Chapter 6 - Pyrrolidine derivatives as TRPV1 blockers.

6.1 Selection of an alternative scaffold with three substitution sites.

The previously synthesized library was comprised of triazine and peptoid compounds and from their biological evaluation no information could be obtained about the role of the triazine scaffold in binding to TRPV1. With this purpose and also aiming to dispose of a second family of molecules with activity against TRPV1, we deemed that compounds with a different scaffold that still satisfy the proposed pharmacophore should be designed, prepared and biologically evaluated.

As mentioned in Chapter 2 (Figure 30, pp. 48), other scaffolds that had been considered for the preparation of TRPV1 anatogonist were those of purine, thiobarbituric acid or pirrolidine. Having a different spatial distribution of atoms and substituents, and different geometries, these could shed light on the triazine mode of interaction with the receptor. After a few initial and unsuccesful synthetic attempts with purine and thiobarbiturate derivatives, the pirrolidine scaffold was chosen since it is also the one that introduces a larger variation relative to the triazine scaffold.

Pyrrolidines are found widespread in natural products, like for example amino acid proline, antibiotics from fungi like anisomicyn, pyrrolizidine alkaloids like the venom from *Crotalaria* genus plants monocrotaline, and drugs like the anticholinergic prociclydine or the calcium channel blocker bepridil (Figure 125).

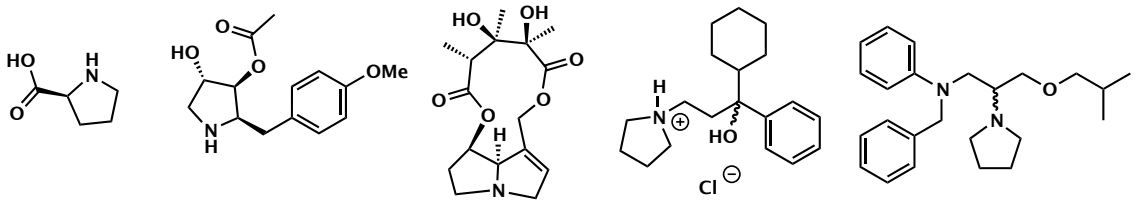


Figure 125. Some relevant natural and synthetic products containing the pyrrolidine ring.

In comparison with triazine, the pyrrolidine ring is smaller and, although not planar, some dynamic symmetry can be achieved by conformational equilibrium between different envelopes. Taking into account the structure of triazine **46**, the most active triazine identified, compounds **86** and **87** could be considered as two possible pyrrolidine analogues (Figure 126). Despite including the same substituents (ie. dimethylaminopropyl and 4-fluorophenethyl), both pyrrolidines would be more flexible than the parent triazine and at least **86** would also show different rotamers in equilibrium due to the presence of the amide groups.

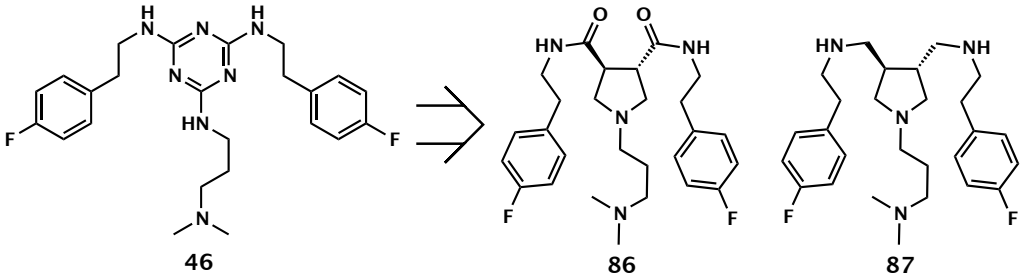


Figure 126. Proposed pyrrolidine analogues of triazine **46**.

6.2 Activity prediction of pyrrolidines 86 and 87.

Previous to carrying out the synthesis of new compounds, a prediction of the activity of the two pyrrolidines was performed using the QSAR models described in the preceding chapter. For that purpose, first, pyrrolidines **86** and **87** were aligned to templates 1 (triazine **46**) and 2 (triazine **72**) using the previously described methods. Figure 127 shows the results of these alignments and reveals that both pyrrolidines show a quite good superposition with both templates, being able to superimpose almost perfectly both aromatic rings and the protonable dimethylamino group with those of template 1. On the other hand, the pyrrolidine ring is slightly shifted, covering only partially the space occupied by the triazine. In compound **86** this space is occupied by a carbonyl and an NH groups of each amide moiety, which are forming an intramolecular hydrogen bond in the conformations aligned to templates 1 and 2.

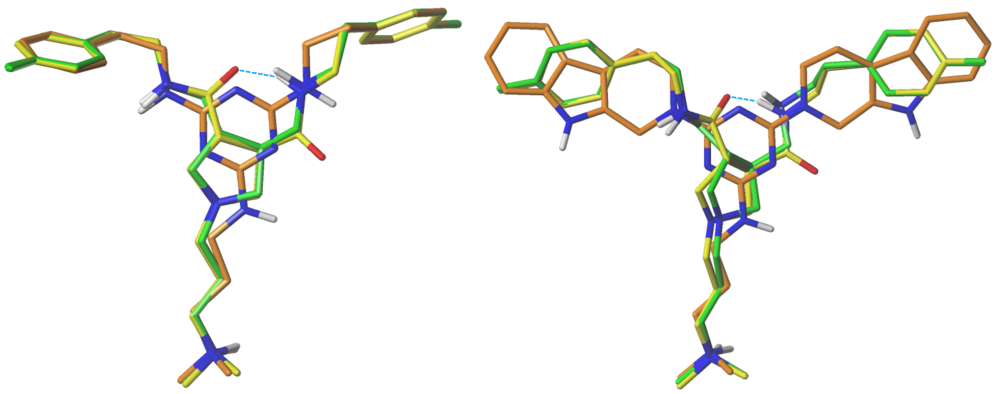


Figure 127. Structures of pyrrolidines **86** and **87** aligned on templates 1 (left) and 2 (right). Color codes: triazines **46** and **72** in orange, pyrrolidine **86** in yellow and pyrrolidine **87** in green.

The CoMSIA donor and electrostatic fields for the aligned conformations of **86** and **87** were calculated (Figure 128), and then they were used to predict the activities of both compounds using the most relevant QSAR models previously developed (ie. CoMSIA-4, 7 and 15).

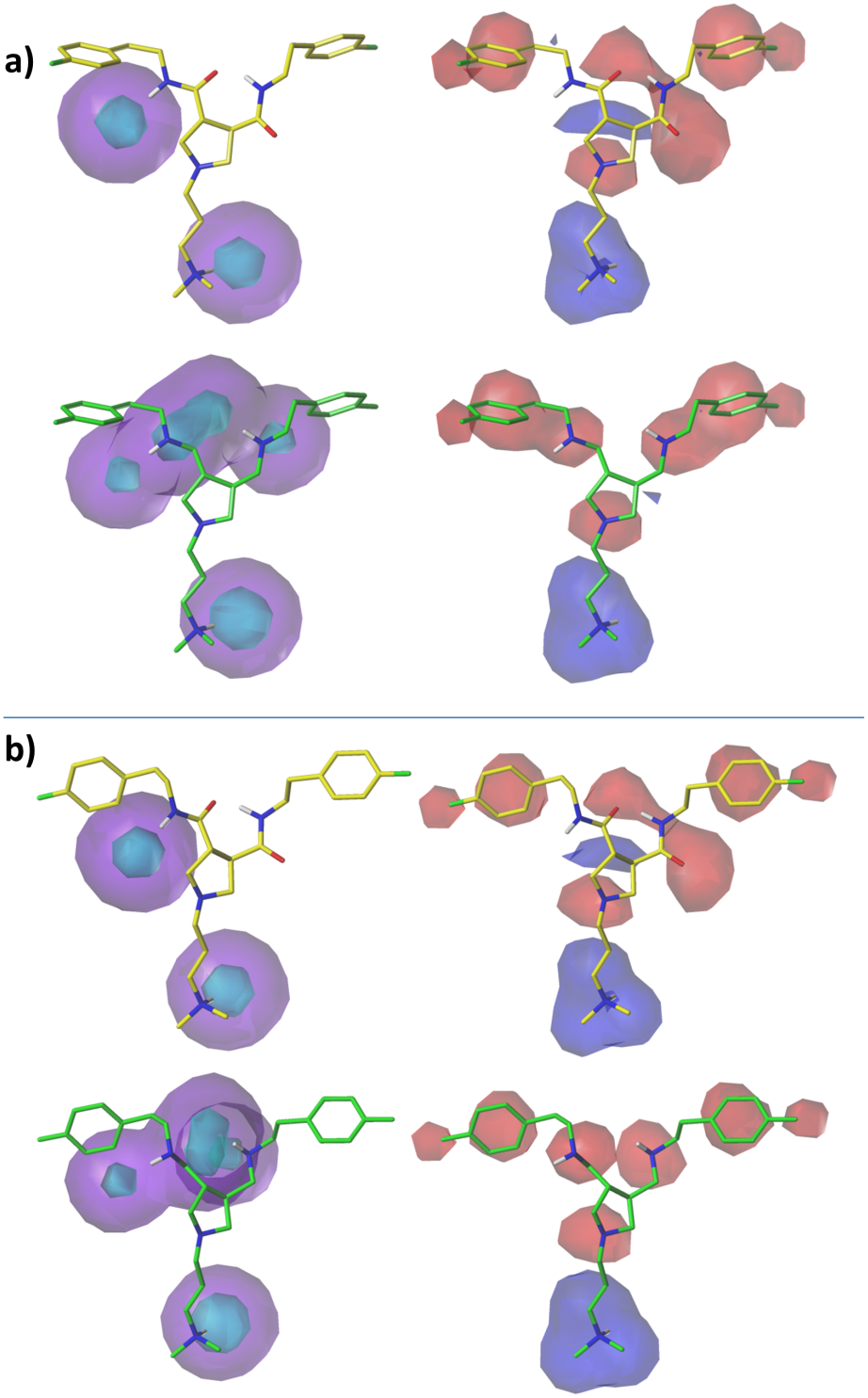


Figure 128. CoMSIA donor (cyan and purple, left) and electrostatic (blue and red, right) fields calculated for pyrrolidines **86** (yellow) and **87** (green) with conformations from alignments to templates 1 (a) and 2 (b). Fields are shown as contours at 80 (cyan and blue) and 20 (purple and red) % contribution.

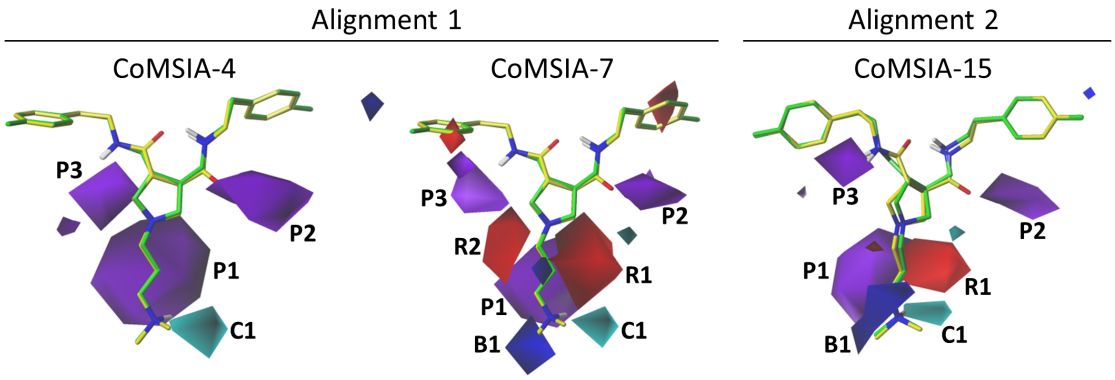


Figure 129. CoMSIA fields representation of 3D-QSAR models COMSIA-4, 7 and 15, and overlaid structures of pyrrolidines **86** (yellow) and **87** (green). Donor (cyan and purple) and electrostatic (blue and red) fields are shown as contours at 80 (cyan and blue) and 20 (purple and red) % contribution. The main contours of each field are labelled.

Figure 129 shows the aligned conformers of pyrrolidines **86** and **87** overlaid on the fields from CoMSIA models 4, 7 (both derived from Alignment 1) and 15 (derived from Alginment 2). As previously mentioned (see Chapter 5), the main positive contribution to activity according to model CoMSIA-4 arises from the presence of donor groups in the molecules close to region C1, while donor groups close to regions represented by contours P1-P3 is detrimental. In alignment 1, both pyrrolidines show favourable donor contours (Figure 128a, cyan) close to their protonated dimethylamino group which overlap region C1. On the other hand, they also show donor contours close to one of the amide-NH groups which would overlap region P3, contributing negatively to their activities. Model CoMSIA-7 is more complex since in addition to the donor field contributions, it also includes contributions from the electrostatic fields from the ligands. So, besides the donor regions C1, P1-P3, with similar shape and location as those observed in model CoMSIA-4, model CoMSIA-7 also includes favourable contributions to activity from electropositive groups located close to region B1 and electronegative groups close to regions R1 and R2. Aside from the donor field contribution, the proximity of the cationic dimethylammonium group of pyrrolidines **86** and **87**, which generates a positive electrostatic field region (Figure 128a, blue), to the B1 contour of the CoMSIA-7 model would favourably contribute to their predicted activity. This activity would also be enhanced by the negative electrostatic fields (Figure 128a, red) centered on the two aromatic moieties, which would overlap smaller electronegative favorable regions of the model (Figure 129, middle). Finally, model CoMSIA-15, derived from Alignment 2, is similar in the distribution of favourable and

unfavourable contours to CoMSIA-7, and both pyrrolidines would show similar interactions with this model to those previosuly mentioned.

The predicted activities for **86** and **87** according to the above three models are summarized in Table 18. From these values, an activity (IC_{50}) close to 10 μM could be expected for both pyrrolidines, turning out that they could be initial hits with a promising activity as TRPV1 antagonists.

Table 18. CoMSIA predicted activities (pIC_{50}, M) for products **86** and **87**.

Model	86	87
CoMSIA-4	4.99	4.92
CoMSIA-7	5.11	4.89
CoMSIA-15	5.00	4.90

6.3 Design of a synthetic route for the preparation of pirrolidines 86 and 87.

After reviewing the literature,[195-201] the retrosynthetic route shown in Figure 130 for the preparation of the desired trisubstituted pyrrolidines was envisioned.

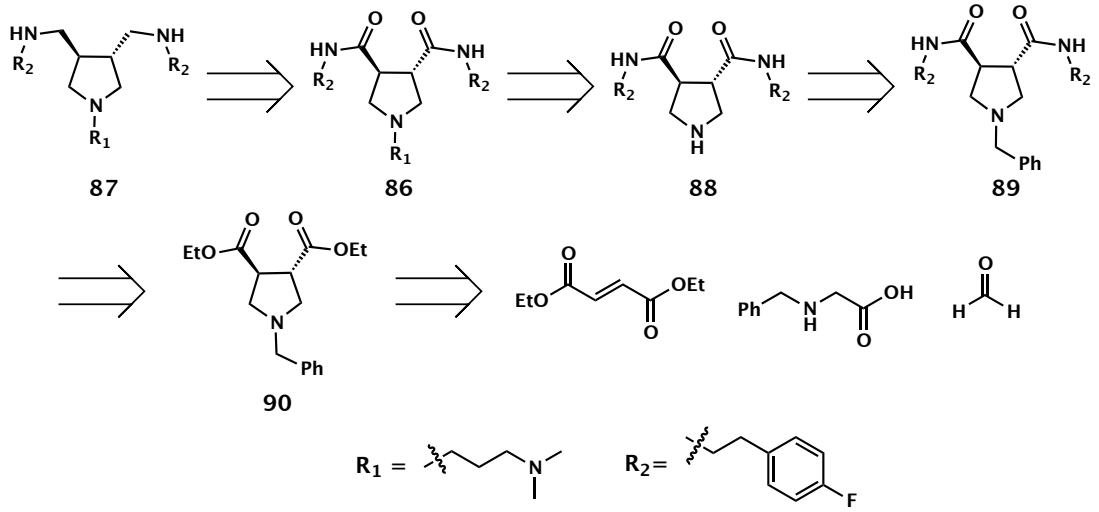


Figure 130. Retrosynthetic scheme for the preparation of pyrrolidines **86** and **87**.

Thus, pyrrolidine **87** could be derived from reduction of the diamide **86**. This would come from alkylation with a conveniently activated dimethylaminopropyl precursor of the pyrrolidine nitrogen of **88**, which in turn would be prepared by hydrogenolysis of **89**. This protected pyrrolidine (**89**) could be prepared from precursor **90** by an ester amination reaction and, finally, **90** could be readily obtained in one step from diethyl fumarate and *N*-benzylglycine, according to Specker et al.[201] It is worth

noting that, playing with the nature of the reagents at the second and fourth key steps, the same scheme could be applied for the preparation of a library of pyrrolidine analogues that would present a diverse range of R_1 and R_2 moieties.

Formation of pyrrolidine **90** involves the stereoselective formation of two stereogenic centers during the cyclization reaction. This implies 3 steps that occur *in-situ* on the same reaction (Figure 131):[201]

a) First, an azomethine ylide formation by reaction between paraformadehyde and *N*-benzyl glycine, with the consequent elimination of a water molecule.
b) Second, an asymmetric 1,3-dipolar cycloadition between the azomethine ylide dienophile and the diethyl fumarate dipolarophile.
c) Finally, an acid decarboxylation generates pyrrolidine **90**.

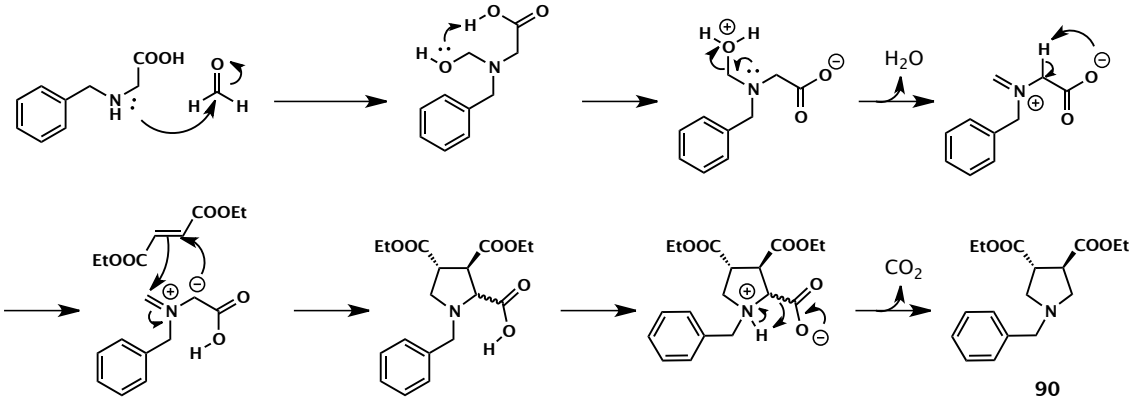


Figure 131. Proposed mechanism for the formation of pyrrolidine **90**.

6.4 Synthesis of pyrrolidine 87.

6.4.1 Preparation of pyrrolidine 90.

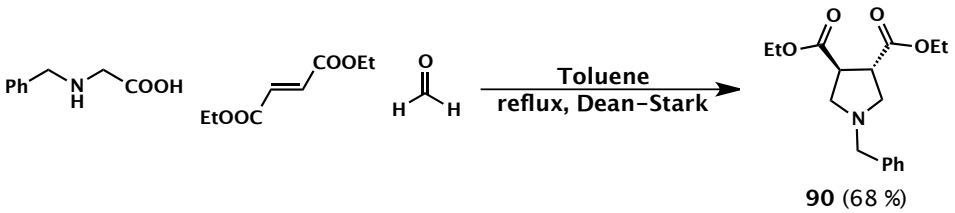


Figure 132. Reaction scheme for the obtention of **90.**

The experimental procedure to obtain **90** (Figure 132) implied the use of a Dean-Stark apparatus and a final distillation at 160 ºC (~1 mbar) to promote the decarboxylation and obtain the desired product. The reaction conditions reported in the work of Specker et al. were performed on a relatively large scale, but we decided to scale down all the reagents to work with easy to handle amounts of materials. The procedure consisted on suspending the reagents N-benzylglycine, diethyl fumarate and paraformaldehyde in toluene, in an almost equimolar ratio, and then heating the mixture to reflux to remove the water generated during formation of the azomethine ylide by azeotropic distillation. Working on a smaller scale made harder to observe how much water was removed by the distillation process. On the other hand, the evolution of the reaction was difficult to control by HPLC or GC due to the low UV-VIS absorption of the products and the complexity of the GC chromatographic traces. Therefore it was necessary to perform several experiments varying the work scale to find the appropriate conditions to obtain the best yields (Table 19). Distillation of the crude reaction mixtures after work-up provided 3 fractions (Figure 133): two of them were identified as unreacted diethyl fumarate and the desired pyrrolidine **90**, and the third was an unknown product that was not identified.

Table 19. Reaction conditions assayed for the synthesis of **90**. The conditions reported in the work of Specker et al. are also included for comparison purposes.

Reaction conditions	N-BnGly mmol	Diethyl fumarate mmol	p-formaldehyde mmol	Toluene mL	Yield %
Specker et al.	907	926,0	1100	1300	78
1	6.0	6.1	7.5	9	68
2	36.0	37.0	45.0	52	49

Under the best conditions assayed, pyrrolidine **90** was obtained in 68 % yield, comparable to the yield reported by Specker et al., as a colorless oil that turned pale

yellow with time, and with a very strong odour, like wet ashes. Its characterization by NMR and HRMS confirmed its identity.

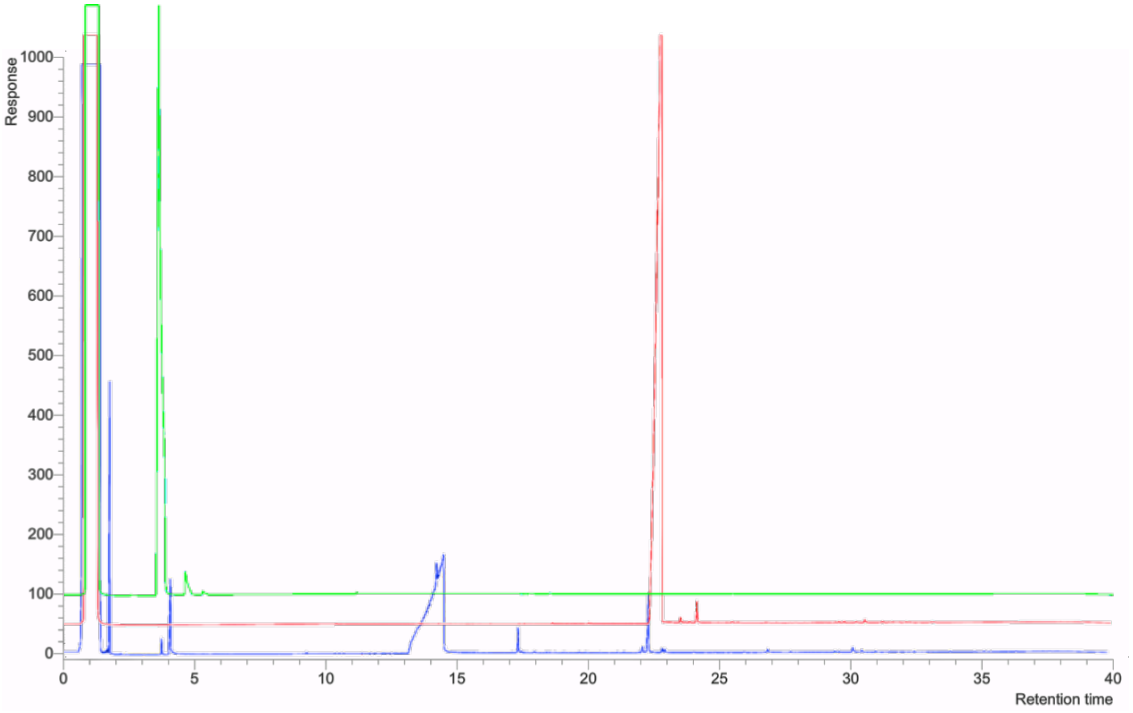


Figure 133. GC-FID traces of the three products obtained from distillation of the crude reaction mixtures. From top to bottom: diethyl fumarate, pyrrolidine **90** and a non characterized product.

6.4.2 Amination of diester 90.

Once the susbtituted pyrroline scaffold was synthesized, the transformation of the ethyl ester groups of **90** into amides of 4-flurophenethylamine (**20c**) was attempted.

Amination of activated esters, like those of *p*-nitrophenol, can be achieved relatively easily,[202] however simple esters (Me/Et esters) are not very reactive and they may require harder conditions, like strong basic catalysts,[203] carbene catalysts[204] and/or high pressure.[205] Nonetheless, milder bases like acetate, [206] or even conditions in the absence of catalysts [207] have also been used to promote the amination of esters.

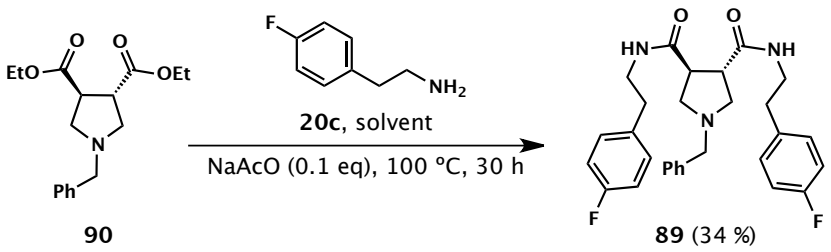


Figure 134. Reaction scheme for the obtention of **89**.

With these antecedents, the preparation of the pyrrolidine diamide **89** was attempted using sodium acetate as catalyst and a large excess of the amine precursor **20c**, which would act as reagent and solvent, heating the reaction mixture at high temperature (100 ºC). Under these conditions, the released EtOH would be removed, pulling the reaction towards the formation of the desired product. Aliquots of the reaction mixture showed complex chromatographic profiles where peaks from monosubstituted and disubstituted pyrrolidines were identified (Figure 135). The reaction was assayed with and without microwave irradiation, leading to similar crude reaction mixtures. Work-up implied, first, partition between organic solvent and acidic water, to remove most of the remaining amine, followed by purification by direct phase flash chromatography. In this last purification step, the eluent included a small amount of commercial anhydrous ammonia in methanol solution to reduce the interaction of the products with the solid support.

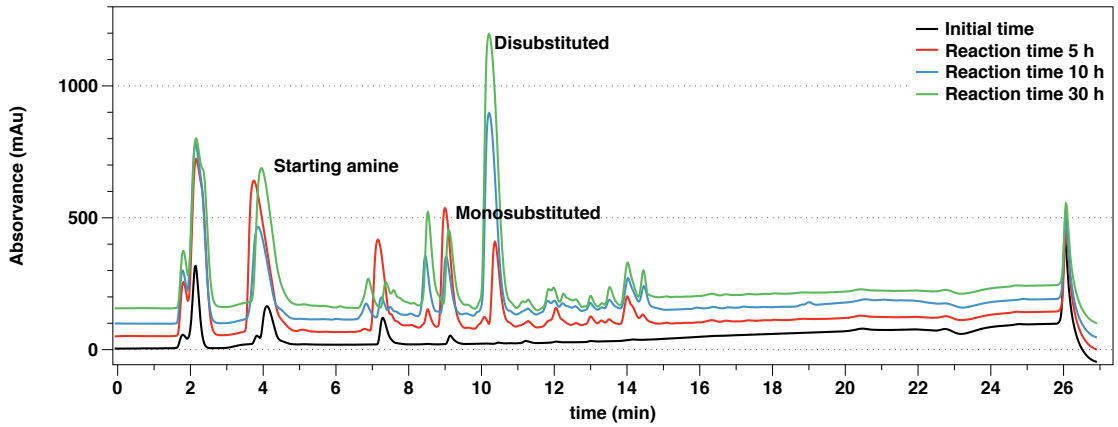


Figure 135. HPLC-UV/Vis traces of the crude reaction mixture to form pyrrolidine **89** at different reaction times.

The final diamide **89** was obtained with a moderate yield of 34 %, and with a high purity, which was confirmed by NMR spectroscopy and HPLC reverse phase chromatography.

6.4.3 Hydrogenation of pyrrolidine 89.

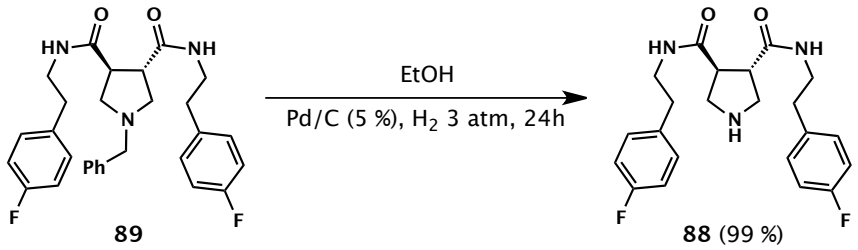


Figure 136. Hydrogenation of **89** to furnish the deprotected pyrrolidine **88**.

Initial hydrogenation attempts of pyrrolidine **89** at 1 atm and room temperature[201] showed that the conversion was incomplete. It required 24 h and 3 atm of H_2 to completely remove the benzyl protecting group. The reaction was clean, with essentially no side-products generated, such that simple filtration through celite and evaporation of the solvent afforded the pure desired product **88** in 99% yield.

6.4.4 Synthesis of tosylate 91.

The tosylate derivative from alcohol **21F** was required to introduce the dimethylaminopropyl moiety in pyrrolidine **88** by nucleophylic substitution of the tosylate.

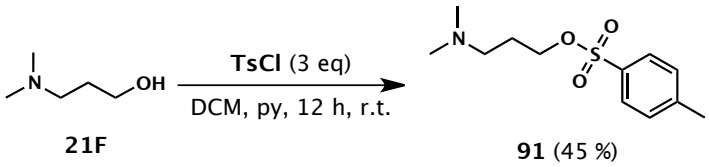


Figure 137. Synthetic scheme of tosylate **91** from alcohol **21F**.

Tosylate **91** was prepared by reaction of **21F** with tosyl chloride under standard conditions.[208] Reaction proceeded slowly at room temperature and 12 h were required in order to achieve total conversion. After work-up, pure tosylate **91** was obtained in 45% yield.

6.4.5 Preparation of trisubstituted pyrrolidine 86.

Nucleophylic attack of pyrrolidine **88** over tosylate **91** was performed in acetonitrile, in the presence of potassium carbonate, at 60 ºC.[209] The insoluble tosylate dissolved completely on heating. After evaporation of the solvent the crude was purified

by reverse phase semipreparative HPLC. Selected interest fracctions were evaporated and after the work up procedure, pyrrolidine **86** was obtained as a colorless oil in 60 % yield.

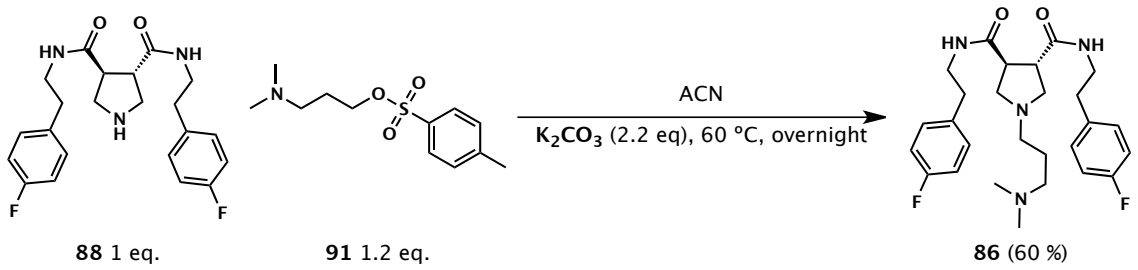


Figure 138. Synthetic scheme for trisubstituted pyrrolidine **86**.

The ^{1}H-,^{13}C-NMR and UPLC-HRMS of the isolated product showed signals compatible with the proposed structure. In addition the protons and carbons in α position from the amide group show no rotameric behavior. ^{13}C-NMR showed the typical and expected C-F couplings, giving place to doublet signals for the aromatic carbon nuclei.

6.4.6 Attempt of reduction of the amide groups of 86 to furnish pyrrolidine 87.

The last synthetic step to obtain pyrrolidine **87** was the reduction of the amide groups present in precursor **86**. To that end, the borane-tetrahydrofuran complex (BTHF) was employed. BHTHF has been used for the reduction of a variety of functional groups, including aldehydes, ketones, carboxylic acids, amides, oximes, imines, and nitriles, as well as for hydroboration reactions with carbon-carbon double and triple bonds.[210] The carboxylic acid group is reduced at a faster rate than most groups, including non-conjugated alkenes.[211,212] Linear alkylic amines have been reduced using this reagent, achieving moderate to high yields.[213-215]

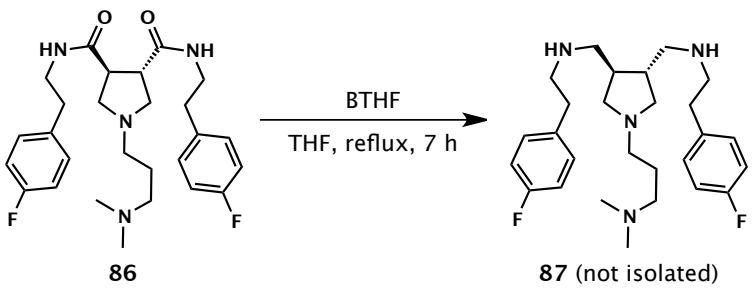


Figure 139. Reduction of secondary amides of **86** to yield **87**.

The reaction was performed in THF at reflux temperature under inert atmosphere. Quenching of the crude reaction mixture with aqueous HCl was required in order to analyse the reaction composition. Aliquots of the quenched crude analysed by HPLC-MS revealed the formation of mixtures of the monoreduced and completely reduced compounds, even after adding more equivalents of BTHF. After several attempts, the best final mono/di reduced ratio achieved was around 1:2 and efforts to purify the desired product **87** by semipreparative-HPLC were unsuccessful. Because of the insuficient resolution of the chromatographic system and the limited amount of crude mixture the synthesis of product **87** was abandoned.

At this point, the "Marato de TV3" research project and the funding had reached the end. Considering that there were no precursors left to reattempt the preparation of pyrrolidine **87** and the amount of effort that would be required to restart the synthesis from the beginning, it was decided to finish the synthetic work of this thesis here, leaving the development of new pyrrolidine based TRPV1 antagonist as future work for our group.

6.5 Evaluation of pyrrolidines 86, 88 and 89 as TRPV1 antagonists.

Although product **87** could not be tested because it could not be obtained in pure enough form, the anti-TRPV1 activity of pyrrolidine **86** and its precursors **88** and **89** was analyzed in the voltage-clamp assay. The 3 products complied totally or partially with the proposed pharmacophore. In addition the activity of **86** had already been predicted with the QSAR models previously developed. Therefore it could be considered as a benchmark to validate the models. If active these compounds would be initial hits of a new family of potential TRPV1 antagonist. Table 20 summarizes the channel blockade and IC_{50} values determined for the three pyrrolidines.

Table 20. Biological evaluation as TRPV1 antagonists of pyrrolidine derivatives **86**, **88** and **89**.

Pyrrolidine	% Blockade at 10 μM	IC_{50} (μM)
86	65	6.2
88	25	40
89	11	192

The three pyrrolidines showed activities (IC_{50}) as TRPV1 antagonist that ranged from low to high μM and which could be correlated with their structural features. Noteworthy, pyrrolidine **86** was the most active of the three, showing an excellent correspondence with the values predicted by our QSAR models. This pyrrolidine included the three features present in the proposed pharmacophoric model and, as previously discussed, the alignment to the structure of the most active triazine **46** showed that it could adopt a conformation where these features could superimpose almost perfectly to those of the triazine. However, some differences in both structures were captured by the developed 3D-QSAR models, such that the predictions indicated that this pyrrolidine would show a lower activity than the triazine, as had been later experimentally confirmed. Overall, these results suggested that this pyrrolidine could interact with the TRPV1 channel in a similar mode as the active triazines synthesized.

Concerning the other two pyrrolidines, the fact that **88** was still moderately active was a bit surprising since it did not include the dimethylaminopropyl moiety present in most active triazines. However, it does contain the secondary amino group of the pyrrolidine, which is susceptible of being protonated at physiological pH, thus being able to interact with negatively charged residues present in the vestibule of the channel. With respect to pyrrolidine **89**, although presenting a tertiary amino group, it could be hypothesized that the *N*-bound benzyl moiety could hamper the interaction with the residues of that vestibule, thus decreasing its binding affinity, or perturb its mode of binding, making it less efficient as TRPV1-channel blocker.

Altogether these results allowed to propose pyrrolidine **86** as a starting point for future development of new anti-TRPV1 compounds. The still very optimizable synthetic scheme used for its preparation would be amenable to modify the nature of the diversity sources introduced, allowing for the construction of a library of compounds for biological testing.

Conclusions.

The vanilloid receptor type 1 (TRPV1) is considered an important integrator of various pain stimuli. As a main constituent of the pain transducing signal, it has become the centre of many medicinal chemistry studies using small molecules as inhibitors. The present thesis aimed to design and synthesize new uncompetitive blockers of TRPV1. Analysis of the molecular features determinant of the TRPV1 blocking activity were also studied. From the results obtained, the following conclusions could be drawn:

1. Based on a previously established pharmacophic hypothesis, consisting on two aromatic moieties and one cationic group, and the structure of two described peptoids with activity as uncompetitive antagonist of TRPV1, a small library of 38 2,4,6-trisubstituted-1,3,5-triazines was designed and synthesized. Different protocols of the *state of the art* in triazine synthetic methodologies were adapted to generate a focused procedure to easily access the desired triazine derivatives without hard or time-consuming purifications and with acceptable yields. This general procedure was based on the use of microwave activation to carry out a double addition of two arylalkylamines to the 2,4,6-trichloro-1,3,5-triazine precursor followed by the addition of the third cation bearing substituent. Modifications on this general procedure were devised for the synthesis of 8 triazines that could not be obtained by that protocol and for the gram scale synthesis of the most active one.
2. The characterization by NMR using different two-dimensional techniques revealed that most triazine derivatives exist in solution as mixtures of rotamers. The energy barriers between the different rotamers were determined using both the EXSY-NMR technique and theoretical DFT calculations at the B3LYP/6-31++G** level, under implicit solvation conditions. Both methodologies yielded similar results, pointing in the direction that although distinct rotamers can be observed at low temperatures in ACN-d_6, the effects of their existence might be negligible under *in vivo* conditions because of their rapid equlibration.
3. Biological evaluation of the triazine compounds revealed activities that range more than 6 orders of magnitude. Ten of them exhibited activities (IC_{50}) under the μM range, confirming the triazine scaffold as a good candidate for the design of TRPV1 blockers. Triazine **46**, 2-(3'-(*N*,*N*-dimethylamino)propylamino)-4,6-bis(4'-fluorophenethylamino)-1,3,5 triazine, with an IC_{50} = 50 nM, was the most potent uncompetitive TRPV1 antagonist identified and one of the most potent so far described.

4. *In vitro* and *in vivo* biological characterization of **46** allowed to classify this triazine as a selective (*vs.* TRPM8 and NMDA channels) polymodal TRPV1 blocker with a low toxicity profile, that interacts deep in the channel vestibule of TRPV1, as deduced from fitting the voltage-dependent blockade data ($I_{blocked}/I_{control}$) to the Woodhull equation. The *in vivo* evaluation of triazine **46** on both normal and acute pain models showed a long effect of pain relief, which lasted for up to 72 h on both models although it had a greater effect on the acute model. The fact that the same compound showed some activity as blocker of the hERG channel limits its potential for systemic use in pain treatment.

5. A 3D-QSAR study using the CoMFA and CoMSIA methodologies was carried out to correlate the structural properties of the library of TRPV1 antagonists with their activity. CoMSIA models based on the donor and electrostatic properties of the compounds showed good internal statistical parameters as well as external predictive capacity. These models evidence the contributions of different regions and chemical groups of the ligands to their activity, revealing the presence of a donor/cationic group at a certain distance from the triazine ring as a main determinant of activity.

6. A new set of pyrrolidine based analogues potentially active against TRPV1 was designed, whose activity was predicted with the above CoMSIA models. Three of these analogues were synthesized and their anti-TRPV1 activity was evaluated, identifying one of them, pyrrolidine **86**, as a micromolar TRPV1 antagonist and confirming the predicted activity value. This provided further validation to the predictive capacity of the 3D-QSAR models, and allowed to propose pyrrolidine **86** as starting point for future development of new anti-TRPV1 compounds

7. The results obtained encouraged us to submit a publication and filling a patent:

Vidal-Mosquera, M., Fernández-Carvajal, A., Moure, A., Valente, P., Planells-Cases, R., González-Ros, J. M., Bujons, J., Ferrer-Montiel, A., Messeguer, A. Triazine-Based Vanilloid 1 Receptor Open Channel Blockers: Design, Synthesis, Evaluation, and SAR Analysis. *J. Med. Chem.*,**54**, 7441-7452 (2011).

Ferrer-Montiel A., Fernández-Carvajal A., Gonzalez J., Belmonte C., Viana F., Gomis A., Messeguer A., Bujons J. Vidal-Mosquera M. Triazine Derivatives and Their Uses as Trpv1 Inhibitors, Patent WO2012136873.

Chapter 7 – Experimental procedures.

7.1 General methods and materials.

All solvents were obtained from VWR (Barcelona, Spain) and were used without further purification. All reagents were from Sigma-Aldrich (St. Louis, Missouri, United States), except amine **21C**, which was from Matrix Scientific (Columbia, SC, USA). Unless otherwise stated, reagents were used without further purification

Reactions activated by microwave irradiation were performed either on a domestic microwave oven, for reactions on solid phase, or on a CEM Discover Microwave apparatus. On the domestic microwave oven, reactions were carried out by repeating short cycles of irradiation at a given power. On the other hand, the CEM microwave oven was controlled by a computer in which reaction conditions were set and reaction variables like pressure inside the vessel or temperature during the heating process were monitored. Normal procedure conditions implied to set up a maximum irradiation power and maximum temperature. If maximum temperature was reached then the irradiation power was changed automatically to maintain the temperature set.

HPLC analyses were carried out on a Hewlett Packard Series 1100 modular system which included a diode array UV detector, using a Xterra MS RP18 (Waters) column (5 µm, 4.6 x 150 mm). Acetonitrile-water mixtures containing TFA (0,07% in ACN, 1% in water) were usually employed as mobile phase. Flux was set at 1 mL/min and the UV detector monitoring at 220 nm and 254 nm. The general analysis method was set up as: min 0, 20% ACN → min 13, 80% ACN → min 20, 100% ACN.

TLC analysis were performed using aluminum supported Merck Kieselgel 60 F_{254}. To visualize the spots, irradiation at 254 or 360 nm as well as development with different stain solutions were used. Stain solutions: phosphomolybdic acid (5% in absolute EtOH), vanillin (5% vanillin 1% H_2SO_4 in absolute EtOH) or p-anisaldehyde (5% p-anisaldehyde, 5% H_2SO_4, 1% AcOH in absolute EtOH) followed by heating at high temperature.

GC-FID analyses were carried out on a Hewlett Packard 5890 Series II coupled to an HP3396 Series II integrator, using a capillary column SPB-5 (15 m, inner diameter 0.25 mm). The analysis program was: min 0, 60 ºC → min 35, 350 ºC.

High resolution mass spectra (HRMS) were recorded at the Mass Spectrometry Service of our institute (IQAC-CSIC) with an Aquity UPLC (Waters) chromatograph, coupled to a LCT Premier Xe TOF detector (Waters) in positive detection mode (ESI+), using an Aquity UPLC BEH C18 (Waters) column (1.7 µm, 2.1x100 mm). Mobile phase

was composed of ACN-H_2O mixtures containing 20 mM of HCOOH. The general analysis method was: min 0, 10% ACN → min 5, 100% ACN.

Two different chromatographic purification methods were employed depending on the crude mixture:

- Semi-preparative HPLC: Compounds were purified using a LC-4000 system (Waters) coupled to an L-4000UV detector (Hitachi) with a X-terra RP18 (Waters) column (15-20 μm, 47 x 300 mm). ACN-H_2O mixtures containing 0.1 % TFA were employed as mobile phases, setting the equipment at a flow rate of 10 mL/min.

- Flash chromatography: It was carried out on an Isolera system (Biotage) using prepacked reverse phase RP-18 SNAP Cartridges (12 g). ACN-H_2O mixtures containing 0.1 % TFA as mobile phases were employed at a flow rate of 10 mL/min.

Unless otherwise stated, routine NMR spectra experiments were recorded using a Varian Mercury-400 apparatus (^{1}H NMR: 400 MHz; ^{13}C NMR: 100 MHz). A Varian Innova-500 apparatus (^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz) was also employed for specific experiments. Chemical shifts (δ) are given in ppm relative to the solvent signal, and coupling constants (*J*) are reported in Hertz (Hz). NMR signals are characterized by their chemical shift, the multiplicity (s=singlet, d=doblet, t=triplet, m=mutiplet, cs=complex signal, bb=broad band, bs=broad singlet…) with the corresponding coupling constants and the relative integral. Spectra acquired in the presence of trifluoroacetic acid as cosolvent showed the corresponding signals appearing as a broad peak at ~10.5 ppm in ^{1}H NMR, or as two quadruplets at 160.00 (*J* = 39 Hz) and 116.72 ppm (*J* = 290 Hz) in ^{13}C NMR. Most of the described compounds exhibit complex NMR spectra due to the presence in solution of different conformers in equilibrium. In those cases, the spectra of the compounds are given as absorption ranges rather than discrete signals. Amine signals were not always indicated because of their common behavior of being very variable in area and chemical shift depending on the solvent media.

7.2 Synthesis of peptoids.

Peptoids were synthesized using 10 mL polypropylene (PP) syringes as reaction vessel to carry out all solid phase steps, following the general procedure reported by our laboratory for *N*-alkylglycine oligomers.[116] Synthesis was performed on a 1% cross-linked polystyrene resin bearing the Fmoc-protected Rink amide linker AM RAM (0.79 mmol/g, Rapp Polymer; Germany).

The first step in the peptoid synthesis scheme was the deprotection of the commercial resin. Then, acylation of the generated free amine with bromoacetic acid leading to an alkyl bromide intermediate, which was then subjected to nucleophilic substitution using an amino building block. Successive cycles of acylation and amination were used to attach units of *N*-alkylglycine. After that, the cleavage procedure released the peptoid from resin. Semi-preparative HPLC was then used to purify the final product. To follow the evolution of the solid phase steps, two qualitative resin dyeing tests were employed.

The 2,4,6-Trinitrobenzenesulfonic acid (TNBS) test was used to check the presence of primary amines in the resins. Thus, 1-3 mg of dry resin were placed over a microscope slide. Then one drop of a solution of DIPEA (10%) in DMF and one drop of a solution of TNBS (1%) in DMF were added. After 1 min at room temperature, resin turned red revealing the presence of primary amine groups in the sample, or it remained uncolored if no primary amine groups were present.

The chloranil (2,3,5,6-tetrachloro-p-benzoquinone) test was used to check the presence of primary and secondary amines in the resin. As above, 1-3 mg of dry resin were placed over a microscope slide, and then one drop of acetaldehyde solution (2%) in DMF and one drop of a chroranil solution (2%) in DMF were added over the resin. After 3 min at room temperature, resin turned light blue if primary amine groups were present on the resin or dark blue/green if secondary amine groups were present, whereas it remained uncolored in absence of both primary and secondary amine groups.[92]

7.2.1 Deprotection of the Rink amide resin.

5 mL of 20% piperidine in DMF were added to a syringe containing 450 mg (0.36 meq) of resin and then the mixture was stirred for 30 min at 20 °C. The solution was drained and the resin washed with DMF (4 × 10 mL), isopropyl alcohol (IPA) (4 x 10 mL), DCM (4 x 10 mL) and DMF (1 x 10 mL), and this treatment was carried out twice. A final wash with DCM (2 x 5 mL) was performed to ensure a good drying of the resin. The TNBS test was performed to confirm the deprotection of the resin.

7.2.2 Acylation protocol.

The resin contained in the syringe was suspended in a 2:1 solution of DCM/DMF (5 mL) containing bromacetic acid (1.64 mmol, 228 mg) and *N*,*N*'-dicyclohexylcarbodiimide (DIC) (1.64 mmol, 257 mg). The suspension was allowed to react for 30 min in a linear shaker at 200 rpm. The reagents solution was drained and the resin washed with DMF (4 × 10 mL), IPA (4 x 10 mL), DCM (4 x 10 mL) and DMF (1 x 10 mL). This treatment was carried out twice. Final wash with DCM (2 x 5 mL) was performed to ensure a good drying of the resin. The chloranil test was performed and if the resin rested colorless the synthesis moved on to the next amination step, otherwise the acylation protocol was repeated.

7.2.3 Amination protocol.

To the resin contained in the syringe, a solution of DIPEA (1.64 mmol, 205 µL) and the corresponding amine (1.64 mmol) in DMF (5 mL) was added. The crude reaction mixture was allowed to react under microwave activation using 6 cycles of 20 seconds at a mean power of 100 W in the domestic microwave oven. Then the resin was drained and washed with DMF (4 × 10 mL), IPA (4 x 10 mL), DCM (4 x 10 mL) and DMF (1 x 10 mL). This treatment was performed twice and then a final wash was carried out using DCM to ensure dryness of the resin. Table 21 shows the amounts of amines used for each amination step. Choranil tests were performed after each amination cycle to confirm that the reaction took place.

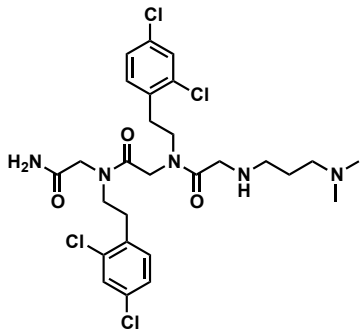
Table 21: Amount and volume of required amines for each amination step.

Product	Amination cycle	Amine	Molecular Weight (g/mol)	mmol	Volume (µL)
12	1	**20a**	190.07	1.64	248
	2	**20a**	190.07		248
	3	**21A**	102.12		133
13	1	**20b**	155.62	1.64	230
	2	**20b**	155.62		230
	3	**21A**	102.12		133
14	1	**20a**	190.07	1.64	248
	2	**20a**	190.07		248
	3	**21N**	75.11		98
15	1	**20a**	190.07	1.64	248
	2	**20a**	190.07		248
	3	**21O**	73.14		95

7.2.4 Cleavage from the resin to obtain the synthesized peptoids.

After the last amination step, the resin was well dried and transferred to a 10 mL pyrex reaction tube with a teflon screw cap resistant to acid. The resin was then suspended in a cleavage cocktail (3 mL, 60:40:2 TFA/DCM/H2O), turning immediately into a red color, and was shaken at 200 rpm in a linear shaker IKA HS501 (Labortecknik) for 30 min at room temperature. Afterwards, the suspension was filtered and the crude reaction mixture was evaporated under vacuum. The residue was redissolved with ACN (5 mL) followed by concentration under vacuum, and this process was repeated three times to remove the excess of TFA and water from the crude reaction mixture. The remaining crude was then purified using semipreparative HPLC to yield the corresponding product.

7.2.5 [*N*-(3'-(*N'*,*N'*-dimethylamino)propyl)glycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (12).



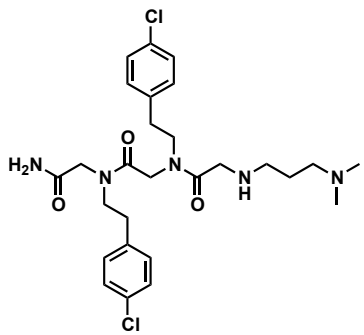
Following the general protocol, 100 mg of **12** were obtained as a white powder, 52 % yield.

HRMS (ESI+): calculated for $C_{27}H_{35}Cl_4N_5O$ (M+H) 618.1589, found 618.1572.

^{1}H NMR (CD_3CN) (confs): 7.50-7.14 (H_{Ar}, cs, 6H), 6.73-6.12 ($CONH_2$, cs, 2H), 4.17-3.67 (3 x CH_2, cs, 6H), 3.59-3.28 (2 x CH_2, cs, 4H), 3.19-2.71 (4 x CH_2 + 2 x CH_3, cs, 14H), 2.29-2.03 (CH_2, bb, 2H).

^{13}C NMR (CD_3CN) (confs): 172.48-169.88 (3 CO), 136.87-135.11 (4 C_{Ar}), 134.19-132.97 (2 C_{Ar} + 2 $C_{Ar}H$), 130.43-129.64 (2 $C_{Ar}H$), 128.79-128.09 (2 $C_{Ar}H$), 70.34 ($CH_2N\underline{Me_2}$), 61.07 ($CH_2N\underline{Me_2}$), 51.47-47.77 (3 $CO\underline{C}H_2$), 46.08-44.92 (3 NCH_2), 43.62 (2 CH_3), 33.00-30.95 (2 $\underline{C}H_2C_{Ar}$), 22.65-19.68 ($CH_2\underline{C}H_2CH_2$).

7.2.6 [*N*-(3'-(*N'*,*N'*-dimethylamino)propyl)glycyl]-[*N*-(4'-chlorophenethyl)glycyl]-*N*-(4'-chlorophenethyl)glycinamide (13).



Following the general protocol, 82 mg of **13** were obtained as a white powder, 45% yield.

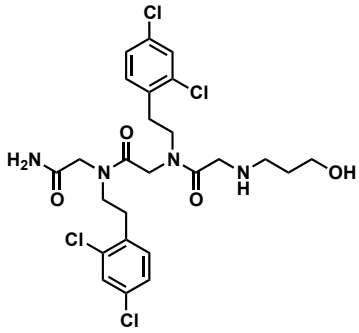
HRMS (ESI+): calculated for $C_{27}H_{37}Cl_2N_5O_3$ (M+H) 550.2374, found 550.2352.

^{1}H NMR (CD_3CN) (confs): 7.39-7.11 (HAr, cs, 8H), 6.71-6.11 ($CONH_2$, 2H), 4.15-3.62 (3 x CH_2, cs, 6H), 3.59-3.25

(2 x CH_2, cs, 4H), 3.19-2.63 (4 x CH_2 + 2 x CH_3, cs, 14H), 2.27-2.05 (CH_2, bb, 2H).

^{13}C NMR (CD_3CN) (confs): 172.59-166.77 (3 CO), 139.27-137.68 (2 C_{Ar}), 133.27-132.18 (2 C_{Ar}), 131.99-131.26 (2 CH_{Ar}), 129.80-129.11 (2 CH_{Ar}), 70.28 ($CH_2N\underline{Me_2}$), 61.04 ($CH_2N\underline{Me_2}$), 51.42-47.83 (3 $CO\underline{C}H_2$), 45.83-44.85 (3 NCH_2), 43.46 (2 CH_3), 34.59-33.05 (2 $\underline{C}H_2C_{Ar}$), 22.79-19.66 ($CH_2\underline{C}H_2CH_2$).

7.2.7 [*N*-(3'-hydroxypropyl)glycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (14).



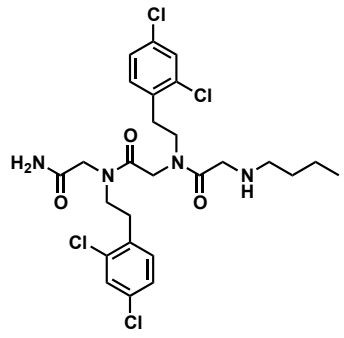
Following the general protocol, 55 mg of **14** were obtained as a white powder, 28% yield.

HRMS (ESI+): calculated for $C_{25}H_{30}Cl_4N_4O_4$ (M+H) 591.1107, found 591.1099

^{1}H NMR (CD_3CN) (confs): 7.52-7.17 (6 x CH_{Ar}, cs, 6H), 6.76-6.07 ($CONH_2$, cs, 2H), 4.17-3.62 (3 x CH_2, cs, 6H), 3.58-3.33 (2 x CH_2, cs, 4H), 3.14-2.76 (4 x CH_2, cs, 8H), 1.89-1.78 (CH_2, cs, 2H).

^{13}C NMR (CD_3CN) (confs): 170.39-166.23 (3 CO), 136.87-135.24 (4 C_{Ar}), 134.29-132.88 (2 C_{Ar} + 2 $C_{Ar}H$), 130.45-129.30 (2 $C_{Ar}H$), 128.14-127.89 (2 $C_{Ar}H$), 61.24 (CH_2OH), 51.07-47.73 (2 $\underline{C}H_2N$ + 3 $CO\underline{C}H_2$), 33.07-29.50 (CH_2NH + 2 $\underline{C}H_2C_{Ar}$), 28.72 ($CH_2\underline{C}H_2CH_2$).

7.2.8 [*N*-butylglycyl]-[*N*-(2',4'-dichlorophenethyl)glycyl]-*N*-(2',4'-dichlorophenethyl)glycinamide (15).



Following the general protocol, 72 mg of **15** were obtained as a white powder, 37% yield.

HRMS (ESI+): calculated for $C_{26}H_{32}Cl_4N_4O_3$ (M+H) 589.1309, found, 589.1311.

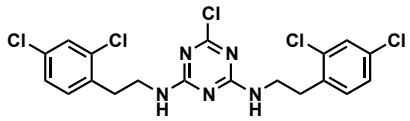
^{1}H NMR (CD_3CN) (confs): 7.54-7.14 (6 x CH_{Ar}, cs, 6H), 6.82-5.97 ($CONH_2$, cs, 2H), 4.27-3.23 (5 x CH_2, cs, 10H), 3.11-2.70 (3 x CH_2, cs, 6H), 1.78-1.49 (CH_2, cs, 2H), 1.43-1.11 ($CH_3C\underline{H_2}$, cs, 2H), 0.99-0.82 (CH_3, cs, 3H).

^{13}C NMR (CD_3CN) (confs): 170.18-167.09 (3 CO), 137.23-135.40 (4 C_{Ar}), 134.56-133.12 (2 C_{Ar} + 2 $C_{Ar}H$), 130.21-129.11 (2 $C_{Ar}H$), 129.11-128.32 (2 $C_{Ar}H$), 50.61-48.15 (2 $\underline{C}H_2N$ + 3 $CO\underline{C}H_2$), 33.29-31.35 (CH_2NH + 2 $\underline{C}H_2C_{Ar}$), 28.81 ($CH_2\underline{C}H_2CH_2$), 20.68 ($C\underline{H_2}CH_3$), 14.14 (CH_3).

7.3 General synthesis of disubstituted triazines.

A solution of 1,3,5-trichloro-2,4,6-triazine (**16**) (90 mg, 0.5 mmol, 1 eq) in THF (4 mL) was allowed to react with the corresponding amine (2 mmol, 4 eq) under microwave activation in the CEM oven for 10 min at 70 ºC (90 W, closed system). Then the crude reaction mixture was poured into H_2O (20 mL), heated for 10 min at 60 ºC and filtered. The precipitate was resuspended in 20 mL of water, heated for another 10 min at 60 ºC and filtered a second time. The insoluble material was subjected to the same treatment a second time then it was washed with water (3 x 20 mL) and cold absolute ethanol (1 x 5 mL), and finally dried to give the pure expected disubstituted triazine.

7.3.1 2-chloro-4,6-bis(2',4'-dichlorophenethylamino)-1,3,5-triazine (18).

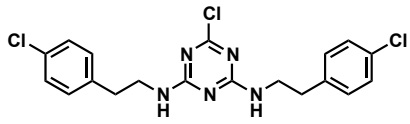


Starting from 101 mg of **16** and 420 mg of amine **20a**, 262 mg of **18** were obtained as a white powder, 97 % yield.

HRMS (ESI+): calculated for $C_{19}H_{17}Cl_5N_5$ (M+H) 489.9927, found 489.9919.

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.38 (H_{Ar}, dd, *J* = 3.7 Hz, 2.1 Hz, 2H), 7.20 (H_{Ar}, ddd, *J* = 8.2 Hz, 2.1 Hz, 0.8 Hz, 2H), 7.17 – 7.13 (H_{Ar}, m, 2H), 3.82 – 3.73 (NCH_2, cs, 2H), 3.03 (CH_2C_{Ar}, t, *J* = 7.2 Hz, 2H).

7.3.2 2-chloro-4,6-bis(4'-chlorophenethylamino)-6-chloro-1,3,5-triazine (22).



Starting from 553 mg of **16** and 1.87 g of amine **20b**, 1.19 g of **22** were obtained as a white powder, 94 % yield.

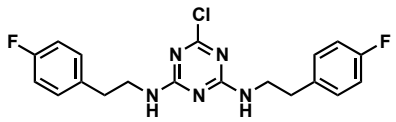
HRMS (ESI+): calculated for $C_{19}H_{18}Cl_3N_5$ (M+H) 422.0706, found 422.0715.

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.32-7.24 (H_{Ar}, cs, 4H), 7.17-7.09 (H_{Ar}, cs, 4H), 3.81-3.69 ($NHC\underline{H_2}$, cs, 4H), 2.94-2.86 (CH_2C_{Ar}, cs, 4H).

7.3.3 2-chloro-4,6-bis(4'-fluorophenethylamino)-1,3,5-triazine (23).

Although the general synthetic protocol could be used to obtain compound **23** in small scale (~100 mg), an adaptation of the protocol was required to obtain gram scale amounts of this product, which were necessary for the preparation of compounds required for in vivo assays. Briefly, this adapted protocol consisted on the following.

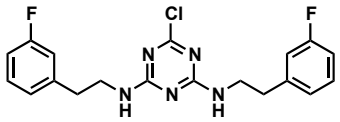
A solution of **16** (1.1 g, 6 mmol, 1 eq) in THF (20 mL) was stirred while the reaction vessel was soaked in an ice-water bath. Then a solution of amine **20c** (3.35 g, 24 mmol, 4 eq) in THF (20 mL) was added dropwise in about 10 min. The white suspension was introduced in the microwave oven with a ball condenser coupled to the reaction vessel. The mixture was heated in the CEM microwave to 70 ºC for 40 min with the irradiation power set to 90 W. The suspension was poured H_2O (200 mL) and the mixture was heated at 70 ºC during 40 min under stirring. Filtering the suspension and washing the white solid with H_2O (3 x 20 mL) and EtOH (3 x 5 mL) allowed the isolation of 2.27 g of **23,** 97% yield.



HRMS (ESI+): calculated for $C_{19}H_{18}ClF_2N_5$ (M+H) 390.1297, found 390.1283

^{1}H NMR ($CDCl_3$, 7% TFA): 7.19-7.12 (CH_{Ar}, cs, 4H), 7.03-6.97 (CH_{Ar}, cs, 4H), 3.80-3.67 ($NHCH_2$, cs, 4H), 2.94-2.86 (CH_2C_{Ar}, cs, 4H).

7.3.4 2-chloro-4,6-bis(3'-fluorophenethylamino)-1,3,5-triazine (24).

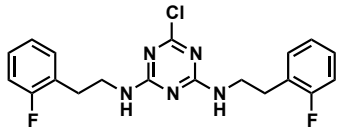


Starting from 101 mg of **16** and 306 mg of amine **20d**, 205 mg of **24** were obtained as a white powder, 95% yield.

HRMS (ESI+): calculated for $C_{19}H_{19}ClF_2N_5$ (M+H) 390.1297, found 390.1302.

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.30-7.25 (H_{Ar}, m, 2H), 7.00-6,88 (H_{Ar}, cs, 6H), 3.81-3.71 ($NHCH_2$, cs, 4H), 2.96 -2.89 (CH_2C_{Ar}, cs, 4H).

7.3.5 2-chloro-4,6-bis(2'-fluorophenethylamino)-1,3,5-triazine (25).

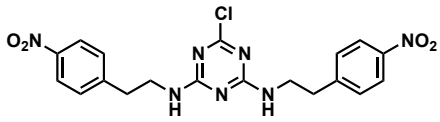


Starting from 101 mg of **16** and 306 mg of amine **20e**, 210 mg of **25** were obtained as white powder, 98% yield.

HRMS (ESI+): calculated for $C_{19}H_{19}ClF_{2s}N_5$ (M+H) 390.1297, found 390.1299

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.27-7.15 (H_{Ar}, cs, 4H), 7.13-6.98 (H_{Ar}, cs, 4H), 3.83-3.74 ($NHCH_2$, m, 4H), 3.01-2.94 (CH_2C_{Ar}, m, 4H).

7.3.6 2-chloro-4,6-bis(4'-nitrophenethylamino)-1,3,5-triazine (26).

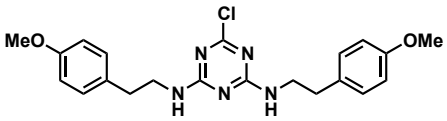


Starting from 110 mg of **16** and 400 mg of amine **20f**, 250 mg of **26** were obtained as white powder, 93% yield.

HRMS (ESI+): calculated for $C_{19}H_{18}ClN_7O_4$ (M+H) 444.1180, found 444.1187

^{1}H-NMR ($CDCl_3$, 7% TFA): 8.22-8.15 (H_{Ar}, cs, 4H), 7.43-7.35 (H_{Ar}, cs, 4H), 3.87-3.78 ($NHCH_2$, m, 4H), 3.06 (CH_2C_{Ar}, q, J = 7.6 Hz, 4H).

7.3.7 2-chloro-4,6-bis(4'-methoxiphenethylamino)-1,3,5-triazine (27).

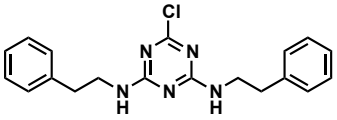


Starting from 100 mg of **16** and 328 mg of amine **20g**, 210 mg of **27** were obtained as white powder, 94% yield.

HRMS (ESI+): calculated for $C_{21}H_{24}ClN_5O_2$ (M+H) 414.1697, found 414.1706

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.13-7.10 (H_{Ar}, cs, 4H), 6.90-6.85 (H_{Ar}, cs, 4H), 3.82 (CH_3O, s, 6H), 3.76-3.70 ($NHCH_2$, m, 4H), 2.90-2.83 (CH_2C_{Ar}, cs, 4H).

7.3.8 2-chloro-4,6-diphenethylamino-1,3,5-triazine (28).

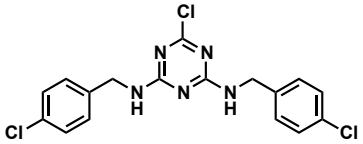


Starting from 100 mg of **16** and 262 mg of amine **20h**, 170 mg of **28** were obtained as white powder, 89% yield.

HRMS (ESI+): calculated for $C_{19}H_{20}ClN_5$ (M+H) 354.1485, found 354.1477

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.35-7.17 (H_{Ar}, cs, 10H), 3.82-3.72 ($NHCH_2$, m, 4H), 2.98-2.88 (CH_2C_{Ar}, m, 4H).

7.3.9 2-chloro-4,6-bis(4'-chlorobenzylamino)-1,3,5-triazine (29).

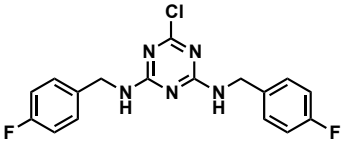


Starting from 101 mg of **16** and 311 mg of amine **20i**, 200 mg of **29** were obtained as white powder, 92% yield.

HRMS (ESI+): calculated for $C_{17}H_{14}Cl_3N_5$ (M+H), 394.0393, found 394.0381.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.29 (H_{Ar}, d, J = 8.0 Hz, 4H), 7.11 (H_{Ar}, d, J = 8.1 Hz, 4H), 4.61 (CH_2, s, 4H).

7.3.10 2-chloro-4,6-bis(4'-fluorobenzylamino)-1,3,5-triazine (30).

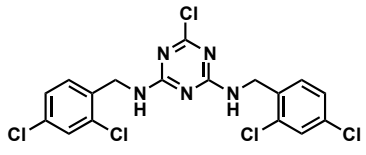


Starting from 101 mg of **16** and 275 mg of amine **20j**, 110 mg of **30** were obtained as white powder, 60% yield.

HRMS (ESI+): calculated for $C_{17}H_{15}ClF_2N_5$ (M+H), 362.0984, found 362.0989.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.33-7.19 (H_{Ar}, cs, 4H), 7.06-7.00 (H_{Ar}, cs, 4H), 4.72-4.63 (CH_2, cs, 4H).

7.3.11 2-chloro-4,6-bis(2',4'-dichlorobenzylamino)-1,3,5-triazine (31).

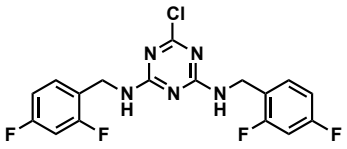


Starting from 111 mg of **16** and 422 mg of amine **20k**, 263 mg of **31** were obtained as white powder, 95% yield.

HRMS (ESI+): calculated for $C_{17}H_{12}Cl_5N_5$ (M+H) 461.9614, found 461.9694

^{1}H-NMR ($CDCl_3$, 7% TFA) (conf): 7.46-7.06 (H_{Ar}, m, 6H), 4.76-4.68 (CH_2, m, 4H).

7.3.12 2-chloro-4,6-bis(2',4'-difluorobenzylamino)-1,3,5-triazine (32).

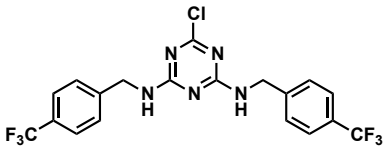


Starting from 110 mg of **16** and 340 mg of amine **20l**, 206 mg of **32** were obtained as white powder, 87% yield.

HRMS (ESI+): calculated for $C_{17}H_{12}ClF_4N_5$ (M+H) 398.0796, found 398.0790

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.38-7.19 (H_{Ar}, cs, 2H), 6.90-6.81 (H_{Ar}, cs, 4H), 4.74-4.65 (CH_2, cs, 4H).

7.3.13 2-chloro-4,6-Bis-(4'-trifluoromethylbenzylamino)-1,3,5-triazine (33).

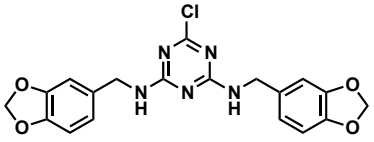


Starting from 200 mg of **16** and 756 mg of amine **20m**, 390 mg were obtained as white powder, 78% yield.

HRMS (ESI+): calculated for $C_{19}H_{14}ClF_6N_5$ (M+H), 462.0920; found, 462.0928.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.54 (H_{Ar}, dd, J = 66.3, 8.2 Hz, 4H), 7.42 (H_{Ar}, dd, J = 105.1 Hz, 8.1 Hz, 4H), 4.72 (CH_2C_{Ar}, dd, J = 33.4, 6.0 Hz, 1H).

7.3.14 2-chloro-4,6-Bis-(5'-methylenedioxyphenyl)methylamino-1,3,5-triazine (34).

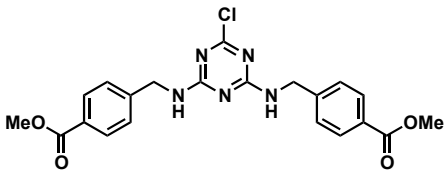


Starting from 200 mg of **16** and 653 mg of amine **20n**, 390 mg of **34** were obtained as white powder, 87% yield.

HRMS (ESI+): calculated for $C_{17}H_{12}ClF_4N_5$ (M+H), 414.0969; found, 414.0958.

^{1}H NMR ($CDCl_3$, 7% TFA): 6.82-6.72 (H_{Ar}, cs, 6H), 5,97 (OCH_2O, s, 4H), 4.61-4.57 (CH_2C_{Ar}, m, 4H).

7.3.15 2-chloro-2,4-Bis-(4'-methoxycarbonylphenyl)methylamino-1,3,5-triazine (35).

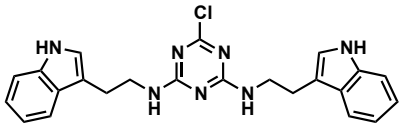


Starting from 200 mg of **16** and 720 mg of amine **20o**, 461 mg were obtained as white powder, 96% yield.

HRMS (ESI+): calculated for $C_{21}H_{20}ClN_5O$ (M + H, 442.1282; found, 442.1291.

^{1}H NMR ($CDCl_3$, 7% TFA): 8.04-7.94 (H_{Ar}, cs, 4H), 7.43-7.24 (H_{Ar}, cs, 4H), 4.82-4.63 (CH_2C_{Ar}, cs, 4H), 3.98 (CH_3O, s, 6 H).

7.3.16 2-chloro-4,6-Bis-2'-(3''-indoyl)ethylamino-1,3,5-triazine (36).

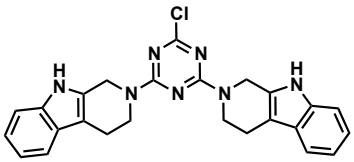


Starting from 202 mg of **16** and 705 mg of amine **20r**, 412 mg of **38** were obtained as white powder, 86% yield.

HRMS (ESI+): calculated for $C_{23}H_{22}ClN_7$ (M+H), 432.1703; found, 432.1703.

^{1}H NMR (ACN-d_6, 7% TFA): 7.75-6.96 (H_{Ar}, cs, 10H), 3.76-3.61 ($NHC\underline{H}_2$, m, 4H), 3.03 (CH_2C_{Ar}, t, J = 7.0 Hz, 4H).

7.3.17 2-chloro-4,6-bis(3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)-1,3,5-triazine (37).

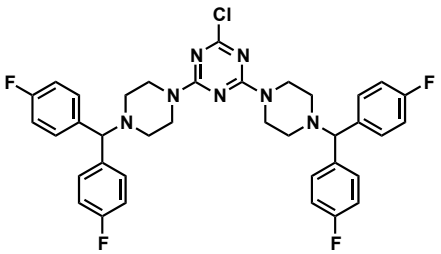


Starting from 100 mg of **16** and 372 mg of amine **20s**, 200 mg of **39** were obtained as white powder, 81% yield.

HRMS (ESI+): calculated for $C_{25}H_{22}ClN_7$ (M+H), 456.1703; found, 456.1708.

^{1}H NMR (ACN-d_6, 7% TFA): 7.50-7.36 (H_{Ar}, cs, 4H), 7.16-7.03 (H_{Ar}, cs, 4H), 5.11-4.91 ($NC\underline{H}_2$, cs, 4H), 4.30-4.07 ($NC\underline{H}_2$, cs, 4H), 2.97-2.83 ($C\underline{H}_2CH_2N$, bb, 4H).

7.3.18 2-chloro-4,6-bis(4-(bis(4-fluorophenyl)methyl)piperazin-1-yl)-1,3,5-triazine (38).



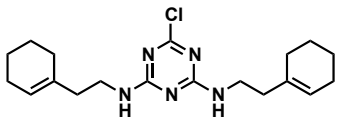
Starting from 101 mg of **16** and 634 mg of amine **20t**, 205 mg of **40** were obtained as white powder, 54% yield.

HRMS (ESI+): calculated for $C_{37}H_{34}ClF_4N_5$ (M+H), 688.2579; found, 688.2570.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.58-7.53 (H_{Ar}, cs, 8H),

7.21-7.12 (H_{Ar}, cs, 8H), 5.17-4.96 (CHC_{Ar}, bb, 2H), 4.78-4.45 (2 x CH_2N, bb, 4H), 3.76-3.42 (4 x CH_2N, bb, 8H), 3.11-2.84 (2 x CH_2N, bb, 4H).

7.3.19 2-chloro-4,6-Bis-2'-(1''-cyclohexenyl)ethylamino-1,3,5-triazine (39).



Starting from 199 mg of **16** and 541 mg of amine **20u**, 340 mg of **41** were obtained as white powder, 87% yield.

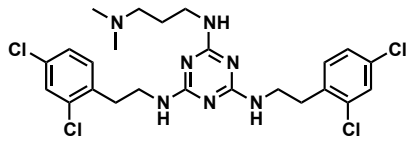
HRMS (ESI+): calculated for $C_{19}H_{28}ClN_5$ (M+H), 362.2111; found, 362.2110.

^{1}H NMR ($CDCl_3$, 7% TFA): 5.50-5.47 (C=CH, m, 2H), 3.62-3.55 ($NHC\underline{H}_2$, m, 4H), 2.30-2.20 ($NHCH_2C\underline{H}_2$, m, 4H), 2.00-1.91 (4 x CH_2, cs, 8H), 1.66-1.50 (4 x CH_2, cs, 8H).

7.4 General synthesis of trisubstituted triazines.

A suspension of the corresponding disubstituted triazine (0.5 mmol, 1 eq) in THF (5 mL) was allowed to react with the corresponding amine (2 mmol, 4 eq) under microwave activation in the CEM oven for 20 min at 100 ºC (110 W, closed system). The crude reaction mixture was diluted with EtOAc (20 mL) and washed with H_2O (20 mL), brine (20 mL) and dried over $MgSO_4$. Elimination of solvents on a rotary evaporator yielded the expected trisubstituted triazine. If higher purity than that obtained by this procedure was required, the product was purified by semipreparative HPLC. Since the HPLC eluents normally contained a proportion of TFA, the eluted triazines were assumed to be in the form of trifluoracetates. To obtain the TFA-free compounds, the collected fractions were evaporated under vacuum, redissolved in EtOAc and washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. Elimination of solvent afforded the pure desired trisubstituted triazines.

7.4.1 2-(3'-(N,N-dimethylamino)propylamino)-4,6-Bis(2',4'-dichlorophenethyl amino)-1,3,5-triazine (17).



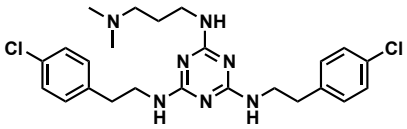
Starting from 100 mg of **18** and 80 mg of amine **21A**, 100 mg of **17** were obtained as yellowish oil, 88% yield.

HRMS (ESI+): calculated for $C_{24}H_{29}N_7Cl_4$ (M+H) 556.1317, found 556.1320.

^{1}H NMR ($CDCl_3$, 7% TFA) (conf): 7.39-7.36 (H_{Ar}, cs, 4H), 7.20-7.12 (H_{Ar}, cs, 2H), 3.73-3.60 ($NHC\underline{H}_2$, cs, 4H), 3.57-3.48 ($NHC\underline{H}_2$, cs, 2H), 3.22-3.14 ($N(CH_3)_2C\underline{H}_2$, cs, 2H), 3.02-2.96 ($CH_2C_{Ar}$, cs, 4H), 2.9 ($CH_3$, s, 6H), 2.11-2.05 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 7% TFA) (conf): 162.93 ($NC_{Ar}N$), 155.72 ($NC_{Ar}N$), 154.89 (C_{Ar}), 134.92 - 131.78 (C_{Ar}), 129.59 ($C_{Ar}H$), 127.58 ($C_{Ar}H$), 127.48 ($C_{Ar}H$), 55.90 ($N(CH_3)_2\underline{C}H_2$), 43.48 ($NHCH_2$), 40.96 ($CH_3$), 37.53 ($NHCH_2$), 32.81 ($\underline{C}H_2C_{Ar}$), 24.19 ($CH_2\underline{C}H_2CH_2$).

7.4.2 2-(3'-(N,N-dimethylamino)propylamino)-4,6-Bis(4'-chlorophenethylamino)-1,3,5-triazine (40).



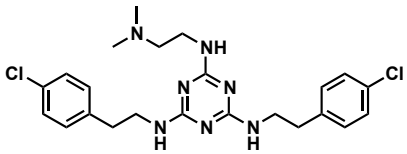
Starting from 125 mg of **22** and 121 mg of amine **21A**, 103 mg of **40** were obtained as white powder, 71% yield.

HRMS (ESI+): calculated for $C_{24}H_{31}N_7Cl_2$ (M+H), 488.2096; found, 488.2103.

^{1}H NMR (CD_3CN_3, 7% TFA): 7.33-7.20 (H_{Ar}, cs, 8H), 3.65-3.52 ($NHC\underline{H}_2$, cs, 4H), 3.48-3.38 ($NHC\underline{H}_2$, cs, 2H), 3.12-3.04 ($N(CH_3)_2C\underline{H}_2$, cs, 2H), 2.89-2.83 ($CH_2C_{Ar}$, cs, 4H), 2.79-2.75 ($CH_3$, cs, 6H), 1.98-1.90 ($CH_2C\underline{H}_2CH_2$, m, 2H);

^{13}C NMR ($CDCl_3$, 7% TFA): 163.32 ($NC_{Ar}N$), 155.57-154.78 ($NC_{Ar}N$), 136.50 (C_{Ar}), 132.71 (C_{Ar}), 129.97 ($C_{Ar}H$), 128.85 ($C_{Ar}H$), 55.93 ($N(CH_3)_2\underline{C}H_2$), 43.57 ($NHCH_2$), 42.34 ($CH_3$), 37.39 ($NHCH_2$), 34.63 ($\underline{C}H_2C_{Ar}$), 24.33 ($CH_2\underline{C}H_2CH_2$).

7.4.3. 2-(2'-(N,N-dimethylamino)ethylamino)-4,6-Bis(4'-chlorophenethylamino)-1,3,5-triazine (41).



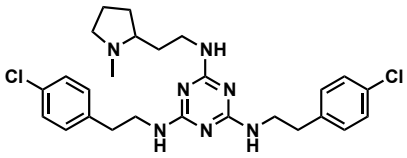
Starting from 125 mg of **22** and 104 mg of amine **21B**, 100 mg of **41** were obtained as white powder, 71% yield.

HRMS (ESI+): calculated for $C_{23}H_{29}N_7Cl_2$ (M+H), 474.1940; found, 474.1946.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.31-7.22 (H_{Ar}, cs, 4H), 7.14-7.05 (H_{Ar}, cs, 4H), 3.80-3.93 ($NHC\underline{H}_2$, cs, 2H), 3.71-3.59 ($NHC\underline{H}_2$, cs, 4H), 3.49-3.36 ($N(CH_3)_2C\underline{H}_2$, cs, 2H), 3.05-2.94 ($CH_3$, cs, 6H), 2.88-2.79 ($CH_2C_{Ar}$, cs, m, 4H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.82 ($NC_{Ar}N$), 155.02 ($NC_{Ar}N$), 136.12 (C_{Ar}), 132.96 (C_{Ar}), 130.05 ($C_{Ar}H$), 129.06 ($C_{Ar}H$), 57.30 ($N(CH_3)_2\underline{C}H_2$), 44.31 ($CH_3$), 42.75 ($NHCH_2$), 36.05 ($NHCH_2$), 34.58 ($\underline{C}H_2C_{Ar}$).

7.4.4 2-(2'-(1''-methylpyrolidin-2''-yl)ethylamino)-4,6-Bis(4'-chlorophenethyl amino)-1,3,5-triazine (42).



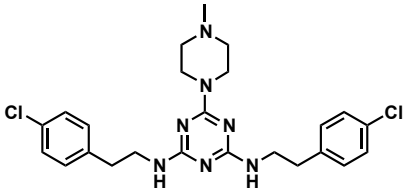
Starting from 125 mg of **22** and 152 mg of amine **21H**, 92 mg of **42** were obtained as colorless oil, 60% yield.

HRMS (ESI+): calculated for $C_{26}H_{33}N_7Cl_2$ (M+H), 514.2253; found, 514.2230.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.32-7.21 (H_{Ar}, cs, 4H), 7.15-7.08 (H_{Ar}, cs, 4H), 3.95-2.95 (4 x $NC\underline{H}_2$ + $NC\underline{H}$, cs, 9H), 2.95-2.75 (2 x CH_2C_{Ar} + NCH_3, cs, 7 H), 2.40-1.75 (2 x $C\underline{H}_2CH$ + $CH_2C\underline{H}_2CH_2$, cs, 6H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.56 ($NC_{Ar}N$), 155.57 ($NC_{Ar}N$), 136.46 (C_{Ar}), 133.03 (C_{Ar}), 130.11 ($C_{Ar}H$), 129.10 ($C_{Ar}H$), 68.39 (CH), 56.94 ($N(CH_3)\underline{C}H_2$), 42.66 ($NHCH_2$), 40.83 (CH_3), 38.17 ($NHCH_2$), 34.77 ($\underline{C}H_2C_{Ar}$), 29.77 ($CH\underline{C}H_2CH_2$), 29.49 ($CH\underline{C}H_2CH_2$), 21.71 ($CH_2\underline{C}H_2CH_2$).

7.4.5 2-(4'-methylpiperazin-1'-yl)-4,6-Bis(4'-chlorophenethylamino)-1,3,5-triazine (43).



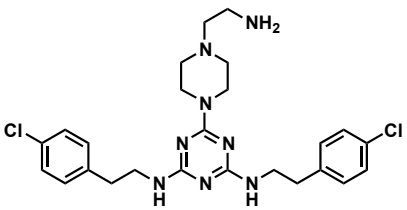
Starting from 125 mg of **22** and 118 mg of amine **21I**, 52 mg of **43** were obtained as colorless oil, 36% yield.

HRMS (ESI+): calculated for $C_{24}H_{29}N_7Cl_2$ (M+H), 486.1940 found, 486.1986.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.25 (H_{Ar}, d, *J* = 7.4 Hz, 4H), 7.10 (H_{Ar}, d, *J* = 7.4 Hz, 4H), 4.74 (NCH_2, d, *J* = 14.9 Hz, 2H), 3.82 (NCH_2, d, *J* = 12.1 Hz, 2H), 3.64 ($N(CH_3)C\underline{H}_2$, m, 4H), 3.40 (NCH_2, t, *J* = 13.3 Hz, 2H), 3.02 (CH_3, s, 3H), 2.88-2.72 (CH_2C_{Ar} + NCH_2, cs, 6H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.18 ($NC_{Ar}N$), 155.44 ($NC_{Ar}N$), 136.72 (C_{Ar}), 132.86 (C_{Ar}), 130.27 ($C_{Ar}H$), 129.00 ($C_{Ar}H$), 54.15 ($N(CH_3)\underline{C}H_2$), 44.24 (CH_3), 42.68 ($NHCH_2$), 40.89 ($N\underline{C}H_2CH_2N(CH_3)$), 34.87 ($\underline{C}H_2C_{Ar}$).

7.4.6 2-(4'-(2"-aminoethyl)piperazin-1'-yl)-4,6-Bis(4'-chlorophenethylamino)-1,3,5-triazine (44).



Starting from 150 mg of **22** and 183 mg of amine **21J**, 116 mg of **44** were obtained as colorless oil, 64% yield.

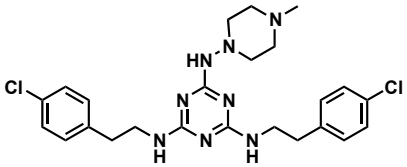
HRMS (ESI+): calculated for $C_{25}H_{32}N_8Cl_2$ (M+H),

515.2205; found, 515.2204.

^{1}H-NMR ($CDCl_3$, 48 °C): 7.57-7.48 (H_{Ar}, cs, 4H), 7.44-7.69 (H_{Ar}, cs, 4H), 5.13 (NH_2, bb, 2H), 4.05 (NCH_2, bb, 4H), 3.91-3.80 ($NHCH_2$, bb, 4H), 3.22-3.04 (3xNCH_2, bb, 6H), 2.86-2-64 (2 x CH_2C_{Ar}, NH_2CH_2, bb, 6H).

^{13}C-NMR ($CDCl_3$, 48 °C): 166.43 ($NC_{Ar}N$), 165.27 ($NC_{Ar}N$), 138.10 (C_{Ar}), 132.30 (C_{Ar}), 130.24 ($C_{Ar}H$), 128.77 ($C_{Ar}H$), 53.37 (NCH_2), 43.28 ($NHCH_2$), 42.05 (NCH_2), 38.87 ($N\underline{C}H_2CH_2NH_2$), 35.71 ($\underline{C}H_2C_{Ar}$), 30.54 ($NH_2CH_2$).

7.4.7 2-(4'-methylpiperazin-1'-ylamino)-4,6-Bis(4'-chlorophenethylamino)-1,3,5-triazine (45).



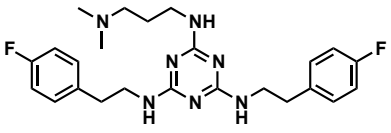
Starting from 125 mg of **22** and 136 mg of amine **21K**, 81 mg of **45** were obtained as white powder, 55% yield.

HRMS (ESI+): calculated for $C_{24}H_{30}N_8Cl_2$ (M+H), 501.2049; found, 501.2058.

^{1}H-NMR ($CDCl_3$, 7% TFA): 7.30-7.24 (H_{Ar}, cs, 4H), 7.15-7.07 (H_{Ar}, cs, 4H), 3.78-3.40 (2x $NHC\underline{H}_2$ + $N(CH_3)C\underline{H}_2$ + 2 x $NC\underline{H}_2CH_2N(CH_3)$, cs, 10H), 3.16-3.08 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.95-2.81 (2 x CH_2C_{Ar} + CH_3, cs, 7H).

^{13}C-NMR ($CDCl_3$, 7% TFA): 157.76 ($NC_{Ar}N$), 154.45-145.15 ($NC_{Ar}N$), 135.62 (C_{Ar}), 133.59 (C_{Ar}), 130.03 ($C_{Ar}H$), 129.36 ($C_{Ar}H$), 53.66 ($\underline{C}H_2N(CH_3)$), 51.76 (NCH_2), 43.78 ($NHCH_2$), 43.40 (CH_3), 34.42-34.19 ($\underline{C}H_2C_{Ar}$).

7.4.8 2-(3'-(*N,N*-dimethylamino)propylamino)-4,6-Bis(4'-fluorophenethylamino)-1,3,5-triazine (46).



Starting from 2.67 g of **23** and 2,80 g of amine **21A**, 2.28 g of **46** were obtained as white powder, 73% yield.

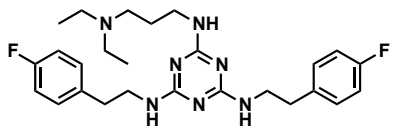
HRMS (ESI+): calculated for $C_{24}H_{31}N_7F_2$ (M+H), 456.2687; found, 456.2668.

^{1}H NMR ($CDCl_3$, 48 °C): 7.19-7.14 (H_{Ar}, cs, 4H), 7.01 – 6.94 (H_{Ar}, cs, 4H), 3.60 ($NHC\underline{H}_2$, cs, 4H), 3.48-3.40 ($NHC\underline{H}_2$, cs, 2H), 2.85 (CH_2C_{Ar}, t, *J* = 7.1 Hz, 4H), 2.46 ($N(CH_3)_2C\underline{H}_2$, t, *J* = 6.8 Hz, 2H), 2.32 (CH_3, s, 6H), 1.78 ($CH_2C\underline{H}_2CH_2$, qn, *J* = 6.7 Hz, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 166.37 ($NC_{Ar}N$), 166.32 ($NC_{Ar}N$), 161.68 (C_{Ar}, d, *J* = 244.1 Hz), 135.23 (C_{Ar}, d, *J* = 3.2 Hz), 130.21 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.31 ($C_{Ar}H$, d, *J* = 21.2

Hz), 57.84 (N(<u>C</u>H$_3$)CH$_2$), 45.54 (CH$_3$), 42.17 (NHCH$_2$), 39.58 (NHCH$_2$), 35.46 (<u>C</u>H$_2$C$_{Ar}$), 27.64 (CH$_2$<u>C</u>H$_2$CH$_2$).

7.4.9 2-(3'-(N,N-diethylamino)propylamino)-4,6-Bis(4'-fluorophenethylamino)-1,3,5 triazine (49).



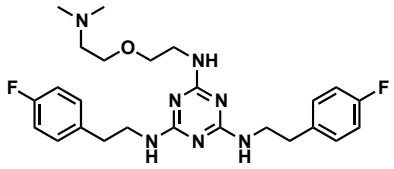
Starting from 110 mg of **23** and 150 mg of amine **21E**, 129 mg of **49** were obtained as white powder, 95% yield.

HRMS (ESI+): calculated for $C_{26}H_{36}N_7F_2$ (M+H), 484,3000; found, 484,3005.

^{1}H NMR (CDCl3, 48 ºC): 7.21-7.10 (H$_{Ar}$, cs, 4H), 7.03-6.92 (H$_{Ar}$, cs, 4H), 3.72-3.43 (NHC<u>H</u>$_2$, cs, 6H), 3.19-3.06 (NC<u>H</u>$_2$, cs, 6H), 2.92-2.81 (CH$_2$C$_{Ar}$, m, 4H), 2.15-2.06 (CH$_2$C<u>H</u>$_2$CH$_2$, cs, 2H), 1.32 (CH$_3$, t, *J* = 7.3 Hz, 6H).

^{13}C NMR (CDCl3, 48 ºC): 163.48 (NC$_{Ar}$N), 163.18 (NC$_{Ar}$N), 161.75 (C$_{Ar}$, d, *J* = 244.7 Hz), 133.71 (C$_{Ar}$), 130.07 (C$_{Ar}$H, d, *J* = 7.9 Hz), 115.45 (C$_{Ar}$H, d, *J* = 21.1 Hz), 49.41 (N<u>C</u>H$_2$CH$_2$), 46.43 (N<u>C</u>H$_2$CH$_3$), 42.55-42.08 (NHCH$_2$, bb), 38.10 (NHCH$_2$), 34.64 (<u>C</u>H$_2$C$_{Ar}$), 23.71 (CH$_2$<u>C</u>H$_2$CH$_2$), 8.19 (CH$_3$).

7.4.10 2-(2'-(2''-(*N,N*-dimethylamino)ethoxy)ethylamino)-4,6-Bis(4'-fluorophenethylamino)-1,3,5-triazine (51).



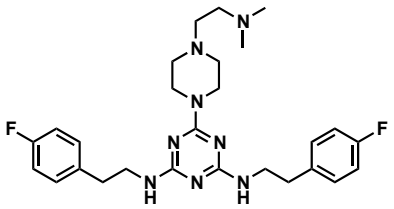
Starting from 170 mg of **23** and 230 mg of amine **21G**, 50 mg of **51** were obtained as white powder, 24% yield.

HRMS (ESI+): calculated for $C_{25}H_{33}N_7F_2O$ (M+H), 486.2793; found, 486.2796.

^{1}H NMR (CDCl$_3$, 7% TFA): 7.17-7.10 (H$_{Ar}$, cs, 4H), 7.02-6.94 (H$_{Ar}$, cs, 4H), 3.84-3.75 (OCH$_2$, bb, 2H), 3.74-3.55 (OCH$_2$ + 3 x NHC<u>H</u>$_2$, cs, 8H), 3.41-3.31 (N(CH$_3$)C<u>H</u>$_2$, bb, 2H), 3.00-2.91 (CH$_3$, bb, 6H), 2.91-2.81 (CH$_2$C$_{Ar}$, cs, 4H).

^{13}C NMR (CDCl$_3$, 7% TFA): 165.54 (NC$_{Ar}$N), 161.81 (C$_{Ar}$, d, *J* = 244.5 Hz), 135.04 (C$_{Ar}$), 130.32 (C$_{Ar}$H, d, *J* = 7.8 Hz), 115.48 (C$_{Ar}$H, d, *J* = 21.2 Hz), 70.13 (OCH$_2$), 68.64 (OCH$_2$), 58.82 (N(CH$_3$)<u>C</u>H$_2$), 45.63 (CH$_3$), 42.24 (NHCH$_2$).

7.4.11 2-(4'-(2''-(*N,N*-dimethylamino)ethyl)piperazin-1'-yl)-4,6-Bis(4'-fluorophenethylamino)-1,3,5-triazine (52).



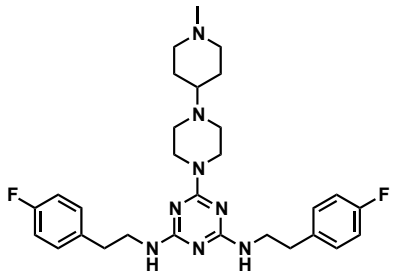
Starting from 200 mg of **23** and 380 mg of amine **21L**, 192 mg of **52** were obtained as white powder, 73% yield.

HRMS (ESI+): calculated for $C_{27}H_{36}N_8F_2$ (M+H), 511.3109; found, 511.3092.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.18-7.13 (H_{Ar}, cs, 4H), 7.01-6.94 (H_{Ar}, cs, 4H), 3.81-3.75 (CH_2N, bb, 4H), 3.59 (NHC<u>H</u>$_2$, dd, *J* = 13.0 Hz, 6.5 Hz, 4H), 2.84 (CH_2C_{Ar}, t, *J* = 7.0 Hz, 4H), 2.55-2.45 (3 x CH_2N + C<u>H</u>$_2$N(CH_3), cs, 8H), 2.29 (CH_3, s, 6H).

^{13}C NMR ($CDCl_3$, 48 ºC): 166.15 ($NC_{Ar}N$), 164.99 ($NC_{Ar}N$), 161.62 ($C_{Ar}F$, d, *J* = 244.0 Hz), 135.12 (C_{Ar}, d, *J* = 3.0 Hz), 130.25 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.38 ($C_{Ar}H$, d, *J* = 21.1 Hz), 56.88 (NCH_2), 56.83 (NCH_2), 53.67 (NCH_2), 45.98 (CH_3), 42.96 ($NHCH_2$), 42.20 (NCH_2), 35.38 (<u>C</u>H_2C_{Ar}).

7.4.12 2-(4'-(1''-methylpiperidin-4''-yl)piperazin-1'-yl)-4,6-Bis(4'-fluorophenethyl amino)-1,3,5-triazine (53).



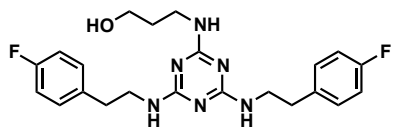
Starting from 200 mg of **23** and 376 mg of amine **21G**, 261 mg of **53** were obtained as white powder, 95% yield.

HRMS (ESI+): calculated for $C_{29}H_{38}N_8F_2$ (M+H), 537.3265; found, 537.3209.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.17-7.14 (H_{Ar}, cs, 4H), 6.99-6.96 (H_{Ar}, cs, 4H), 3.82-3.77 (NCH_2, bb, 4H), 3.58 ($NHCH_2$, dd, *J* = 13.1 Hz, 6.6 Hz, 4H), 3.36-3.29 (N(CH_3)C<u>H</u>$_2$, bb, 2H), 2.85 (CH_2C_{Ar}, t, *J* = 7, 4H), 2.65-2.45 (CH_3 + 2 x NCH_2 + CH + N(CH_3)C<u>H</u>$_2$, cs, 10 H), 2.06-1.94 (CHC<u>H</u>$_2$, cs, 4H).

^{13}C NMR ($CDCl_3$, 48 ºC): 163.47 ($NC_{Ar}N$), 161.84 ($C_{Ar}F$, d, *J* = 244.4 Hz), 134.67 (C_{Ar}), 130.29 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.49 ($C_{Ar}H$, d, *J* = 21.2 Hz), 59.22 (CH), 54.03 (NCH_2), 49.09 (N(CH_3)<u>C</u>H_2), 44.32 (CH_3), 43.83 (NCH_2), 42.30 ($NHCH_2$), 35.13 (<u>C</u>H_2C_{Ar}), 26.19 (CH<u>C</u>H_2).

7.4.13 2-(3'-hydroxypropylamino)-4,6-Bis(4'-fluorophenethylamino)-1,3,5-triazine (54).



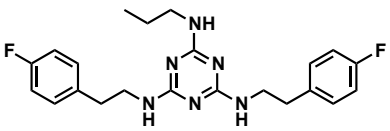
Starting from 100 mg of **23** and 73 mg of amine **21N**, 100 mg of **54** were obtained as white powder, 91% yield.

HRMS (ESI+): calculated for $C_{22}H_{26}N_6OF_2$ (M+H), 429.2214; found, 429.2207.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.17-7.10 (H_{Ar}, cs, 4H), 6.99-6.91 (H_{Ar}, cs, 4H), 4.08 (OH, bb, 1H) 3.63-3.44 ($C\underline{H}_2OH$ + 3 x $NHCH_2$, cs, 8H), 2.80 (CH_2C_{Ar}, t, *J* = 6.9 Hz, 4H), 1,74-1.63 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 ºC): 166.67 ($NC_{Ar}N$), 166.00 ($NC_{Ar}N$), 161.87 ($C_{Ar}F$, d, *J* = 244.3 Hz), 135.05 (C_{Ar}, d, *J* = 3.0 Hz), 130.33 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.51 ($C_{Ar}H$, d, *J* = 21.2 Hz), 58.63 (CH_2OH), 42.25 ($NHCH_2$), 36.87 ($NHCH_2$), 35.47 ($\underline{C}H_2C_{Ar}$), 33.34 ($CH_2\underline{C}H_2CH_2$).

7.4.14 2,4-Bis(4'-fluorophenethylamino)-6-propylamino-1,3,5-triazine (55).



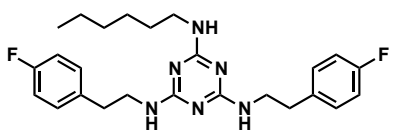
Starting from 200 mg of **23** and 121 mg of amine **21P**, 113 mg of **55** were obtained as white powder, 53% yield.

HRMS (ESI+): calculated for $C_{22}H_{26}N_6F_2$ (M+H), 413.2265; found, 413.2279.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.18-7.11 (H_{Ar}, cs, 4H), 7.03-6.96 (H_{Ar}, cs, 4H), 3.51-3.36 ($NHCH_2$, bb, 4H), 3.48-3.40 ($NHCH_2$, cs, 2H), 2.91 (CH_2C_{Ar}, t, *J* = 7.3 Hz, 4H), 1.72-161 ($C\underline{H}_2CH_3$, m, 2H), 1.01-0.94 (CH_3, m, 3H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.21 ($C_{Ar}F$, d, *J* = 245.7 Hz), 157.64 ($NC_{Ar}N$), 154.51 ($NC_{Ar}N$), 152.62 ($NC_{Ar}N$), 133.10 (C_{Ar}), 130.24 ($C_{Ar}H$, d, *J* = 7.9 Hz), 115.87 ($C_{Ar}H$, d, *J* = 21.4 Hz), 44.24 ($NHCH_2$), 43.57 ($NHCH_2$), 34.30 ($\underline{C}H_2C_{Ar}$), 22.15 ($\underline{C}H_2CH_3$), 11.17 (CH_3).

7.4.15 2,4-Bis(4'-fluorophenethylamino)-6-hexylamino-1,3,5-triazine (56).



Starting from 103 mg of **23** and 99 mg of amine **21Q**, 85 mg of **56** were obtained as white powder, 73% yield.

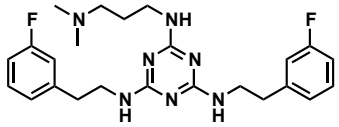
HRMS (ESI+): calculated for $C_{25}H_{32}N_6F_2$ (M+H), 455.2734; found, 455.2739.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.19-7.10 (H_{Ar}, cs, 4H), 7.04-6.94 (H_{Ar}, cs, 4H), 3.76-3.62 ($NHCH_2$, cs, 4H), 3.51-3.34 ($NHCH_2$, cs, 2H), 2.93-2.83 (CH_2C_{Ar}, cs, 4H), 1.67-1.54

($NHCH_2\underline{C}H_2CH_2$, cs, 2H), 1.40-1.24 ($CH_3\underline{C}H_2$ + $CH_3CH_2\underline{C}H_2$ + $CH_3CH_2CH_2\underline{C}H_2$, cs, 6H), 0.94-0.84 ($CH_3$, cs, 3H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.36 ($C_{Ar}F$, d, *J* = 245.6 Hz), 155.13 ($NC_{Ar}N$), 153.62 ($NC_{Ar}N$), 153.23 ($NC_{Ar}N$), 133.38 (C_{Ar}), 130.24 ($C_{Ar}H$, d, *J* = 7.9 Hz), 115.86 ($C_{Ar}H$, d, J = 21.5 Hz), 43.13 ($NHCH_2$), 42.31 ($NHCH_2$), 34.50 ($\underline{C}H_2C_{Ar}$), 31.47 ($CH_3CH_2CH_2\underline{C}H_2$), 29.02 ($CH_3CH_2\underline{C}H_2$), 26.57 (**I**), 22.61 ($CH_3\underline{C}H_2$), 14.00 (CH_3).

7.4.16 2-(3'-(*N,N*-dimethylamino)propylamino)-4,6-Bis(3'-fluorophenethylamino)-1,3,5-triazine (57).



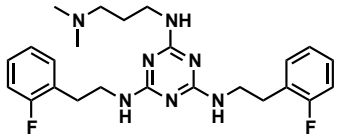
Starting from 118 mg of **24** and 261 mg of amine **21A**, 133 mg of **57** were obtained as white powder, 53% yield.

HRMS (ESI+): calculated for $C_{24}H_{31}N_7F_2$ (M+H), 456.2687; found, 456.2705.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.28-7.23 (H_{Ar}, cs, 2H), 6.99-6.87 (H_{Ar}, cs, 6H), 3.74-3.58 ($NHCH_2$, cs, 4H), 3.58-3.44 ($NHCH_2$, cs, 2H), 3.23-3.09 ($N(CH_3)\underline{C}H_2$, cs, 2H), 2.93-2.82 (CH_2C_{Ar} + CH_3, cs, 10H), 2.15-2.02 ($CH_2\underline{C}H_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 7% TFA): 163.88 ($NC_{Ar}N$), 163.18 ($C_{Ar}F$, d, *J* = 246.2 Hz), 140.73 (C_{Ar}), 130.40 ($C_{Ar}H$, d, *J* = 8.3 Hz), 124.48 ($C_{Ar}H$, d, *J* = 2.3 Hz), 115.66 ($C_{Ar}H$, d, *J* = 21.2 Hz), 113.85 ($C_{Ar}H$, d, *J* = 20.9 Hz), 55.60 ($N(CH_3)\underline{C}H_2$), 43.26 (CH_3), 41.18 ($NHCH_2$), 37.11 ($NHCH_2$), 35.16 ($\underline{C}H_2C_{Ar}$), 24.32 ($CH_2\underline{C}H_2CH_2$).

7.4.17 2-(3'-(*N,N*-dimethylamino)propylamino)-4,6-Bis(2'-fluorophenethylamino)-1,3,5-triazine (58).



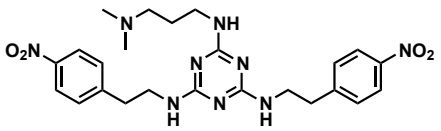
Starting from 214 mg of **25** and 224 mg of amine **21A**, 143 mg of **58** were obtained as white powder, 57% yield.

HRMS (ESI+): calculated for $C_{24}H_{31}N_7F_2$ (M+H), 456.2687; found, 456.2701.

^{1}H NMR ($CDCl_3$, 48 °C): 7.26-6.99 (H_{Ar}, cs, 8H), 3.77-3.64 ($NHCH_2$, cs, 4H), 3.64-3.48 ($N(CH_3)\underline{C}H_2$, cs, m, 2H), 3.29-3.17 ($NHCH_2$, cs, 2H), 2.99-2.89 (CH_2C_{Ar} + CH_3, cs, 10H), 2.17-2.06 ($CH_2\underline{C}H_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 161.42 ($C_{Ar}F$, d, *J* = 245.0 Hz), 131.18 ($C_{Ar}H$, d, *J* = 4.6 Hz), 128.95 (C_{Ar}), 124.77 ($C_{Ar}H$), 124.49 ($C_{Ar}H$), 115.54 ($C_{Ar}H$, d, J = 22.0 Hz), 56.27-55.75 ($N(CH_3)\underline{C}H_2$), 43.74 (CH_3), 42.18-41.43 ($NHCH_2$), 37.92 ($NHCH_2$), 29.01 ($\underline{C}H_2C_{Ar}$), 24.27 ($CH_2\underline{C}H_2CH_2$).

7.4.18 2,4-Bis(4'-nitrophenethylamino)-6-(3'-(*N*,*N*-dimethylamino)propylamino)-1,3,5-triazine (59).



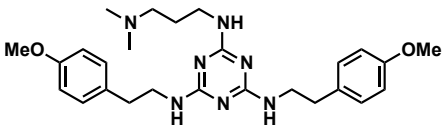
Starting from 200 mg of **26** and 184 mg of amine **21A**, 223 mg of **59** were obtained as white powder, 97% yield.

HRMS (ESI+): calculated for $C_{24}H_{31}N_9O_4$ (M+H), 510.2577; found, 510.2594.

^{1}H NMR ($CDCl_3$, 48 °C): 8.13-8.07 (H_{Ar}, cs, 4H), 7.37-7.31 (H_{Ar}, cs, 4H), 3.66-3.56 ($NHCH_2$, cs, 4H), 3.44-3.36 ($NHCH_2$, cs, 2H), 2.96 (CH_2C_{Ar} t, *J* = 6.9 Hz, 4H), 2.47 ($N(CH_3)C\underline{H}_2$, t, *J* = 6.9 Hz, 2H), 2.31 (CH_3, s, 6H), 1.77 ($CH_2C\underline{H}_2CH_2$, m, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 166.26 ($NC_{Ar}N$), 147.53 (C_{Ar}), 147.03 (C_{Ar}), 129.82 ($C_{Ar}H$), 123.85 ($C_{Ar}H$), 57.59 ($N(CH_3)\underline{C}H_2$), 45.23 (CH_3), 41.65 ($NHCH_2$), 39.41 ($NHCH_2$), 36.31 ($\underline{C}H_2C_{Ar}$), 27.20 ($CH_2\underline{C}H_2CH_2$).

7.4.19 2,4-Bis(4'-methoxyphenethylamino)-6-(3'-(*N*,*N*-dimethylamino)propyl amino)-1,3,5-triazine (60).



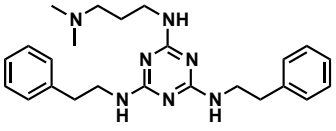
Starting from 200 mg of **27** and 184 mg of amine **21A**, 224 mg of **60** were obtained as white powder, 97% yield.

HRMS (ESI+): calculated for $C_{26}H_{37}N_7O_2$ (M+H), 480.3087; found, 480.3090.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.13-7.06 (H_{Ar}, cs, 4H), 6.89-6.84 (H_{Ar}, cs, 4H), 3.80 (CH_3O, s, 6H), 3.72-3.65 ($NHCH_2$, cs, 4H), 3.62-3.45 ($NHCH_2$, cs, 2H), 3.26-3.10 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.95-2.75 ($N(CH_3)$ + CH_2C_{Ar}, cs, 10H), 2.20-2.00 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 7% TFA): 162.18 ($NC_{Ar}N$), 158.58 ($NC_{Ar}N$), 154.71 (C_{Ar}), 130.23 (C_{Ar}), 129.82 ($C_{Ar}H$), 114.54 ($C_{Ar}H$), 56.10 ($N(CH_3)_2\underline{C}H_2$), 55.56 ($CH_3O$), 43.75 ($N(CH_3)_2$), 43.10 ($NHCH_2$), 37.80 ($NHCH_2$), 34.41 ($\underline{C}H_2C_{Ar}$), 24.34 ($CH_2\underline{C}H_2CH_2$).

7.4.20 2,4-Bis(phenethylamino)-6-(3'-(*N*,*N*-dimethylamino)propylamino)-1,3,5-triazine (61).



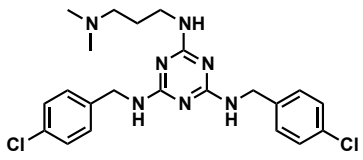
Starting from 120 mg of **28** and 139 mg of amine **21A**, 105 mg of **61** were obtained as white powder, 74% yield.

HRMS (ESI+): calculated for $C_{24}H_{33}N_7$ (M+H), 432.1703; found, 432.1703.

^{1}H NMR ($CDCl_3$, 48 °C): 7.32-7.16 (H_{Ar}, cs, 10H), 3.61 ($NHCH_2$, cs, 4H), 3.47-3.39 ($NHCH_2$, sc, 2H), 2.86 (CH_2C_{Ar}, t, *J* = 7.0 Hz, 4H), 2.46-2.39 ($N(CH_3)C\underline{H}_2$, m, 2H), 2.28 (CH_3, s, 6H), 1.79-1.70 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 166.13 ($NC_{Ar}N$), 139.58 (C_{Ar}), 128.93 ($C_{Ar}H$), 128.67 ($C_{Ar}H$), 126.43 ($C_{Ar}H$), 57.79 ($N(CH_3)_2\underline{C}H_2$), 45.36 ($N(CH_3)_2$), 42.21 ($NHCH_2$), 39.54 ($NHCH_2$), 36.33 ($\underline{C}H_2C_{Ar}$), 27.44 ($CH_2\underline{C}H_2CH_2$).

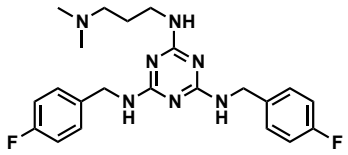
7.4.21 2,4-Bis(4'-chlorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (62).



Starting from 138 mg of **29** and 143 mg of amine **21A**, 110 mg of **62** were obtained as white powder, 68% yield.
HRMS (ESI+): calculated for $C_{22}H_{27}N_7Cl_2$ (M+H), 460.1783; found, 460.1779.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.35-7.24 (H_{Ar}, cs, 4H), 7.23-7.08 (H_{Ar}, cs, 4H) 4.66-4.53 (CH_2C_{Ar}, bs, 4H), 3.59-3.51 ($NHCH_2$, cs, 2H), 3.26-3.04 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.94-2.83 ($N(CH_3)_2$, cs, 6H), 2.24-2.01 ($CH_2C\underline{H}_2CH_2$, cs, 2H).
^{13}C NMR ($CDCl_3$, 7% TFA): 162.54 ($NC_{Ar}N$), 155.65 ($NC_{Ar}N$), 134.42 (C_{Ar}), 129.29-128.78 (C_{Ar} + 2 x $C_{Ar}H$), 56.34 ($N(CH_3)_2\underline{C}H_2$), 45.15 ($\underline{C}H_2C_{Ar}$), 43.90 ($N(CH_3)_2$), 38.31 ($NHCH_2$), 24.25 ($CH_2\underline{C}H_2CH_2$).

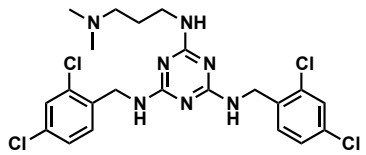
7.4.22 2,4-Bis(4'-fluorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (63).



Starting from 123 mg of **30** and 139 mg of amine **21A**, 105 mg of **63** were obtained as white powder, 72% yield.
HRMS (ESI+): calculated for $C_{22}H_{27}N_7F_2$ (M+H), 428.2374; found, 428.2394.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.26-7.10 (H_{Ar}, cs, 4H), 7.07-6.95 (H_{Ar}, cs, 4H), 4.66-4.51 (CH_2C_{Ar}, bs, 4H), 3.64-3.51 ($NHCH_2$, cs, 2H), 3.27-3.12 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.96-2.86 ($N(CH_3)_2$, cs, 6H), 2.20-2.00 ($CH_2C\underline{H}_2CH_2$, cs, 2H).
^{13}C NMR ($CDCl_3$, 7% TFA): 162.80 ($NC_{Ar}N$), 154.73-153.80 ($NC_{Ar}N$), 131.49 (C_{Ar}), 129.49 ($C_{Ar}H$), 115.93 ($C_{Ar}H$, d, J = 21.7 Hz), 56.17 ($N(CH_3)_2\underline{C}H_2$), 45.21 ($\underline{C}H_2C_{Ar}$), 43.87 ($N(CH_3)_2$), 38.45 ($NHCH_2$), 24.26 ($CH_2\underline{C}H_2CH_2$).

7.4.23 2,4-Bis(2',4'-dichlorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (64).



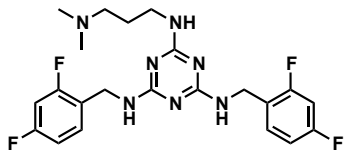
Starting from 50 mg of **31** and 44 mg of amine **21A**, 53 mg of **64** were obtained as white powder, 93% yield.

HRMS (ESI+): calculated for $C_{22}H_{25}N_7Cl_4$ (M+H), 528.1004; found, 528.0992.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.44-6.98 (H_{Ar}, cs, 6H), 4.71-4.54 (CH_2C_{Ar}, cs, 4H), 3.60-3.42 ($NHCH_2$, cs, 2H), 3.22-3.00 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.95-2.75 ($N(CH_3)_2$, cs, 6H), 2.15-2.00 ($CH_2C\underline{H}_2CH_2$, cs, 2H)

^{13}C NMR ($CDCl_3$, 7% TFA): 163.81 ($NC_{Ar}N$), 156.11 ($NC_{Ar}N$), 134.57 (C_{Ar}), 134.04 (C_{Ar}), 132.75 (C_{Ar}), 130.01 ($C_{Ar}H$), 129.64 ($C_{Ar}H$), 127.44 ($C_{Ar}H$), 56.08 ($N(CH_3)_2\underline{C}H_2$), 43.70 ($\underline{C}H_2C_{Ar}$), 42.34 ($N(CH_3)_2$), 37.74 ($NHCH_2$), 24.39 ($CH_2\underline{C}H_2CH_2$).

7.4.24 2,4-Bis(2',4'-difluorobenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (65).



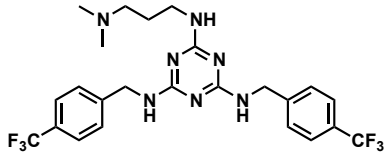
Starting from 110 mg of **32** and 113 mg of amine **21A**, 52 mg of **65** were obtained as white powder, 40% yield.

HRMS (ESI+): calculated for $C_{22}H_{25}N_7F_4$ (M+H), 464.2186; found, 464.2199.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.31-7.10 (H_{Ar}, cs, 2H), 6.91-6.75 (H_{Ar}, cs, 4H), 4.70-4.55 (CH_2C_{Ar}, cs, 4H), 3.64-3.46 ($NHCH_2$, cs, 2H), 3.26-3.14 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.95-2.86 ($N(CH_3)_2$, cs, 6H), 2.15-2.03 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 7% TFA): 163.06 ($NC_{Ar}N$), 163.40 ($C_{Ar}F$, dd, J = 198.6 Hz, 11.5), 160.91 ($C_{Ar}F$, dd, J = 198.6 Hz, 11.6 Hz), 155.42 (NCN), 130,91 ($C_{Ar}H$), 119.54 (C_{Ar}), 111.74 ($C_{Ar}H$, d, J = 21.0 Hz), 104.33 ($C_{Ar}H$, t, J = 25.5 Hz), 56.20 ($N(CH_3)_2\underline{C}H_2$), 43.82 ($N(CH_3)_2$), 39.29-38.88 ($\underline{C}H_2C_{Ar}$), 37.96 ($NHCH_2$), 24.29 ($CH_2\underline{C}H_2CH_2$).

7.4.25 2,4-Bis(4'-trifluoromethylbenzylamino)-6-(3'-(*N,N*-dimethylamino)propyl amino)-1,3,5-triazine (66).



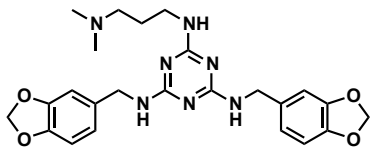
Starting from 200 mg of **33** and 354 mg of amine **21A**, 220 mg of **66** were obtained as colorless oil, 96% yield.

HRMS (ESI+): calculated for $C_{24}H_{27}N_7F_6$ (M+H), 528.2310; found, 528.2292.

^{1}H-NMR ($CDCl_3$, 48 °C): 7.54-7.47 (H_{Ar}, cs, 4H), 7.39-7.28 (H_{Ar}, cs, 4H), 4.61-4.50 (CH_2C_{Ar}, cs, 4H), 3.38-3.31 ($NHCH_2$, cs, 2H), 2.38-2.29 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.21 ($N(CH_3)_2$, s, 6H) 1.71-1.61 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 166.31 ($NC_{Ar}N$), 144.00 (C_{Ar}), 129.50 (C_{Ar}, q, J = 32.4 Hz), 127.60 ($C_{Ar}H$), 125.47 ($C_{Ar}H$, dd, J = 7.5 Hz, 3.7 Hz), 124.32 (CF_3, q, J = 272.0 Hz), 57.64 ($N(CH_3)_2\underline{C}H_2$), 45.34 ($N(CH_3)_2$), 44.25 ($\underline{C}H_2C_{Ar}$), 39.50 ($NHCH_2$), 27.22 ($CH_2\underline{C}H_2CH_2$).

7.4.27 2,4-Bis(3'-4'-methylenedioxybenzylamino)-6-(3'(*N*,*N*-dimethylamino)propyl amino) -1,3,5-triazine (67).



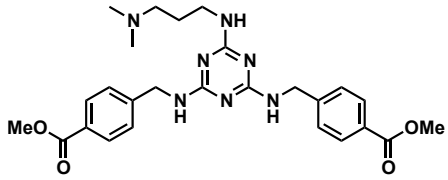
Starting from 207 mg of **34** and 204 mg of amine **21A**, 226 mg of **67** were obtained as colorless oil, 94% yield.

HRMS (ESI+): calculated for $C_{24}H_{29}N_7O_4$ (M+H), 480.2359; found, 480.2365.

^{1}H NMR ($CDCl_3$, 48 °C): 6.84-6.79 (H_{Ar}, cs, 2H), 6.79-6.70 (H_{Ar}, cs, 4H), 5.95-5.91 (OCH_2O, cs, 4H), 4.52-4.43 (CH_2C_{Ar}, cs, 4H), 3.50-3.40 ($NHCH_2$, cs, 2H), 2.61-2.52 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.43-2.34 ($N(CH_3)_2$, cs, 6H), 1.86-1.75 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 165.37 ($NC_{Ar}N$), 147.99 (C_{Ar}), 146.93 (C_{Ar}), 133.34 (C_{Ar}), 120.90 ($C_{Ar}H$), 108.41 ($C_{Ar}H$), 108.35 ($C_{Ar}H$), 101.11 (OCH_2O), 57.45 ($N(CH_3)_2\underline{C}H_2$), 44.82 ($N(CH_3)_2$), 44.66 ($\underline{C}H_2C_{Ar}$), 39.34 ($NHCH_2$), 26.65 ($CH_2\underline{C}H_2CH_2$).

7.4.28 2,4-Bis(4'-methoxycarbonylbenzylamino)-6-(3'-(*N*,*N*-dimethylamino)propyl amino) -1,3,5-triazine (68).



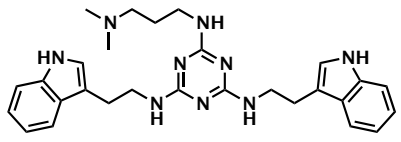
Starting from 200 mg of **35** and 180 mg of amine **21A**, 97 mg of **68** were obtained as colorless oil, 42% yield.

HRMS (ESI+): calculated for $C_{28}H_{33}N_7O_4$ (M+H), 508.2630; found, 508.2633.

^{1}H NMR ($CDCl_3$, 48 °C): 7.99-7.88 (H_{Ar}, cs, 4H), 7.39-7.26 (H_{Ar}, cs, 4H), 4.64-4.52 (CH_2C_{Ar}, cs, 4H), 3.94-3.86 (OCH_3, s, 6H), 3.42-3.34 ($NHCH_2$, cs, 2H), 2.38-2.28 ($N(CH_3)_2$, s, 6H), 2.25-2.17 ($N(CH_3)_2\underline{C}H_2$, cs, 2H), 1.73-1.63 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 °C): 167.05 (CO), 166.51-166.46 ($NC_{Ar}N$), 145.13 (C_{Ar}), 129.94 ($C_{Ar}H$), 129.17 (C_{Ar}), 127.33 ($C_{Ar}H$), 57.89 ($N(CH_3)_2\underline{C}H_2$), 52.13 ($OCH_3$), 45.54 ($N(CH_3)_2$), 44.55 ($\underline{C}H_2C_{Ar}$), 39.74 ($NHCH_2$), 27.44 ($CH_2\underline{C}H_2CH_2$).

7.4.29 2-(3'-(*N*,*N*-dimethylamino)propylamino)-4,6-Bis(2'-(3''-indolyl)ethylamino)-1,3,5-triazine (71).



Starting from 50 mg of **38** and 47 mg of amine **21A**, 20 mg of **71** were obtained as brown solid, 34% yield.

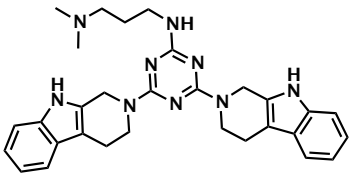
HRMS (ESI+): calculated for $C_{28}H_{35}N_9$ (M+H), 498.3094; found, 498.3084.

^{1}H NMR (CD_3CN, 48 °C): 9.13 ($C\underline{H}NH$, s, 2H), 7.62 (H_{Ar}, d, *J* = 7.9 Hz, 2H), 7.49 (H_{Ar}, d, *J* = 8.2 Hz, 2H), 7.13 (H_{Ar}, t, *J* = 7.5 Hz, 6H), 7.09-6.98 (H_{Ar}, cs, 4H), 3.69-3.57

($NHCH_2$, cs, 4H), 3.40-3.30 ($NHCH_2$, bb, 2H), 2.99 (CH_2C_{Ar}, cs, 4H), 2.30 ($N(CH_3)_2\underline{C}H_2$, cs, 2H), 2.16 ($N(CH_3)_2$, s, 6H), 1.73-1.63 ($CH_2\underline{C}H_2CH_2$, m, 2H).

^{13}C NMR (CD_3CN, 48 ºC): 167.86 ($NC_{Ar}N$), 138.03 (C_{Ar}), 129.04 (C_{Ar}), 123.77 ($C_{Ar}H$), 122.71 ($C_{Ar}H$), 120.05 ($C_{Ar}H$), 119.88 ($C_{Ar}H$), 114.38 (C_{Ar}), 112.55 ($C_{Ar}H$), 58.76 ($N(CH_3)_2\underline{C}H_2$), 45.95 ($N(CH_3)_2$), 42.38 ($NHCH_2$), 40.45 ($NHCH_2$), 28.76 ($\underline{C}H_2C_{Ar}$), 26.82 ($CH_2\underline{C}H_2CH_2$).

7.4.30 2,4-Bis(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indol-2(9*H*)-yl)-6-(3'-(*N*,*N*-dimethylamino)propylamino-1,3,5-triazine (72).



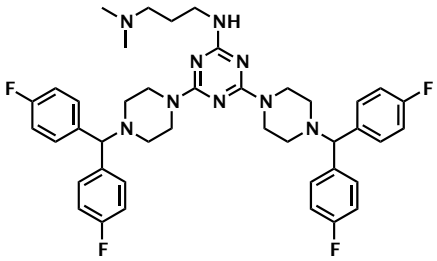
Starting from 110 mg of **39** and 99 mg of amine **21A**, 80 mg of **72** were obtained as brownish oil, 64% yield.

HRMS (ESI+): calculated for $C_{30}H_{35}N_9$ (M+H), 522.3094; found, 522.3079.

^{1}H NMR (CD_3CN, 48 ºC): 9.00 ($C\underline{H}NH$, s, 2H), 7.47-6.98 (H_{Ar}, cs, 8H), 5.02-4.85 ($C\underline{H}_2CH$, m, 4H), 4.22-4.03 ($C_{Ar}CH_2C\underline{H}_2N$, cs, 4H), 3.47-3.32 ($NHCH_2$, cs, 2H), 2.84-2.72 ($C_{Ar}C\underline{H}_2CH_2N$, cs, 4H), 2.37-2.27 ($N(CH_3)_2C\underline{H}_2$, cs, 2H), 2.22-2.13 ($N(CH_3)_2$, cs, 6H), 1.76-1.64 ($CH_2C\underline{H}_2CH_2$, m, 2H).

^{13}C NMR (CD_3CN, 48 ºC): 167.70 ($NC_{Ar}N$), 167.00 ($NC_{Ar}N$), 137.60 (C_{Ar}), 133.27(C_{Ar}), 128.38 (C_{Ar}), 122.18 ($C_{Ar}H$), 120.07 ($C_{Ar}H$), 118.71 ($C_{Ar}H$), 112.06 (C_{Ar}), 109.45 ($C_{Ar}H$), 58.68 ($N(CH_3)_2\underline{C}H_2$), 45.90 ($N(CH_3)_2$), 42.48 ($NCH_2$), 42.35 ($NCH_2$), 40.36 ($NHCH_2$), 28.64 ($CH_2\underline{C}H_2CH_2$), 21.97 ($C_{Ar}\underline{C}H_2CH_2N$).

7.4.31 2,4-Bis(4'-(bis(4''-fluorophenyl)methyl)piperazin-1'-yl)-6-(3'-(*N*,*N*-dimethyl amino)propylamino-1,3,5-triazine (73).



Starting from 138 mg of **40** and 82 mg of amine **21A**, 120 mg of **73** were obtained as white powder, 80% yield.

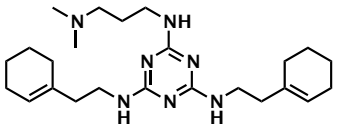
HRMS (ESI+): calculated for $C_{42}H_{47}N_9F_4$ (M+H), 754.3969; found, 754.3958.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.38-7.29 (H_{Ar}, cs, 8H), 7.01-6.29 (H_{Ar}, cs, 8H), 4.23 (CH, s, 2H), 3.75-3.65 ($C_{Ar}NC\underline{H}_2$, cs, 8H), 3.41 ($NHCH_2$, cs, 2H), 2.69 ($N(CH_3)_2C\underline{H}_2$, cs, 2H), 2.48 ($N(CH_3)_2$, s, 6H), 2.38-2.27 ($CHNC\underline{H}_2$, cs, 8H), 1.93-1.83 (**k**, m, 2H).

^{13}C NMR ($CDCl_3$, 48 ºC): 166.18-164.71 ($NC_{Ar}N$), 162.09 ($C_{Ar}F$, d, J = 245.8 Hz), 138.11 (C_{Ar}, d, J = 3.0 Hz), 129.51 ($C_{Ar}H$, d, J = 7.9 Hz), 115.60 ($C_{Ar}H$, d, J = 21.3 Hz),

74.66 (CH), 56.87 ($N(CH_3)_2\underline{C}H_2$), 51.85 ($C_{Ar}N\underline{C}H_2$), 44.21 ($N(CH_3)_2$), 43.51 ($CHN\underline{C}H_2$), 38.60 ($NHCH_2$), 26.39 ($CH_2\underline{C}H_2CH_2$).

7.4.32 2-(3'-(*N,N*-dimethylamino)propylamino)-4,6-Bis(2'-(1''-cyclohexenyl) ethylamino)-1,3,5-triazine (74).



Starting from 100 mg of **41** and 272 mg of amine **21A**, 220 mg of **74** were obtained as white solid, 95% yield.

HRMS (ESI+): calculated for $C_{24}H_{41}N_7$ (M+H), 428.3502; found, 428.3505.

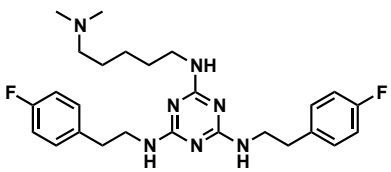
^{1}H NMR ($CDCl_3$, 48 ^{o}C): 5.46 (CCH, cs, 2H), 3.47-3.35 (3 x $NHCH_2$, cs, 6H), 2.34 ($N(CH_3)_2\underline{C}H_2$, cs, 2H), 2.22 ($N(CH_3)_2$, s, 6H), 2.16 ($CC\underline{H}_2CH_2NH$, t, J = 6.8, 4H), 2.02-1.89 ($C\underline{H}_2CHCC\underline{H}_2$, cs, 8H), 1.77-1.67 ($NHCH_2\underline{C}H_2CH_2$, m, 2H), 1.65-1.50 ($CH_2C\underline{H}_2C\underline{H}_2CH_2$, cs, 8H).

^{13}C NMR ($CDCl_3$, 48 ^{o}C): 166.17 ($NC_{Ar}N$), 135.01 ($\underline{C}CH$), 123.43 ($C\underline{C}H$), 57.88 ($N(CH_3)_2\underline{C}H_2$), 45.58 ($CH_3$), 39.59 ($NHC\underline{H}_2$), 38.77 ($NHC\underline{H}_2$), 38.19 ($C\underline{C}H_2CH_2NH$), 28.22 ($\underline{C}H_2CH$), 27.72 ($CH_2\underline{C}H_2CH_2$), 25.41 ($\underline{C}H_2CCH$), 23.06 ($\underline{C}H_2CH_2CHC$), 22.57 ($\underline{C}H_2CH_2CH$).

7.5 Synthesis of triazines with specific protocols.

7.5.1 2,4-Bis(4'-fluorophenethylamino)-6-(5'-(*N,N*-dimethylamino)pentylamino)-1,3,5-triazine (47).

A suspension of disubstituted triazine **23** (170 mg, 0.44 mmol, 1 eq) in THF (4 mL) was allowed to react with amine **21C** (178 μL, 1.32 mmol, 3 eq) for 3 h at 85 ºC under microwave activation (95 W, closed system). The reaction crude was diluted with water (20 mL) and extracted 3 times with EtAcO. The organic layer was evaporated and the residue was subjected to reverse phase chromatography to yield **47** as a yellow oil (48 mg, 23% yield).



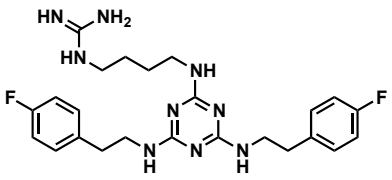
HRMS (ESI+): calculated for $C_{26}H_{35}N_7F_2$ (M+H), 484.3000; found, 484.2992.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.21-7.15 (H_{Ar}, cs, 4H), 7.02-6.96 (H_{Ar}, cs, 4H), 3.66-3.54 $NHCH_2$, cs, 4H), 3.43-3.31 ($NHCH_2$, bb, 2H), 2.90-2.82 (CH_2C_{Ar}, cs, 4H), 2.30 ($N(CH_3)C\underline{H}_2$, t, J = 7.0 Hz, 2H), 2.25 (CH_3, s, 6H), 1.66-1.47 ($C\underline{H}_2CH_2C\underline{H}_2$, m, 4H), 1.45-1.32 ($CH_2C\underline{H}_2CH_2$, m, 2H).

^{13}C NMR ($CDCl_3$, 48 ºC): 166.69-166.19 ($NC_{Ar}N$), 161.84 ($C_{Ar}F$, d, *J* = 244.2 Hz), 135.21 (C_{Ar}, d, *J* = 3.2 Hz), 130.35 ($C_{Ar}H$, d, *J* = 7.7 Hz), 115.49 ($C_{Ar}H$, d, *J* = 21.3 Hz), 59.82 ($N(CH_3)\underline{C}H_2$), 45.48 ($N(CH_3)_2$), 42.26 ($NHCH_2$), 40.76 ($NHCH_2$), 35.56 ($\underline{C}H_2C_{Ar}$), 30.01 ($\underline{C}H_2CH_2CH_2$), 27.41($CH_2CH_2\underline{C}H_2$), 24.96($CH_2\underline{C}H_2CH_2$).

7.5.2 2,4-Bis(4'-fluorophenethylamino)-6-(4'-guanidylbutylamino)-1,3,5-triazine (48).

A solution of 2,4,6-trichlorotriazine (**16**) (100 mg, 0.54 mmol) and mono-Fmoc-1,4-diaminobutane hydrochloride (208 mg, 0.60 mmol) in THF (6 mL) was allowed to react for 20 min at 100 ºC under microwave activation (100 W, closed system). Then, amine **20c** was added (350 µL, 2.70 mmol, 5 eq) to the crude and then it was heated again for 6 h at 110 ºC under microwave activation (150 W, closed system). The resulting crude reaction mixture was diluted with THF (20 mL), sedimented by centrifugation for 15 minuts, and the supernatant was poured off. The residue was suspended in THF (10 mL) and centrifuged a second time and the supernatant was separated as before. The joined supernatant fractions were evaporated under vacuum to give a crude residue containing the triazine intermediate **78**. This mixture was allowed to react with a solution of piperidine (20 %) in THF for 1.5 h at room temperature, diluted with AcOEt (20 mL), washed with 2 x H_2O (20 mL) and brine (20 mL) and dried over anhydrous $MgSO_4$. Finally, the crude reaction mixture obtained after elimination of solvents was dissolved in DCM (6 mL) containing triethylamine (40 µL) and treated with 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine (**80**) (130 mg, 0,33 mmol) for 2 h at room temperature. The crude reaction mixture was diluted with DCM (20 mL), washed with 2 M $NaHSO_3$ (20 mL), saturated solution of $NaHCO_3$ (20 mL), and brine (20 mL), and dried over anhidrous $MgSO_4$. The residue obtained after elimination of solvent was purified by HPLC at semipreparative scale (10 mL/min, 30 to 80 % ACN containing 0.1 % TFA in 50 min). The collected fractions were evaporated under vacuum, redissolved in a 1:1 DCM:TFA mixture and stirred for 30 min at room temperature. After addition of DCM (20 mL), the solution was washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL) and dried. Elimination of solvent afforded the expected pure triazine **48** as colorless oil. (60 mg, 23% yield).



HRMS (ESI+): calculated for $C_{24}H_{31}N_9F_2$ (M+H), 484.2749; found, 484.2729.

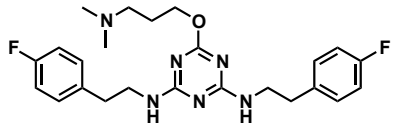
^{1}H NMR (CD_3CN_3, 7% TFA): 7.17-7.09 (H_{Ar}, cs, 4H), 7.03-6.95 (H_{Ar}, cs, 4H), 3.74-3.60 ($NHCH_2$, cs, 4H),

3.54-3.15 ($NHCH_2$ + $CNHCHC\underline{H}_2$, cs, 4H), 2.94-2.83 (CH_2C_{Ar}, cs, 4H), 1.76-1.59 ($CH_2C\underline{H}_2C\underline{H}_2CH_2$, cs, 4H).

^{13}C NMR (CD_3CN, 7% TFA): 162.84 ($C_{Ar}F$, d, *J* = 242.0), 158.30 ($C(NH)_3$), 156.54 (NCN), 136.14 (C_{Ar}), 131.75 ($C_{Ar}H$, d, *J* = 6.6 Hz), 116.20 ($C_{Ar}H$, d, *J* = 21.3 Hz), 43.30 ($NHCH_2$), 42.47-41.27 ($CNH\underline{C}H_2$ + $NHCH_2$), 35.37 ($\underline{C}H_2C_{Ar}$), 30,16 ($CH_2\underline{C}H_2CH_2CH_2$), 26.83($CH_2CH_2\underline{C}H_2CH_2$).

7.5.3 2-(3'-(*N,N*-dimethylamino)propoxy)-4,6-Bis(4'-fluorophenethylamino)-1,3,5-triazine (50).

A suspension of **23** (700 mg, 3.8 mmol) in aminoalcohol **21F** (4 mL) was heated at 120 ºC for 12 h in presence of 600 mg of anhydrous Na_2CO_3. The residue obtained after elimination of solvent was purified by semipreparative HPLC using mixtures of ACN and H_2O containing 0.1% TFA. The collected fractions were evaporated under vacuum, redissolved in EtOAc and washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$ to yield pure triazine **50** as a colorless oil (90 mg, 38% yield).



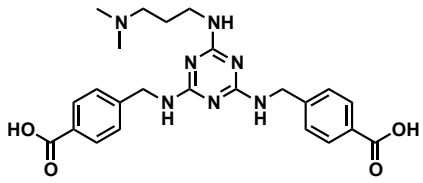
HRMS (ESI+): calculated for $C_{24}H_{30}N_6F_2O$ (M+H), 457.2527; found, 457.2515.

^{1}H NMR ($CDCl_3$, 48 ºC): 7.17-7.09 (H_{Ar}, cs, 4H), 7.00-6.92 (H_{Ar}, cs, 4H), 4.37-4.25 (H_{Ar}, cs, 2H), 3.67-3.55 ($NHC\underline{H}_2$, cs, 4H), 2.88-2.76 (CH_2C_{Ar}, cs, 4H), 2.61-2.52 ($N(CH_3)C\underline{H}_2$, cs, 2H), 2.34 ($N(CH_3)_2$, s, 6H), 2.25-1.92 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 ºC): 171.20 (NCN), 167.38 (NCN), 161.87 ($C_{Ar}F$, d, *J* = 244.7 Hz), 134.81 (C_{Ar}), 130.34 ($C_{Ar}H$, d, *J* = 7.9 Hz), 115.56 ($C_{Ar}H$, d, *J* = 21.2 Hz), 60.52 (CH_2O), 56.46 ($N(\underline{C}H_3)_2CH_2$), 45.11 ($CH_3$), 42.28 ($NHCH_2$), 35.33 ($\underline{C}H_2C_{Ar}$), 26.78 ($CH_2\underline{C}H_2CH_2$).

7.5.4 2,4-Bis(4'-carboxybenzylamino)-6-(3'-(*N,N*-dimethylamino)propylamino)-1,3,5-triazine (69).

Triazine **68** (279 mg, 0.55 mmol) was submitted to a hydrolysis protocol using 20 mL of 0.1 N NaOH in a mixture of MeOH/H_2O (60:40) at 40 ºC during 4 h. The crude reaction mixture was concentrated and purified by semipreparative HPLC to yield pure triazine **69** as colorless oil (160 mg, 61% yield).



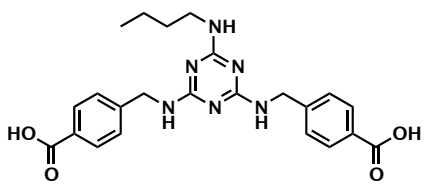
HRMS (ESI+): calculated for $C_{24}H_{29}N_7O_4$ (M+H), 480.2395; found, 480.2384.

^{1}H NMR (C_5D_5N, 48 ºC): 9.45-8.80 (OH, bb, 2H), 8.38 (H_{Ar}, d, *J* = 8.0 Hz, 4H), 7.63 (H_{Ar}, d, *J* = 7.7 Hz, 4H), 4.87 (CH_2C_{Ar}, s, 4H), 3.61 (NHC$\underline{H}_2$, t, *J* = 6.3 Hz, 2H), 3.09 (N(C$\underline{H}_3$)$_2$CH$_2$, t, *J* = 7.5, 2H), 2.70 (CH_3, s, 6H), 2.18 ($CH_2C\underline{H}_2CH_2$, m, 2H).

^{13}C NMR (C_5D_5N, 48 ºC): 169.28 (CO), 164.91 (NCN), 145.54 (C_{Ar}), 131.85 ($C_{Ar}H$), 131.12 (C_{Ar}), 128.38 ($C_{Ar}H$), 56.31 (N($\underline{C}H_3$)$_2$CH$_2$), 45.15 (CH_3), 43.19 ($\underline{C}H_2C_{Ar}$), 38.74 (NHCH$_2$), 25.81 ($CH_2\underline{C}H_2CH_2$).

7.5.5 2,4-Bis(4'-hydroxycarbonylbenzylamino)-6-(butylamino)-1,3,5-triazine (70).

The general procedure to synthesize trisubstituted triazines was employed using disubstituted triazine **35** and butylamine (**21O**). Then the solvent from the crude reaction mixture was evaporated and over this residue a similar hydrolytic protocol to that used for the preparation of **69** was performed. For that purpose 20 mL of 0.1 N NaOH in a mixture of MeOH/H_2O (60:40) were added over the residue and heated at 40 ºC during 4 h. The crude reaction mixture was concentrated and purified by semipreparative HPLC to yield pure triazine **70** as colorless oil (25 mg, 27% yield).



HRMS (ESI+): calculated for $C_{23}H_{26}N_6O_4$ (M+H), 451.2094; found, 451.2087.

^{1}H NMR (C_5D_5N, 48 ºC): 8.42-8.33 (H_{Ar}, d, *J* = 8.0 Hz, 4H), 7.66-7.57 (H_{Ar}, d, *J* = 7.8 Hz, 4H), 4.88 (CH_2C_{Ar}, s, 4H), 3.56 (NHC$\underline{H}_2$, t, *J* = 7.0, 2H), 1.3 ($CH_3CH_2C\underline{H}_2$, m, 2H), 1.44-1.35 ($CH_3C\underline{H}_2$, m, 2H), 0.90 (CH_3, t, *J* = 7.4 Hz, 3H).

^{13}C NMR (C_5D_5N, 48 ºC): 169.32 (CO), 166.61 (NCN), 146.20 (C_{Ar}), 131.73-131.12 ($C_{Ar}H$), 130.47 (C_{Ar}), 128.40-128.05 ($C_{Ar}H$), 45.21 (CH_3), 41.30 (NHCH$_2$), 32.76 ($CH_3CH_2\underline{C}H_2$), 20.83 ($CH_3\underline{C}H_2$), 14.42 (CH_3).

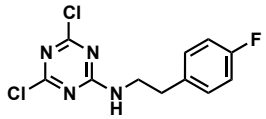
7.5.6 2-(4'-(Allylcarbamoyl)benzylamino)-4-(3'-(N,N-dimethylamino)propylamino)-6-(4'fluorophenethylamino)-1,3,5-triazine (83).

The synthesis was carried out in several steps, namely: three sequential and selective chlorine substitutions of **16** to obtain trisubstituted derivative **82** followed by the introduction of the allylamine moiety to afford **83**.

1,3,5-trichloro-2,4,6-triazine (**16**) (1 g, 5,42 mmol, 1 eq) was dissolved in THF (20 mL) at 0ºC. Then a solution of amine **20c** (1,5 g, 10.84 mmol, 2 eq) in THF (20 mL) was added dropwise in about 10 min under strong stirring. A precipitate slowly appeared. Once all the amine was added, the mixture was allowed to react for 2 h at

room temperature. Afterwards, the suspension was poured on water (200 mL) and the mixture was evaporated under vacuum to remove most of the THF. The solid in suspension was filtered, washed first with water (20 mL) then with cold methanol (3 x 5 mL), and finally dried to afford 1.38 g of a white solid essentially constituted by intermediate **85** (85 % yield).

7.5.6.1 2,4-dichloro-6-(4-fluorophenethylamino)-1,3,5-triazine (85).



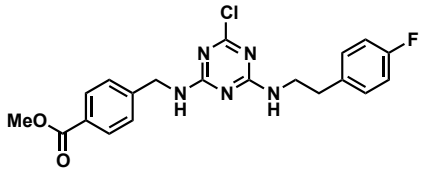
HRMS (ESI+) calculated for $C_{11}H_8Cl_2N_4F$ (M+H) 287.0267**,** found 287.0277.

^{1}H NMR ($CDCl_3$): 7.19-7.11 (H_{Ar}, m, 2H), 7.03-6.95 (H_{Ar}, m, 2H), 3.73 ($NHC\underline{H_2}CH_2$, q, *J* = 6.8 Hz, 2H), 2.89 ($NHCH_2C\underline{H_2}$**,** t, *J* = 7.0 Hz, 2H).

^{13}C NMR ($CDCl_3$): 170.03 ($C_{Ar}NH$), 166.10 ($C_{Ar}Cl$), 162.07 ($C_{Ar}F$, d, *J* = 245), 133.51 (C_{Ar}, d, *J* = 3.3), 130.35 ($C_{Ar}H$, d, *J* = 8), 115.79 ($C_{Ar}H$, d, *J* = 21), 42.79 ($NH\underline{C}H_2CH_2$), 34.57 ($NHCH_2\underline{C}H_2$).

To a solution of 950 mg of this solid in THF (5 mL), amine **20o** (1.90 mL, 6,6 mmol, 2 eq) was added. The reaction mixture was allowed to react under microwave activation for 10 min at 70 ºC (90 W, closed system). The crude reaction mixture was poured into H_2O (20 mL), heated for 10 min at 60 ºC and filtered. The precipitate was resuspended in water (20 mL), heated for 10 min at 60 ºC and filtered again, and the insoluble material was washed with cold absolute ethanol (3 x 5 mL) and dried, to give the expected disubstituted triazine **84** (900 mg, 65% yield) as a white powder which was used without further purification.

7.5.6.2 2-Chloro,4-(4'fluorophenethylamino)-6-(3'-(N,N-dimethylamino) propylamino) -1,3,5-triazine (84).

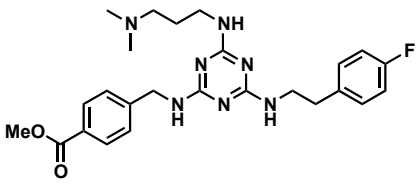


HRMS (ESI+): calculated for $C_{20}H_{17}N_5O_2ClF$ (M+H), 416.1290; found, 416.1286.

^{1}H NMR ($CDCl_3$, 7% TFA): 7.43-7.37 (H_{Ar}, m, 2H), 7.19-7.16 (H_{Ar}, cs, 2H), 7.09-6.96 (H_{Ar}, cs, 4H) 4.77-4.72 ($C_{Ar}C\underline{H_2}NH$, cs, 2H), 3.98 (CH_3O, s, 3H), 3.80-3.67 ($NHC\underline{H_2}CH_2$, cs, 2H), 2.92-2.82 ($NHCH_2C\underline{H_2}$, cs, 2H).

To a suspension of **84** (900 mg, 2.16 mmol, 1 eq) in THF (6 mL), amine **21A** (544 μL, 4.32 mmol, 2 eq) were added. The reaction mixture was allowed to react under microwave irradiation (20 min, 100 ºC, 110 W) in a sealed tube. Water (10 mL) and EtOAc (20 mL) were added to the crude reaction mixture and, after separation, the organic phase was washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. Elimination of solvent afforded product **82** as a colorless oil (940 mg, 90% yield) in essentially pure form.

7.5.6.3 2-(4'-(Methylcarboxy)benzylamino),4-(4'fluorophenethylamino)-6-(3'-(N,N-dimethylamino)propylamino)-1,3,5-triazine (82).



HRMS (ESI+): calculated for $C_{25}H_{32}N_7O_2F$ (M+H), 482.2680; found, 482.2686.

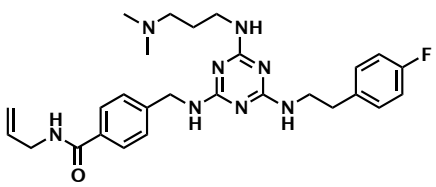
^{1}H NMR (CDCl3, 48 ºC): 8.02-7.92 (H_{Ar}, cs, 2H), 7.43-7.33 (H_{Ar}, cs, 2H), 7.21-7.04 (H_{Ar}, cs, 2H), 7.00-6.89 (H_{Ar}, cs, 2H), 4.67-4.57 ($C_{Ar}C\underline{H_2}NH$, cs, 2H), 3.90 (OCH_3, s, 3H), 3.63-3.50 ($NHC\underline{H_2}CH_2$, cs, 2H), 3.48-3.31 ($NHC\underline{H_2}CH_2$, cs, 2H), 2.87-2.74 ($NHCH_2C\underline{H_2}C_{Ar}$, cs, 2H), 2.40-2.31 ($N(CH_3)_2C\underline{H_2}$, cs, 2H), 2.23 ($N(CH_3)_2$, s, 6H), 1.76-1.65 ($CH_2C\underline{H_2}CH_2$, cs, 2H).

^{13}C NMR (CDCl3, 48 ºC): 166.93 (CO), 166.31 (NCN), 161.62 ($C_{Ar}F$, d, J = 243.9 Hz), 145.32 (C_{Ar}), 135.13 (C_{Ar}, d, J = 2.9 Hz), 130.16 ($C_{Ar}H$, d, J = 7.8 Hz), 129.80 ($C_{Ar}H$), 129.02 (C_{Ar}), 127.21 ($C_{Ar}H$), 115.24 ($C_{Ar}H$, d, J = 21.3 Hz), 57.67 ($N(CH_3)_2\underline{C}H_2$), 51.97 ($CH_3O$), 45.39 ($N(CH_3)_2$), 44.38 ($C_{Ar}C\underline{H_2}NH$), 42.11($NH\underline{C}H_2CH_2$), 39.46 ($NH\underline{C}H_2CH_2$), 35.34 ($NHCH_2C\underline{H_2}C_{Ar}$), 27.44 ($CH_2\underline{C}H_2CH_2$).

For the formation of the allylamide, **82** (370 mg, 0.77 mmol, 1 eq) and a catalytic amount of sodium acetate (3 mg, 0.037 mmol, 5 % eq) in allyl amine (2 mL, 26.6 mmol) were allowed to react for 1 h under microwave irradiation (110 W) at 130 ºC. Then, 10 mL of 2N HCl were added to the crude reaction mixture and it was extracted with EtOAc (20 mL). After separation, the organic phase was washed with water (3 x 20 mL) and brine (20 mL), dried over magnesium sulphate and concentrated under vacuum. The concentrated crude was purified by semipreparative HPLC using a solvent gradient (ACN-H_2O with 1% TFA: 20:100 → 100:0 in 30 min). The fractions of interest were joined, and 10 mL of 2N $NaHCO_3$ and 20 mL of EtOAc were added. After separation, the organic phase was washed with water (3 x 20 mL) and brine (1 x 20

mL), dried over magnesium sulphate and concentrated under vacuum to afford product **83** as a colorless oil (67 mg 17 % yield).

7.5.6.4 2-(4'-(Allylcarbamoyl)benzylamino)-4-(3'-(N,N-dimethylamino)propylamino)-6-(4'fluorophenethylamino)-1,3,5-triazine (83).



HRMS (ESI+): calculated for $C_{27}H_{36}N_8OF$ (M+H), 507.2996; found, 507.3001.

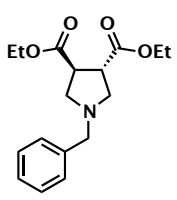
^{1}H NMR ($CDCl_3$, 48 ºC): 8.01-7.96 (**f**, m, 2H), 7.40-7.34 (H_{Ar}, cs, 2H), 7.19-7.06 (H_{Ar}, cs, 2H), 7.01-6.92 (H_{Ar}, cs, 2H), 6.00-5.87 ($CH_2C\underline{H}CH_2$, cs, 1H), 5.30-5.14 ($C\underline{H}_2CHCH_2NHCO$, cs, 2H), 4.70-4.53 ($C_{Ar}C\underline{H}_2NH$, cs, 2H), 3.91 ($CONHC\underline{H}_2$, s, 2H), 3.62-3.52 ($NHC\underline{H}_2CH_2C_{Ar}$, cs, 2H), 3.51-3.40 ($NHC\underline{H}_2CH_2$, cs, 2H), 2.88-2.76 ($NHC\underline{H}_2CH_2$, cs, 2H), 2.75-2.39 ($NHCH_2C\underline{H}_2C_{Ar}$ + $N(CH_3)_2$, cs, 8 H), 1.89-1.78 ($CH_2C\underline{H}_2CH_2$, cs, 2H).

^{13}C NMR ($CDCl_3$, 48 ºC): 167.00 (CO), 163.46 (NCN), 161.82 ($C_{Ar}F$, d, J = 245.4 Hz), 144.48 (C_{Ar}), 134.79 (C_{Ar}), 130.30 ($C_{Ar}H$, d, J = 7.8 Hz), 130,17 ($CONHCH_2\underline{C}HCH_2$), 130.02 ($C_{Ar}H$), 129.38 ($C_{Ar}$), 127.35 ($C_{Ar}H$), 115.48 ($C_{Ar}H$, d, J = 21.2 Hz), 57.02 ($N(CH_3)_2\underline{C}H_2$), 52.19 ($CONH\underline{C}H_2CHCH_2$), 44.56 ($NH\underline{C}H_2CH_2C_{Ar}$), 44.34 ($N(CH_3)_2$), 42.29 ($NH\underline{C}H_2CH_2C_{Ar}$), 35.22 ($NHCH_2\underline{C}H_2C_{Ar}$), 25.99 ($CH_2\underline{C}H_2CH_2$).

7.6 Synthesis of pyrrolidines.

7.6.1 rac-(3S,4S)-(1-Benzyl-pyrrolidine-3,4-dicarboxylic acid diethylester (90).

N-Benzyl-glycine (1.00 g, 6 mmol), diethyl fumarate (1.06 g, 6.2 mmol), and paraformaldehyde (222 mg, 7.5 mol) were suspended in 9 mL of toluene and heated to reflux with a Dean–Stark trap. After 1 h the solution was filtered, concentrated in a rotary evaporator and the residual liquid distilled at 159 ºC (0.9 mbar) to give the desired product (**90**) (1.23 g 67 % yield).



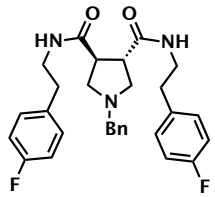
HRMS (ESI+): calculated for $C_{17}H_{23}NO_4$ (M + H), 306.1705; found, 306.1717.

^{1}H NMR ($CDCl_3$): 7.30 (H_{Ar}, s, 2H), 7.29 (H_{Ar}, s, 2H), 7.26 – 7.23 (H_{Ar}, m, 1H), 4.14 ($CH_3C\underline{H}_2CO$, q, J = 7.1 Hz, 4H), 3.61 ($NC\underline{H}_2C_{Ar}$, s, 2H), 3.48 – 3.39 ($C\underline{H}CO$, m, 2H), 2.90 ($NC\underline{H}_2CH$, t, J = 8.6 Hz, 2H), 2.79 ($NC\underline{H}_2CH$, dd, J = 9.4, 5.7 Hz, 2H), 1.23 (CH_3, t, J = 7.1 Hz, 6H).

^{13}C NMR (CDCl3): 173.48 (CO), 128.97 (C_{Ar}), 128.56 ($C_{Ar}H$), 128.25 ($C_{Ar}H$), 127.22 (C_{Ar}), 61.15 (CH_2O), 59.45 ($C_{Ar}\underline{C}H_2N$), 56.70 ($CH\underline{C}H_2N$), 45.63 ($\underline{C}HCH_2$), 14.33 (CH_3).

7.6.2 rac-(3*S*,4*S*)-1-benzyl-*N*^{3},*N*^{4}-bis(4-fluorophenethyl)pyrrolidine-3,4-dicarboxamide (89).

Pyrrolidine **90** (1.2 g, 3.93 mmol, 1 eq) was dissolved in amine **20c** (4.12 mL, 31.3 mmol, 8 eq) and then a catalytic amount of NaOAc (32 mg, 0.39 mmol, 0.1 eq) was added to the mixture. The reaction mixture was allowed to react at 100 ºC for 30 h. Then the crude was poured in a decantation flask containing EtAcO (30 mL) and HCl (20 mL, 1N). After separation, the organic layer was washed with HCl (2 x 30 mL, 1N) and brine (1 x 20 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography using as eluent a mixture of Hexane/DCM/MeOH containing 7N ammonia (50:45:5) to afford pyrrolidine **89** (650 mg, 34 % yield).



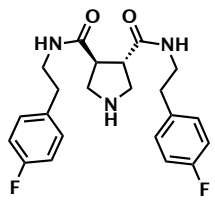
HRMS (ESI+): calcd for $C_{29}H_{31}N_3O_2F$ (M + H), 492.2463; found, 492.2442.

^{1}H NMR ($CDCl_3$): 7.36 - 7.29 (CH_{Ar}, m, 3H), 7.25-7.22 (CH_{Ar}, m, 2H), 7.16 - 7.10 (CH_{Ar}, m, 4H), 6.98 - 6.92 (CH_{Ar}, m, 4H), 3.70 ($C_{Ar}CH_2N$, s, 2H), 3.51 - 3.38 (NHC$\underline{H}_2$, m, 4H), 2.96 - 2.79 (NC$\underline{H}_2$C$\underline{H}$, m, 6H), 2.76 ($CH_2C\underline{H}_2C_{Ar}$, t, *J* = 7.1 Hz 4H).

^{13}C NMR ($CDCl_3$): 173.10 (CO), 161.75 ($C_{Ar}F$, d, *J* = 244.5 Hz), 136.87 (C_{Ar}), 134.55 (C_{Ar}, d, *J* = 3.2 Hz), 130.32 ($C_{Ar}H$, d, *J* = 7.8 Hz), 129.04 ($C_{Ar}H$), 128.80 ($C_{Ar}H$), 128.04 (C_{Ar}), 115.46 ($C_{Ar}H$, d, *J* = 21.2 Hz), 59.52 (NCH_2C_{Ar}), 55.75 ($\underline{C}HCO$), 48.24 ($N\underline{C}H_2CH$), 40.85 ($\underline{C}H_2CH_2C_{Ar}$), 34.94 ($CH_2\underline{C}H_2C_{Ar}$).

7.6.3 rac-(3S,4S)-N3,N4-bis(4-fluorophenethyl)pyrrolidine-3,4-dicarboxamide (88).

150 mg of pyrrolidine **89** (0.30 mmol, 1 eq) and 30 mg of 5% Pd/C (15 μmol, 5% eq) catalyst were suspended on EtOH (30 mL) under a positive pressure of hydrogen (3 atm) and the mixture was allowed to react for 24 h. The reaction was monitored by TLC. Once the reaction was completed the crude mixture was filtered through a small pad of celite, and the solvent was removed from the filtrate at low pressure to afford the desired product (**88**) (121 mg, 99 % yield).



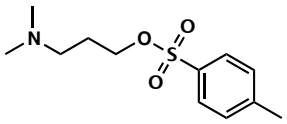
HRMS (ESI+): calcd for $C_{22}H_{25}N_3O_2F$ (M + H), 402.1993; found, 402.1992.

^{1}H NMR ($CDCl_3$): 7.15 - 7.07 ($C_{Ar}H$, m, 4H), 7.00 - 6.93 ($C_{Ar}H$, m, 4H), 3.53 - 3.36 ($C\underline{H}_2CH_2C_{Ar}$, m, 4H), 3.15 ($NC\underline{H}_2CH$, dd, *J* = 11.2, 7.3 Hz, 2H), 3.01 ($NC\underline{H}_2CH$, dd, *J* = 11.2, 6.0 Hz, 2H), 2.89 ($NCH_2C\underline{H}$, p, *J* = 6.5 Hz, 2H), 2.76 ($CH_2C\underline{H}_2C_{Ar}$, t, *J* = 7.1 Hz, 4H).

13C NMR ($CDCl_3$): 173.46 (CO), 161.79 ($C_{Ar}F$, d, *J* = 244.6 Hz), 134.41 (C_{Ar}, d, *J* = 3.4 Hz), 130.25 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.55 ($C_{Ar}H$, d, *J* = 21.2 Hz), 51.64 (CHCO), 50.03 (NCH_2CH), 40.91($CH_2CH_2C_{Ar}$), 34.99 ($CH_2CH_2C_{Ar}$).

7.6.4 3-(dimethylamino)propyl tosylate (91).

570 μL of 3-(dimethylamino)propanol (0.50 g, 4.8 mmol, 1 eq) and 1.16 mL of pyridine (1.14 g, 14.4 mmol, 3 eq) were dissolved in DCM (2.5 mL). Tosyl chloride (1.85 g, 9.70 mmol, 2 eq) was added as a solid under inert atmosphere and the mixture was allowed to react at room temperature overnight. A white solid was formed which was filtered and washed with DCM (3 x 8 mL) yielding the desired tosylate (**91**) (560 mg, 45 % yield).



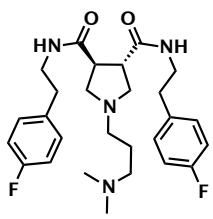
HRMS (ESI+): calcd for $C_{12}H_{19}NSO_3$ (M + H), 258.1164; found, 258.1157.

^{1}H NMR ($CDCl_3$): 7.76 ($C_{Ar}H$, d, *J* = 8.4 Hz, 4H), 7.36 ($C_{Ar}H$, d, *J* = 8.0 Hz, 4H), 4.12 (OCH_2, t, *J* = 5.7 Hz, 2H), 3.19 – 3.08 (NCH_2, m, 2H), 2.80 ($N(CH_3)_2$, d, *J* = 4.9 Hz, 6H), 2.45 (CH_3C_{Ar}, s, 3H), 2.37 – 2.26 ($CH_2CH_2CH_2$, m, 2H).

^{13}C NMR ($CDCl_3$): 145.61 (C_{Ar}), 132.26 (C_{Ar}), 130.26 ($C_{Ar}H$), 128.06($C_{Ar}H$), 67.27 (OCH_2), 55.16 (NCH_2), 43.38 ($N(CH_3)_2$), 27.30 ($CH_2CH_2CH_2$), 21.80 ($C_{Ar}CH_3$).

7.6.5 rac-(3*S*,4*S*)-1-(3-dimethylamino)propyl-*N*^{3},*N*^{4}-bis(4-fluorophenethyl) pyrrolidine-3,4-dicarboxamide (86).

Pyrrolidine **88** (50 mg, 0.12 mmol, 1 eq) and potassium carbonate (38 mg, 0.27 mmol, 2.2 eq) were mixed in 7.50 mL of ACN and the suspension was heated at 60 ºC overnight. After this time the crude was allowed to cool and, once at room temperature, tosilate **91** (38 mg, 0.15 mmol, 1.2 eq) was added as a solid. Temperature was raised to 60 ºC and the mixture was allowed to react for 12 h. Once the reaction was complete as shown by TLC, the solvent was removed at reduced pressure and the crude reaction mixture was subjected to semipreparative-HPLC purification using water-ACN mixtures containing 0.1% TFA as eluent. The fractions of interest were collected and the solvent was evaporated affording the purified pyrrolidine as TFA salt. To recover the free pyrrolidine, the product was dissolved in EtOAc (20 mL) and wahed with saturated $NaHCO_3$ (20mL), water (3 x 20 mL), and brine (1 x 20 mL) and dried over anhidrous $MgSO_4$. Finally, the solvent was removed on a rotary evaporator to yield the desired pyrrolidine **86** (36 mg, 60 % yield).



HRMS (ESI+): calcd for $C_{27}H_{36}N_4O_2F_2$ (M + H), 487.2885; found, 487.2877.

^{1}H NMR ($CDCl_3$): 7.18 - 7.13 ($C_{Ar}H$, m, 4H), 6.99 - 6.94 ($C_{Ar}H$, m, 4H), 3.45 ($C\underline{H}_2CH_2C_{Ar}$, dd, *J* = 13.4, 6.8 Hz, 4H), 2.96 - 2.90 ($CH_2C\underline{H}CO$, m, 2H), 2.87 - 2.68 ($NC\underline{H}_2CH$, m, 4H), 2.79 ($CH_2C\underline{H}_2C_{Ar}$, t, *J* = 7.3 Hz, 4H), 2.65 - 2.49 ($C\underline{H}_2CH_2C\underline{H}_2$, m, 4H), 2.40 ($CH_3$, s, 6H), 1.75 - 1.65 ($CH_2C\underline{H}_2CH_2$, m, 2H).

^{13}C NMR ($CDCl_3$): 173.42 (CO), 161.69 ($C_{Ar}F$, d, *J* = 244.2 Hz), 134.76 (C_{Ar}, d, *J* = 3.2 Hz), 130.36 ($C_{Ar}H$, d, *J* = 7.8 Hz), 115.37 ($C_{Ar}H$, d, *J* = 21.1 Hz), 57.73 ($N(CH_3)_2\underline{C}H_2$), 55.63 ($\underline{C}HCO$), 53.44 ($N(CH_3)_2CH_2CH_2\underline{C}H_2$), 48.14 ($N\underline{C}H_2CH$), 44.93 ($N(CH_3)_2$), 40.89 ($\underline{C}H_2CH_2C_{Ar}$), 34.98 ($CH_2\underline{C}H_2C_{Ar}$), 25.15 ($CH_2\underline{C}H_2CH_2$).

7.6.6 rac-(3*S*,4*S*)-1-(3-dimethylamino)propyl-*3,4*-bis(4-fluorophenethylamino methyl)pyrrolidine (87).

20 mg of diamide **86** (0,04 mmol, 1 eq) were dissolved in 1 mL of anhydrous THF under innert atmosphere. Once the diamide was dissolved, 330 µL of a 1N borane solution in THF (0,33 mmol, 8 eq) were added dropwise. Once the borane solution was added reaction mixture was hold at 75 ºC for 7 h. Then, the crude reaction mixture was cooled in an ice bath and quenched with 80 µL water. The solvent was removed at low pressure and 800 µL of a 6 N HCl solution were added over the reaction residue. Crude reaction mixture was refluxed for 4 h. After this period the solvent was removed at low pressure yielding 15 mg of a mixture of both mono and di reduced products in their corresponding chlorhidrate form. Attempts to isolate the reduced amide **87** by preparative HPLC were unsuccessful.

7.7 Biological Methods.

All cloning, expression and electrophysiological TRPV1 assays were carried out in collaboration with the group of Professor Ferrer-Montiel, from the Universidad Miguel Hernandez (Elche), using procedures developed by the same group.[146] Male Wistar rats (250–300 g) used in *in vivo* experiments were obtained from Janvier, Holland. Experimental procedures were approved by the Ethics Committee and met European Union guidelines for care and management of experimental animals.

7.7.1 Recombinant rat TRPV1 channels expression in Xenopus oocytes and channel blockade.

All the procedures have been described in detail elsewhere.[83,216] Whole-cell currents from oocytes expressing rat TRPV1 were recorded in Mg^{2+}-Ringer's solution (10 mM Hepes pH 7.4, 115 mM NaCl, 2.8 mM KCl, 0.1 mM $BaCl_2$, 2.0 mM $MgCl_2$) with a two-microelectrode voltage-clamp amplifier at 20 °C. TRPV1 channels were activated by application of 10 µM capsaicin in absence or presence of individual compounds at a holding potential (V_h) of -60 mV. Dose-response curves for individual products were fitted to a Hill equation:

$$\frac{I}{I_{max}} = \frac{1}{1+\left(\frac{[blocker]}{IC_{50}}\right)^{n_H}}$$

Equation ix: Hill equation. I/I_{max} is the ratio of current in the presence and absence of blocker, IC_{50} is the 50 % inhibitory concentration, and n_H denotes the Hill coefficient, which is an estimate of the number of drug binding sites.

I-V characteristics were recorded using a ramp protocol.[216,217] Oocytes were depolarized from -80 mV to 20 mV in 5 s (20 mV/s). Leak currents were measured in the absence of agonist in the external bath medium and subtracted from the ionic current recorded in the presence of the ligand. Voltage dependence of channel blockade was studied as described by Ferrer-Montiel et al.[217] Experimental data were fitted to either the Hill or Woodhull equations[217] with a nonlinear least-squares regression algorithm using GraphPad Prism 5 software.

7.7.2 MTT Cytotoxicity Assay.

Cell viability was assessed by the detection of mitochondrial activity in living cells using a modified colorimetric analysis of Thiazolyl Blue Tetrazolium Bromide (MTT).[218] Briefly, HEK293 cells (2×10^4 cells/well) were subcultured in 96-well plates, grown until 80-90% confluence, and incubated with increasing concentrations of the product of interest for 24 h (100 µM to 1nM). Following treatment, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well and further incubated for 4 h at 37°C. Subsequently, 100 µL of DMSO was added to each well to dissolve any deposited formazan resulting from cleavage and reduction of MTT by active mitochondrial dehydrogenases. The optical density of each well was measured at 540 nm with a microplate reader (Polastar BMG LABTECH, Offenberg, Germany).

7.7.3 Rat knee joint nociceptor fiber preparation and in vivo recording.

Adult male Wistar rats (270-370 g; n = 10) were initially anesthetized with an intraperitoneal (i.p.) injection of ketamine (75 mg/kg) and xilacine (10 mg/kg). Supplementary doses of 40 mg/Kg of sodium pentobarbital were supplied in order to maintain a deep level of anesthesia throughout the experiment. The trachea, the left femoral vein and the femoral artery were cannulated. Arterial blood pressure was continuously monitored. An additional catheter was inserted into the right saphenous artery for local intra-arterial injection of substances into the joint area. The rats were paralyzed by intravenous administration of gallamine triethiodide (1 mg/100 g of bodyweight). The right femur was fixed by a special grip and a pool was formed by skin flaps and filled with warm paraffin oil. The saphenous nerve was dissected and fine filaments were subdissected from the peripheral end. Nerve fibres innervating the knee joint were identified by the location of their receptive field that was determined by the firing response to probing the structures in and around the knee joint with a hand-held glass.[219] The mechanical stimuli consisted of normal and noxious outward and inward rotation of the knee joint lasting 10 s. Activation of the units by close intra-arterial injection of KCl (0.1 mM, 0.1 ml) was used to ascertain that solutions reached the sensory endings in the knee joint. Successful experiments included complete recordings in 20 multiunits filaments containing between two and five identifiable units.

7.7.4 Thermal sensitivity (Hot Plate test).

To assess the sensitivity to thermal stimulation, triazine **46** was administered through an i.p. injection at 10 mg/kg to a control group of mice (sham operated) and a group of mice whose bile duct had been ligated. Thereafter, mice were located over a hot plate at 52 ºC (LE7406 Hot-Plate, Harvard Apparatus) and the latency to one of the following nocifensive behaviours was measured: licking, biting, lifting, guarding or shaking of the hind paws, or jumping. Once scored, the heating was switched off. A cut-off time of 30 s was established to avoid tissue injury. The time that the mice spent over the hot plate without exhibiting a nocifensive behavior over the plate was recorded as Paw Withdrawal Latency (PWL).[69]

7.7.5 CFA Inflammatory Model.

Complete Freund's adjuvant (CFA) emulsion (1:1 oil/saline, 0.5 mg/ml) was injected into the plantar surface (50 µl) of the left hind paw.[83] Compounds were intravenously (i.v.) administered at 10 mg/kg 24 h after CFA injection. Thermal

hyperalgesia was monitored 24 h after CFA injection and up to 4 h after administering the compounds with an Ugo Basile Plantar Test (Hargreaves Apparatus). In brief, rats were habituated to an apparatus consisting of individual Perspex boxes on an elevated glass table. A mobile radiant heat source was located under the table and focused on the hind paw. Paw withdrawal latencies were defined as the time taken by the rat to remove its hind paw from the heat source. A cutoff point of 25 s was set to prevent tissue damage.

7.8 Computational Methods.

7.8.1 Molecular modeling and alignment of data set.

The structures of the compounds included in the 3D-QSAR study were built and energy minimized using the program suite MOE.[193] The implemented MMFF94x force field, a modified version of the MMFF94s force field,[191,192] was used for all energy calculations. Ionizable groups were protonated or unprotonated according to their predicted ionization state at physiological pH, as calculated with Epik (see Annex 3).[220-222] That is, all compounds that include a dimethylamine moiety were modeled in the protonated state, all compounds with pKa values under 7 were considered to be unprotonated. Compounds were structurally aligned using two minimum energy extended conformations for compounds **46** and **72** as templates and the Flexible Alignment module implemented in MOE. The alignment algorithm parameters were adjusted to achieve the best overlapping of the triazine core (for compounds of triazine family compounds) and of features such as aromatic rings, hydrophobic moieties, hydrogen bond donors-acceptors and molecular volume.

7.8.2 Generation of CoMFA and CoMSIA fields.

The program Sybyl[181] was used for the 3D-QSAR analysis. Potential fields for CoMFA (steric and electrostatic) and CoMSIA (steric, electrostatic, hydrophobic, donor and acceptor) were calculated using the default Sybyl settings, except for the grid spacing. Thus, 3D-cubic grids with 2, 1 and 0.5 Å grid spacings, which enclosed the aligned compounds and extended 4 Å beyond any of their atoms, were defined. MMFF94 partial atomic charges were calculated for every compound. The steric and

electrostatic CoMFA fields were calculated at each point of the grids from the Lennard-Jones and coulombic terms of the interaction potential with the default sp3 charged C+ atom probe. Similarly, the CoMSIA fields were derived according to Klebe et al.[179] using the default probe with charge +1, radius 1 Å and hydrophobicity +1.

7.8.3 PLS-based CoMFA and CoMSIA model derivation.

Model derivation employing partial least-squares (PLS) regression was carried out as implemented in Sybyl. Leave-one-out (LOO) cross-validation, as determined by the SAMPLS method,[184] was used for the fast determination of statistical significance (ie. q^2 and standard error of prediction, SEP) of different combination of fields and parameters (ie. column filtering). Random groups cross validation using 10 groups was repeated 10 times and average statistical values were calculated to further assess the robustness and statistical confidence of the best models. The final non cross-validated models were developed using the optimal number of components that had both the highest q^2 and smallest SEP values. Quality of these models was assessed through their statistics (ie. squared correlation coefficient (r^2), standard error of estimate (SEE) and F-value) and by prediction of the activities of the compounds in the test set.