J_{2,F}$ = 50.5 Hz, $J_{2,3}$ = 9.6 Hz, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 4.54-4.46 (m, 1H, H-1'), 4.40 (s, 1H, H-2'), 4.35-4.22 (m, 2H, H-1'', CH-Fmoc), 4.24-4.02 (m, 5H, CH_2-Fmoc, H-6a, H-6b, H-5), 2.05, 2.03, 1.95 (s, 9H, 3CH_3, Ac); **^{19}F NMR** (MeOD, 376.5 MHz) δ in ppm: –203.0 (dd, $J_{F,2}$ = 49.1 Hz, $J_{F,3}$ = 11.9 Hz, F-2); **^{13}C NMR** (MeOD, 100.6 MHz) δ in ppm: 170.9, 170.3, 169.8, (C=O, Ac, Ser), 144.0, 143.8, 141.2, 141.17 (C-Ar, Fmoc), 127.41, 127.37, 126.8, 125.0, 124.8, 119.55, 119.52, (CH-Ar, Fmoc), 96.8 (d, *J* = 20.5 Hz, C-1), 87.2 (d, *J* = 193.9 Hz, C-2), 70.7 (d, *J* = 19.3 Hz, C-3), 69.4 (CH_2-Fmoc), 68.2 (d, *J* = 7.1 Hz, C-4), 67.5 (C-5), 66.6 (C-1'), 61.7 (C-6), 47.9 (C-2'), 47.0 (CH-Fmoc), 19.24, 19.22, 19.13 (CH_3, OAc); **HRMS (TOF ES$^-$)** for $(M-H)^-$ $C_{30}H_{31}FNO_{12}^-$ (m/z): calc. 616.1836; found 616.1848; spectroscopic data was identical to the previously described.[25]

[25] Maschauer, S.; Pischetsrieder, M.; Kuwert, T.; Prante, O. *J. Label. Compd. Radiopharm.* **2005**, *48*, 701-719.

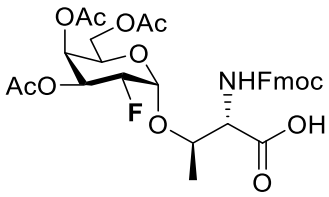
N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-mannopyranosyl)-L-serine *tert*-butyl ester (6.9)



The general procedure was applied to **6.3α** (50.4 mg, 0.075 mmol) using trifluoroacetic acid (1.35 mL) and distilled water (150 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.9α** as a white foam (41.1 mg, 89%).

R$_f$ (95:5 CH_2Cl_2:AcOH): 0.44; **[α]$^{D}_{25}$**: + 23.0 (0.59, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.76 (d, *J* = 7.4 Hz, 2H, CH-Ar), 7.60 (m, 2H, CH-Ar), 7.39 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.30 (t, *J* = 7.1 Hz, 2H, CH-Ar), 6.32 (d, $J_{NH,2'}$ = 7.0 Hz, 1H, NH'), 5.35-5.22 (m, 2H, H-3, H-4), 5.00 (d, $J_{1,F}$ = 6.2 Hz, 1H, H-1), 4.71 (d, $J_{2,F}$ = 50.2 Hz, 1H, H-2), 4.64 (bs, 1H, H-2'), 4.44 (dd, $J_{1',1''}$ = 10.3 Hz, $J_{1',2'}$ = 7.4 Hz, 1H, H-1'), 4.35 (dd, $J_{1',1''}$ = 10.3 Hz, $J_{1',2'}$ = 7.2 Hz, 1H, H-1''), 4.26-3.98 (m, 6H, CH_2-Fmoc, H-5, CH-Fmoc, H-6a, H-6b), 2.08, 2.05, 1.97 (s, 9H, $3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –204.0 (dd, $J_{F,2}$ = 49.0 Hz, $J_{F,3}$ = 27.1 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.9, 170.87, 169.6, (C=O, Ac, Fmoc), 156.1 (C=O, Ser), 143.7, 141.3 (C-Ar, Fmoc), 127.8, 127.1, 125.1, 125.0, 120.0, (CH-Ar, Fmoc), 97.8 (d, *J* = 29.6 Hz, C-1), 86.6 (d, *J* = 179.6 Hz, C-2), 70.1 (C-1'), 69.9 (C-4), 69.2 (C-3), 69.1 (C-5), 67.4 (CH_2-Fmoc), 65.8 (C-2'), 62.0 (C-6), 47.1 (CH-Fmoc), 20.8, 20.7, 20.6 (CH_3, OAc); **FT-IR:** 1748, 1718, 1684, 1539, 1521, 1369, 1231, 1086, 1050; **HRMS (TOF ES^{-})** for $(M-H)^{-}$ $C_{30}H_{31}FNO_{12}^{-}$ (m/z): calc. 616.1836; found 616.1847.

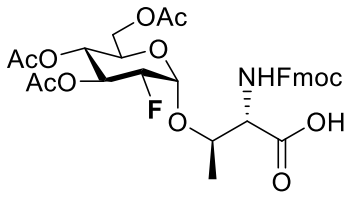
N^{α}-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-galactopyranosyl)-L-threonine (6.10)



The general procedure was applied to **6.4α** (30.1 mg, 0.044 mmol) using trifluoroacetic acid (0.8 mL) and distilled water (87 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.10α** as a white solid (18.7 mg, 68%).

***R**f* (95:5 CH_2Cl_2:AcOH): 0.31; **$[\alpha]^{D}_{25}$**: + 50.0 (0.58, MeOH); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.76 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.63 (m, 2H, CH-Ar), 7.40 (t, *J* = 7.1 Hz, 2H, CH-Ar), 7.32 (td, *J* = 7.5 Hz, *J* = 3.0 Hz, 2H, CH-Ar), 5.81 (d, $J_{NH,2'}$ = 8.8 Hz, 1H, NH'), 5.49 (bs, 1H, H-4), 5.36 (dt, $J_{3,F}$ = 11.4 Hz, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 2.6 Hz, 1H, H-3), 5.24 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.71 (ddd, $J_{2,F}$ = 50.2 Hz, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 4.52-4.29 (m, 4H, H-1', H-2', CH_2-Fmoc), 4.35-4.30 (m, 1H, H-5), 4.25 (t, *J* = 7.6 Hz, 1H, CH-Fmoc), 4.08 (d, $J_{5,6}$ = 6.3 Hz, 2H, H-6a, H-6b), 2.13, 2.03, 2.02 (s, 9H, $3CH_3$, Ac), 1.35 (d, $J_{CH3,1'}$ = 5.8 Hz, 3H, CH_3, Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –207.1 (dd, $J_{F,2}$ = 49.6 Hz, $J_{F,3}$ = 11.2 Hz, F-2); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.4, 170.36, 170.0, (C=O, Ac, Fmoc), 156.8 (C=O, Thr), 143.8, 143.7, 141.3 (C-Ar, Fmoc), 127.7, 127.1, 125.2, 120.0, (CH-Ar, Fmoc), 97.9 (d, *J* = 21.5 Hz, C-1), 87.2 (d, *J* = 191.3 Hz, C-2), 77.6 (C-1'), 68.6 (d, *J* = 7.0 Hz, C-4), 68.2 (d, *J* = 18.9 Hz, C-3), 67.5 (C-5), 67.0 (CH_2-Fmoc), 61.6 (C-6), 58.5 (C-2'), 47.1 (CH-Fmoc), 20.66, 20.60, 20.52 (CH_3, OAc), 18.1 (CH_3, Thr); **FT-IR:** 1749, 1733, 1717, 1684, 1558, 1540, 1507, 1374, 1232, 1074, 1036; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{34}FNNaO_{12}^+$ (m/z): calc. 654.1957; found 654.1954.

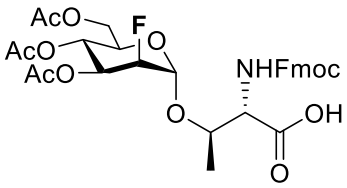
N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-threonine (6.11)



The general procedure was applied to **6.5α** (34.5 mg, 0.05 mmol) using trifluoroacetic acid (0.9 mL) and distilled water (100 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.11α** as a white solid (16.4 mg, 60%).

R$_f$ (95:5 CH_2Cl_2:AcOH): 0.45; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.78 (d, *J* = 7.3 Hz, 2H, CH-Ar), 7.60 (m, 2H, CH-Ar), 7.41 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.33 (m, 2H, CH-Ar), 5.72 (d, $J_{NH,2'}$ = 7.0 Hz, 1H, NH'), 5.38 (dt, $J_{3,F}$ = 11.0 Hz, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 1H, H-3), 5.21 (s, 1H, H-1), 4.99 (t, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 4.58-4.35 (m, 5H, H-2, H-2', CH_2-Fmoc, H-1'), 4.32-4.19 (m, 2H, CH-Fmoc, H-6a), 4.14-4.05 (m, 2H, H-5, H-6b), 2.07, 2.05 (s, 9H, $3CH_3$, Ac), 1.36 (d, $J_{CH3,1'}$ = 5.9 Hz, 3H, CH_3-Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –200.0 (dd, $J_{F,2}$ = 49.9 Hz, $J_{F,3}$ = 11.0 Hz, F-2); **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{34}FNNaO_{12}^+$ (m/z): calc. 654.1957; found 654.1957; spectroscopic data was identical to the previously described.[25]

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-mannopyranosyl)-L-serine *tert*-butyl ester (6.12)

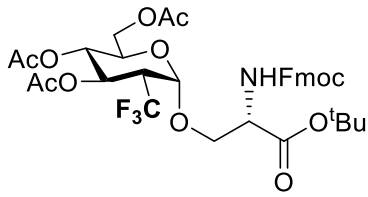


The general procedure was applied to **6.6α** (35 mg, 0.048 mmol) using trifluoroacetic acid (0.85 mL) and distilled water (100 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.12α** as a white solid (19.8 mg, 65.3%).

***R**$_f$* (95:5 CH_2Cl_2:AcOH): 0.52; **[α]$^{D}_{25}$**: + 37.2 (0.42, MeOH); **^{1}H NMR** ($CHCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.64 (dd, *J* = 7.2 Hz, *J* = 3.8 Hz, 2H, CH-Ar), 7.41 (dd, *J* = 7.3 Hz, *J* = 2.7 Hz, 2H, CH-Ar), 7.34 (m, 2H, CH-Ar), 5.82 (d, $J_{NH,2'}$ = 9.6 Hz, 1H, NH), 5.31 (t, $J_{3,4}$ = $J_{4,5}$ = 10.0 Hz, 1H, H-4), 5.20 (ddd, $J_{3,F}$ = 28.2 Hz, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 2.2 Hz, 1H, H-3), 5.09 (d, $J_{1,F}$ = 6.5 Hz, 1H, H-1), 4.65 (ddd, $J_{2,F}$ = 49.7 Hz, $J_{2,3}$ = $J_{1,2}$ = 2.1 Hz, 1H, H-2), 4.52 (dd, $J_{NH,2'}$ = 9.6 Hz, $J_{1',2'}$ = 2.2 Hz, 1H, H-2'), 4.49-4.39 (m, 3H, H-1', CH_2-Fmoc), 4.25 (dd, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6a}$ = 5.7 Hz, 1H, H-6a), 4.29-4.21 (m, 1H, CH-Fmoc), 4.25 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 2.2 Hz, 1H, H-6b), 4.10-4.02 (m, 1H, H-5), 3.35 (m, 1H, H-2'), 2.05, 2.03, 1.95 (s, 9H, $3CH_3$, Ac), 1.34 (d, $J_{1',CH3}$ = 6.3 Hz, 3H, CH_3, Thr); **^{19}F NMR** ($CHCl_3$, 376.5 MHz) δ in ppm: –203.1 (dd, $J_{F,2}$ = 49.7 Hz, $J_{F,3}$ = 28.1 Hz, $J_{F,1}$ = 6.8 Hz, F-2); **^{13}C NMR** ($CHCl_3$, 100.6 MHz) δ in ppm: 171.0, 170.9, 169.6 (C=O, Ac), 157.0 (C=O, Thr), 143.9, 143.8, 141.4 (C-Ar, Fmoc), 128.0, 127.3, 125.3, 120.2 (CH-Ar, Fmoc), 98.4 (d, *J* = 28.2 Hz, C-1), 86.6 (d, *J* = 181.8 Hz, C-2), 77.7 (C-1'), 70.2 (d, *J* = 17.2 Hz, C-3), 69.4 (C-5), 67.6 (CH_2-Fmoc), 65.8 (C-4), 62.3 (C-6), 58.5 (C-2'), 47.3 (CH-Fmoc), 20.93, 20.84, 20.75, (CH_3, OAc), 18.0 (CH_3, Thr); **FT-IR:** 2924, 1748, 1508, 1369, 1340, 1221, 1181, 1158, 1082, 1048; **HRMS (TOF ES^{+})** for $(M+Na)^+$ $C_{31}H_{34}FNNaO_{12}^+$ (m/z): calc. 654.1957; found 654.1957.

6.5.6. Synthesis of trifluoromethylated analogues of the Tn antigen

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucopyranosyl)-L-serine-*tert*-butyl ester (6.13)



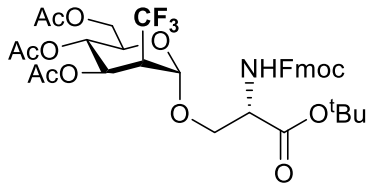
The general procedure of bromination was applied using glucosyl acetate **4.7** (45 mg, 0.112 mmol), 33% HBr/AcOH (0.6 mL) in CH_2Cl_2 (0.6 mL). After 3 h of stirring at room temperature, TLC indicated the reaction had finished. After standard work-up, the crude glucosyl bromide **4.7-Br** was azeotropically dried twice with toluene and maintain under vacuum for 2 h. The general procedure of glycosylation was applied to the crude using Fmoc-Ser-O^{t}Bu (51.5 mg, 0.134 mmol), preactivated 4 Å MS, Ag_2O (26.0 mg, 0.112 mmol) and TfOH (2 µL, 0.022 mmol) in toluene (2.4 mL). After 3h of stirring at -10 °C, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 42% yield of **6.13** obtaining an α:β ratio of 1.5:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.13α** as a white foam (12.1 mg, 15%), a mixture of **6.13α/β** (5.1 mg, 1:1 (α:β), 6.3%), and **6.13β** as a white foam (8.1 mg, 10%).

Data for **6.13α*: R_f*** (1:1 EtOAc/hexane): 0.62; **[α]$^{D}_{25}$**: + 29.3 (0.48, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.6 Hz, 2H, CH-Ar), 7.64 (dd, *J* = 7.3 Hz, *J* = 3.0 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.5 Hz, 2H, CH-Ar), 7.33 (td, *J* = 7.4 Hz, *J* = 1.1 Hz, 2H, CH-Ar), 5.76 (d, $J_{2',NH'}$ = 7.7 Hz, 1H, NH'), 5.61 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 5.09 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 5.00 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.45-4.37 (m, 3H, H-2', CH_2-O-Fmoc), 4.24 (t, *J* = 7.0 Hz, 1H, CH-Fmoc), 4.18-4.19 (m, 1H, H-1'), 4.11-3.96 (m, 4H, H-1'', H-5, H-6a, H-6b), 2.83 (dqd, $J_{2,3}$ = 11.3 Hz, $J_{2,CF3}$ = 7.6 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.06, 2.03, 2.00 (s, 9H, $3CH_3$, OAc), 1.49 (s, 9H, $3CH_3$, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –64.9 (d, $J_{2,CF3}$ = 7.6 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 169.8, 169.4, 168.3 (C=O,

Ac, Fmoc), 155.8 (C=O, Ser), 143.8, 143.7, 141.3 (C-Ar, Fmoc), 127.8, 127.7, 127.1, 125.2, 125.1 (CH-Ar, Fmoc), 123.9 (q, *J* = 282.0 Hz, CF_3), 120.0, 119.97 (CH-Ar, Fmoc), 95.7 (q, *J* = 4.6 Hz, C-1), 83.3 (O-C-$(CH_3)_3$, O^{t}Bu), 69.2 (C-1'), 68.7 (C-5), 68.0 (C-4), 67.2 (CH_2-Fmoc), 66.7 (C-3), 61.7 (C-6), 54.6 (C-2'), 48.4 (q, *J* = 26.9 Hz, C-2), 47.1 (CH-Fmoc), 27.9 (3xCH_3, O^{t}Bu), 20.65, 20.58, 20.56 (3xCH_3, OAc); **FT-IR (neat)** *ν* in cm^{-1}: 2923, 2852, 1747, 1451, 1369, 1342, 1223, 1180, 1157, 1140, , 1106, 1044; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{40}F_3NNaO_{12}^+$ (m/z): calc. 746.2395; found 746.2384.

Data for **6.13β*: R_f** (1:1 EtOAc/hexane): 0.50; **$[\alpha]^D_{25}$**: + 14.6 (0.31, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.61 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.41 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.33 (td, *J* = 7.4 Hz, *J* = 3.5 Hz, 2H, CH-Ar), 5.64 (d, $J_{2',NH'}$ = 8.1 Hz, 1H, NH'), 5.43 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.1 Hz, 1H, H-3), 5.00 (t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 4.62 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1), 4.42 (dt, $J_{2',NH'}$ = 8.1 Hz, $J_{1',2'}$ = $J_{1'',2'}$ = 2.4 Hz, 1H, H-2'), 4.45-4.19 (m, 5H, CH_2-O-Fmoc, CH-Fmoc, H-1', H-6a), 4.13 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.4 Hz, 1H, H-6b), 3.82 (dd, $J_{1',1''}$ = 9.7 Hz, $J_{1'',2'}$ = 2.8 Hz, 1H, H-1''), 3.68 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 2.8 Hz, 1H, H-5), 2.66 (dquint, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = $J_{2,CF3}$ = 8.0 Hz, 1H, H-2), 2.07, 2.03 (s, 9H, 3CH_3, OAc), 1.48 (s, 9H, 3CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –65.5 (d, $J_{2,CF3}$ = 7.6 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 169.7, 169.5, 168.3 (C=O, Ac, Fmoc), 155.8 (C=O, Ser), 143.9, 143.7, 141.3 (C-Ar, Fmoc), 127.7, 127.2, 125.1 (CH-Ar, Fmoc), 124.3 (q, *J* = 281.4 Hz, CF_3), 120.0 (CH-Ar, Fmoc), 98.4 (C-1), 82.8 (O-C-$(CH_3)_3$, O^{t}Bu), 71.4 (C-5), 70.0 (C-1'), 68.7 (C-4), 67.9 (C-3), 67.2 (CH_2-Fmoc), 61.9 (C-6), 54.4 (C-2'), 47.1 (CH-Fmoc), 49.5 (q, *J* = 23.8 Hz, C-2), 27.9 (3xCH_3, O^{t}Bu), 20.65, 20.56 (3xCH_3, OAc); **FT-IR (neat)** *ν* in cm^{-1}: 1748, 1508, 1369, 1221, 1181, 1158, 1082, 1048; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{40}F_3NNaO_{12}^+$ (m/z): calc. 746.2395; found 746.2379.

N$^{\alpha}$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-mannopyranosyl)-L-serine *tert*-butyl ester (6.14)

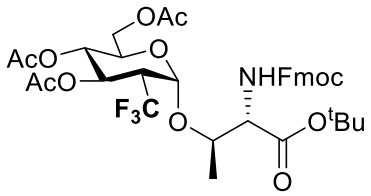


The general procedure of bromination was applied using mannosyl acetate **4.8** (54.9 mg, 0.137 mmol), 33% HBr/AcOH (0.7 mL) in CH_2Cl_2 (0.7 mL). After 3 h of stirring at room temperature, TLC indicated the reaction had finished. After standard work-up, the crude mannosyl bromide **4.8-Br** was azeotropically dried twice with toluene and maintain under vacuum for 2 h. The general procedure of glycosylation was applied to the crude using Fmoc-Ser-O^{t}Bu (63.0 mg, 0.164 mmol) and preactivated 4 Å MS in CH_2Cl_2 (1.4 mL), then AgOTf (70.4 mg, 0.274 mmol) was added in toluene (1.4 mL). After 3h of stirring at -80 °C, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 67% yield of **6.14** obtaining an α:β ratio of 1:0. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.14α** as a white foam (33.2 mg, 33.5%).

R$_f$ (3:7 EtOAc/hexane): 0.23**; [α]$^{D}_{25}$**: + 43.4 (0.35, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.62 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.32 (t, *J* = 7.3 Hz, 2H, CH-Ar), 5.74 (d, $J_{2',NH'}$ = 7.7 Hz, 1H, NH'), 5.38-5.28 (m, 2H, H-3, H-4), 5.15 (s, 1H, H-1), 4.46 (td, $J_{2',NH'}$ = 7.7 Hz, $J_{1',2'}$ = $J_{1'',2'}$ = 3.2 Hz, 1H, H-2'), 4.42 (d, *J* = 7.4 Hz, 2H, CH_2-O-Fmoc), 4.24 (t, *J* = 7.0 Hz, 1H, CH-Fmoc), 4.19-4.13 (m, 2H, H-6a, H-6b), 4.01-3.95 (m, 3H, H-1', H-1'', H-5), 3.11 (qd, $J_{2,CF3}$ = 9.6 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-2), 2.06, 2.05 (s, 9H, 3CH_3, OAc), 1.50 (s, 9H, 3CH_3, O^{t}Bu); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –62.8 (d, $J_{2,CF3}$ = 9.6 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.9, 169.4, 168.5 (C=O, Ac, Fmoc), 155.8 (C=O, Ser), 143.7, 141.3 (C-Ar, Fmoc), 127.8, 127.1, 125.1 (CH-Ar, Fmoc), 124.5 (q, *J* = 280.4 Hz, CF_3), 120.0 (CH-Ar, Fmoc), 96.0 (q, *J* = 4.5 Hz, C-1), 83.2 (O-C-(CH_3)$_3$, O^{t}Bu), 69.4 (C-1'), 68.9 (C-5), 67.5 (C-4), 67.2 (CH_2-

Fmoc), 65.7 (C-3), 62.2 (C-6), 54.6 (C-2'), 47.1 (CH-Fmoc), 46.0 (q, *J* = 26.1 Hz, C-2), 28.0 (3xCH_3, O^{t}Bu), 20.68, 20.61 (3xCH_3, OAc); **FT-IR (neat)** *v* in cm^{-1}: 1747, 1520, 1508, 1451, 1370, 1340, 1267, 1228, 1157, 1121, 1049; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{35}H_{40}F_3NNaO_{12}^+$ (m/z): calc. 746.2395; found 746.2409.

N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucopyranosyl)-L-threonine *tert*-butyl ester (6.15)



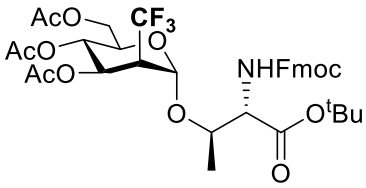
The general procedure of bromination was applied using glucosyl acetate **4.7** (50.2 mg, 0.125 mmol), 33% HBr/AcOH (0.65 mL) in CH_2Cl_2 (0.65 mL). After 3 h of stirring at room temperature, TLC indicated the reaction had finished. After standard work-up, the crude glucosyl bromide **4.7-Br** was azeotropically dried twice with toluene and maintain under vacuum for 2 h. The general procedure of glycosylation was applied to the crude using Fmoc-Thr-O^{t}Bu (59.8 mg, 0.151 mmol), preactivated 4 Å MS, Ag_2O (29.0 mg, 0.125 mmol) and TfOH (2.2 µL, 0.025 mmol) in toluene (2.5 mL). After 3h of stirring at -10 °C, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 38% yield of **6.15** obtaining an α:β ratio of 1:1. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.15α** as a white foam (10.9 mg, 12%), a mixture of **6.15α/β** (4.6 mg, 1.2:1 (α:β), 5%), and **6.15β** as a white foam (11.3 mg, 12,3%).

Data for **6.15α:** R_f (1:1 EtOAc/hexane): 0.50; **$[\alpha]^D_{25}$**: + 59.0 (0.56, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.77 (d, *J* = 7.6 Hz, 2H, CH-Ar), 7.62 (t, *J* = 8.3 Hz, 2H, CH-Ar), 7.40 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.33 (m, 2H, CH-Ar), 5.66 (dd, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 5.48 (d, $J_{2',NH'}$ = 9.4 Hz, 1H, NH'), 5.30 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 5.02 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4), 4.45-4.19 (m, 6H, H-1', H-2', CH_2-O-Fmoc, CH-Fmoc, H-6a), 4.12 (ddd, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-5), 4.10 (dd, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-6b), 2.83 (dqd, $J_{2,3}$ = 11.3 Hz,

$J_{2,CF3}$ = 7.7 Hz, $J_{1,2}$ = 3.4 Hz, 1H, H-2), 2.08, 2.06, 2.04 (s, 9H, 3CH_3, OAc), 1.48 (s, 9H, 3CH_3, O^{t}Bu), 1.34 (d, $J_{1',CH3}$ = 6.5 Hz, 1H, CH_3, Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –64.4 (d, $J_{2,CF3}$ = 7.7 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.8, 169.6, 169.0 (C=O, Ac, Fmoc), 156.6 (C=O, Thr), 143.9, 143.8, 141.3 (C-Ar, Fmoc), 127.7, 127.1, 125.3 (CH-Ar, Fmoc), 124.5 (q, J = 281.6 Hz, CF_3), 120.0, 119.98 (CH-Ar, Fmoc), 94.6 (q, $J_{1,CF3}$ = 4.7 Hz, C-1), 83.0 (O-C-(CH_3)$_3$, O^{t}Bu), 75.7 (C-1'), 68.9 (C-4), 68.2 (CH_2-Ph), 67.5 (C-3), 66.7 (CH_2-Fmoc), 61.9 (C-6), 58.9 (C-2'), 48.5 (q, $J_{2,CF3}$ = 25.4 Hz, C-2), 47.1 (CH-Fmoc), 27.9 (3xCH_3, O^{t}Bu), 20.7, 20.64 (3xCH_3, OAc), 18.8 (CH_3, Thr); **FT-IR (neat)** ν in cm^{-1}: 1747, 1516, 1508, 1451, 1368, 1308, 1223, 1180, 1157, 1123, 1091, 1029; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{36}H_{42}F_3NNaO_{12}^+$ (m/z): calc. 760.2551; found 760.2547.

Data for **6.15β:** R_f (1:1 EtOAc/hexane): 0.42; **$[\alpha]^D_{25}$**: + 5.4 (0.37, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.76 (d, J = 7.5 Hz, 2H, CH-Ar), 7.61 (d, J = 7.0 Hz, 2H, CH-Ar), 7.40 (t, J = 7.5 Hz, 2H, CH-Ar), 7.31 (tt, J = 7.5 Hz, J = 1.3 Hz, 2H, CH-Ar), 5.50 (d, $J_{2',NH'}$ = 9.6 Hz, 1H, NH'), 5.45 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.9 Hz, 1H, H-3), 5.00 (t, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-4), 4.72 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.52 (qd, $J_{1',CH3}$ = 6.0 Hz, $J_{1',2'}$ = 2.3 Hz, 1H, H-1'), 4.41 (dd, J = 10.4 Hz, J = 7.8 Hz, 1H, CH-Fmoc), 4.37-4.22 (m, 4H, H-2', CH_2-O-Fmoc, H-6a), 4.12 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.5 Hz, 1H, H-6b), 3.69 (ddd, $J_{4,5}$ = 10.2 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 2.3 Hz, 1H, H-5), 2.66 (dquint, $J_{2,3}$ = 10.8 Hz, $J_{1,2}$ = $J_{2,CF3}$ = 7.9 Hz, 1H, H-2), 2.06, 2.04, 2.03 (s, 9H, 3CH_3, OAc), 1.47 (s, 9H, 3CH_3, O^{t}Bu), 1.23 (d, $J_{1',CH3}$ = 6.3 Hz, 3H, CH_3, Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –65.3 (d, $J_{2,CF3}$ = 7.5 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.5, 169.7, 169.6, 168.8 (C=O, Ac, Fmoc), 156.6 (C=O, Ser), 144.2, 143.7, 141.3 (C-Ar, Fmoc), 127.7, 127.0, 125.2 (CH-Ar, Fmoc), 124.5 (q, CF_3), 120.0 (CH-Ar, Fmoc), 94.6 (q, J = 5.1 Hz, C-1), 82.3 (O-C-(CH_3)$_3$, O^{t}Bu), 73.4 (C-1'), 71.4 (C-5), 68.7 (C-4), 68.0 (C-3), 67.3 (CH_2-Fmoc), 61.7 (C-6), 58.6 (C-2'), 49.6 (q, J = 23.8 Hz, C-2), 47.1 (CH-Fmoc), 27.9 (3xCH_3, O^{t}Bu), 20.62, 20.56 (3xCH_3, OAc), 15.1 (CH_3, Thr); **FT-IR (neat)** ν in cm^{-1}: 1747, 1515, 1509, 1450, 1369, 1321, 1294, 1210, 1181, 1159, 1125, 1087, 1043; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{36}H_{42}F_3NNaO_{12}^+$ (m/z): calc. 760.2551; found 760.2543.

N^{α}-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-mannopyranosyl)-L-threonine *tert*-butyl ester (6.16)

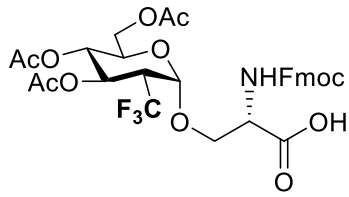


The general procedure of bromination was applied using mannosyl acetate **4.8** (44 mg, 0.110 mmol), 33% HBr/AcOH (0.55 mL) in CH_2Cl_2 (0.55 mL). After 3 h of stirring at room temperature, TLC indicated the reaction had finished. After standard work-up, the crude mannosyl bromide **4.8-Br** was azeotropically dried twice with toluene and maintain under vacuum for 2 h. The general procedure of glycosylation was applied to the crude using Fmoc-Thr-O^{t}Bu (52.5 mg, 0.132 mmol), preactivated 4 Å MS, Ag_2O (25.5 mg, 0.110 mmol) and TfOH (2 µL, 0.022 mmol) in toluene (2.2 mL). After 3h of stirring at -10 °C, the TLC indicated the reaction had finished. After standard work-up, quantitative ^{19}F NMR analysis indicated 50% yield of **6.16** obtaining an α:β ratio of 1:0. The residue was purified by flash column chromatography (SiO_2, from hexane to 1:1 EtOAc/Hex) to afford **6.16α** as a white foam (25.4 mg, 32%).

***R*$_f$** (1:1 EtOAc/hexane): 0.52; **[α]$^D_{25}$**: +26.4 (0.41, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 7.78 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.65 (d, *J* = 7.5 Hz, 2H, CH-Ar), 7.41 (t, *J* = 7.4 Hz, 2H, CH-Ar), 7.33 (td, *J* = 7.3 Hz, *J* = 3.3. Hz, 2H, CH-Ar), 5.49 (d, $J_{2',NH'}$ = 9.4 Hz, 1H, NH'), 5.41-5.29 (m, 2H, H-3, H-4), 5.26 (s, 1H, H-1), 4.52-4.11 (m, 8H, H-2', CH_2-O-Fmoc, CH-Fmoc, H-1', H-1'', H-6a, H-6b), 4.08-4.01 (m, 1H, H-5), 3.11 (qd, $J_{2,CF3}$ = 9.6 Hz, $J_{2,3}$ = 2.6 Hz, 1H, H-2), 2.09, 2.08, 2.07 (s, 9H, $3CH_3$, OAc), 1.48 (s, 9H, $3CH_3$, O^{t}Bu), 1.33 (d, $J_{2',CH3}$ = 6.4 Hz, 3H, CH_3, Thr); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: –62.5 (d, $J_{2,CF3}$ = 9.6 Hz, 3F, CF_3); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 170.0, 169.5, 168.1 (C=O, Ac, Fmoc), 156.7 (C=O, Thr), 143.7, 141.3 (C-Ar, Fmoc), 127.8, 127.1, 125.2 (CH-Ar, Fmoc), 120.0 (CH-Ar, Fmoc), 96.0 (q, *J* = 4.3 Hz, C-1), 83.0 (O-C-$(CH_3)_3$, O^{t}Bu), 68.9 (C-1'), 67.47 (C-5), 67.43 (C-4), 67.2 (CH_2-Fmoc), 65.9 (C-3), 62.5 (C-6), 58.9 (C-2'), 47.2 (CH-Fmoc),

46.3 (m, C-2), 27.9 (3xCH_3, O^{t}Bu), 20.74, 20.64 (3xCH_3, OAc), 18.3 (CH_3, Thr); **FT-IR (neat)** *v* in cm^{-1}: 1746, 1524, 1508, 1450, 1369, 1308, 1267, 1228, 1157, 1115, 1092, 1045; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{36}H_{42}F_3NNaO_{12}^+$ (m/z): calc. 760.2551; found 760.2559.

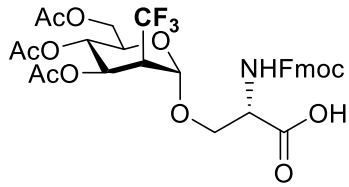
N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucopyranosyl)-L-serine (6.17)



The general procedure was applied to **6.13α** (12.1 mg, 0.017 mmol) using trifluoroacetic acid (0.3 mL) and distilled water (50 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.17α** as a white solid.

R$_f$ (95:5 CH_2Cl_2:AcOH): 0.58; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{32}F_3NNaO_{12}^+$ (m/z): calc. 690.1769; found 690.1767.

N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-mannopyranosyl)-L-serine (6.18)

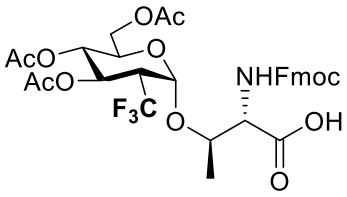


The general procedure was applied to **6.14α** (33.2 mg, 0.046 mmol) using trifluoroacetic acid (0.85 mL) and distilled water (100 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard

work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.18α** as a white solid.

***R*$_f$** (95:5 CH_2Cl_2:AcOH): 0.60; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{31}H_{32}F_3NNaO_{12}^+$ (m/z): calc. 690.1769; found 690.1772.

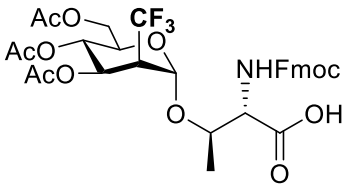
N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-glucopyranosyl)-L-threonine (6.19)



The general procedure was applied to **6.15α** (10.9 mg, 0.015 mmol) using trifluoroacetic acid (0.27 mL) and distilled water (30 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.19α** as a white solid.

***R*$_f$** (95:5 CH_2Cl_2:AcOH): 0.45; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{32}H_{34}F_3NNaO_{12}^+$ (m/z): calc. 704.1925; found 704.1934.

N$^\alpha$-(9-fluorenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-D-mannopyranosyl)-L-threonine (6.20)



The general procedure was applied to **6.16α** (25.4 mg, 0.034 mmol) using trifluoroacetic acid (0.6 mL) and distilled water (70 µL). After 3 h of stirring at room temperature, the TLC indicated the reaction had finished. After standard

work-up, the residue was purified by flash column chromatography (SiO_2, from CH_2Cl_2 to 95:3:2 CH_2Cl_2/MeOH/AcOH) to afford **6.20α** as a white solid.

R_f (95:5 CH_2Cl_2:AcOH): 0.46; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{32}H_{34}F_3NNaO_{12}^+$ (m/z): calc. 704.1925; found 704.1933.

CHAPTER VII

INCORPORATION OF FLUORINATED AND TRIFLUOROMETHYLATED SUGARS IN RECOMBINANT MONOCLONAL ANTIBODIES

Chapter VII

7.1. INTRODUCTION

Antibodies play key roles in host defence against pathogens by recognizing microorganisms or infected cells, controlling and eradicating infections. They drive the clearance of bacteria, viruses, fungi, and parasites by binding to specific antigens, and connecting the adaptive immune response to the innate immune system. The ability of antibodies to produce such immune response has been attributed to their unique structure, that has been successfully exploited to develop new treatments for cancer, inflammatory disorders, and autoimmune diseases.[1] Antibodies are formed by two identical heavy chains and two identical light chains, which are linked by disulphide bonds forming a "Y"-shaped structure. They are composed of two functional domains; the variable domain (Fab) and the constant domain (Fc). The Fab domain is responsible for the recognition of the antigen and is formed by variable domains of light and heavy chains. The Fc region is responsible of the effector functions, interacting with different Fc receptors (FcRs) and proteins, and it is formed entirely by constant domains of heavy chain. Human immunoglobulins are divided into five classes (IgG, IgA, IgD, IgE and IgM) and each antibody has distinct circulation profiles and effector functions. Among them, IgGs are the most abundant class of antibodies in serum, comprising the 75% of all them. They are further categorized into 4 subclasses (IgG1-4), and they present a single N-linked glycan in the Fc portion, which is essential for initiating many IgG effector functions (**Figure 7.1, A**).[2]

Recombinant monoclonal antibodies (rmAbs) have emerged as potent therapeutics for the treatment of infectious and autoimmune diseases and cancer since they present excellent single antigen specificity.[3] Human IgGs are the most common class of rmAbs, and the oligosaccharides present in the Fc portion are essential to the mode of action. Glycosylation has appeared as a

[1] a) Scott, A. M.; Wolchok, J. D.; Old, L. J. *Nat. Rev. Cancer* **2012**, *12*, 278-287; b) Hudis, C. A. *N. Engl. J. Med.* **2007**, *357*, 39-51.
[2] a) Shade, K.-T. C.; Anthony, R. M.; *Antibodies* **2013**, *2*, 392-414; b) Irvine, E. B.; Alter, G. *Glycobiology*, **2020**, *30*, 241-253.
[3] Ecker, D. M.; Jones, S. D.; Levine, H. L. *mAbs* **2015**, *7*, 9-14.

common strategy to improve antibody-based therapeutics and allow the development of rmAb with optimized specificity and effector functions.[4] The mechanism of action of cancer antibodies is normally related to the destruction of tumoural cells or enhancement of tumour-specific T cell immunity. In this field, antibody-drug conjugates (ADCs) are one of the fastest growing classes of oncology therapeutics. ADCs consist of a recombinant monoclonal antibody (mAb) covalently bounded via a linker to a small cytotoxic molecule. These immunoconjugates combine the antitumoural potency of the highly cytotoxic small molecule drug with the high selectivity, stability, and favourable pharmacokinetic profile of mAbs (**Figure 7.1, B**).[5]

A common strategy for the modification of antibodies is the engineered introduction of non-canonical antibody residues at specific locations in the protein sequence, suitable for subsequent chemical modifications. Among native amino acids, cysteine has become the main choice for site-selective modifications, because it has a relatively low abundance, and the sulfhydryl group presents enhanced nucleophilicity. Most common chemical methods for the modification of cysteine include formation of disulfide bridges, alkylations with α-halocarbonyls and Michael acceptors (e.g. maleimides). To date, maleimides constitute one of the most used moieties to achieve site-selective cysteine modification in proteins.[6] The synthetic value of these bioconjugates has been validate by their use in approved and under development ADCs therapeutics.[5] Cysteine modification occurs rapidly and almost quantitatively under physiological conditions, allowing the incorporation of higher levels of cytotoxic drug and tackling heterogeneity problems. However, thiosuccinimide adducts can undergo retro-Michael additions and other thiol exchange reactions under physiological conditions (**Figure 7.1, C**).[7] The low thiosuccinimide stability has motivated the development of new maleimide

[4] Jefferis, R. *Nat. Rev. Drug Discov.* **2009**, *8*, 226-234.
[5] a) Zolot, R. S.; Basu, S.; Million, R. P. *Nat. Rev. Drug Discov.* **2013**, *12*, 259; b) Beck, A.; Goetsch, L.; Dumontet, C.; Corvaïa, N. *Nat. Rev. Drug Discov.* **2017**, *16*, 315-337.
[6] Ravasco, J. M.; Faustino, H.; Trindade, A.; Gois, P. M. *Chem. Eur. J.* **2019**, *25*, 43-59.
[7] Szijj, P. A.; Bahou, C.; Chudasama, V. *Drug Discov. Today Technol.* **2018**, *30*, 27-34.

reagents that overcome these drawbacks, either by attenuating thiol exchange reactions or by the development of alternative more stable structures.

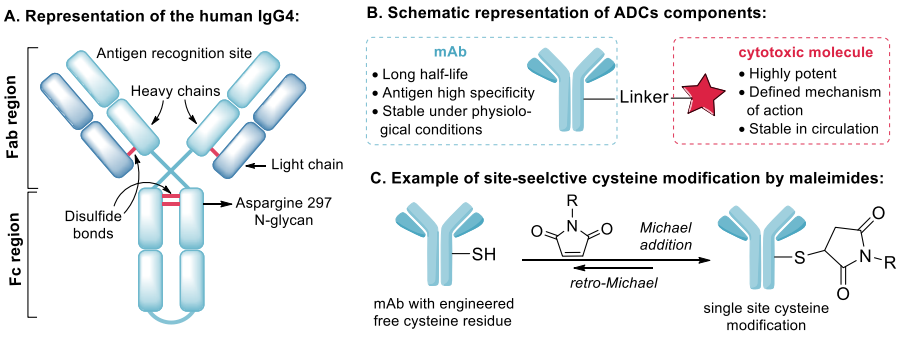


Figure 7.1. A) Schematic representation of the human igG4 antibody structure, indicating two main regions, Fab and Fc regions. B) Schematic representation of antibody-drug conjugates (ADCs) components. C) Example of site-selective cysteine modification by Michael addition with maleimides, and retro-Michael reaction that can occur under physiological conditions.

Glycosylation of peptides and proteins has been often correlated with enhancement of the pharmacokinetic properties and cellular uptake. However, the study of the role of protein-bounded carbohydrates has been challenging due to the difficult preparation of well-defined glycoconjugates. Methods to access to pure synthetic glycoproteins are therefore of great interest. Many methodologies include the use of labels to study carbohydrate function and prove their binding interactions *in vitro* and *in vivo*.[8] In this regard, fluorine has proved to be a powerful probe in NMR, MRI, and positron emission tomography (PET) techniques and has found several applications in medicinal chemistry.[9] Indeed, fluorosugar 2-deoxy-2-[^{18}F]fluoroglucose (^{18}FDG) is the most widely used radiopharmaceutical for imaging with PET.[10] Despite the great potential of fluorinated compounds for imaging and biochemical studies, few methods are available for site-controlled incorporation of fluorine into proteins

[8] Park, S.; Lee, M. R.; Shin, I. *Chem. Soc. Rev.*; **2008**, *37*, 1579.
[9] Gillis EP, Eastman KJ, Hill MD, Donnelly DJ, Meanwell NA, *J. Med. Chem.* **2015**, *58*, 8315-8359.
[10] Ametamey, S.; Honer, M.; Schubiger, P. *Chem. Rev.*; **2008**, *108*, 1501.

or glycoproteins. Recent advances in selective chemical protein modification[11] have stimulated the selective incorporation of synthetic, unnatural F-oligosaccharide mimetics into peptides and proteins[12] mainly using Cu(I)-mediated azide–alkyne cycloadditions[13] and thiol chemistry. [14] Recently, Davis and co-workers reported an elegant method for site-selective incorporation of fluorosugars into proteins that allows the obtention of homogenenous fluoroglycoproteins.[13a] The method involves the site-selective incorporation of the unnatural amino acid bearing a triple bond homopropargylglycine (Hpg), followed by conjugation with a fluorine-labelled glycosyl azide via Copper(I)-catalyzed alkyne–azide cycloaddition reaction. These methodologies have emerged as versatile and powerful synthetic tools for the preparation of well-defined F-carbohydrate-based vaccines and other detection/diagnostic elements.[15] However, avoiding the use of detrimental reaction conditions that may lead to toxicity[16] and/or reactivity[17] problems is an important issue still to be addressed.

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[11] a) Boutureira, O.; Bernardes, G. J. L. *Chem. Rev.* **2015**, *115*, 2174-2195; b) Krall, N.; da Cruz, F. P.; Boutureira, O.; Bernardes, G. J. L. *Nat. Chem.* **2015**, *8*, 103-113; c) Spicer, C. D.; Davis, B. G. *Nat. Commun.* **2014**, *5*, 4740.

[12] Orwenyo, J.; Huang, W.; Wang, L. X. *Bioorg. Med. Chem.* **2013**, *21*, 4768-4777.

[13] a) Boutureira, O.; D'Hooge, F.; Fernández-González, M.; Bernardes, G. J. L.; Sánchez-Navarro, M.; Koeppe, J. R.; Davis, B. G. *Chem. Commun.* **2010**, *46*, 8142-8144; b) Fernández-González, M.; Boutureira, O.; Bernardes, G. J. L.; Chalker, J. M.; Young, M. A.; Errey J. C.; Davis, B. G.; *Chem. Sci*. **2010**, *1*, 709-715; c) Maschauer, S.; Prante, O. *Carbohydr. Res.* **2009**, *344*, 753-761; d) Maschauer, S.; Einsiedel, J.; Haubner, R.; Hocke, C.; Ocker, M.; Hübner H.; Kuwert, T.; Gmeiner, P.; Prante, O. *Angew. Chem. Int. Ed.* **2010**, *49*, 976-979.

[14] a) Salvadó, M.; Amgarten, B.; Castillón, S.; Bernardes, G. J. L.; Boutureira, O. *Org. Lett.* **2015**, *17*, 2836-2839; b) Boutureira, O.; Bernardes, G. J. L.; Fernández-González, M.; Anthony, D. C.; Davis, B. G. *Angew. Chem. Int. Ed.* **2012**, *51*, 1432-1436; c) Boutureira, O.; Bernardes, G. J. L.; D'Hooge, F.; Davis, B. G. *Chem. Commun*. **2011**, *47*, 10010-10012.

[15] a) Collet, C.; Maskali, F.; Clément, A.; Chrétien, F.; Poussier, S.; Karcher, G.; Marie, P. Y.; Chapleur, Y.; Lamandé-Langle, S. *J. Label. Compd. Radiopharm.* **2015**, *59*, 54-62; b) Fischer, C. R.; Müller, C.; Reber, J.; Müller, A.; Krämer, S. D.; Ametamey, S. M.; Schibli, R. *Bioconjug. Chem.* **2012**, *23*, 805-813; c) Wuest, F.; Berndt, M.; Bergmann, R.; van den Hoff, J.; Pietzsch, J. *Bioconjug. Chem.* **2008**, *19*, 1202-1210; d) Namavari, M.; Cheng, Z.; Zhang, R.; De, A.; Levi, J.; Hoerner, J. K.; Yaghoubi, S. S.; Syud, F. A.; Gambhir, S. S. *Bioconjug. Chem.* **2009**, *20*, 432-436.

[16] a) Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem. Int. Ed.* **2009**, *48*, 9879-9883; b) Besanceney-Webler, C.; Jiang, H.; Zheng, T.; Feng, L.; Soriano del Amo, D.; Wang, W.; Klivansky, L. M.; Marlow, F. L.; Liu, Y.; Wu, P. *Angew. Chem. Int. Ed.* **2011**, *50*, 8051-8056.

In this chapter we envisioned a chemical method that would allow simultaneous formation of a well-defined glycoprotein and incorporation of fluoro-labelled sugars (with additional radiolabelling potential). For that, an approach for site-selective chemical synthesis of proteins bearing fluorinated sugars has been studied. The strategy is based on a "tag-and modify" approach, that exploits the introduction of a linker bearing a side chain thar contains a functional group that can be chemoselectively modified. This chapter describes the utilization of a two-step protein-modification protocol for the development of synthetic homogeneous fluorinated glycoproteins. First step involves the incorporation of strained alkynes into proteins by maleimide *S*-alkylation[18] followed by copper-free strain-promoted azide–alkyne cycloaddition (SPAAC)[19] using two different antibodies (Trastuzumab and Gemtuzumab) and a variety of native, fluorinated, and trifluoromethylated sugars. Studying the reactivity of novel partners and conditions to access fluorinated (glyco)peptides and proteins will provide valuable information to further explore the biological implications of these emerging therapeutics and imaging agents *in vivo*.

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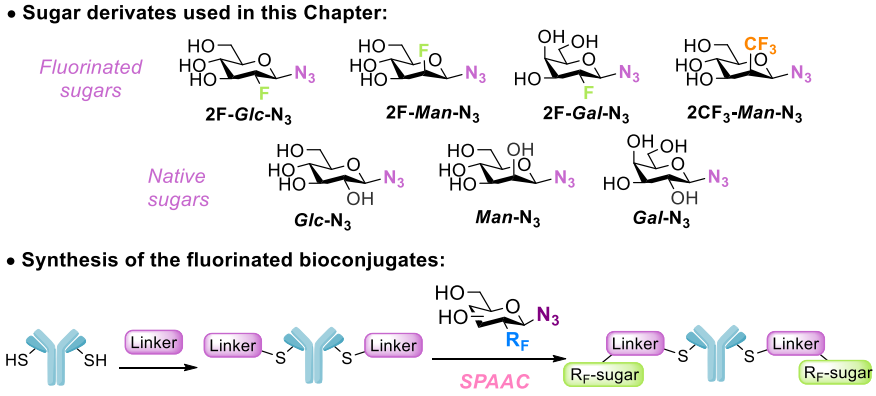
[18] a) Renault, K.; Fredy, J. W.; Renard P. Y.; Sabot, C. *Bioconjug. Chem.* **2008**, *29*, 2497-2513; b) Ravasco, J. M.; Faustino, H.; Trindade, A.; Gois, P. M. P. *Chem. Eur. J*. **2008**, *25*, 43-59.

[19] a) Pickens, C. J.; Johnson, S. N.; Pressnall, M. M.; Leon, M. A.; Berkland, C. J. *Bioconjug. Chem.* **2018**, *29*, 686-701; b) Oliveira, B. L.; Guo, Z.; Bernardes, G. J. L. *Chem Soc Rev* **2017**, *46*, 4895-4950.

7.2. OBJECTIVES

We expect that the incorporation of fluorinated sugars in antibodies could have an influence in some of their properties, such as their stability or other physicochemical parameters. The specific objectives of this chapter are:

1) The synthesis of 2-deoxy-2-fluorinated and trifluoromethylated glycosyl azides with different sugar configurations.
2) To study the reaction kinetics of the cooper-free strain-promoted azide-alkyne cycloaddition (SPAAC) using different alkyne azides and selection of the best performer.
3) Selection of the proteins and the linker used in this study
4) Optimization of the bioconjugation and SPAAC reactions in antibodies.
5) Study of the bioconjugation step using different maleimides
6) Modification of two different antibodies and extension of the scope with synthesised fluorinated sugars and native glycosides.
7) Flow cytometry studies to prove the specificity of fluorinated and native antibodies to the surface CD-33 and HER2 receptors.
8) Confocal microscopy studies to prove the specific binding to cell receptors and localization in cells.



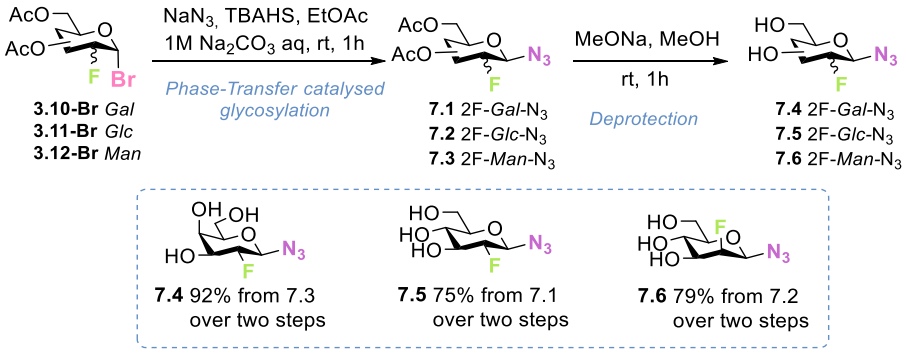
Scheme 7.1. Top: Sugar derivates used in this study. Bottom: Bioconjugation step using a linker and an antibody followed by SPAAC reaction to obtain the desired fluorinated antibodies.

7.3. RESULTS AND DISCUSSION

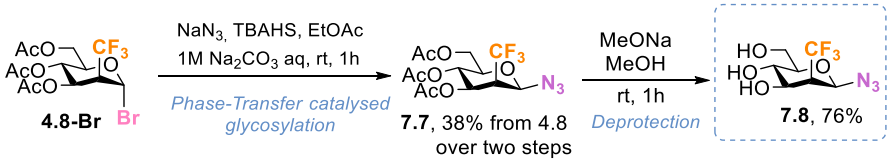
7.3.1. Synthesis of fluorinated glycosyl azides

The first step was the preparation of the fluorinated and trifluoromethylated glycosyl azides. The protocol to synthetize 2-deoxy-2-fluoro glycosyl azides was previously described,[13a] and the desired products were easily obtained in two steps (**Scheme 7.2**). Starting from the glycosyl bromide, the first step was a phase-transfer catalysed glycosylation to obtain the protected glycosyl azide, which treated under Zemplén deacetylation conditions gave the desired products in high yields and as sole β-anomers.

- **Synthesis of 2-deoxy-2-fluoro glycosyl azides:**



- **Synthesis of 2-deoxy-2-trifluoromethyl glycosyl azides:**



Scheme 7.2: Top: synthesis of 2-deoxy-2-fluoro glycosyl azides. Bottom: Synthesis of 2-deoxy-2-trifluoromethyl glycosyl azide.

Our initial aim was to achieve the synthesis of fluorinated and trifluoromethylated derivates of *gluco* and *manno* sugar configurations. However, due to the synthetic difficulties and poor stability observed for 2-deoxy-2-trifluoromethyl glucosyl azide, this compound was discarded from the study. Many different approaches were tried, however, all of them gave the

elimination product 2-trifluoromethyl glucal. Thus, 2-deoxy-2-trifluoromethyl mannoside was used as a single probe to study the effect of the trifluoromethyl moiety together with the fluorinated derivates of *gluco, manno* and *galacto* configurations (**Scheme 7.2**).

7.3.2. Study of the click kinetics with different strained alkynes

Considering that the SPAAC reaction is going to be applied in antibodies, our objective is to achieve a fast and effective reaction to avoid decomposition and denaturalization of the antibody. A variety of strained alkynes have been used for the SPAAC reaction. This field is still flourishing with the discovery of new products with enhanced reactivity and stability.[20] DIBO, BCN and DBCO were selected for this study. These commercially available compounds are formed by different cyclooctyne structures that affect to its reactivity and lipophilicity.[21]

Reaction rate of the SPAAC reaction was studied for the selected strained cycloalkynes and 2-deoxy-2-fluoroglucosyl azide **7.5** to determine the best performer. Since cycloalkynes were soluble in organic solvents and glycosyl azides were highly soluble in water, a mixture of D_2O and *d*-ACN was used to increase the solubility of the starting materials. To determinate the second-order rate constants (k_t), the SPAAC reaction was followed by ^{19}F NMR using trifluoroethanol (TFE) as the internal standard, following the protocol described in **Section 7.5.3**. Each reaction was carried out by triplicate and between 10 to 15 points were taken. The second-order rate constant for the reaction was determined by plotting 1/[glycosyl azide] versus time, followed by subsequent analysis by linear regression analysis (**Scheme 7.3**). Second-order rate constants (k_t, M^{-1} s^{-1}) corresponds to the average of the determined slopes and the errors represent standard derivations from the three replicate experiments.

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[20] a) Debets, M. F., Prins, J. S., Merkx, D., van Berkel, S. S., van Delft, F. L., van Hest, J. C., Rutjes, F. P. *Org. Biomol. Chem*. **2014**, *12*, 5031-5037; b) Gröst, C.; Berg, T. *Org. Biomol. Chem*. **2015**, *13*, 3866-3870.

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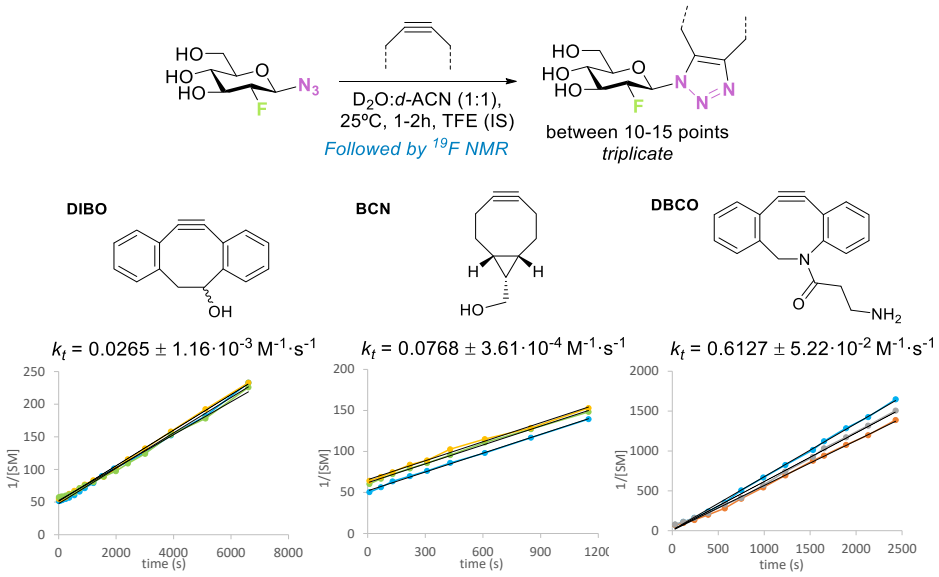
Results obtained for the SPAAC reaction of the three selected cycloalkynes are shown in **Scheme 7.3**. DIBO showed the lowest rate, followed by BCN, which were in concordance with the previous published studies. Results showed that the DBCO derivate was the best performer, obtaining much higher reactivity compared with the other strained cycloalkynes.



Scheme 7.3. Results obtained in the determination of the second-order rate constants (k_t) of the SPAAC reaction between different strained alkynes (DIBO, BCN and DBCO) and 2-deoxy-2-fluoro-glucosyl azide.

7.3.3. Selection of the linker and the proteins used in the study

As previously introduced, maleimides are the most commonly used reagents in cysteine bioconjugation and have proven to be very selective. DBCO-maleimide was commercially available and it was chosen as the linker in our study. Gemtuzumab and Trastuzumab were the antibodies chosen in this study. Gemtuzumab (KD1, hP67.6 antibody) is a recombinant monoclonal antibody (mAb) and consists of a human IgG4 kappa framework with putative complementarity determining region (CDR) grafted mouse sequences, which form the antigen-binding site. The theoretical molecular masses (average) for the predominant *N*-linked glycoforms, assuming *C*-terminal G residues in both

H chains, and full disulfide bond connectivity, is 148 KDaltons. Gemtuzumab recognises human Siglec-3/CD33, which is a transmembrane receptor expressed on predominantly on cells of the myeloid lineage, but it can also be found on some lymphoid cells. Trastuzumab is a recombinant humanized anti-HER2 monoclonal antibody (mAb), which consists in a highly purified recombinant DNA-derived humanized monoclonal igG1 kappa framework that binds with high affinity and specificity to the extracellular domain of the HER2 receptor. Trastuzumab was found to inhibit the growth of human cancer cells and tumor xenografts overexpressing HER2. HER2 is overexpressed in 25-30% of breast cancers and is suggested to have a direct role in the pathogenesis and clinical aggressiveness of HER2 overexpressing tumors.

The antibodies used in this study were provided from the laboratories of AstraZeneca in Cambridge.[22] These antibodies were genetically modified to have a free cysteine residue in both light chains and the sequences of the selected antibodies are described in **Section 7.5.6.** Therefore, the antibodies used had two free cysteines that can be modified and would react with the maleimide motif of the selected linker, DBCO-maleimide. After the bioconjugation, the DBCO motif linked to the antibody, would react in a SPAAC reaction with the azide moiety of the sugars giving the desired antibody bioconjugates (**Figure 7.2**).

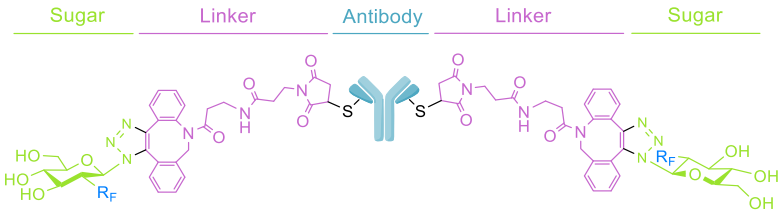


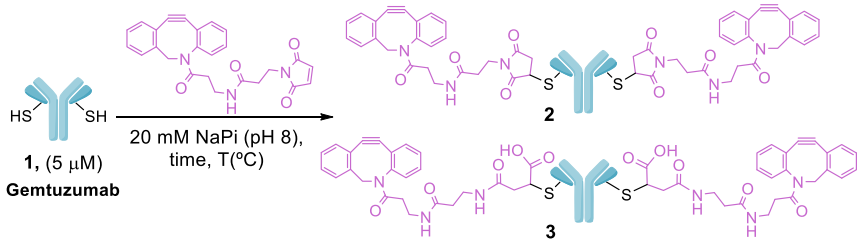
Figure 7.2. Design of the antibody bioconjugates used in this study.

[22] Acknowledgments to Dr. Victor Laserna for providing the modified antibodies

7.3.4. Optimization of bioconjugation and SPAAC reactions

Before obtaining the desired bioconjugates, optimization of the bioconjugation step and the SPAAC reaction was studied with both antibodies. Antibodies were used freshly refolded following the protocol described in **Section 7.5.7**, and the reactivity of the free cysteine was evaluated by its reaction with methyl maleimide. Once the reactivity of the free cysteine antibodies was demonstrated, bioconjugation reaction using our selected linker was studied and results are shown in **Table 7.1**.

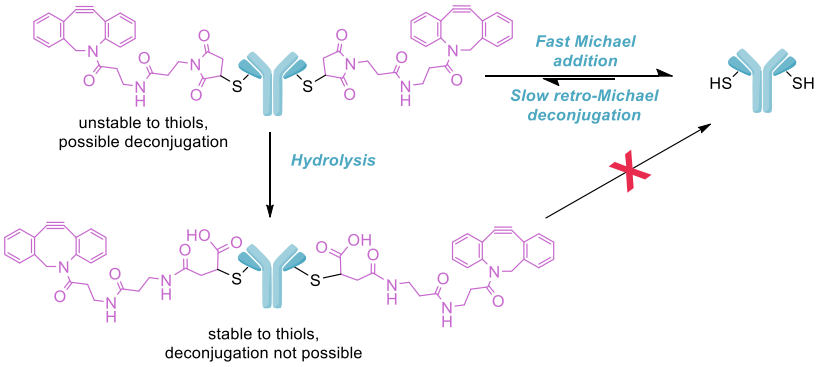
Table 7.1: Optimization of the bioconjugation reaction using Gemtuzumab and DBCO-maleimide



Entry	T (ºC)	time (h)	DBCO-Mal eq/Ab	[c] Ab final (μM)	**1** (%)	**2** (%)	**3** (%)
1	25	4	20	4.22	15	85	0
2	25	6	20	4.00	10	85	5
3	25	6	50	4.32	10	80	10
4	25	16	50	4.66	2	20	78
5	37	2	20	4.59	15	35	50
6	37	4	20	4.92	10	0	90
7	37	6	20	4.40	7	0	93
8	37	16	50	4.14	5	0	95

Starting from the refolded antibody, bioconjugation reaction with DBCO-maleimide was studied at different conditions. First attempts showed good conversion when using just 20 equivalents of the linker, however, total conversion was not achieved and in all the attempts a small amount of the starting Gemtuzumab was observed. By further investigating this reaction, we were able to determinate that the small amount of unreactive Gemtuzumab is

remanent from the refolding step, which is not complete and a small amount (1-5%) of oxidised Gemtuzumab ("free" cysteines forming a disulfide bridge involving cysteine 205) is present. When studying the reaction at 25 °C for 4 h the Gem-DBCO-Maleimide **2** bioconjugate was mainly obtained (Entry 1). However, when heating at 37 °C, the opening of the maleimide to form the acid **3** was observed (Entry 6). Considering that maleimides could give a retro-Michael reaction that forms the native antibody, we decided to optimize the bioconjugation to favour the opening of the maleimide to form the acid form **3**, since this product is more stable and does not give a retro-Michael reaction.



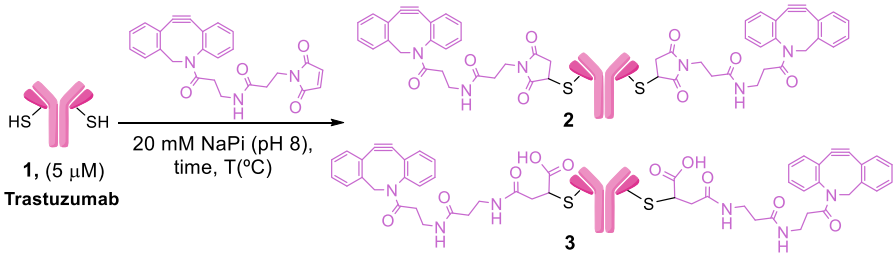
Scheme 7.4. Opening mechanism of the maleimide bonded to a cysteine residue, catalysed by an amino acid residue.

When increasing the reaction time at 25 °C, the formation of the acid product **3** is slightly favoured, but the maleimide form **2** is mainly obtained. However, when the reaction was incubated overnight at the same temperature, the acid form is obtained favourably (entry 4). Finally, optimal conditions were achieved when increasing the temperature to 37 °C, since the formation of the acid form **3** is highly favoured. In just 4 h of reaction the desired bioconjugate was mainly obtained in good yield, with no decomposition observed. When increasing the reaction times, we were able to observe a slight increase of the conversion, however, concentration of the bioconjugate was reduced and some decomposition was observed in the LC-MS spectra.

The bioconjugation reaction was then studied for Trastuzumab to see if there are any differences between both antibodies and to determine the best

conditions to obtain the acid form **3**. Results obtained for the reaction of Trastuzumab and DBCO-maleimide are shown in **Table 7.2**. At 25 °C the maleimide product **2** was clearly favoured even incubating the reaction overnight (entries 1 and 2). These results are in contrast with those obtained in the reaction with Gemtuzumab reaction at 25 °C, in which the maleimide product **2** was obtained mainly when using short reaction times, but the acid **3** was favoured when increasing the reaction time to 16 h. Therefore, when using Thiomab the formation of the acid derivate **2** is less favoured than when Gemtuzumab is used. At 37 °C results for Thiomab and Gemtuzumab were similar, and the acid product was favoured regardless of the reaction times. These results indicate the possibility to control the formation of the two products, either the maleimide **2** or the acid **3** products by only controlling the reaction temperature and time.

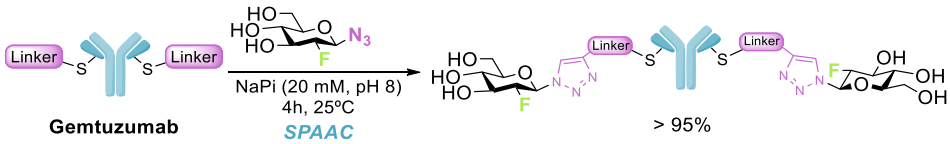
Table 7.2: Optimization of the bioconjugation reaction using Trastuzumab and DBCO-maleimide.



Entry	T (ºC)	time (h)	DBCO-Mal eq/Ab	[c] Ab final (μM)	**1** (%)	**2** (%)	**3** (%)
1	25	6	50	4.32	10	90	0
2	25	16	50	4.33	2	88	10
3	37	6	50	4.04	5	0	95
4	37	16	50	3.92	2	0	98

Once the conditions of the bioconjugation step were successfully determined to obtain the opening of the maleimide, SPAAC reaction was studied for Gemtuzumab and model 2-deoxy-2-fluoro-glucopyranosyl azide. Reaction was carried out at 25 °C and 250 rpm, and aliquots were taken each hour and analysed by LC-MS. Total conversion to form the glycosyl

bioconjugate was obtained after 4 h of reaction at the conditions showed in **Scheme 7.5**. These conditions were chosen as the optimal conditions to develop the SPAAC reaction, since short reaction times and low temperature were needed.



Scheme 7.5: Optimization of the SPAAC reaction using Gemtuzumab-DBCO-maleimide and 2F-*Glc*-N_3.

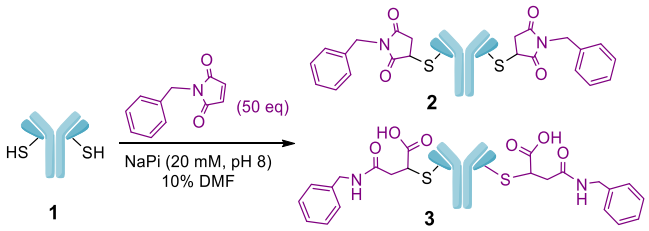
7.3.5. Study of maleimides reactivity and stability

Considering the results obtained in the bioconjugation of the selected antibodies and the linker DBCO-maleimide, the bioconjugation of these antibodies with different maleimide was studied. We envisioned the possibility to control the bioconjugation reaction and obtain either the maleimide form or the acid form just by changing the reaction time and the temperature. Reaction of Gemtuzumab and Trastuzumab with bezylmaleimide was studied at different temperatures and reaction times. Results obtained are shown in **Table 7.3** and the LC-MS spectra are shown in the experimental section.

Results suggest that although both antibodies have slightly different reactivity kinetics, the final product composition is similar. Bioconjugation of Gemtuzumab and benzylmaleimide at 25 °C for 8 h gave the maleimide product **2** favourably. However, when conducting the reaction overnight (16 h), the acid product **3** is mainly obtained. By increasing the reaction time, formation of the acid **3** is favoured, and same pattern is observed when the temperature is increased from 25 to 37 °C. Bioconjugation of Trastuzumab and benzylmaleimide at 25 °C for 8 h gives similar results to the ones obtained for Gemtuzumab, however, when increasing reactivity times, the formation of the acid product **3** was slightly favoured, and a mixture 1:1 of the maleimide:acid products were obtained. Reaction of Trastuzumab and benzylmaleimide at 37 °C gave the acid product favourably, however, 16 h of reaction were needed to

avoid the formation of the maleimide form **2**. In contrast, when using Gemtuzumab the acid product was obtained solely regardless of the time employed. Despite the differences obtained in the reactivity, the general patterns observed are similar to the results obtained with DBCO-maleimide.

Table 7.3: Reaction of Gemtuzumab and Trastuzumab with benzylmaleimide:



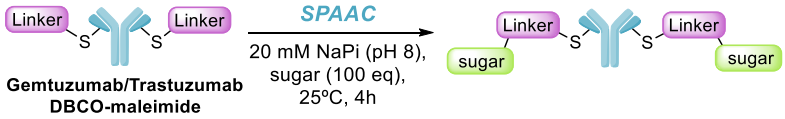
Entry	Antibody	T°C	time (h)	**1** (%)	**2** (%)	**3** (%)
1	Gemtuzumab	25	8	20	70	10
2	Gemtuzumab	25	16	5	5	90
3	Gemtuzumab	37	8	10	0	90
4	Gemtuzumab	37	16	10	0	90
5	Trastuzumab	25	8	0	75	20
6	Trastuzumab	25	16	5	45	50
7	Trastuzumab	37	8	5	30	65
8	Trastuzumab	37	16	5	0	95

Considering the results obtained in **Section 7.3.4,** the optimization of the bioconjugation between the antibodies and DBCO-maleimide, and the results obtained in its reaction with benzylmaleimide (**Table 7.3**), it can be concluded that the products formed in the bioconjugation step between antibodies and maleimides can be controlled by changing the temperature and reaction times. Small differences were obtained for the two antibodies, which indicate that bioconjugation reaction should be studied for each antibody and there is not a general rule that could be applied to different antibodies. Despite general patterns were observed for both antibodies, at 25 °C and short reaction times, the maleimide conjugate was mainly obtained, in contrast, increasing the reaction time and temperature favoured the formation of the acid conjugate.

7.3.6. Bioconjugation and SPAAC reactions with sugar derivatives

Optimized bioconjugation and SPAAC reactions were applied to Gemtuzumab and Trastuzumab following the protocol described in **Section 7.5.8**. The desired bioconjugates were obtained with high purity and yield and the LC-MS spectra of all the products is included in the experimental section. Results are shown in **Table 7.4** for Gemtuzumab (entries 1-7) and Trastuzumab modifications (entries 8-14).

Table 7.4: Scope of the SPAAC reaction between Gemtuzumab and Trastuzumab and the sugars selected in this study.



Entry	Ab	sugar	[c] Ab final (μM)	Calculated mass	Observed mass
1	Gemtuzumab	*Glc*-N_3	3.80	24483	24482
2	Gemtuzumab	*Man*-N_3	3.52	24483	24483
3	Gemtuzumab	*Gal*-N_3	3.22	24483	24482
4	Gemtuzumab	2F-*Glc*-N_3	3.25	24485	24485
5	Gemtuzumab	2F-*Man*-N_3	3.43	24485	24485
6	Gemtuzumab	2F-*Gal*-N_3	3.68	24485	24484
7	Gemtuzumab	$2CF_3$-*Man*-N_3	3.22	24535	24534
8	Trastuzumab	*Glc*-N_3	2.93	24097	24097
9	Trastuzumab	*Man*-N_3	2.69	24097	24097
10	Trastuzumab	*Gal*-N_3	2.98	24097	24098
11	Trastuzumab	2F-*Glc*-N_3	2.25	24099	24099
12	Trastuzumab	2F-*Man*-N_3	2.88	24099	24099
13	Trastuzumab	2F-*Gal*-N_3	3.03	24099	24099
14	Trastuzumab	$2CF_3$-*Man*-N_3	2,82	24149	24148

7.3.7. Stability studies of modified antibodies

Once the desired antibodies were obtained, we aimed to study if the introduction of the fluorinated sugars affects to the stability of the antibody. Linker stability is an essential requirement for the development of useful protein conjugates for therapeutic and diagnostic applications since systemic, nontargeted release of the conjugated payload could result in undesired side-toxicity and nonspecific imaging, respectively. Stability of the different bioconjugates shown in **Figure 7.3** was studied in human plasma and reduced glutathione.

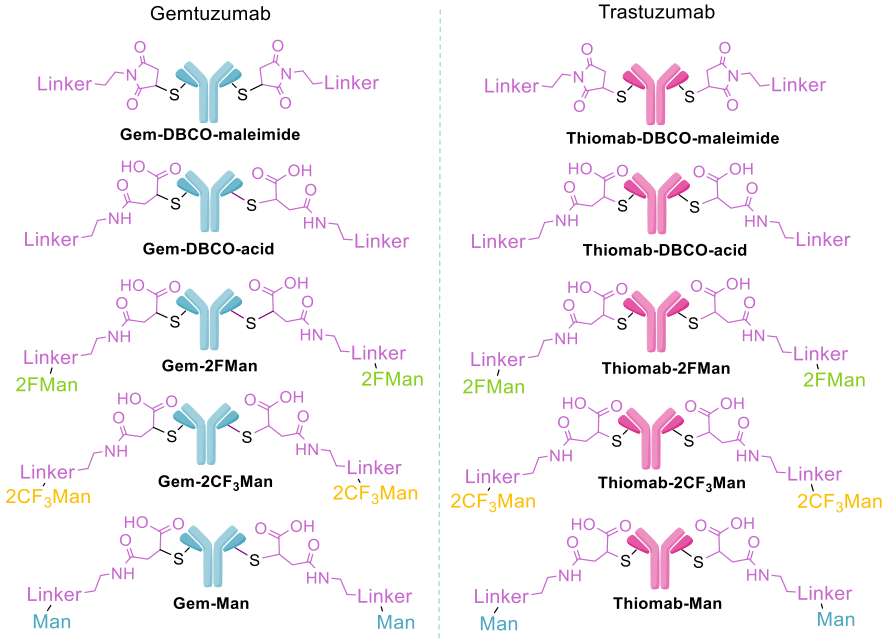


Figure 7.3: Bioconjugates used in the study of the stability in plasma and reduced glutathione.

Each bioconjugate was incubated in human plasma and glutathione at 37 °C for 24 h and then analysed by LC-MS following the protocol described in **Section 7.5.9** and **Section 7.5.10**. **Figure 7.4** shows the results obtained for the different bioconjugates indicating the respective stability using GAR (green-amber-red) analysis.

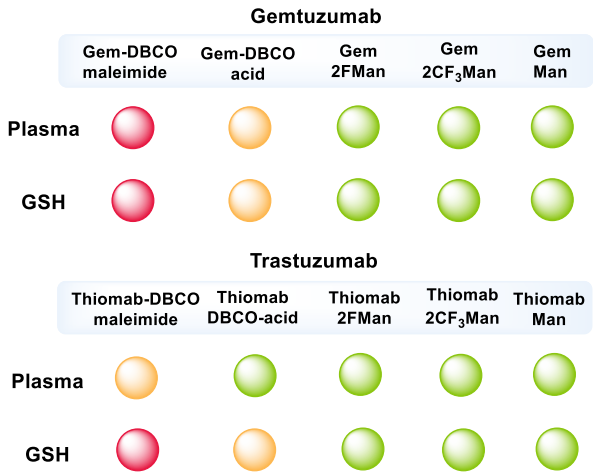


Figure 7.4: GAR (green-amber-red) analysis of the stability of the bioconjugates in human plasma and reduced glutathione (GSH).

Poor stability was observed for the bioconjugates bearing a DBCO-maleimide group either in plasma or GSH. In all the cases, the native antibody was observed by LC-MS analysis, indicating that the maleimide-cysteine bond breaks under these conditions. A slightly increased stability was observed for antibodies bearing a DBCO-acid group in the presence of human plasma. This result indicates that the cysteine-acid bond formed by the opening of the maleimide, is more stable and gives lower decomposition to form the native antibody. Similar results were observed in the presence of reduced glutathione, however higher decomposition was observed for both antibodies.

Importantly, no detectable degradation of the bioconjugates bearing a sugar moiety was observed after incubation with human plasma at 37 °C for 24 h, demonstrating the stability of the conjugates under these conditions. Additionally, when bioconjugates were incubated under reducing conditions (20 mM glutathione at 37 °C for 24 h), the conjugate remained intact, further demonstrating its stability in biologically relevant conditions.

7.3.8. Flow cytometry studies

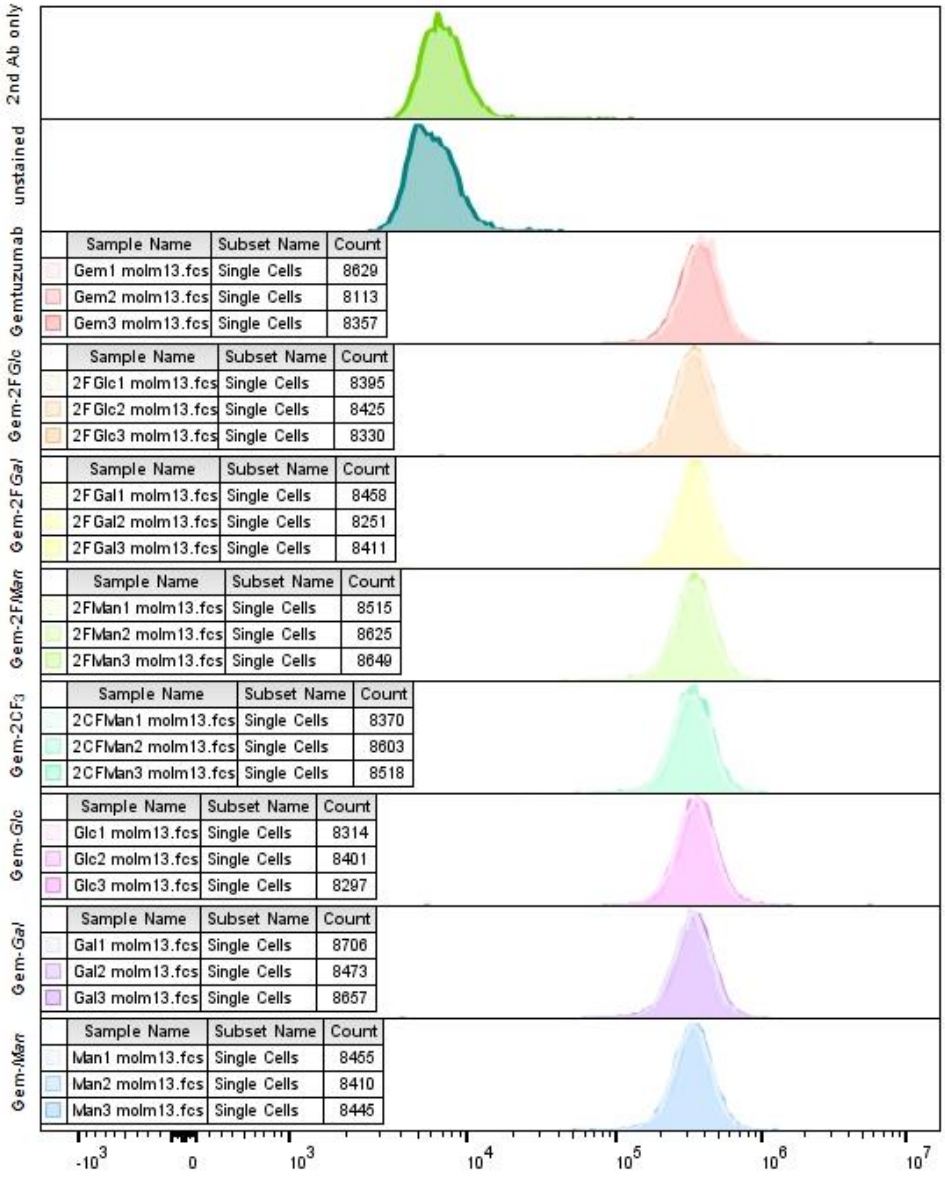
Once the stability of the bioconjugates was demonstrated in reduced glutathione and human plasma, the binding to the protein receptor was studied. Our aim was to determine if the modifications affect to their specificity to the protein receptors and determine if there is any difference between the modified bioconjugates and the native antibodies. To this end, flow cytometry experiments were carried out, using the modified and native antibodies and the corresponding cells.

- *Gemtuzumab antibodies*

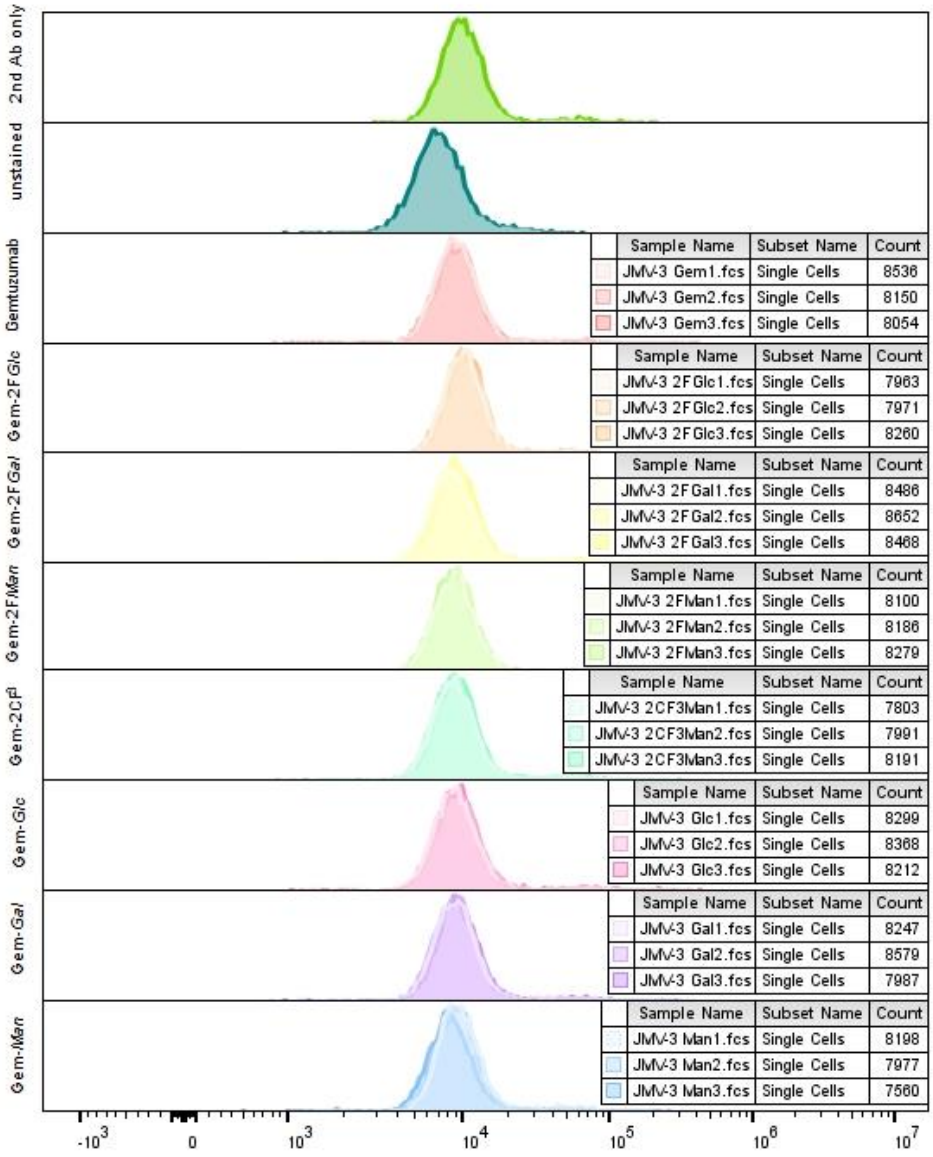
Binding of modified and native Gemtuzumab antibodies to the surface CD-33 receptor was analysed by flow cytometry. Two different cell lines were selected to carry out flow cytometry experiments, one with high expression of CD-33 and one with low expression of CD-33. MOLM-13 cells are acute myeloid leukaemia cells that have a high expression of CD-33 and JVM-3 cells are chronic B cell leukaemia cells that have a low expression of CD-33. MOLM-13 cells and JVM-3 cells were selected to carry out flow cytometry experiments and were routinely cultured at 37 °C with 5% CO_2 in RPMI-1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine as described in **Section 7.5.11**. Cell samples were prepared in a 96 well plate following the flow cytometry protocol described in **Section 7.5.12**, and each sample was prepared by triplicate. The primary antibody was detected by using a commercial Goat Alexa 647-conjugated anti-human (H + L) antibody (Thermo Fisher Scientific).

Results obtained for both cell lines and the modified and native antibodies are shown in **Scheme 7.6** and **Scheme 7.7**. **Scheme 7.6** shows the results obtained for MOLM-13 cells, in which it is observed a clear difference between the control cells and the cells treated with the antibodies. In addition, no difference is observed between the native antibody (green line) and the modified antibodies. In contrast, when using cells that have low expression of CD-33, JVM-3 cells (**Scheme 7.7**), the differences between control and treated cells are despicable. These results demonstrate that modified Gemtuzumab

antibodies maintain its high specificity to CD-33, and the modifications does not affect to its capacity to bind CD-33.



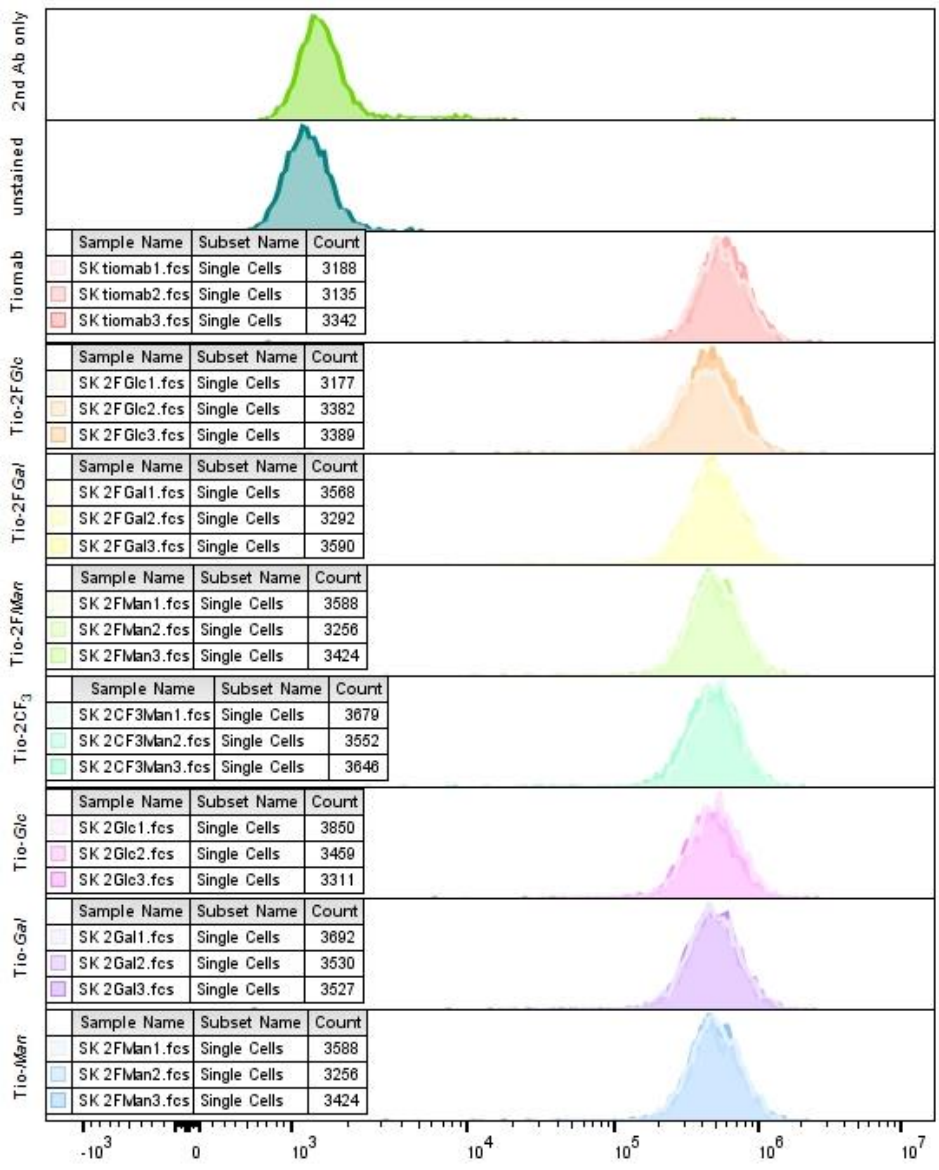
Scheme 7.6: Results obtained in the flow cytometry experiment using Gemtuzumab antibodies in MOLM-13 cells.



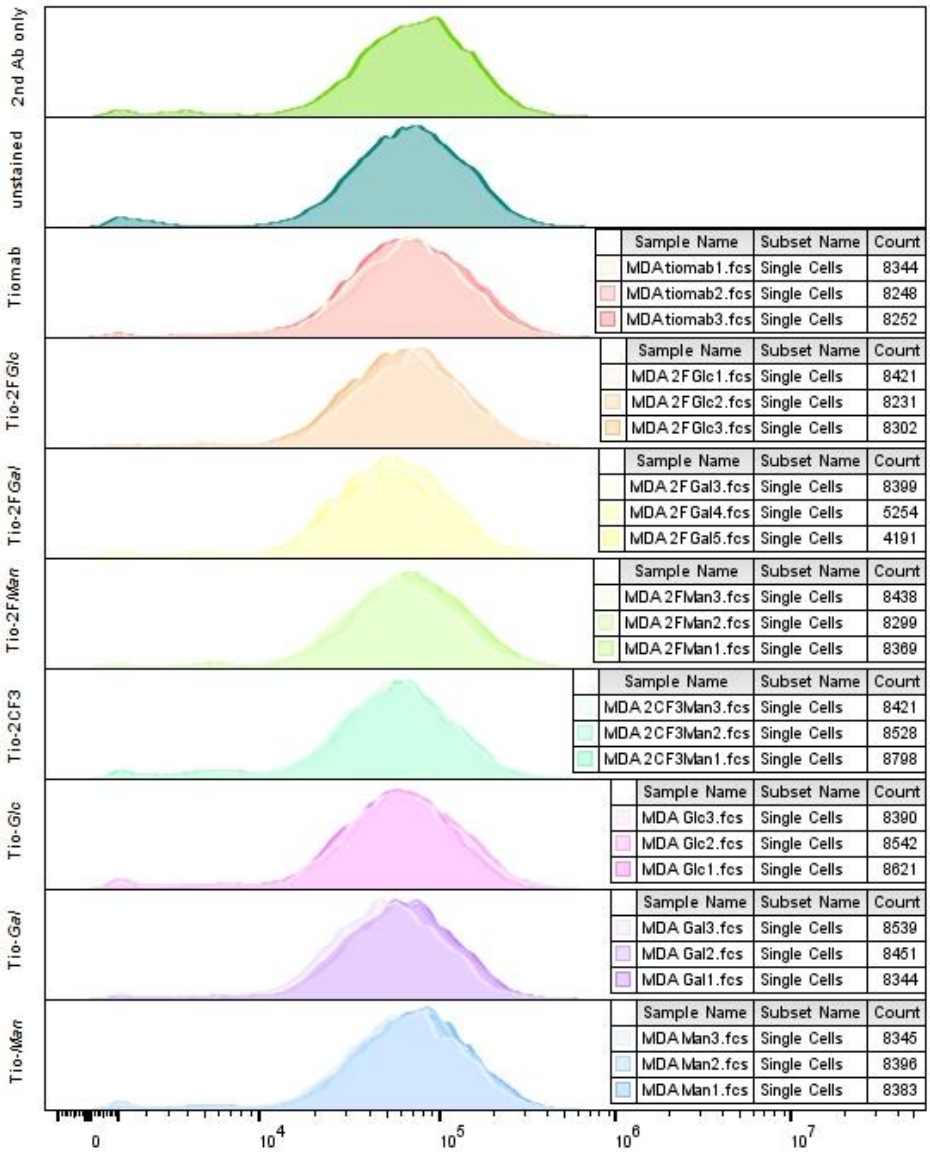
Scheme 7.7: Graphic 2: Results obtained in the flow cytometry experiment using Gemtuzumab antibodies in JVM-3 cells.

- *Trastuzumab antibodies*

Binding of fluorinated and native Trastuzumab antibodies to the surface HER2 receptor was analysed by flow cytometry. Two different cell lines were selected to carry out flow cytometry experiments, one which express HER2 and one that does not express HER2. SK-BR-3 cells are breast carcinoma cells that express HER2 and MDA-MB-231 cells are breast carcinoma cells that do not express HER2 protein receptor. SK-BR-3 cells and MDA-MB-231 cells were routinely cultured at 37 °C with 5% CO_2 in DMEM medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine as described in **Section 7.5.11**. Cell samples were prepared in a 96 well plate following the flow cytometry protocol described in **Section 7.5.12**, and each sample was prepared by triplicate. The primary antibody was detected by using a commercial Goat Alexa 647-conjugated anti-human (H + L) antibody. Samples were acquired with a FACS Fortessa I/II (BD Bioscience) and analysed with FlowJo (Tree Star). Results obtained for both cell lines and the modified and native antibodies are shown in **Scheme 7.8** and **Scheme 7.9**. **Scheme 7.8** shows the results obtained for SK-BR-3 cells, in which it is observed a clear difference between the control cells samples (green and red lines) and the cells treated with the antibodies. In addition, no difference is observed between the native antibody (pink line) and the modified antibodies. In contrast, when using cells does not express HER2, MDA-MB-231 cells (**Scheme 7.9**), the differences between control and treated cells are despicable. These results demonstrate that modified Trastuzumab antibodies maintain its high specificity to HER2, and the modifications does not affect to its capacity to bind HER2.



Scheme 7.8: Results obtained in the flow cytometry experiment using Trastuzumab antibodies and SK-BR-3 cells.



Scheme 7.9: Results obtained in the flow cytometry experiment using Gemtuzumab antibodies and MDA-MB-231 cells.

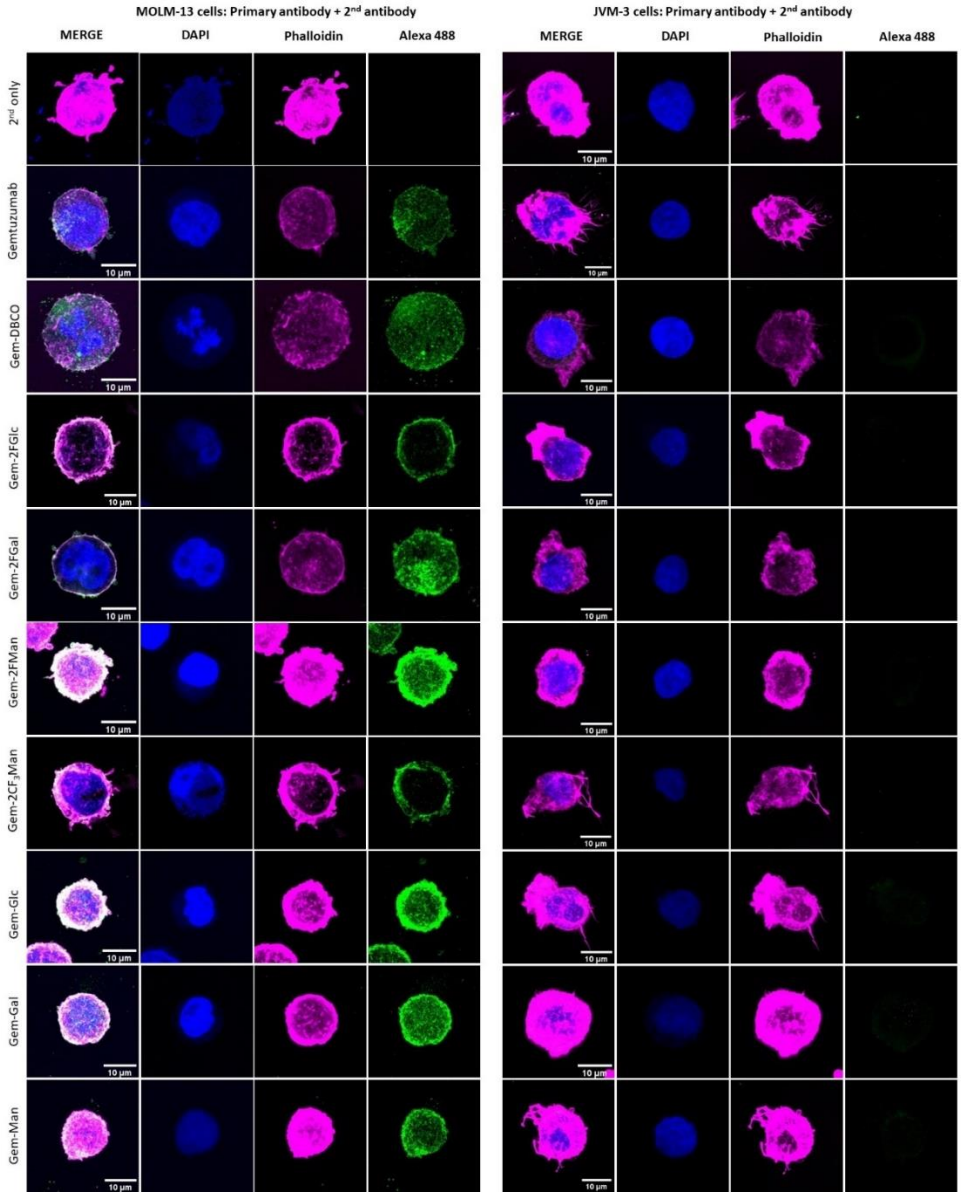
7.3.9. Confocal microscopy

Confocal microscopy experiments were carried out to study the binding dynamics of the modified antibodies and to determinate its disposition in cells. Experiments were carried out as indicated in **Section 7.5.13.** Modified antibodies bearing the linker and the different sugar moieties were imaged together with the native antibody.

- *Gemtuzumab antibodies*

Two different cell lines were selected to carry out microscopy confocal experiments, one with high expression of CD-33 (MOLM-13 cells) and one with low expression of CD-33 (JVM-3 cells). MOLM-13 cells and JVM-3 cells were routinely cultured as described in **Section 7.5.11**. Cell samples were prepared in a 24 well plate following confocal microscopy protocol described in **Section 7.5.13**. Control experiments of each cell line were carried out, in which no secondary antibody was added, and no positive results were observed (Experimental Section).

Results of the confocal microscopy experiments for Gemtuzumab antibodies in both cells lines are shown in **Scheme 7.10**. Prolong™ Diamond Antifade Mountant with DAPI was used as mounting media and as marker of cell's nucleus (blue), phalloidin was used as marker of the cell's membrane (magenta) and Goat F(ab')2 Anti-Human IgG. Alexa Fluor ® 488 Conjugate was used as secondary antibody (green). **Scheme 7.10** shows the three different channels used to mark the cell's nucleus (DAPI, blue), the cell's membrane (Phalloidin, magenta) and the secondary antibody (Alexa Fluor 488, green), and the merge of the three channels together. Results obtained for MOLM-13 cells indicate that the primary antibody together with the secondary antibody is bond to the cell's receptor CD-33. In contrast, in JVM-3 cells which had low expression of CD-33, no binding of the primary antibody was observed, which indicates that the modified antibodies are selective to CD-33 protein receptor. In addition, no differences were observed for the native primary Gemtuzumab and the modified versions of Gemtuzumab, which indicates that the modification does not influence to its specificity and the binding dynamics.

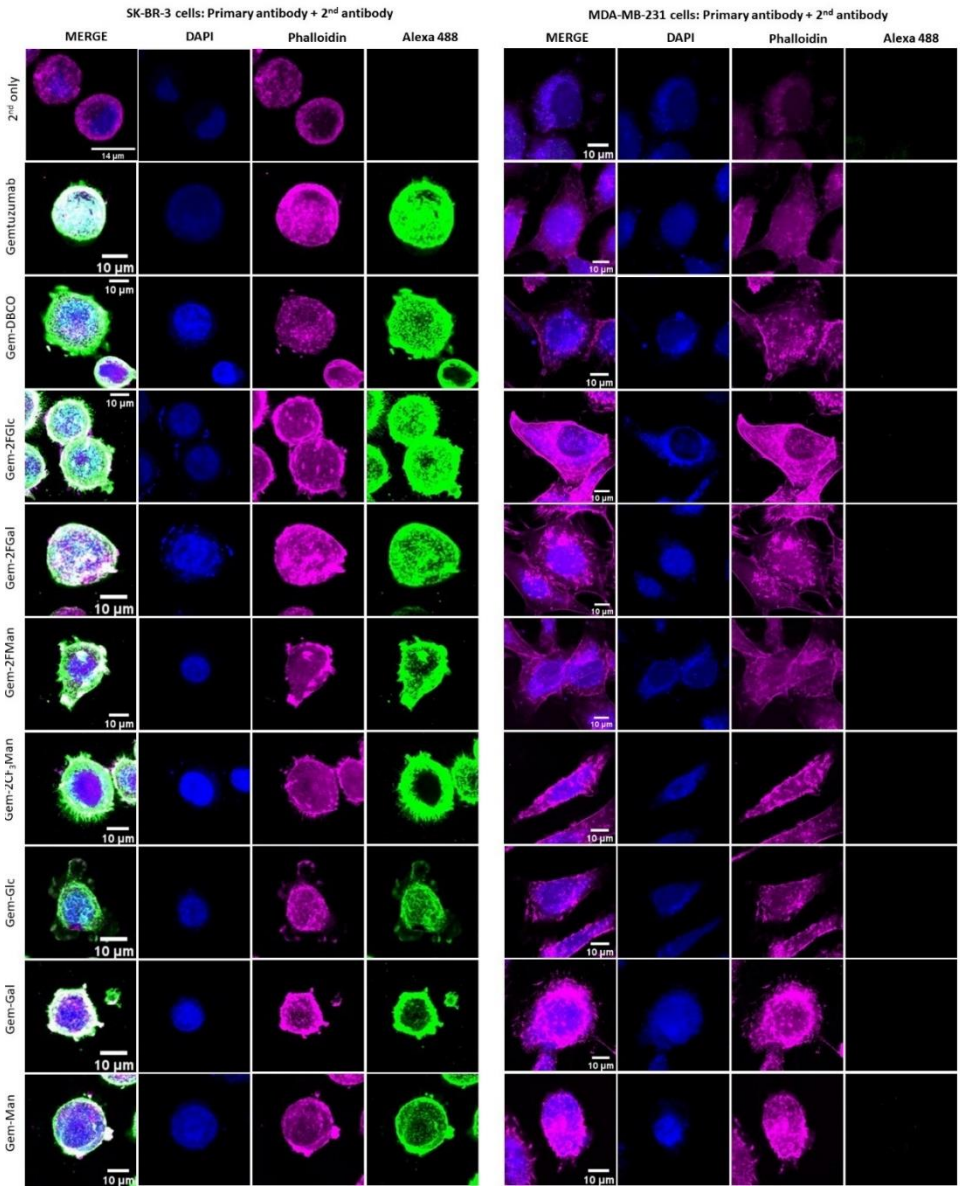


Scheme 7.10: Left: Confocal microscopy images of MOLM-13 cells treated with modified and native Gemtuzumab primary antibodies and 2nd antibody. Right: Confocal microscopy images of JVM-3 cells treated with modified and native Gemtuzumab primary antibodies and 2nd antibody.

- *Trastuzumab antibodies*

Two different cell lines were selected to carry out microscopy confocal experiments, one which express HER2 (SK-BR-3 cells) and one that does not express HER2 (MDA-MB-231 cells). SK-BR-3 cells and MDA-MB-231 cells were routinely cultured as described in **Section 5.10**. Cell samples were prepared in a 24 well plate following confocal microscopy protocol described in **Section 5.12**. Control experiments of each cell line were carried out, in which no secondary antibody was added, and no positive results were observed (see Experimental Section).

Results of the confocal microscopy experiments for Gemtuzumab antibodies in both cells lines are shown in **Scheme 7.11**. Prolong™ Diamond Antifade Mountant with DAPI was used as mounting media and as marker of cell's nucleus (blue), phalloidin was used as marker of the cell's membrane (magenta) and Goat F(ab')2 Anti-Human IgG. Alexa Fluor ® 488 Conjugate was used as secondary antibody (green). **Scheme 7.11** shows the three different channels used to mark the cell's nucleus (DAPI, blue), the cell's membrane (Phalloidin, magenta) and the secondary antibody (Alexa Fluor® 488, green), and the merge of the three channels together. Results obtained for SK-BR-3 cells indicate that the primary antibody together with the secondary antibody is bond to the cell's receptor HER2. In contrast, in MDA-MB-23 cells that does not express HER2, no binding of the primary antibody was observed, which indicates that the modified antibodies are selective to HER2 protein receptor. In addition, no differences were observed for the native primary Trastuzumab and the modified versions of Trastuzumab, which indicates that the modification does not influence to its specificity and the binding dynamics.



Scheme 7.11: Left: Confocal microscopy images of SK-BR-3 cells treated with modified and native primary Trastuzumab antibodies and 2nd antibody. Right: Confocal microscopy images of MDA-MB-231 cells treated with modified and native primary Trastuzumab antibodies and 2nd antibody.

7.4. CONCLUSIONS

In summary, an effective protocol for the synthesis of 2-deoxy-2-trifluoromethyl glycosyl azides was described and the same protocol was also applied to the synthesis of 2-deoxy-2-fluoro glycosyl azides. A series of trifluoromethylated, fluorinated, and native glycosyl azides were synthetised. Evaluation of the SPAAC kinetics with different strained alkynes allowed the selection DBCO-maleimide as the best linker.

Trastuzumab and Gemtuzumab were chosen as model antibodies for this study. Optimization of the bioconjugation step using both antibodies and the selected linker was achieved. In addition, control of the bioconjugation step to obtain the ring opening of the maleimide to form the more stable acid conjugate was achieved by changing the temperature and the reaction times. The bioconjugation step was applied to other maleimide structure (benzyl maleimide), and control of the products was achieved by tuning both the temperature and reaction times. Optimized bioconjugation and SPAAC reactions were applied to both antibodies and a series of sugar azides, obtaining the desired bioconjugates with high purity.

Stability of the bioconjugates in plasma and GSH was addressed. Bioconjugates containing the maleimide form, proved to be unstable under these conditions and total decomposition was observed. In contrast, the bioconjugate containing the acid form, proved to be more stable and, in the case of Thiomab, no decomposition was observed. Additionally, the bioconjugates bearing the acid linker and the sugars proved to be completely stable under these conditions.

Flow cytometry experiments prove that the modified antibodies bearing the acid linker and the sugars maintain its specificity to the cell's receptors, CD-33 for Gemtuzumab and HER2 for Trastuzumab. In addition, no significant differences were observed for the modified linker versus the native antibody. Confocal microscopy experiments demonstrate that the modified bioconjugates maintain the specificity and binding dynamics to cell's receptors.

7.5. EXPERIMENTAL SECTION

7.5.1. General considerations

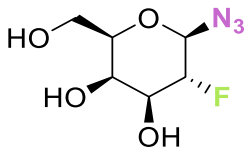
Proton (^{1}H NMR), carbon (^{13}C NMR) and fluorine (^{19}F NMR) nuclear magnetic resonance spectra were recorded on a Varian Mercury spectrometer or a Bruker Avance Ultrashield (400 MHz for ^{1}H, 100.6 MHz for ^{13}C and 376.5 MHz for ^{19}F). Spectra were fully assigned using COSY, HSQC, HMBC and NOESY. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^{1}H NMR: $CDCl_3$ = 7.26 and ^{13}C NMR: $CDCl_3$ = 77.00) and $CFCl_3$. Coupling constants (J) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and app = apparent. Infrared (IR) spectra were recorded on a Jasco FT/IR-600 Plus ATR Specac Golden Gate spectrophotometer. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (*c*) are given in g/100 mL. High resolution mass spectra (HRMS) were recorded on an Agilent 1100 Series LC/MSD mass spectrometer with electrospray ionization (ESI) by the Servei de Recursos Científics (URV). Nominal and exact *m/z* values are reported in Daltons. Thin layer chromatography (TLC) was carried out using commercial aluminum backed sheets coated with $60F_{254}$ silica gel. Visualization of the silica plates was achieved using a UV lamp (max = 254 nm) and 6% H_2SO_4 in EtOH. Flash column chromatography was carried out using silica gel 60 Å (230-400 mesh). Mobile phases are reported in relative composition (e.g. 1:1 EtOAc/hexane v/v). All solvents were used as supplied (Analytical, synthesis or HPLC grade), without prior purification. All reagents were used as received from commercial suppliers. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate ($MgSO_4$) was used as drying agent after reaction work-up, as indicated.

7.5.2. Synthetic procedures to obtain glycosyl azides

General procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides

Sodium azide (2 mmol) was added to a mixture of the corresponding peracetylated 2-deoxy-2-fluoroglycosyl bromide (1 mmol), TBAHS (2 mmol), and 1 M aqueous Na_2CO_3 (4 mL) in EtOAc (20 mL) at room temperature. The reaction mixture was stirred at the same temperature and the progress monitored by TLC. The crude was then diluted with EtOAc and extracted. The combined organic layers were dried over MgSO4, filtered, and concentrated under vacuum. The residue was purified by flash column chromatography. The product obtained was redissolved in methanol (10mL), NaOMe (0.2 mmol) was added, and the reaction was stirred at room temperature until total conversion was observed by TLC. Dowex (H^+ 50WX8-200) was then added, and the mixture stirred for 30 minutes at room temperature. The crude was filtered, washed with MeOH and concentrated under vacuum. The residue was purified by flash column chromatography to give the desired compounds.

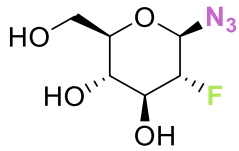
2-Deoxy-2-fluoro-β-D-galactopyranosyl azide (7.4)



The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide **3.10-Br** (207.2 mg, 0.585 mmol), NaN_3 (76.1 mg, 1.17 mmol), TBAHS (397.2 mg, 1.17 mmol) and 1 M aqueous Na_2CO_3 (2.4 mL) in EtOAc 11.7 mL). The reaction mixture was stirred at room temperature for 2 h. After standard work-up, the residue was purified by column chromatography (1:2 EtOAc/hexane) to afford **7.1** (187.2 mg, 96%) as a white solid. R_f (2:8 EtOAc/hexane): 0.26. Then the product was dissolved in MeOH and NaOMe was added. Reaction was stirred at room temperature for 1 h. After work up, the residue was purified by flash column chromatography (1:9 MeOH:CH_2Cl_2) to afford **7.4** (111.5 mg, 96 %) as white solid.

***R**f* (7:1 EtOAc/MeOH): 0.43; **^{1}H NMR** (CD_3OD, 400 MHz) δ in ppm: 4.83 (dd, $J_{1,2}$ = 8.4 Hz, $J_{1,F}$ = 3.7 Hz, 1H, H-1), 4.29 (ddd, $J_{2,F}$ = 52.0 Hz, $J_{1,2}$ = $J_{2,3}$ = 8.4 Hz, 1H, H-2), 3.92 (m, 1H, H-4), 3.80–3.66 (m, 4H, H-3, H-5, H-6a, H-6b); **^{13}C NMR** (CD_3OD, 100.4 MHz) δ in ppm: 92.5 (d, $J_{2,F}$ = 183.1 Hz, C-2), 89.6 (d, $J_{1,F}$ = 23.8 Hz, C-1), 79.2 (C-5), 73.3 (d, $J_{3,F}$ = 17.2 Hz, C-3), 71.0 (d, $J_{4,F}$ = 8.6 Hz, C-4), 62.4 (C-6); **^{19}F NMR** (CD_3OD, 376.5 MHz) δ in ppm: –207.9 (dddd, $J_{2,F}$ = 52.0 Hz, $J_{3,F}$ = 13.9 Hz, $J_{1,F}$ = 3.7 Hz, $J_{4,F}$ = 3.5 Hz, F-2); spectroscopic data was identical to that previously reported.[23]

2-Deoxy-2-fluoro-β-D-glucopyranosyl azide (7.5)



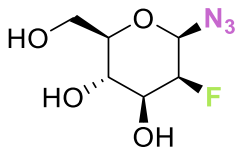
The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide **3.11-Br** (277 mg, 0.748 mmol), NaN_3 (97.0 mg, 1.5 mmol), TBAHS (509.3 mg, 1.5 mmol) and 1 M aqueous Na_2CO_3 (3 mL) in EtOAc (15 mL). The reaction mixture was stirred at room temperature for 2 h. After standard work-up, the residue was purified by column chromatography (1:2 EtOAc/hexane) to afford **7.2** (197.2 mg, 79%) as a white solid. R_f (1:1 EtOAc/hexane): 0.48. Then the product was dissolved in MeOH and NaOMe was added. Reaction was stirred at room temperature for 1 h. After work up, the residue was purified by flash column chromatography (1:9 MeOH:CH_2Cl_2) to afford **7.5** (115.3 mg, 94%) as white solid.

***R**f* (7:1 EtOAc/MeOH): 0.42; **^{1}H NMR** (CD_3OD, 400 MHz) δ in ppm: 4.83 (dd, $J_{1,2}$ = 8.8 Hz, $J_{1,F}$ = 2.3 Hz, 1H, H-1), 3.93 (ddd, $J_{2,F}$ = 51.6 Hz, $J_{1,2}$ = $J_{2,3}$ = 8.8 Hz, 1H, H-2), 3.88 (dd, $J_{6a,b}$ = 12 Hz, $J_{5,6a}$ = 2.2 Hz, 1H, H-6a), 3.71-3.60 (m, 2H, H-3,6b), 3.45-3.33 (m, 2H, H-4, H-5); **^{13}C NMR** (CD_3OD, 100.4 MHz) δ in ppm: 93.3

[23] Boutureira, O.; D'Hooge, F.; Fernandez-Gonzalez, M.; Bernardes, G. J.; Sanchez-Navarro, M.; Koeppe, J. R.; Davis, B. G. *Chem. Comm.* **2010**, *46*, 8142-8144.

(d, $J_{2,F}$ = 186.9 Hz, C-2), 89.2 (d, $J_{1,F}$ = 22.9 Hz, C1), 80.4 (C-5), 76.4 (d, $J_{3,F}$ = 16.2 Hz, C-3), 70.9 (d, $J_{4,F}$ = 8.6 Hz, C-4), 62.4 (C-6); **^{19}F NMR** (CD_3OD, 376.5 MHz) δ in ppm: -199.4 (ddd, $J_{2,F}$ = 51.6 Hz, $J_{3,F}$ = 14.9 Hz, $J_{1,F}$ = 2.3 Hz, F-2); spectroscopic data was identical to that previously reported.[24]

2-Deoxy-2-fluoro-β-D-mannopyranosyl azide (7.6)

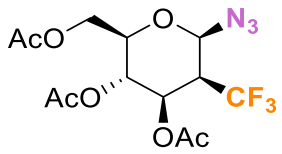


The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranosyl bromide **3.12-Br** (166.1 mg, 0.447 mmol), NaN_3 (58.2 mg, 0.895 mmol), TBAHS (303.9 mg, 0.895 mmol) and 1 M aqueous Na_2CO_3 (2 mL) in EtOAc (10 mL). The reaction mixture was stirred at room temperature for 2 h. After standard work-up, the residue was purified by column chromatography (1:2 EtOAc/hexane) to afford **7.3** (122.8 mg, 83%) as a white solid. R_f (2:8 EtOAc/hexane): 0.20. Then the product was dissolved in MeOH and NaOMe was added. Reaction was stirred at room temperature for 1 h. After work up, the residue was purified by flash column chromatography (1:9 MeOH:CH_2Cl_2) to afford **7.6** (73.0 mg, 95%) as white solid.

R_f (7:1 EtOAc/MeOH): 0.33; **^{1}H NMR** (CD_3OD, 400 MHz) δ in ppm: 4.71 (d, $J_{2,F}$ = 51.9 Hz, 1H, H-2), 4.63 (d, $J_{1,F}$ = 22.2 Hz, 1H, H-1), 3.93 (d, $J_{6a,6b}$ = 12.1 Hz, H-6a), 3.73 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 6.3 Hz, 1H, H-6b), 3.62–3.50 (m, 2H, H-3, H-4), 3.38 (m, 1H, H-5); **^{13}C NMR** (CD_3OD, 100.4 MHz) δ in ppm: 93.2 (d, $J_{2,F}$ = 185.1 Hz, C-2), 86.6 (d, $J_{1,F}$ = 15.9 Hz, C-1), 80.8 (C-5), 74.0 (d, $J_{3,F}$ = 17.5 Hz, C-3), 68.2 (d, $J_{4,F}$ = 1.9 Hz, C-4), 62.7 (C-6); **^{19}F NMR** (CD_3OD, 376.5 MHz) δ in ppm: -220.2 (m, F-2); spectroscopic data was identical to that previously reported.[23]

[24] S. Maschauer, O. Prante, Carbohydr. Res. 2009, 344, 753-761.

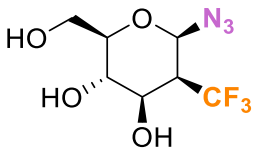
3,4,6,-Tri-O-acetyl-2-deoxy-2-trifluoromethyl-β-D-mannopyranosyl azide (7.7)



To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoromethyl-α-D-glucopyranoside **4.8** (40.5 mg, 0.101 mmol) in 0.51 mL of CH_2Cl_2 were added 0.51 mL of 33% of HBr in AcOH at 0 °C. The reaction was stirred and slowly allowed to warm up to room temperature for 4 h. The crude was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ at 0 °C. The two layers were separated, and the aqueous layer was successively extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated to afford the crude bromide which was used without further purification. 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-α-D-glucopyranosyl bromide **4.8-Br** (42.8 mg, 0.102 mmol) was dissolved in EtOAc (2 mL), then NaN_3 (13.3 mg, 0.205 mmol), TBAHS (72.0 mg, 0.205 mmol) and 1 M aqueous Na_2CO_3 (0.4 mL) were added. The reaction mixture was stirred at room temperature for 1 h. After standard work-up, the residue was purified by column chromatography (1:2 EtOAc/hexane) to afford **7.7** (14.4 mg, 37.5%) as a white solid.

R_f (3:7 EtOAc/hexanes): 0.21; **$[\alpha]^{D}_{25}$**: - 76.2 (0.29, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ in ppm: 5.22 (d, $J_{1,2}$ = 2.5 Hz, 1H, H-1), 5.20-5.11 (m, 2H, H-3, H-4), 4.34 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6a}$ = 4.4 Hz, 1H, H-6a), 4.28 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 5.9 Hz, 1H, H-6b), 3.88 (td, $J_{5,6b}$ = $J_{4,5}$ = 6.0 Hz, $J_{5,6a}$ = 4.4 Hz, 1H, H-5), 3.04 (m 1H, H-2), 2.04, 2.03 (s, 9H, $3CH_3$, Ac); **^{13}C NMR** ($CDCl_3$, 100.6 MHz) δ in ppm: 170.6, 169.8, 169.1 (3C=O, Ac), 124.2 (q, J = 282.0 Hz, CF_3), 83.8 (C-1), 74.2 (C-5), 67.0 (C-3), 65.2 (C-4), 62.3 (C-6), 43.1 (q, $J_{C2,CF3}$ = 26.0 Hz, C-2), 20.75, 20.72, 20.69 ($3CH_3$, Ac); **^{19}F NMR** ($CDCl_3$, 376.5 MHz) δ in ppm: -61.6 (bs, 3F, CF_3); **FT-IR (neat)** ν in cm^{-1}: 2122, 1748, 1370, 1226, 1173, 1157, 1132, 1079, 1049; **HRMS (TOF ES$^+$)** for $(M+Na)^+$ $C_{13}H_{20}F_3N_3NaO_7^+$ (m/z): calc. 406,0833; found 406,0830.

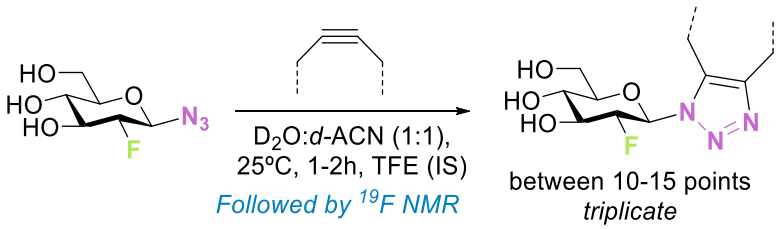
2-Deoxy-2-trifluoromethyl-β-D-mannopyranosyl azide (7.8)



To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoromethyl-β-D-mannopyranosyl azide **7.7** (14.4 mg, 0.036 mmol) in MeOH (0.5 mL), NaOMe (1.4 mg, 0.025 mmol) was added, and the reaction stirred at room temperature for 1 h. After standard work up, the residue was purified by flash column chromatography (1:9 MeOH:CH_2Cl_2) to afford **7.8** (6.6 mg, 76 %) as white solid.

R_f (1:9 MeOH/CH_2Cl_2): 0.24; **[α]$^{D}_{25}$**: - 49.1 (0.55, MeOH); **^{1}H NMR** (CD_3OD, 400 MHz) δ in ppm: 5.14 (dd, $J_{1,2}$ = 2.7 Hz, $J_{1,3}$ = 1.2 Hz, 1H, H-1), 3.88 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 2.7 Hz, 1H, H-6a), 3.83 (ddd, $J_{3,4}$ = 8.8 Hz, $J_{2.3}$ = 5.2 Hz, $J_{1,3}$ = 1.2 Hz, 1H, H-3), 3.70 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 6.2 Hz, 1H, H-6b), 3.63 (t $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, 1H. H-4), 3.44 (ddd, $J_{4,5}$ = 8.7 Hz, $J_{5,6b}$ = 6.2 Hz, $J_{5,6a}$ = 2.7 Hz, 1H, H-5), 3.04 (qdd, $J_{2,CF3}$ = 9.9 Hz, $J_{2,3}$ = 5.2 Hz, $J_{1,2}$ = 2.7 Hz, 1H, H-2); **^{13}C NMR** (CD_3OD, 100.6 MHz) δ in ppm: 125.6 (q, *J* = 282.4 Hz, CF_3), 85.0 (C-1), 80.4 (C-5), 71.1 (C-3), 67.0 (C-4), 61.5 (C-6), 46.6 (q, $J_{C2,CF3}$ = 23.4 Hz, C-2); **^{19}F NMR** (CD_3OD, 376.5 MHz) δ in ppm: -59.5 (bs, 3F, CF_3); **FT-IR (neat)** *ν* in cm^{-1}: 3359, 2122, 1254, 1138, 1097, 1067, 1043; **HRMS (TOF ES$^-$)** for $(M+Cl)^-$ $C_7H_{10}ClF_3N_3O_4^-$ (m/z): calc. 292.0317; found 292.0319.

7.5.3. Procedure for study SPAAC reaction rates using different strained alkynes



2-Deoxy-2-fluoro-β-D-glucopyranosyl azide was weighted in an NMR tube, then D_2O and *d*-ACN were added using a syringe and the tube was sealed

using a rubber septum. TFE was added using a Hamilton syringe and the mixture was vigorously mixed. The NMR robot was then syntonised to the sample in ^{19}F NMR (D_2O syntonised, SW = -20 to -250 ppm, OP1 = -138 ppm, nt=10, d_1=1, adquisition time = 18 s, lb=1, gain = 26) and the temperature set at 25 °C. Once the temperature was reached and the settings were applied, the strained alkyne was added to the sample in the NMR tube, closed with a rubber septum, vigorously mixed for 20 seconds, and then analysed by ^{19}F NMR. Each reaction was carried out by triplicate and between 10 to 15 points were taken. The second-order rate constants for the reaction were determine by plotting 1/[glycosyl azide] versus time, followed by subsequent analysis by linear regression. Second-order rate constants (k_t, M^{-1} s^{-1}) correspond to the average of the determined slopes and error bars represent standard derivations from the three replicate experiments.

7.5.4. Bioconjugation materials and reagents

All reagents and solvents (analytical or HPLC grade) are used as received from commercial suppliers without prior purification. Milli-QR purified water is used for protein manipulations. Follow all waste disposal regulations when disposing waste materials.

1. 20 mM NaPi buffer pH 8.
2. N,N-Dimethylformamide (DMF).
3. DBCO-maleimide was purchased from Sigma-Aldrich.
4. Nanodrop
5. Eppendorf tube: 0.5 mL and 1.5 mL.
6. Zeba™ Spin desalting column: Thermo Fisher Scientific, 7K MWCO, 0.5 mL.
7. Vivaspin® 500 centrifugal concentrator: Sigma-Aldrich, 5K MWCO, 0.5 mL.
8. Laboratory centrifuge.
9. Vortex shaker.
10. Eppendorf incubator shaker.

7.5.5. Protein Mass Spectrometry Equipment

Liquid chromatography–mass spectrometry (LC–MS) is performed on a Xevo G2-S TOF mass spectrometer coupled to an Acquity UPLC system (Acquity UPLC BEH300 C4 column, 1.7 µm, 2.1 mm x 50 mm). Water with 0.1% formic acid (solvent A) and 70% acetonitrile and 29.925% water with 0.075% formic acid (solvent B) are used as the mobile phase at a flow rate of 0.2 mL/min. The gradient is programmed as follows: from 72% A to 100% B for 25 min then 100% B for 2 min and 72% A for 18 min. The electrospray source is operated with a capillary voltage of 2.0 kV and a cone voltage of 40 V. Nitrogen is used as the desolvation gas at a total flow of 850 L/h. Total mass spectra are reconstructed from the ion series using the MaxEnt algorithm preinstalled on MassLynx software (v. 4.1 from Waters) according to the manufacturer's instructions.

7.5.6. Antibody sequences

- ***Sequence of Gemtuzumab V205C (modified residue underlined and bold).***

LC V205C: DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQQKPGK APKLLMYAASNQGSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQTKEVPWSFGQGT KVEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP**C**TKSFNRGEC

HC 239: EVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVRQAPGQSLEW IGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDTAFYYCVNGNPWLAYW GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPE FLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG

- ***Sequence of Trastuzumab V205C (modified residue underlined and bold).***

LC V205C: DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP**C**TKSFNRGEC

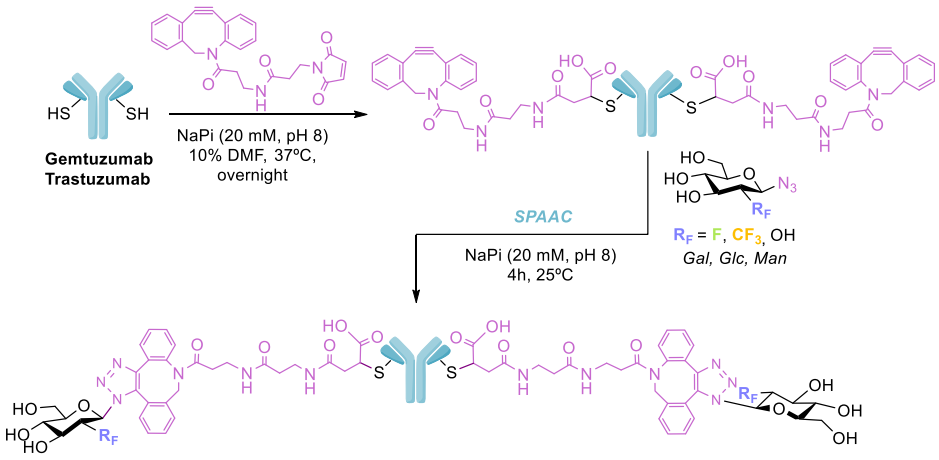
HC: EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVA RIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMD YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK//THT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

7.5.7. General procedure for the refolding of antibodies

Antibodies were stored in a solution of PBS pH 7.2 + 1 mM EDTA buffer at -80 °C and were previously defrosted in an ice bath for 2 h. The refolding procedure was carried out prior reaction. First, antibodies were reduced with a solution of 50 mM tris (2-carboxyethyl)phosphine hydrochloride (TCEP) in DMF (20 eq./Ab to a final concentration of 10% DMF) for 30 min at 37 °C. Then antibodies were desalted using Zeba™ Spin desalting columns (2 min, 15000 x g, ThremoScientific) into PBS pH 7.2 + 1 mM EDTA. Then, antibodies were oxidized using a solution of 50 mM (L)-dehydroascorbic acid in DMF (50 eq./Ab to a final concentration of 10% DMF) overnight at 25 °C. After oxidation, antibodies were desalted using Zeba™ Spin desalting columns (0.5 mL, 10 kDa MWCO, size exclusion columns, ThermoFisher, 2 min, 15000 x g) into sodium phosphate (NaPi) 20 mM pH 8 buffer. A sample of the refolded antibody (2 µM in 10 µL + 100 eq TCEP) was subjected to reduced LC-MS analysis and the concentration was determined by UV absorbance at 280 nm (A280), measured using a SpecrtaDrop reader (MIniMax i3x Imager, Molecular Devices).

Refolding and reactivity of the antibody was assessed by reaction of the freshly refolded sample (10 µM in 10 µL in NaPi pH 8) with methylmaleimide (5 eq/cysteine + 1 µL of a stock in DMF) for 30 minutes at 37 °C. Then a sample of conjugate (2 µM in 10 µL + 100 eq TCEP) was subjected to reduced LC-MS analysis to assess conversion.

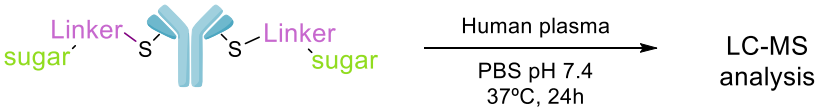
7.5.8. General procedure for the bioconjugation of the linker followed by SPAAC reaction with sugar derivatives



First step was the bioconjugation reaction of the antibody and the linker (DBCO-maleimide). In a 0.5 mL Eppendorf tube was added 8 µL of the refolded antibody (25 µM Ab solution in 20 mM NaPi pH 8) and 28 µL of 20 mM NaPi pH 8 to a final volume of 36 µL. Then a freshly prepared solution of DBCO-maleimide in DMF (5 mM in DMF, 20eq./Ab) and DMF (to a final concentration of 10% DMF) were added. The resulting mixture was vortex for 10 seconds, spin down and incubated at 25 °C and shaking at 500 rpm. Reaction progress was monitored by LC-MS until no starting material was detected. Once the reaction has finished, small molecules were removed from the reaction mixture by loading the sample onto a Zeba™ Spin desalting column (Thermo Fisher Scientific, 10K MWCO, 2 min, 1500 x g), previously equilibrated with 20 mM NaPi pH 8. The sample was recovered and the concentration of the bioconjugate was determined by UV absorbance at 280 nm (A280), as measured using a SpecrtaDrop reader (MIniMax i3x Imager, Molecular Devices).

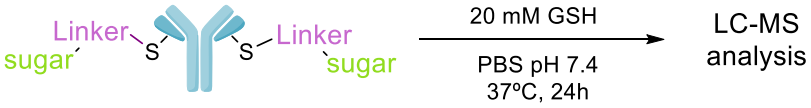
Next step is the SPAAC reaction with a glycosyl azide derivate. In a 0.5 mL Eppendorf 40 µL of the freshly prepared bioconjugate (Ab-DBCO-maleimide, 5 µM in 20 mM NaPi pH 8) and 100 eq of the glycosyl azide (50 mM solution in H_2O) were added. The resulting mixture was vortex for 10 seconds, spin down and incubated at 25 °C and shaking at 500 rpm. Reaction progress was monitored by LC-MS until no starting material was detected. Once the reaction has finished, small molecules were removed from the reaction mixture by loading the sample onto a Zeba™ Spin desalting column (Thermo Fisher Scientific, 10K MWCO, 2 min, 1500 x g), previously equilibrated with 20 mM NaPi pH 8. The sample was recovered and the concentration of the bioconjugate was determined by UV absorbance at 280 nm (A280), measured using a SpecrtaDrop reader (MIniMax i3x Imager, Molecular Devices). Then the sample was flash frozen with liquid nitrogen and stored at -80°C until use.

7.5.9. Stability studies in plasma



To a 10 µL aliquot of the antibody (2.5-4.5 µM in PBS pH 7.4) in a 0.2 mL Eppendorf was added 1 µL of reconstituted human plasma (Sigma-Aldrich) at room temperature. The resulting mixture was vortexed for 10 seconds and incubated at 37 °C shaking at 250 rpm. After 24 h, a 4 µL aliquot was analysed by LC-MS (2 µM in 10 µL (NaPi 20 mM pH 8) + 100 eq TCEP (1 mM in H_2O)).

7.5.10. Stability studies in reduced glutathione



To a 10 µL aliquot of the antibody (2.5-4.5 µM in PBS pH 7.4) in a 0.2 mL Eppendorf was added 1 µL of 20 mM glutathione (6 mg in 1 mL of PBS buffer at pH 7.4) at room temperature. The mixture was vortexed for 10 seconds and incubated at 37 °C shaking at 250 rpm. After 24 h, a 4 µL aliquot was analysed by LC-MS (2 µM in 10 µL (NaPi 20 mM pH 8) + 100 eq TCEP (1 mM in H_2O)).

7.5.11. Cell lines and culture data

All the primary cells were obtained at the Department of Chemistry in the University of Cambridge. Cells were defrosted and maintain under the conditions described below for 2 weeks before the development of the experiments.

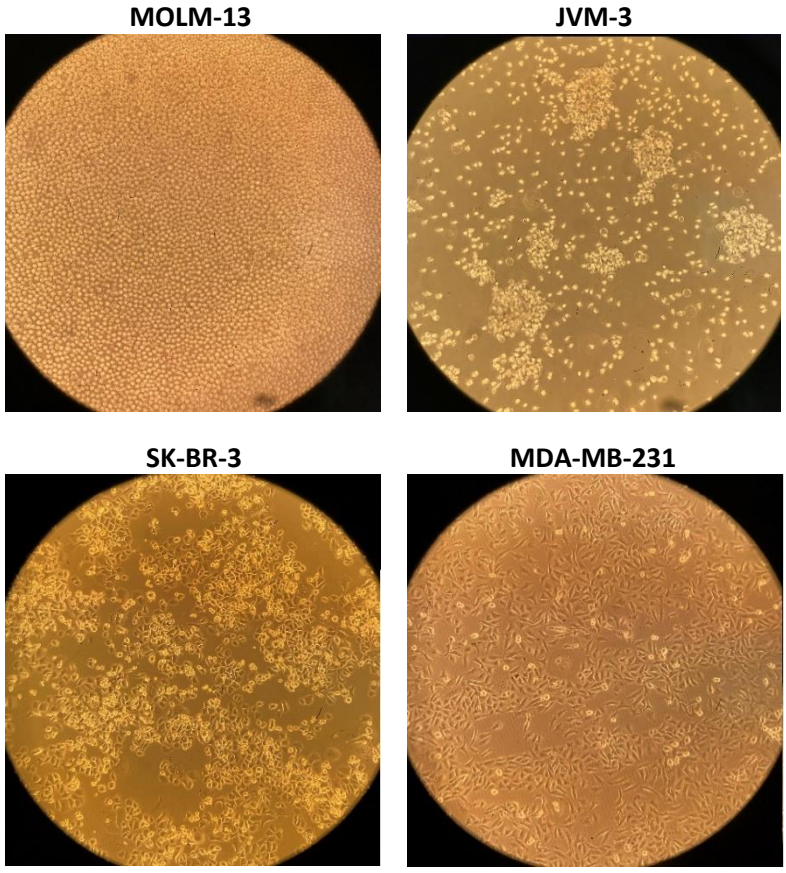


Figure 7.5. Images of the cells used in this chapter through a microscope.

- *MOLM-13 cells*
  - Disease: Chronic B-cell leukaemia
  - Specie: human
  - Growth properties: suspension
  - Morphology: round single cells in suspension

- Culture medium: 90% RPMI-1640 + 10% h.i. FBS
- Subculture: seed out at $1 \cdot 10^6$ cells/mL, maintain at $0.4\text{-}2 \cdot 10^6$ cells/mL, split saturated culture 1:2 to 1:2 every 2-3 days.
- Incubation: 37 °C with 5% of CO_2
- Doubling time: 50 h
- Storage: frozen with 70% medium, 20% FBS, 10% DMSO

- *JVM-3 cells*

  - Disease: Chronic B-cell leukaemia
  - Specie: human
  - Growth properties: suspension
  - Morphology: lymphoblastoid cells growing singly or in clumps.
  - Culture medium: 90% RPMI-1640 + 10% h.i. FBS
  - Subculture: seed out at $0.5 \cdot 10^6$ cells/mL, maintain at $0.1\text{-}0.5 \cdot 10^6$ cells/mL, split saturated culture 1:3 to 1:4 every 3 or 4 days.
  - Incubation: 37°C with 5% of CO_2
  - Doubling time: about 40 h
  - Storage: frozen with 70% medium, 20% FBS, 10% DMSO

- *SK-BR-3 cells*

  - Disease: breast carcinoma
  - Specie: human
  - Growth properties: adherent
  - Morphology: adherent cells growing in monolayers changing from round shape after seeding to epithelial-like cells
  - Culture medium: 80% McCoy's 5a + 10% h.i. FBS
  - Subculture: seed out at $1\text{-}2 \cdot 10^6$ cells/mL, maintain at $0.5\text{-}2.5 \cdot 10^6$ cells/mL, split semi-confluent culture 1:3 to 1:5 once or twice a week
  - Incubation: 37 °C with 5% of CO_2
  - Doubling time: 2-3 days
  - Storage: frozen with 70% medium, 20% FBS, 10% DMSO

- *MDA-MB-231 cells*

  - Disease: breast carcinoma
  - Specie: human
  - Growth properties: adherent
  - Morphology: epithelial cells growing adherently as monolayer, sometimes forming spindle-like extensions
  - Culture medium: 90% DMEM + 10% h.i. FBS
  - Subculture: seed out at 0.5-1·10^6 cells/mL, maintain at 0.1-0.5·10^6 cells/mL, split semi-confluent culture 1:5 to 1:20 every 3-5 days using trypsin-EDTA.
  - Incubation: 37 °C with 5% of CO_2
  - Doubling time: 25-30 h
  - Storage: frozen with 70% medium, 20% FBS, 10% DMSO

7.5.12. Flow cytometry protocol

- *Preparation of cells samples*

MOLM-13 cells and JVM-3 cells were routinely cultured at 37 °C with 5% CO_2 in RPMI-1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. SK-BR-3 cells were routinely cultured at 37 °C with 5% CO_2 in 80% McCoy's 5a supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. MDA-MB-231 cells were routinely cultured at 37 °C with 5% CO_2 in DMEM medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. Prior to the experiments, cells were counted, washed and a solution of 2·10^6 cells/mL in ice cold FACS buffer (PBS, 3% FBS 0.1% NaN_3) was prepared.

- *Reagents*
  - Solution of 2·10^6 cells /mL in ice cold FACS buffer (PBS, 3% FBS 0.1% NaN_3)
  - 20 nM solution of the primary antibodies in FACS buffer
  - Secondary antibody: Goat F(ab')2 Anti-Human IgG. Alexa Fluor ® 488 Conjugate
  - Solution of 2% paraformaldehyde (PFA) in PBS buffer pH 7.4

- *Preparation of flow cytometry samples in a 96 well plate*

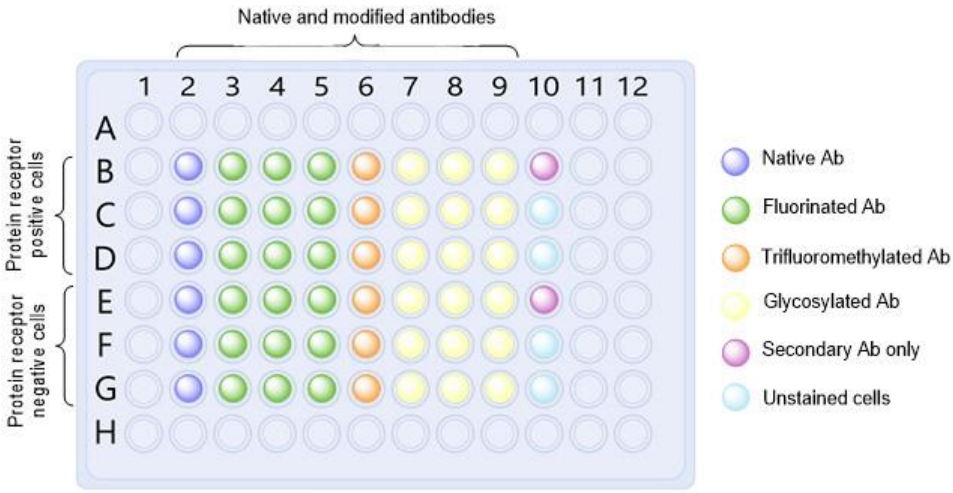


Figure 7.6. Flow cytometry experiment settings in a 96 well plate.

The experiment settings are shown in the figure X. 100 µL of ice-cold cell suspension in FACS buffer were added a V-bottom 96 well plate, then centrifuged 2 min at 1500 rpm at 4 °C and the medium discarded. 100 µL of 20nM primary antibodies in FACS Buffer were added to the corresponding wells (as indicated in Figure 7.X), pipet up and down to mix and incubated 1 h on ice. After this time, cells were centrifuge (2 min, 1500 rpm, 4 °C), media was discarded, and cells were washed with 100 µL FACS buffer (2 min, 1500 rpm, 4 °C). 100 µL of the secondary antibody in FACS Buffer (diluted 1:100) were added to the corresponding wells and cells were incubated for 30 min at room temperature under darkness. After completion of this time, cells were centrifuge (2 min, 1500 rpm, 4 °C), media discarded, and cells washed with 100 µL FACS buffer (2 min, 1500 rpm, 4 °C). Then, 100 µL of 2% PFA in PBS were added to all the wells and maintain 10 minutes at rt under darkness. Then, cells were centrifuge (2 min, 1500 rpm, 4 °C), and resuspend in 200 µL of ice-cold FACS buffer. Fixation will inactivate most biohazardous agents, minimize deterioration and help to maintain the integrity of the samples. Samples were then transferred to a 1.5 mL Eppendorf, acquired with a FACS Fortessa I/II (BD Bioscience) and analysed with FlowJo (Tree Star).

7.5.13. Confocal microscopy

- *Preparation of cells samples*

MOLM-13 cells and JVM-3 cells were routinely cultured at 37 °C with 5% CO_2 in RPMI-1640 medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. SK-BR-3 cells were routinely cultured at 37 °C with 5% CO_2 in 80% McCoy's 5a supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. MDA-MB-231 cells were routinely cultured at 37 °C with 5% CO_2 in DMEM medium supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM L-glutamine. Prior to the experiments, cells were counted, washed, and a solution of $0.2 \cdot 10^6$ cells /mL in their corresponding media was prepared.

- *Reagents*
  - Solution of the cells at $0.1 \cdot 10^6$ cells/mL in corresponding media
  - Poly-L-lysine solution (20 µg/mL)
  - Fixation buffer: 4% Paraformaldehyde (PFA) in PBS
  - Blocking buffer: 3% BSA in PBS
  - Mounting media for samples with 2nd antibody: Prolong™ Diamond Antifade Mountant with DAPI
  - 20 nM solution of the primary antibodies in PBS buffer pH 7.4
  - Secondary antibody: Goat F(ab')2 Anti-Human IgG. Alexa Fluor ® 488 Conjugate
  - Phalloidin
- *Preparation of the coverslips in a 26 well plate:*

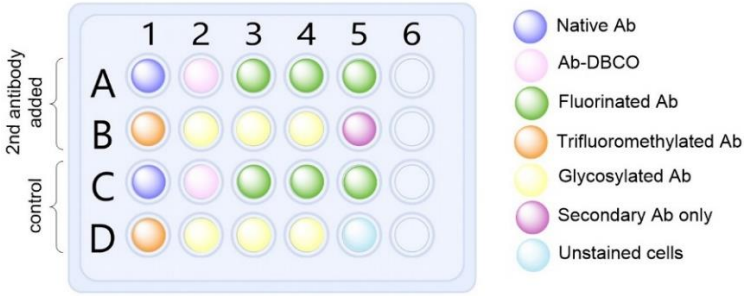


Figure 7.7. Confocal microscopy experiment settings in a 24 well plate.

- *Protocol*

In a 24 well plate, a 13 mm round coverslip (thickness 1.5) were put in 20 wells as indicated in the Figure X. To make the coverslips sticky, 500 µL of poly-L-lysine (20 µg/mL in PBS buffer pH 7.4) were added and incubated at 37 °C for 1 h. Then coverslips were washed with 500 µL of PBS buffer pH 7.4 three times and 500 µL of $0.2 \cdot 10^6$ cells /mL cells solution were added and incubated at 37 °C with 5% CO_2 overnight. Media was removed and cells were fixed with 500 µL of fixation buffer (4% PFA in PBS) and let 30 min a rt. Fixation buffer was removed and 500 µL of blocking buffer was added and incubated 30 min at rt. Blocking buffer was removed and 100 µL of primary antibody solution (20 nM in PBS pH 7.4) were added to the corresponding wells containing the coverslip and cells. Cells were incubated at rt for 1 h then antibody solutions were removed, and cells washed 3 times with PBS pH 7.4. 100 µL of a solution of secondary antibody and phalloidin in PBS (1:100 secondary antibody and 1:20 phalloidin) were added to the corresponding wells and 500 µL of PBS were added to the remaining wells. Cells were incubated at rt for 30 min in darkness and then washed with PBS. To a glass slide was added a drop of mounting media, then coverslips were transferred and place in the slide with the side of the cells facing the drop. The coverslips were let dry for 1 h and then imaged in the microscope.

- *Imagen acquisition*

Images were recorded using a Leica Microscope operating at x10, x20, x40 and x63 magnification with immersion mode. Coverslips were imaged using a 63x oil immersion objective. Laser lines at 458nm, 488nm and 555nm were used to excite the fluorophores. For all images, tile scans and z-stacks were acquired with a step size of 2.5 µm and a pinhole of 1 airy unit. Images were taken at 1024x1024 voxel density with a line averaging of 8. Fluorophores and autofluorescence were unmixed into separate channels using the unmixing algorithm provided in the Leica software (LAS X). Single stained slides were used to obtain the reference spectra of the different fluorophores.

CHAPTER VIII

GENERAL CONCLUSIONS

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The present doctoral work is focused on the development of new synthetic methodologies for the stereoselective preparation of fluorinated sugars, the study of their key physicochemical properties, and the application of such methodologies for the stereoselective synthesis of biologically relevant fluorinated glycosyl derivatives. The main objectives proposed in this PhD thesis have been fulfilled.

CHAPTER III

In Chapter III a microwave-assisted method for the stereoselective synthesis of 2-deoxy-2-fluoro-α-glycosides was developed. Different parameters of the glycosylation reaction were studied and their influence on stereoselectivity was evaluated. Temperature has a strong effect on glycosylation stereoselectivity and the increase of the temperature reduces reaction times and favours the formation of the most stable α-isomer for *galacto*, *gluco* and *manno* configurations. Regarding glycosyl donors, glycosyl iodides proved to be more reactive than analogous bromides. The leaving group seemed to affect stereoselectivity only at low temperatures, and at high temperatures, no differences in stereoselectivity were observed between bromide and iodide glycosyl donors. Moreover, glycosyl donor configuration and protecting groups have a crucial effect on stereoselectivity. *Manno* glycosides gave the higher α-selectivities, followed by *galacto* and *gluco* glycosides, and strongly inductive protecting groups favour the formation of the α-isomer. Glycosyl acceptor size has a crucial effect in stereoselectivity and large nucleophiles favour the formation of the α-isomer. However, nucleophilicity seemed to have modest impact on stereoselectivity.

Results obtained in the variable-temperature ^{19}F NMR and ^{19}Sn NMR experiments suggested that two distinct mechanisms are operative depending on the glycosylation temperature. At low temperatures (<70°C), a S_N2-like mechanism could be favoured, and at high temperatures (>100°C), a S_N1-like mechanism could be operative, with the anomeric effect governing the

outcome of the glycosylation reaction. The stereocontrol of the reaction seemed to be in accordance with the Anh-Einsenstein 1,2-induction model and a Curtin-Hammett scenario where the stereoselectivities are reinforced or diminished depending on the combination of C-2 fluorine configuration and the inductive nature of the protecting groups.

Finally, the method proves to be effective for the synthesis of fluorinated derivatives of biologically important glycosides, that were obtained in high yields and good stereoselectivies, achieving the isolation of the α-isomer in all the cases.

CHAPTER IV

In Chapter IV a method to access previously uncharted 2-deoxy-2-trifluoromethyl-glycosides has been described. In addition, the utility of the synthetized 2-CF_3-glycosides as glycosyl donors was demonstrated in the glycosylation with primary and secondary alcohols, including primary and secondary aliphatic-OH, sugars, and complex aglycones. The study of the stereoelectronic properties of the CF_3 group highlighted the selectivity control of glycosylation affording 1,2-*trans* glycosides. Comparison of these results with a seminal report of Ryan Gilmour, revealed a higher steric contribution of the trifluoromethyl group in the glycosylation stereoselectivity in comparison with fluorine. Moreover, structural screening indicated that best stereoselectivities are reinforced by the electronic nature of protecting groups (OBn/CF_3^{gluco} as β-selective; OAc/CF_3^{manno} as α-selective).

Computational and experimental (NMR) conformational analysis of resulting CF_3-glycosides showed no perturbations in the molecular geometry, which may stimulate the development of new fluorosugar mimetics with interesting physicochemical properties. Finally, the method proved successful for preparing biologically relevant CF_3-glycosides including amino acids, cholesterol, and sphingosine/phytosphingosine analogues.

CHAPTER V

In Chapter V, deprotected 2-deoxy-2-fluoro and 2-trifluoromethyl glycosides were efficiently synthetized and used as proves to study and compare some important physicochemical properties. First, experimental (by NMR) and computational evaluation of 2-F and 2-CF_3 glycosides demonstrated that they maintain the $^4C_1$ chair conformation, and minimal distortion is observed for the sterically demanding trifluoromethyl group. Differences between F and CF_3 were more important for *manno* glycosides in which high differences in dipole moments and dihedral angles were observed. In contrast, *gluco* derivatives presented similar electronic and steric properties.

Experimental determination of the lipophilicity was achieved following a protocol employing ^{19}F NMR analysis. Results showed that the trifluoromethyl moiety induces higher lipophilicity increase compared with the fluorine atom, and values differ on 0.65-0.85 log *P* units. The anomeric configuration had a slightly effect on lipophilicity, and 1,2-*cis* glycosides presented higher lipophilicity than 1,2-*trans*-analogues. Importantly, a huge increment on log *P* value is observed while introducing one CF_3 group (log P_{glc} = -3.24). These results lead us to consider 2CF_3-glycosides as promising compounds for biological studies since they present positive log *P* values, which are close to optimal values for membrane permeability (log *P* = 1-3).

Fluorinated and trifluoromethylated glycosides proved stable under standard physiological conditions. Moreover, evaluation of the acid-hydrolysis resistance demonstrated that fluorinated compounds are stable under extreme acid conditions (H_2SO_4 [0.5 M] at up to 60°C). Finally, hydrolyzation of 2F- and 2CF_3-glycosides was observed under H_2SO_4 [0.5 M] at 70 °C. Experimental first-order hydrolysis rate-constants and half-life times were similar for 2F- and 2-CF_3-glycosides, indicating that F and CF_3 have similar stabilizing effect, and both improve the resistance to acid-catalyzed hydrolysis in a similar manner.

CHAPTER VI

In Chapter VI the methodologies described in Chapters III and IV were evaluated to obtain fluorinated and trifluoromethylated analogues of the Tn antigen. The stereoselective synthesis of 2-F and 2-CF_3 analogues of the Tn-antigen using *galacto, gluco* and *manno* configurations and serine and threonine amino acids was achieved, obtaining pure α-isomers.

CHAPTER VII

In Chapter VII, a two-step protein-modification protocol for the development of synthetic homogeneous fluorinated glycoantibodies was developed. First step involves the incorporation of strained alkynes into proteins by maleimide *S*-alkylation followed by copper-free strain-promoted azide–alkyne cycloaddition (SPAAC) using two different antibodies (Trastuzumab and Gemtuzumab) and a variety of native, fluorinated, and trifluoromethylated sugars. First, 2-deoxy-2-trifluoromethyl and 2-fluoro glycosyl azides were synthesized from the glycosyl bromide in two steps that included phase-transfer catalyzed glycosylation followed by Zemplén deacetylation. Optimization of the bioconjugation step and SPAAC reaction was achieved using the selected antibodies (Gemtuzumab and Trastuzumab), DBCO-maleimide, and a fluorinated sugar and it was then applied to both antibodies and the variety of sugars azides, obtaining the desired bioconjugates with high purity. Evaluation of the bioconjugation reaction time and temperature allow the control of the opening of the maleimide to form the more stable acid conjugate.

The stability of the synthetized fluorinated bioconjugates in plasma and reduced glutathione was demonstrated by incubating them in both media (human serum and GSH) at 37°C overnight. The specificity and binding dynamics of the fluorinated modified antibodies to the cell receptors, CD-33 for Gemtuzumab and HER2 for Trastuzumab, was demonstrated by flow cytometry and confocal microscopy experiments. In addition, no significant differences were observed for the modified linker versus the native antibody.

ANNEX

Annex

SCIENTIFIC MEETINGS

1) **1ª Jornada de Jóvenes Investigadores del GEQOR** organized by the Grupo Especializado de Química Orgánica, 16th June 2021, on-line. *Poster presentation*. Title: Unlocking the CF_3 Group in the Synthesis of 2-Modified Glycosides. Number: PA5.

2) **VII Young Researchers Symposium** organized by the Spanish Society of Medicinal Chemisrty (SEQT), 18th June 2021, on-line. *Poster presentation*. Title: Unlocking the CF_3 Group in the Synthesis of 2-Modified Glycosides. Number: P4.

3) **Centre for Misfolding Diseases International Conference 2021**, 6th-8th December 2021, The Cambridge Union, Cambridge. *Oral presentation*.

4) **4th GDRI-HC3A Meeting**, 20th and 21st January 2022, Barcelona. *Oral presentation*. Title: Unlocking the CF_3 Group in the Synthesis of 2-Modified Glycosides. Number TS-11.

5) **Summer School in Biomedical Glycoscience**, 8th-10th June 2022, Jaca. *Poster presentation*. Title: Trifluoromethyl-Directed Glycosylation. Number: P19.

6) **XXXVIII Reunión Bienal de la Sociedad Española de Química (RSEQ)**, 27th-30th June 2022, Granada. *Poster presentation*. Title: CF3-Directed Glycosylation for the stereoselective synthesis of 2-deoxy-2-trifluoromethyl glycosides. Number: PP-285

7) **XXVIII Reunión Bienal del Grupo Especializado de Química Orgánica**, 1st July 2022, Granada. *Poster presentation.* Title: Trifluoromethyl-Directed Glycosylation. Number: P8

8) **13th Spanish-Italian Symposium on Organic Chemistry**, 4-6th September 2022, Tarragona. *Poster presentation.* Title: Trifluoromethyl-Directed Glycosylation. Number: P-5

PUBLICATIONS BASED ON THE CONTENT OF THE THESIS

1. **Trifluoromethyl-Directed 1,2-Trans Glycosylation.** Isabel Bascuas, Jordi Mestre, Miguel Bernús, Sergio Castillón, Omar Boutureira. Submitted to *Angew. Chem. Int. Ed.* on August 22, 2022. Manuscript number: 202212405.

2. **Trifluoromethyl-Directed 1,2-Trans Glycosylation.** Isabel Bascuas, Jordi Mestre, Miguel Bernús, Sergio Castillón, Omar Boutureira. Posted online August 22, 2022. *ChemRvix*. DOI: 10.26434/chemrxiv-2022-vd4dt.

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