Design and Synthesis of Specific Probes for Human 5-HT 4 Receptor Dimerization Studies

Jean-Louis Soulier, Olivier Russo, Mireille Giner, Lucie Rivail, Magali Berthouze, Sandrine Ongeri, Bernard Maigret, Rodolphe Fischmeister, Frank Lezoualc'H, Sames Sicsic, et al.

Title : Design and Synthesis of Specific Probes for Human 5-HT_4 Receptor Dimerization Studies.

Authors : Jean-Louis Soulier,[♠] Olivier Russo,[♠] Mireille Giner,[♠] Lucie Rivail,[♠] Magali Berthouze[♦], Sandrine Ongeri,[♠] Bernard Maigret[♣]; Rodolphe Fischmeister[♦], Frank Lezoualc'h[♦], Sames Sicsic[♠] and Isabelle Berque-Bestel[♠*].

Université de Paris XI, Biocis, [♠]UMR C8076 (CNRS), Laboratoire de Reconnaissance Moléculaire et Synthèses, [♦]INSERM U-446, Laboratoire de Cardiologie Moléculaire et Cellulaire , IFR-75 ISIT, Faculté de Pharmacie, 5, rue Jean-Baptiste Clément, 92296 Châtenay-Malabry 92296, France.

[♣] Equipe de Dynamique des Assemblages Membranaires, UMR-7565, Université Henri Poincaré, 54506 Vandœuvre les Nancy, France.

[*] To whom correspondence should be addressed : Faculté de Pharmacie, 5, rue J. B. Clément, 92296 Châtenay-Malabry Cedex, France. Phone: 33(0)146835743, fax: 33(0)146835740, isabelle.bestel@cep.u-psud.fr

Abstract :

Recently, human 5-HT_4 receptors have been demonstrated to form constitutive dimers in living cells. In order to evaluate the role of dimerization on the 5-HT_4 receptor function, we investigated the conception and the synthesis of bivalent molecules able to influence the dimerization process. Their conception is based on a model of the 5-HT_4 receptor dimer derived from protein /protein docking experiments. These bivalent ligands are constituted by two ML10302 units, a specific 5-HT_4 ligand, linked through a spacer of different sizes and natures. These synthesized bivalent ligands were evaluated in binding assays and cyclic AMP production on the 5-$HT_{4(e/g)}$ receptor isoform stably transfected in C6 glial cells. Our data showed that bivalent ligands conserved a similar affinity compared to the basal ML10302 unit. Nevertheless, according to the nature and the size of the spacer, the pharmacological profile of ML10302 is more or less conserved. In view of the interest of bivalent ligands for investigating GPCR dimerization process, these 5-HT_4 specific bivalent ligands constitute valuable pharmacological tools for the study of 5-HT_4 receptor dimerization.

Introduction

The concept that most G protein coupled receptors (GPCRs) can exist as dimeric entities is now largely accepted. However the reason for this dimerization remains elusive. Although dimerization or oligomerization of GPCR has been proved to participate in receptor function, including agonist affinity, potency and efficacy and G protein specificity, it is difficult to conclude whether dimerization or oligomerization is functionally essential for all GPCRs.[1-5]

Elucidating the role of dimerization in the activation process of GPCR hardly became a new challenge which might lead to develop novel pharmaceutical agents able to promote activation or inhibition of GPCR signaling.

Recently, constitutive human 5-HT_4 receptor dimers were observed in living cells and membrane preparations of CHO and HEK293 cells as detected by co–immunoprecipitation and BRET experiments. 5-HT_4 receptor dimerization was not influenced by the binding of classical 5-HT_4 ligands: agonists such as serotonin, prucalopride, ML10302 or antagonists such as GR113808.[6] Considering the ubiquitous involvement of the 5-HT_4 receptor in major diseases such as memory disorders, gastrointestinal disorders, hypertension, atrial arrhythmia and dysfunction of the urinary tract, the research of 5-HT_4 related new drugs is fundamental.[7] Moreover, recent data have underlined a role of the 5-HT_4 receptor in the amyloid precursor protein (APP) metabolism, a key gene involved in Alzheimer's disease (AD).[8] Together with its role in learning and memory, these results suggest that the 5-HT_4 receptor may constitute a new pharmacological target for the treatment of AD.[9]

Although many 5-HT_4 ligands were synthesized during this last decade, only two drugs indicated for the irritable bowel syndrome became commercially available.[7]

It appears now evident that a better knowledge of GPCRs signaling pathways as well as the characterization of the physiological role of GPCR dimerization process could open new avenues for the design of efficient drugs.

In this context, bivalent ligands, which were proved to modulate the dimerization process by binding dimeric receptors may represent valuable pharmacological tools. In a recent study, Bushan *et al.* demonstrated the importance of bivalent ligands approach in the study of δ – κ opioid receptor heterodimers. Their heterobivalent ligands selective for heterodimeric opioid receptors constituted probes for targeting different tissues.[10,11]

In this paper, we describe the design and the synthesis of specific 5-HT_4 bivalent ligands, based on molecular modeling studies and more particularly on receptor-receptor docking experiments. Those bivalent ligands are constituted by two 5-HT_4 pharmacophoric parts linked with a spacer. Many spacers were used varying in nature and in size. The pharmacological properties of the synthesized bivalent ligands were evaluated.

Identification of dimer interfaces

In order to understand the functional role of dimerization, and the structural mechanism for cross-talk between receptors in a dimeric complex we planed to design 5-HT_4 bivalent ligands as chemical tools. For that we focused first on the identification of the dimer interfaces.

Molecular modeling studies were performed and more particularly protein-protein docking experiments for investigating likely dimer arrangements. Docking was realized from the three dimensional model of 5-HT_4 receptor recently published, based on the crystallized bovine rhodopsin structure,[12] and the specialized software GRAMM (Global Range Molecular Matching), which considers the proteins as rigid entities was used.[13] The procedure implements an exhaustive grid search for the receptor-receptor structure matches. For quantifying possible interactions between two receptors, GRAMM places each monomer in a three-dimensional grid. Then, an exhaustive research in the three dimensional spaces is realized using translations and rotations. Finally, this software evaluates the surface complementarities, penalizes covering and orders the obtained results.

In this study we have applied the low-resolution docking with a 6.8 Å grid step, using the special mode for helix in privileging the hydrophobic interactions. One of the two monomers was rotated with 10°-angle intervals. For each complex, the 100 lowest-energy matches were sorted from low to high energy and then analyzed visually. The values of the different parameters were determined as quasi optimal after many experiments of validation and based to the results of Vasker *et al*. [14,15]

From the 100 structures generated by GRAMM with the optimized parameters, two main structures of dimers emerged with 40% of II-IV/II-IV complexes including helices II and IV in their dimer interfaces and 40% of IV-VI/IV-VI complexes including helices IV and VI

(Figure 1). So, the two most favorable complexes obtained by this docking procedure both involve helix IV. This result agrees well with results of literature which established that in GPCR A family, helix IV is likely to be involved in receptor-receptor contacts. Different biological experiments have identified this transmembrane domain as potential interfaces for dopamine D_2 receptors dimerization,[16] γ-opioid receptors or C5a receptors.[17] The most convincing argument derives from the application of atomic force microscopy to the organization of rhodopsin in its native membrane.[18] Models based on these pictures were inferred to indicate that receptor-receptor links might be provided by contacts between helices IV and V. Moreover, there is some evidence in the literature that similar dimerization motifs in transmembrane domains may be involved in dimerization interfaces. Thus in several mammalian GPCRs, (AGSTP)XXX(GAS) sequence types in transmembrane domains are known to play a key role in receptor dimerization and can be used as a consensus motif to identify potential dimerization sequences.[19] In the 5-HT_4 receptor, analysis of the receptor sequence revealed the presence of a A4.43XXXG4.47 motif in helix IV and visualization of our molecular model indicated a correct orientation of this motif to allow receptor-receptor interactions.

Design of bivalent ligands

Bivalent ligands should be constituted by two 5-HT_4 ligands linked through a spacer. ML10302,[20] a 5-HT_4 receptor specific ligand synthesized in our laboratory was selected (scheme 1). This molecule allows different anchor points for the spacer. For example, a spacer could be introduced on the methoxy group affording an ether bivalent ligand, or on the amino function giving rise to bivalent ligands linked through the anilic position of ML10302. Finally, a link from the 4 position of the piperidine ring of ML10302 can also be afforded.

The two complexes suggested by the GRAMM docking procedure can be used to orient the design of bivalent ligands. For example, in the hypothesis of receptor dimers interfaced by

helices II and IV, a convenient positioning of bivalent ligands suggests to link ML10302 parts through the 4 position of the piperidine ring of ML10302 (Scheme 2). In contrary, the hypothesis of complexes IV-VI/IV-VI should require bivalent ligands linked through the methoxy or the amino function of the aromatic ring of ML10302.

We first focused on II-IV/II-IV complexes and on bivalent ligands linked through the 4 position of the piperidine ring of ML10302. Previous studies on ML10302 molecules had underlined the possibility of substitution of the basic amino part with a voluminous group such as fluorescent probes without penalizing the affinity of molecules.[21] These results were supported by the site directed mutagenesis and molecular modeling results which showed a large pocket between helices III and VI extended to helix VII.[12] Altogether, these observations indicated that the substitution of the spacer on this part of ML10302 should not affect the affinity of bivalent ligands (Scheme 2).

The theoretical II-IV/II-IV complexes obtained were used as templates in order to evaluate the optimal length of the spacer. In order to identify compounds specifically interacting with receptor dimers, we synthesized bivalent ligands possessing different spacer lengths: short length spacers, insufficient for allowing the two pharmacophoric parts to bind to receptor dimers, longer spacers, allowing this binding *via* the lipid bilayer and excessively long spacer to permit bridging of receptor dimers. The influence of the chemical nature of the spacers was also examined with polar oxygenated chains, hydrophobic alkyl chains and alkyl chains including a central cyclic core. The spacers were connected through an amidic function to the 4 position of the piperidine ring of ML10302. In order to introduce some flexibility at this level, the amidic function was first introduced directly on the piperidine ring and then through one and two methylene groups. Taken as a whole, three different series of bivalent ligands were synthesized according to the nature of the spacer: (i) a polar oxygenated spacer series (compounds **6a-c**); (ii) a hydrophobic spacer series (compounds **10a-d**, **11a-d**, **12a-d**, **13a-d**,

16a-d and **18a-b**); (iii) a central cyclic core alkyl spacer series (compounds **23**, **24**, **27** and **32**). In hydrophobic spacer series, two ligands possessing only one pharmacophore head linked to capped spacers were also synthesized, for comparison with bivalent ligands.

Synthesis of bivalent ligands

Molecules **6a-c** were prepared as described in Scheme 3. In the first step, condensation of piperidine-4-carboxylic acid *t*-butyl ester with 4-amino-5-chloro-2-methoxy-benzoic acid 2-bromo-ethyl ester[20] in presence of diisopropylethylamine at 40-50 °C in dry DMF afforded **2**. The acidic hydrolysis of **2** with excess of CF_3COOH in dry CH_2Cl_2 gave the acid **3** with a good yield. Coupling of **3** with Fmoc-2-(2-aminoethoxy)-ethylamine hydrochloride or Fmoc-1-amino-3,6-dioxa-8-octanamine or Fmoc-1-amino-4,7,10-trioxa-13-tridecamine hydrochloride using a general procedure (EDC, HOBt, and NEt_3 in dry CH_2Cl_2) provided respectively **4a-c** with moderate yields. Removal of the Fmoc protecting group using piperidine 20% in DMF and subsequent treatment with MeOH/HCl gave compounds **5a-c.** Compounds **5a-c** were then coupled to **3** by the general coupling reaction as previously described to yield dimers **6a-c**.

Synthesis of molecules of hydrophobic spacer series **10a-d**, **11a-d**, **12a-d**, **13a-d** and **16a-d**, is described in Scheme 4. The synthesized molecules differ in the length of the alkyl spacer n, in the direction of the amidic function (CONH or NHCO) and in the distance between the ML10302 part and the amidic function (m), constituting 5 groups of hydrophobic bivalent ligands.

For the group which possesses the NHCO----NHCO motif, directly fixed on the ML10302 pharmacophoric part, the bivalent molecules **10a-d** were synthesized from compound **7a**[21] by a first coupling reaction with appropriate N-Boc protected amino acids activated with EDC,

HOBt as previously described, and, after deprotection of the obtained compounds **8a-d**, a second coupling reaction between the resulted compounds **9a-d** and molecule **3.**

For the group which possesses the NHCO----OCNH motif fixed on the ML10302 pharmacophoric part directly or through a chain of one and two carbons respectively, bivalent molecules **11a-d**, **12a-d**, **13a-d** were yielded by direct coupling of **7a-c** [21] with suitable acids.

Bivalent ligands **16a-b, d**, which possess the CONH----HNCO motif directly fixed on ML10302 pharmacophore, were obtained by direct coupling of **3** according to the general procedure with the suitable diamine. This procedure applied to the synthesis of compound **16c** gave no product, for this reason this compound was obtained after two steps. In a first step coupling of **3** with *tert*-Butyl *N*-(6-aminohexyl)carbamate using the general procedure afforded compound **14**, followed by a classical deprotection with MeOH/HCl and coupling of the resulted compound **15** with **3** to afford the bivalent ligand **16c**.

The two bivalent ligands **18a,b**, possessing a piperazine ring instead of a piperidine ring were synthesized according to Scheme 5, using appropriate diacides by the same way.

For comparison of activities of the bivalents ligands, compound **20**, an analogue of compound **11d**, swapping one ML10302 unit for a benzylpiperidine group was synthesized (Scheme 6). Coupling of the commercially available 1-benzyl-piperidine-4-ylamine with dodecanedioic acid monoethyl ester[22] using the general coupling conditions gave **19** in a moderate yield. The ester **19** was saponified with LiOH in H_2O/dioxane and used without purification in a second coupling step with **7a**[21] using the general coupling protocol except that CH_2Cl_2 was replaced by anhydrous DMF as solvent.

In the third series, four molecules were synthesized. The bivalent ligand **23** containing a benzyl core in the middle of the spacer was synthesized from the 3-(Boc-aminomethyl)-benzylamine hydrochloride as described in Scheme 7. A first coupling step with **3** using the

general procedure afforded compound **21**. Classical removal of the Boc protecting group with HCl/MeOH gave the hydrochloride amine salt **22** which was further coupled with **3** according to the general procedure.

The preparation of the bivalent ligand **24**, outlined in Scheme 8, resulted from the coupling reaction (general procedure) of 3-[6-(2-carboxy-ethyl)-dibenzofuran-4-yl]-propionic acid[23] with **7a**.[21]

Compound **27** was obtained *via* the route depicted in Scheme 9. Reaction of benzyl-(4,6-dichloro-[1,3,5]triazin-2-yl)-amine[24] with a slight excess of 11-amino-undecanoic acid methyl ester[25] in refluxing DMF in the presence of DIEA afforded diester **25** with a good yield. Saponification of the resulting compound with NaOH in H_2O/dioxane and acidification with concentrated HCl gave the desired diacid **26**. Coupling of compound **26** with **7a** using the general procedure provided the bivalent ligand **27** with a good yield.

Finally the bivalent ligand **32** was synthesized as shown in Scheme 10 from the 1-Boc-4-aminomethyl-piperidine hydrochloride. The general coupling reaction with Z-Gly-OH yielded compound **28** which was further hydrogenated affording **29**. The alkylation by methyl 11-bromo-undecanoic acid methyl ester in presence of Cs_2CO_3 in DMF gave **30**, with a moderate yield. The methyl ester was then hydrolyzed with lithium hydroxide and the resulting acid was coupled with **7a**[21] according to the general procedure to yield the bivalent ligand **31** which was classically deprotected to afford the bivalent ligand **32**.

Biological results and discussion

All compounds were evaluated on C6 glial cells stably transfected with the human 5-$HT_{4(e/g)}$ receptor isoform. For some ligands, the pharmacological evaluation was completed by studies on CHO cell lines transfected with the same 5-$HT_{4(e/g)}$ receptor isoform. The affinities on the 5-HT_4 bivalent ligands were determined in binding studies using the 5-HT_4 receptor

antagonist [^{3}H]-GR113808, and their functional properties were investigated by measuring their ability to regulate cAMP production.

The interest of mono-oxygenated spacer (compound **6a**), or poly-oxygenated spacer (compounds **6b-c**) was to introduce a hydrophilic character at the spacer chain level. These compounds showed good affinities (table 1), more particularly compounds **6b-c** with nanomolar Ki close to that of ML10302 (Ki = 5 nM). However in contrast to ML10302, compounds **6b-c** shared a clear antagonist profile. Compared to analogous more hydrophobic compounds **16c-d** which possess equivalent spacer length chains (table 1), no difference appeared on both the affinity and the activity of bivalent ligands. Compounds **10a-d**, **11a-d** and **16a-d** have their spacer chain attached to the pharmacophoric ML10302 part, directly through NHCO or CONH functions. In this set of compounds the spacer was a polyalkyl chain of variable size from 2 to 10 methylene units. All these compounds have good nanomolar affinities (table 1), with a slight superiority for a spacer size of 9 atoms. However we observed differences in their activity profiles. Thus, the partial agonist tendency of ML10302 is more or less conserved when the two functional groups through which the 4 position of piperidine ring is attached to the spacer are NHCO (compounds **11a-d**), since an antagonist profile is favored when these two functional groups are CONH (compounds **16a-d**). A mixed situation occurred when the attachment groups are NHCO and CONH (compounds **10a-d**). In this case compounds **10b** and **10c** behave as partial agonists since **10d** is an antagonist with Kb = 7 nM (Figures 2 and 3). This phenomenon is confirmed by the observation that compounds **9d** (Ki = 13 nM, cAMP = 50%) and **20** (Ki = 14 nM, and cAMP = 35%) which both contain only one pharmacophoric part with the 4 position of the piperidine ring substituted with a chain through a NHCO function, conserve the partial agonist profile of ML10302 (Figure 3A). Taken into account that a partial agonist can behave as an antagonist in a different cellular system, the pharmacological profiles were also evaluated on CHO cells

and similar results were obtained (Figure 3B). It was also interesting to examine if the flexibility at the attachment point level between the spacer chain and the piperidine ring plays a sensitive role on the biological properties of the bivalent ligands. For that, we introduced one (compounds **12a-d**) or two (compounds **13a-d**) methylene units between the attachment function NHCO and the piperidine ring. All those compounds shown nanomolar affinities and we did not find any differences in their biological activity (table 2). These results indicate that up to 18 atoms the size of a polyalkyl spacer could not clearly differentiate the biological properties of bivalent ligands. Previous results have shown that replacement of piperidine ring with piperazine led to 5-HT_4 ligands with antagonist profile.[26] Piperazine ring is interesting since it allows easy derivatization of the ring in the 4 position. However, in contrast to the piperidine based ligands, the synthesized bivalents compounds **18a-b** showed weak affinities (table 3). Finally, in order to introduce more important modulations of the spacer size up to 29 atoms, molecules **23**, **24**, **27** and **32** were designed and synthesized. Independently of the chemical structure of the central core of these molecules, results of table 3 indicate that increasing spacer size from 9 atoms (compound **23**) to 29 atoms (compound **27**) is detrimental to affinity, since no correlation of their activities can be established. Taken together, these data show that, compared to ML10302, the bivalent piperidine based ligands have generally good nanomolar affinities for the 5-HT_4 receptor excepted for those with large spacer sizes (15 or more atoms). However these compounds have different activity profiles. So, compounds **11a-d** having a polyalkyl chain attached symmetrically by NHCO function to the pharmacophoric parts behave like ML10302 as partial agonists, since their analogues attached symmetrically by CONH function (compounds **16a-d**), and the oxygenated spacer containing compounds **6a-c** behave clearly as antagonists.

In conclusion, since none of the bivalent ligands displayed a higher affinity than ML10302[10] these results do not provide clear information about the possibility for any bivalent ligand to

bind its two pharmacophoric parts to the 5-HT_4 receptor in its dimeric form. It would be now very interesting to evaluate these ligands on more pertinent biological models including notably BRET experiments. Taken into account that classical monovalent ligands of the 5-HT_4 receptor do not influence the receptor dimerization process,[6] these bivalent ligands could provide new useful information concerning the structure of 5-HT_4 receptor dimers. Co-immunoprecipitation and BRET experiments have revealed the presence of constitutive dimers but the monomer-dimer proportion is not clear.[6] The binding of bivalent ligands to dimeric receptors could enhance the dimer proportion which would be revealed by BRET signal modification.

Directed mutagenesis studies are also in progress in order to identity the dimer interfaces. The results of these studies and of BRET evaluation of our ligands will orient the conception and the synthesis of other bivalent ligands for example by linking the ML10302 parts through its methoxy or amino group. Moreover, the study of heterodimers has revealed the possible dimerization process between adrenergic receptors and 5-HT_4 receptors. In this context, the synthesis of bivalent ligands including adrenergic and 5-HT_4 pharmacophoric parts is also envisaged.

Conclusion

In order to understand the functional importance of 5-HT_4 receptor dimerization process, we synthesized a family of bivalent ligands possessing two 5-HT_4 pharmacophoric parts linked through a spacer to the amino basic function. Their conception was based on the molecular modeling of the 5-HT_4 receptor dimer from our published model of the monomer receptor using protein/protein docking experiments. In this context, the basal monomer was ML10302, and several spacers differing in their nature and in their size were chosen and linked by an amidic function to the fourth position of the piperidine ring of ML10302. Three series of

bivalent ligands were then defined, synthesized and pharmacologically evaluated. However no synthesized bivalent ligand shown biological properties which could reflect a specific interaction with a receptor dimer, which would mean binding of each ML10302 part in a distinct monomer of receptor dimers. The evaluation of the synthesized bivalent ligands in BRET experiments is now envisaged.

Experimental Section

Chemistry. Melting points were determined on a Kofler melting point apparatus. NMR spectra were performed on a Bruker AMX 200 (^{1}H, 200 MHz ; ^{13}C, 50 MHz) or Bruker AVANCE 400 (^{1}H, 400 MHz ; ^{13}C, 100 MHz). Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used : singlet (s), doublet (d), triplet (t), multiplet (m), quintet (q), broad doublet (bd), broad multiplet (bm), broad triplet (bt) and broad singlet (bs). Elemental analyses (C, H, N) were performed at the microanalyses Service of the Faculty of Pharmacy at Châtenay-Malabry (France) and were within 0.4 % of the theorical values otherwise stated. Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus.

Materials. DMF distilled from BaO, CH_2Cl_2 distilled from calcium hydride, and usual solvents were purchased from SDS (Paris , France). Liquid chromatography was performed on Merck silica gel 60 (70/30 mesh), and TLC was performed on silica gel, 60F-254 (0.26 mm thickness) plates. Visualisation was achieved with UV light and Dragendorff reagent unless otherwise stated. Boc-β-Ala-OH, Boc-4-aminobutyric acid, Boc-6-aminocaproic acid, Boc-11-aminoundecanoic acid, 3-(Boc-aminomethyl)-benzylamine hydrochloride, 1-Boc-4-aminomethyl-piperidine hydrochloride, succinic acid, glutaric acid, pimelic acid, dodecanedioïc acid, Fmoc-2-(2-aminoethoxy)-ethylamine hydrochloride, Fmoc-1-amino-3,6-dioxa-3-octanamine, Fmoc-1-amino-4,7,10-trioxa-13-tridecamine hydrochloride, ethylenediamine, 1,3-diaminopropane, *tert*-Butyl *N*-(6-aminohexyl carbamate, 4-amino-1-benzylpiperidine dihydrochloride and 1, 10-diaminodecane and methyl 11-bromodecane were purchased from commercial sources. *tert*-Butyl 4-piperidine carboxylate,[27] dodecanedioic acid monoethyl ester,[22] N-benzyl-4,6-di-chloro-1,3,5-triazin-2-amine,[24] methyl 11-aminododecanoate[25] and 3-[6- (3-hydroxy-3-oxopropyl)dibenzo[b,d]furan-4-yl]propanoic acid[23] were prepared according to methods reported in the literature. 4-amino-5-chloro-2-

methoxy-benzoic acid 2-bromo-ethyl ester[20], com pounds **7a-c**[21]**,** and **17**[26] were synthesized as previously described.

***tert*-Butyl 1-{2-[(4-(amino-5-chloro-2-methoxybenzoyl)oxy]ethyl}piperidine-4-carboxylate (2).** A mixture of *tert*-Butyl 4-piperidinecarboxylate[27] (2.54 g, 13.7 mmol, 1 eq), DIEA (2.65 g, 20.5 mmol, 1.5 eq) and 4-amino-5-chloro-2-methoxy-benzoic acid 2-bromo-ethyl ester[20] (5.1 g, 16.5 mmol, 1.2 eq) in dry DMF (60 mL) was stirred for 24 h at 40-50 °C. After concentration in vacuo, the residue was taken up with CH_2Cl_2 (100 mL), washed with saturated aqueous NaCl (100 mL), dried ($MgSO_4$) and concentrated. The crude oil was chromatographed on silica gel (CH_2Cl_2/AcOEt 20:80 then CH_2Cl_2/MeOH 90:10) to give 2.86 g (51%) of **2** as a white solid. R*f* (CH_2Cl_2/MeOH 90:10) 0.14; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.27 (s, 1H), 4.45 (bs, 2H), 4.35 (t, *J* = 6.1 Hz, 2H), 3.83 (s, 3H), 2.93 (m, 2H), 2.67 (t, *J* = 6.1 Hz, 2H), 2.16 (m, 3H), 1.92-1.60 (m, 4H), 1.43 (s, 9H). Anal. ($C_{20}H_{29}ClN_2O_5$), C, H, N.

1-{2-[(4-Amino-5-chloro-2-methoxybenzoyl)oxy]ethyl}piperidine-4-carboxylic acid (3). To a solution of **2** (2.86 g, 6.9 mmol) in dry CH_2Cl_2 (114 mL) was added CF_3COOH (13 mL). The mixture was stirred for 24 h at room temperature. After adding *i*Pr_2O to the reaction mixture, the trifluoroacetate salt of **3** precipitate to afford 2.42 g (74%) of a white solid. mp 226° C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.35 (bs,1H), 9.47(bs, 1H), 7.68 (s, 1H), 6.47 (s, 1H), 6.22 (bs, 2H), 4.43 (m, 2H), 3.73 (s, 3H), 3.72-2.89 (m, 6H), 2.07 (m, 3H), 1.79 (m, 2H). Anal. ($C_{16}H_{21}ClN_2O_5 \cdot CF_3COOH \cdot 0.25H_2O$), C, H, N.

General procedure for the synthesis of compounds 4a-c, 8a-d, 14, 19 and 21. Preparation of 2-{4-[11-(9*H*-fluoren-9-yl)-9-oxo-5,10-dioxa-2,8-diazaundec-1-anoyl]ethylpiperidino 4-amino-5-chlo-2-methoxy benzoate (4a). To a solution of the trifluoroacetate salt of **3** (0.4 g, 0.85 mmol, 1 eq) in dry CH_2Cl_2 (60-70mL) and cooled at 0 °C were added HOBt (0.22 g, 1.66 mmol, 1.95 eq), EDC (0.21 g, 85 mmol, 1.3 eq) and NEt_3 (0.30 g, 2.97 mmol, 3.5 eq). The reaction mixture was stirred at this temperature for 5-10 min and then Fmoc-2-(2-

aminoethoxy)-ethylamine hydrochloride (0.31 g, 0.11 mmol, 1 eq) was added. After maintaining the mixture for 24 h at room temperature, the organic layer was washed with 10% aqueous $KHSO_4$ (25 mL), saturated $NaHCO_3$ (25 mL), brine (25 mL), and dried ($MgSO_4$). A gum which separated from CH_2Cl_2 layer when washing with $KHSO_4$ was immediately taken up in MeOH, and the methanolic solution was dried ($MgSO_4$). This methanolic solution was added to the dried CH_2Cl_2 organic phase. After concentration of the combined organic layers, the residue was purified by chromatography (CH_2Cl_2/MeOH 90:10) to afford 0.31 g (55%) of **4a** as a resinous amber solid. R*f* (CH_2Cl_2/MeOH 90:10) 0.30; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 7.75 (m, 2H), 7.58 (m, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 6.25 (s, 1H), 5.88 (bs, 1H), 5.11 (bs, 1H), 4.41 (m, 4H), 4.33 (t, *J* = 5.9 Hz, 2H), 4.22 (t, *J* = 6.07 Hz, 1H), 3.82 (s, 3H), 3.60-3.25 (m, 8H), 3.00 (m, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.19-1.99 (m, 3H), 1.88-1.58 (m, 4H). Anal. ($C_{35}H_{41}ClN_4O_7$·0.75H_2O), C, H, N.

2-{4-[14-(9*H*-Fluoren-9-yl)-12-oxo-5,8,13-trioxa-2,11-diazatetradec-1-anoyl]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (4b). Same procedure as described for **4a** from **3** and Fmoc-1-amino-3,6-dioxa-8-octanamine hydrochloride. CH_2Cl_2/*i*PrOH 90:10 then CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.15; 69% yield; white foam; ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 7.75 (m, 2H), 7.58 (m, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 6.26 (s, 1H), 5.93 (bs, 1H), 5.52 (bs, 1H), 4.43 (m, 4H), 4.33 (t, *J* = 5.9 Hz, 2H), 4.22 (t, *J* = 6.07 Hz, 1H), 3.82 (s, 3H), 3.63-3.20 (m, 12H), 3.20 (m, 2H), 2.60 (t, *J* = 5.9 Hz, 2H), 2.19-1.81 (m, 3H), 1.90-1.58 (m, 4H). Anal. ($C_{37}H_{45}ClN_4O_8$·H_2O), C, H, N.

2-{4-[19-(9*H*-Fluoren-9-yl)-17-oxo-6,9,12,18-tetraoxa-2,16-diazanonadec-1-anoyl]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (4c). Same procedure as described for **4a** from **3** and Fmoc-1-amino-4,7,10-trioxa-13-tridecamine hydrochloride. CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.18; 77% yield; amber resinous oil; ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 7.75 (m, 2H), 7.59 (m, 2H), 7.40 (m, 2H), 7.30 (m, 2H),

6.24 (s, 1H), 6.16 (bs, 1H), 5.41 (bs, 1H), 4.40 (m, 4H), 4.35 (t, *J* = 5.9 Hz, 2H), 4.21 (t, *J* = 6.07 Hz, 1H), 3.82 (s, 3H), 3.67-3.44 (m, 12H), 3.32 (m, 4H), 2.99 (m, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.19-1.94 (m, 3H), 1.89-1.56 (m, 8H). Anal. ($C_{41}H_{53}ClN_4O_9 \cdot 0.75H_2O$), C, H, N.

Synthesis of amino compounds 5a-c: General procedure. 2-[4-({[2-(2-aminoethoxy) ethyl]amino}carbonyl)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (5a). A solution of **4a** (0.5 g, 0.75 mmol) was treated at room temperature with 20% piperidine (v/v) in DMF (15 mL) for 10-15 min. The organic phase was evaporated. The oily residue obtained was taken up with MeOH and the resulting white solid formed was eliminated by filtration. After acidification of the filtrate with MeOH/HCl 4N and concentration, 0.34 g (88%) of very hygroscopic dihydrochloride salt of amine **5a** was afforded as a beige foam which was used in next step without further purification. ^{1}H NMR (200 MHz): (free base) δ 7.79 (s, 1H), 6.27 (s,1H), 6.06 (bs, 1H), 4.33 (bs, 2H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.56-3.40 (m, 6H), 3.02 (m, 2H), 2.85 (m, 2H), 2.66 (t, *J* = 5.9 Hz, 2H), 2.20-1.99 (m, 3H), 1.89-1.66 (m, 4H), 0.9 (bs, 2H).

2-{4-[({2-[2-(2-(Aminoethoxy)ethoxy]ethoxy]ethyl}amino)carbonyl]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (5b). Same procedure as described for **5a**. 95% yield; very hygroscopic beige foam; ^{1}H NMR (200 MHz): δ 7.77 (s, 1H), 6.26 (s,1H), 6.34 (bs, 1H), 4.53 (bs, 2H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.80 (s, 3H), 3.60-3.38 (m, 8H), 3.01 (m, 2H), 2.84 (m, 2H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.51 (m, 2H), 2.17-1.95 (m, 3H), 1.88-1.55 (m, 4H), 0.98 (bs, 2H).

2-[4-(15-Amino-6,9,12-trioxa-2-azapentadec-1-anoyl)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (5c). Same procedure as described for **5a**. 90% yield; very hygroscopic beige foam; ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 6.46 (bs, 1H), 6.27 (s,1H), 4.49 (bs, 2H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.65-3.40 (m, 12H), 3.34 (m, 2H), 3.01 (m, 2H), 2.84

(m, 2H), 2.78 (m, 2H), 2.71 (t, *J* = 5.9 Hz, 2H), 2-1.96 (m, 3H), 1.89-1.60 (m, 6H), 1.32 (bs, 2H).

Dimer 6a. Method described for **4a**, from **5a** and **3**. CH_2Cl_2/MeOH 90:10, then CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.2; 33% yield; clear amber foam; ^{1}H NMR (200 MHz): δ 7.79 (s, 2H), 6.28 (s, 2H), 5.96 (bt, 2H), 4.50 (bs, 4H), 4.35 (t, *J* = 5.7 Hz, 4H), 3.82 (s, 6H), 3.56-3.6 (m, 8H), 3.00 (m, 4H), 2.72 (t, *J* = 5.7 Hz, 4H), 2.21-2.03 (m, 6H), 1.92-1.66 (m, 8H). ^{13}C NMR (50 MHz): δ 175.6, 164.9, 160.6, 148.4, 133.5, 110.2, 109.8, 98.6, 70.0, 62.3, 57.1, 56.3, 53.7, 43.3, 39.4, 29.2. ESI m/z 781.3 ($M + H^+$), 803.3 ($M + Na^+$). Anal. ($C_{36}H_{50}Cl_2N_6O_9 \cdot 0.75H_2O$), C, H, N.

Dimer 6b. Method described for **4a**, from **5b** and **3**, except that the dihydrochloride salt of amine **5b** was dissolved in anhydrous DMF before adding to the reaction. CH_2Cl_2/MeOH 90:10, then CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 90:10:2) 019; 15% yield; pale yellow foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.77 (s, 2H), 6.48 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.82 (s, 6H), 3.62 (s, 4H), 3.56 (t, *J* = 5.4 Hz, 4H), 3.38 (t, *J* = 5.4 Hz, 4H), 3.08 (m, 4H), 2.77 (t, *J* = 5.7 Hz, 4H), 2.34-2.1 (m, 6H), 1.88-1.72 (m, 8H). ^{13}C NMR (50 MHz, CD_3OD): δ 178.7, 167.3, 162.8, 152.3, 134.9, 111.2, 109.4, 99.6, 72.2, 71.5, 63.6, 58.8, 57.1, 55.3, 44.6, 41.1, 30.5. ESI m/z 825.3 ($M + H^+$). Anal. ($C_{38}H_{54}Cl_2N_6O_{10} \cdot H_2O$), C, H, N.

Dimer 6c. Method described for **4a,** from **5c** and **3**, except that the dihydrochloride salt of amine **5c** was dissolved in anhydrous DMF before adding to the reaction. CH_2Cl_2/MeOH 70:30 R*f* (CH_2Cl_2/MeOH 70:30) 0.09; 34% yield; beige foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.79 (s, 2H), 6.49 (s, 2H), 4.45 (t, *J* = 5.7 Hz, 4H), 3.84 (s, 6H), 3.63 (m, 8H), 3.54 (t, *J* = 6.23 Hz, 4H), 3.38-3.2 (m, 8H), 3.06 (t, *J* = 5.7 Hz, 4H), 2.67-2.45 (m, 4H), 2.45-2.23 (m, 2H), 1.98-1.67 (m, 12H). ^{13}C NMR (50 MHz, CD_3OD): δ 176.2, 166.6, 162.5, 149.9, 135.3,

112.9, 110.6, 101.5, 72.3, 72.0, 70.7, 60.6, 58.0, 57.5, 54.7, 41.7, 38.7, 31.2, 28.4, 25.9. ESI m/z 897.4 ($M + H^+$), 919.4 ($M + Na^+$).

2-[4-({3-[(*tert*-Butoxycarbonyl)amino]propanoyl}amino)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (8a). Method described for **4a**, from **7a**[21] and Boc-β-Ala-OH. CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.27; 31% yield; beige foam; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.28 (s,1H), 5.57 (bs, 1H), 5.12 (bs, 1H), 4.44 (bs, 2H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.83 (s, 3H), 3.78 (m, 1H), 3.38 (m, 2H), 2.91 (m, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.37 (m, 2H), 2.24 (m, 2H), 1.88 (m, 2H), 1.47 (m, 2H), 1.43 (s, 9H). Anal. ($C_{23}H_{35}ClN_4O_6 \cdot 0.25H_2O$), C, H, N.

2-[4-({4-[(*tert*-Butoxycarbonyl)amino]butanoyl}amino)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (8b). Method described for **4a**, from **7a**[21] and Boc-4-aminobutyric acid. CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.38; 31% yield; white foam; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.28 (s, 1H), 6.06 (bs, 1H), 4.71 (bs, 1H), 4.44 (bs, 2H), 4.37 (t, *J* = 5.9 Hz, 2H), 3.83 (s, 3H), 3.78 (m, 1H), 3.15 (m, 2H), 2.94 (m, 2H), 2.75 (t, *J* = 5.9 Hz, 2H), 2.29 (m, 2H), 2.18 (t, *J* = 7 Hz, 2H), 1.92 (m, 2H), 1.80 (m, 2H), 1.53 (m, 2H), 1.43 (s, 9H). Anal. ($C_{24}H_{37}ClN_4O_6 \cdot 0.5H_2O$), C, H, N.

2-[4-({6-[(*tert*-Butoxycarbonyl)amino]hexanoyl}amino)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (8c). Method described for **4a**, from **7a**[21] and Boc-4-aminocaproic acid. CH_2Cl_2/MeOH 90:5; R*f* (CH_2Cl_2/MeOH 90:5) 0.49; 78% yield; beige foam; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.28 (s,1H), 5.32 (bd, 1H), 4.51 (bs, 1H), 4.46 (bs, 2H), 4.35 (t, *J* = 5.9 Hz, 2H), 3.83 (s, 3H), 3.78 (m, 1H), 3.09 (m, 2H), 2.93 (m, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.26 (m, 2H), 2.14 (t, *J* = 7 Hz, 2H), 1.93 (m, 2H), 1.64 (m, 2H), 1.56-1.25 (m, 15H). Anal. ($C_{26}H_{41}ClN_4O_6 \cdot 0.5\ H_2O$), C, H, N.

2-[4-({11-[*tert*-Butoxycarbonyl)amino]undecanoyl}amino)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (8d). Method described for **4a**, from **7a**[21] and Boc-11-

aminodecanoic acid. CH_2Cl_2/MeOH 90:5; R*f* (CH_2Cl_2/MeOH 90:5) 0.18; 73% yield; white solid; mp 112-114 °C; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.28 (s, 1H), 5.29 (bd, 1H), 4.46 (bs, 3H), 4.35 (t, *J* = 5.9 Hz, 2H), 3.83 (s, 3H), 3.80 (m, 1H), 3.09 (m, 2H), 2.93 (m, 2H), 2.74 (t, *J* = 5.9 Hz, 2H), 2.26 (m, 2H), 2.13 (t, *J* = 7 Hz, 2H), 1.94 (m, 2H), 1.64-1.15 (m, 27H). Anal ($C_{31}H_{51}ClN_4O_6$), C, H, N.

Synthesis of amino compounds 9a-d: General procedure. 2-{4-[(3-aminopropanoyl) amino]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (9a). 8a (0.31 g, 0.62 mmol) was dissolved in MeOH (25-20 mL), and 20 mL of MeOH/HCl 4N was added. After stirring 4-5 h at room temperature, 0.29 g (99%) of **9a** was obtained as a very hygroscopic beige foam after concentration and dryness under vacuo. ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 6.89 (bs,1H), 6.27 (s 1H), 4.46 (bs, 2H), 4.34 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.80 (m, 1H), 3.01 (t, *J* = 5.9 Hz, 2H), 2.96 (m, 2H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.27 (m, 4H), 1.85 (m, 2H), 1.47 (m, 2H), 1.0 (bs, 2H). Anal. ($C_{18}H_{27}ClN_4O_5 \cdot 2HCl \cdot 2H_2O$), C, H, N.

2-{4-[(4-Aminobutanoyl)amino]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (9b). Same procedure as described for 9a. Very hygroscopic white foam; 90% yield; ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 6.27 (s,1H), 5.92 (bd, 1H), 4.49 (bs, 2H), 4.33 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.76 (m, 1H), 2.90 (m, 2H), 2.72 (m, 4H), 2.22 (m, 4H), 1.89 (m, 2H), 1.75 (q, *J* = 7.25 Hz, 2H), 1.44 (m, 2H), 1.00 (bs, 2H). Anal. ($C_{19}H_{29}ClN_4O_4 \cdot 2HCl \cdot 2.5H_2O$), C, H, N.

2-{4-[(6-Aminohexanoyl)amino]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (9c). Same procedure as described for **9a**. Very hygroscopic beige foam; 99% yield; ^{1}H NMR (200 MHz): δ 7.79 (s, 1H), 6.27 (s,1H), 5.53 (bd, 1H), 4.48 (bs, 2H), 4.33 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.78 (m, 1H), 2.90 (m, 2H), 2.69 (m, 4H), 2.22 (m, 2H), 2.14 (m, 2H), 1.89 (m,

2H), 1.62 (m, 2H), 1.56-1.25 (m, 6H), 0.95 (bs, 2H). Anal. ($C_{21}H_{33}ClN_4O_4 \cdot 2HCl \cdot 3.75\ H_2O$), C, H, N.

2-{4-[(11-Aminoundecanoyl)amino]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (9d). Same procedure as described for **9a**. Very hygroscopic foam; 92% yield; ^{1}H NMR (200 MHz): δ 7.77 (s, 1H), 6.26 (s,1H), 5.45 (bd, 1H), 4.52 (bs, 2H), 4.32 (t, *J* = 5.9 Hz, 2H), 3.80 (s, 3H), 3.78 (m, 1H), 2.89 (m, 2H), 2.70 (m, 2H), 2.64 (m, 2H), 2.22 (m, 2H), 2.10 (m, 2H), 1.89 (m, 2H), 1.58 (m, 2H), 1.56-1.20 (m, 18H), 0.98 (bs, 2H). Anal. ($C_{26}H_{45}ClN_4O_4 \cdot 2HCl \cdot 3H_2O$), C, H, N.

Dimer 10a. Method described for **4a,** from **9a** and **3**. CH_2Cl_2/MeOH 80:20; R*f* (CH_2Cl_2/MeOH 80:20) 0.28; 24% yield; beige foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.48 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.71 (m, 1H), 3.44 (t, *J* = 6.7 Hz, 2H), 3.07 (m, 4H), 2.79 (t, *J* = 5.7 Hz, 4H), 2.39 (t, *J* = 6.7 Hz, 2H), 2.32-2.12 (m, 5H), 1.96-1.72 (m, 6H), 1.61 (m, 2H). ^{13}C NMR (50 MHz, CD_3OD): δ 178.6, 173.9, 167.3, 162.8, 152.3, 135.0, 111.2, 109.3, 99.6, 63.6, 63.5, 58.8, 58.6, 57.1, 55.2, 54.6, 48.4, 44.5, 37.8, 37.6, 33.2, 30.4. ESI m/z 759.3 ($M + Na^+$). Anal. ($C_{34}H_{46}Cl_2N_6O_8 \cdot 1.5H_2O$), C, H, N.

Dimer 10b. Method described for **4a,** from **9b** and **3**. CH_2Cl_2/MeOH 80:20; R*f* (CH_2Cl_2/MeOH 80:20) 0.37; 16% yield; beige foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (m, 4H), 3.83 (s, 6H), 3.72 (m, 1H), 3.21 (m, 2H), 3.09 (m, 4H), 2.86 (m, 4H), 2.35 (m, 5H), 2.25 (m, 2H), 2.00-1.72 (m, 8H), 1.61 (m, 2H). ^{13}C NMR (50 MHz, CD_3OD): δ 178.4, 175.7, 167.4, 162.9, 152.4, 135.0, 111.2, 109.5, 99.6, 63.4, 63.2, 58.7, 58.5, 57.1, 55.2, 54.5, 44.3, 40.6, 35.3, 33.0, 30.15, 27.7. ESI m/z 751.4 ($M + H^+$), 773.4 ($M + Na^+$). Anal. ($C_{35}H_{48}Cl_2N_6O_8 \cdot 2.5H_2O$), C, H, N.

Dimer 10c. Method described for **4a,** from **9c** and **3**. CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2) 0.39; 30% yield; beige foam; ^{1}H NMR (200 MHz,

CD_3OD): δ 7.74 (s, 2H), 6.46 (s, 2H), 4.34 (t, *J* = 5.7 Hz, 4H), 3.80 (s, 6H), 3.70 (m, 1H), 3.15 (m, 2H), 3.04 (m, 4H), 2.75 (t, *J* = 5.7 Hz, 4H), 2.33-2.08 (m, 7H), 1.92-1.66 (m, 6H), 1.66-1.40 (m, 6H), 1.40-1.24 (m, 2H). ^{13}C NMR (50 MHz, CD_3OD): δ 177.7, 175.4, 166.5, 161.0, 151.5, 134.1, 110.3, 108.5, 98.8, 62.8, 62.7, 57.9, 57.7, 56.3, 54.5, 53.8, 47.6, 44.0, 40.1, 39.9, 32.4, 30.1, 29.7, 27.4, 26.7. ESI m/z 779.4 ($M + H^+$), 801.3 ($M + Na^+$). Anal. ($C_{37}H_{52}Cl_2N_6O_8$), C, H, N.

Dimer 10d. Method described for **4a**, from **9d** and **3**. CH_2Cl_2/MeOH/NH_4OH 20% 90:7:3; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 90:7:3) 0.4; 41% yield; beige foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.73 (m, 1H), 3.18 (m, 2H), 3.07 (m, 4H), 2.79 (t, *J* = 5.7 Hz, 4H), 2.40-2.10 (m, 7H), 2.00-1.75 (m, 6H), 1.70-1.40 (m, 6H), 1.34 (bs, 12H). ^{13}C NMR (50 MHz, CD_3OD): δ 177.7, 175.7, 166.6, 162.0, 151.5, 134.2, 110.4, 108.6, 98.8 62.9, 62.8, 58.0, 57.9, 56.3, 54.5, 53.9, 47.6, 44.0, 40.3, 37.2, 32.5, 30.6, 30.6, 30.4, 30.3, 30.2, 29.7, 28.0, 27.1. ESI m/z 849.5 ($M + H^+$), 871.5 ($M + Na^+$). Anal. ($C_{42}H_{62}Cl_2N_6O_8 \cdot 0.75H_2O$), C, H, N.

Dimer 11a. Method described for **4a,** from **7a**[21] (2 eq), succinic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 90:10 then CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.20; 57% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.38 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.69 (m , 2H), 3.02 (m, 4H), 2.78 (t , *J* = 5.7 Hz, 4H), 2.48 (s, 4H), 2.44-2.33 (m, 4H), 1.99-1.71 (m, 4H), 1.69-1.54 (m, 4H). ^{13}C NMR (50 MHz, CD_3OD): δ 175.4, 166.5, 162.0, 151.5, 134.1, 110.3, 108.5, 98.7, 62.9, 57.7, 56.3, 53.9, 47.5, 36.8, 32.4. ESI m/z 737.3 ($M + H^+$), 759.3 ($M + Na^+$). Anal. ($C_{34}H_{46}Cl_2N_6O_8 \cdot H_2O$), C, H, N.

Dimer 11b. Method described for **4a**, from **7a**[21] (2 eq), glutaric acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 90:10 then CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.42; 27% yield; white foam; ^{1}H NMR

(200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.38 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.70 (m , 2H), 3.03 (m, 4H), 2.79 (t , *J* = 5.7 Hz, 4H), 2.26 (m, 4H), 2.21 (m, 4H), 2.00-1.80 (m, 6H), 1.66-1.43 (m, 4H). ^{13}C NMR (50 MHz, CD_3OD): δ 175.6, 167.4, 162.9, 151.3, 135.0, 111.2, 109.4, 99.6, 63.7, 58.6, 57.1, 54.7, 48.5, 37.1, 33.3, 24.2. ESI m/z 751.3 ($M + H^+$), 773.3 ($M + Na^+$). Anal. ($C_{35}H_{48}Cl_2N_6O_8$), C, H, N.

Dimer 11c. Method described for **4a,** from **7a**[21] (2 eq), pimelic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 90:10 then CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.24; 46% yield; white foam; ^1H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.70 (m , 2H), 3.03 (m, 4H), 2.78 (t , *J* = 5.7 Hz, 4H), 2.48-2.11 (m, 8H), 1.78 (m, 4H), 1.75-1.44 (m, 6H), 1.43-1.19 (m, 4H). ^{13}C NMR (50 MHz, CD_3OD): δ 175.4, 166.5, 162.0, 151.5, 134.4, 110.3 108.5, 98.7, 62.9, 57.8, 56.3, 53.8, 47.5, 36.9, 32.4, 29.6, 26.7. ESI m/z 779.3 ($M + H^+$), 801.3 ($M + Na^+$). Anal. ($C_{37}H_{52}Cl_2N_6O_8 \cdot 1.5H_2O$), C, H, N.

Dimer 11d. Method described for **4a**, from **7a**[21] (2 eq), dodecanedioic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 90:10 then CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f*(CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.3; 47% yield; white foam; ^1H NMR (200 MHz, CD_3OD): δ 7.79 (s, 2H), 6.49 (s, 2H), 4.38 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.71 (m , 2H), 3.03 (m, 4H), 2.79 (t , *J* = 5.7 Hz, 4H), 2.45-2.12 (m, 8H), 1.89 (m, 4H), 1.68-1.48 (m, 8H), 1.42-1.21 (m, 12H). ^{13}C NMR (50 MHz, CD_3OD): δ 175.7, 166.5, 162.0, 151.5, 134.4, 110.4, 108.5, 98.8, 62.9, 57.8, 56.2, 53.9, 47.5, 37.1, 32.4, 30.5, 30.3, 30.2, 27.1. ESI m/z 849.4 ($M + H^+$), 871.4 ($M + Na^+$). Anal. ($C_{42}H_{62}Cl_2N_6O_8 \cdot 0.5H_2O$), C, H, N.

Dimer 12a. Method described for **4a**, from **7b**[21] (2 eq), succinic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq) EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.23; 50% yield; white foam; ^1H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.03 (m, 8H),

2.78 (t, *J* = 5.7 Hz, 4H), 2.46 (s, 4H), 2.10 (m, 4H), 1.77–1.60 (m, 4H), 1.49 (m, 2H), 1.35–1.20 (m, 4H). ^{13}C RMN (50 MHz, CD_3OD): δ 174.7, 166.5, 162.0, 151.5, 134.0, 110.4, 108.6, 98.8, 62.8, 58.0, 56.3, 54.9, 45.9, 37.0, 32.4, 30.6. ESI m/z 765.2 (M + H^+). Anal. ($C_{36}H_{50}Cl_2N_6O_8 \cdot 2H_2O$), C, H, N.

Dimer 12b. Method described for **4a**, from **7b**[21] (2 eq), glutaric acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.30; 34% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.15–2.99 (m, 8H), 2.78 (t, *J* = 5.7 Hz, 4H), 2.35–2.07 (m, 8H), 1.73 (m, 4H), 1.65-1.44 (m, 2H), 1.44–1.22 (m, 6H). ^{13}C RMN (50 MHz, CD_3OD): δ 175.4, 166.5, 162.0, 151.5, 134.1, 110.3, 108.5, 98.7, 62.7, 58.0, 56.2, 54.8, 45.7, 37.2, 36.2, 30.6, 23.4. ESI m/z 779.3 (M + H^+). Anal. ($C_{37}H_{52}Cl_2N_6O_8 \cdot 1H_2O$), C, H, N.

Dimer 12c. Method described for **4a**, from **7b**[21] (2 eq), pimelic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.34; 25% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.11 (m, 8H), 2.73 (t, *J* = 5.7 Hz, 4H), 2.35–2.11 (m, 8H), 1.70 (m, 4H), 1.61 (m, 4H), 1.53–1.44 (m, 2H), 1.31 (m, 6H). ^{13}C RMN (50 MHz, CD_3OD): δ 176.1, 166.5, 162.0, 151.5, 134.0, 110.4, 108.5, 98.7, 62.8, 58.0, 56.2, 54.8, 45.8, 37.0, 36.9, 30.6, 29.8, 26.7. ESI m/z 807.4 (M + H^+). Anal. ($C_{39}H_{56}Cl_2N_6O_8 \cdot 2.5H_2O$), C, H, N.

Dimer 12d. Method described for **4a**, from **7b**[21](2 eq), dodecanedioic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.38; 44% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (t, J = 5.7 Hz, 4H), 3.83 (s, 6H), 3.11 (m, 8H), 2.83

(t, *J* = 5.7 Hz, 4H), 2.20 (m, 8H), 1.88–1.46 (m, 10H), 1.46–1.19 (m, 16H). ^{13}C RMN (50 MHz, CD_3OD): δ 176.3, 166.4, 162.0, 151.5, 134.1, 110.3, 108.4, 98.8, 62.5, 57.9, 56.3, 54.8, 45.7, 37.1, 36.8, 30.5, 30.4, 27.1. ESI m/z 877.3 (M + H^+). Anal. ($C_{44}H_{66}Cl_2N_6O_8 \cdot H_2O$), C, H, N.

Dimer 13a. Method described for **4a**, from **7c**[21] (2 eq), succinic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.28; 52% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.24 (m, 4H), 3.06 (m, 4H), 2.77 (t, *J* = 5.7 Hz, 4H), 2.49 (s, 4H), 2.16 (m, 4H), 1.76 (m, 4H), 1.56–1.23 (m, 10H). ^{13}C RMN (50 MHz, CD_3OD): δ 174.4, 166.4, 162.0, 151.5, 134.0, 110.3, 108.5, 98.6, 62.7, 58.1, 56.2, 55.1, 40.0, 36.9, 34.1, 32.7, 32.3. ESI m/z 793.4 (M + H^+). Anal. ($C_{38}H_{54}Cl_2N_6O_8 \cdot 0.5H_2O$), C, H, N.

Dimer 13b. Method described for **4a**, from **7c**[21] (2 eq), glutaric acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.34; 29% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.24 (m, 4H), 3.06 (m, 4H), 2.77 (t, *J* = 5.7 Hz, 4H), 2.32–2.06 (m, 8H), 1.76 (m, 4H), 1.55–1.22 (m, 12H). ^{13}C RMN (50 MHz, CD_3OD): δ 175.2, 166.4, 162.0, 151.5, 133.9, 110.3, 108.5, 98.7, 62.7, 58.0, 56.2, 55.1, 37.9, 37.0, 36.3, 34.0, 32.7, 23.4. ESI m/z 807.4 (M + H^+). Anal. ($C_{39}H_{56}Cl_2N_6O_8 \cdot 3.5H_2O$), C, H, N.

Dimer 13c. Method described for **4a**, from **7c**[21] (2 eq), pimelic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.39; 28% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.24 (m, 4H), 3.07 (m, 4H), 2.79 (t, *J* = 5.7 Hz, 4H), 2.33–2.06 (m, 8H), 1.78 (m, 4H), 1.66 (m, 4H), 1.47

(m, 4H) 1.40–1.31 (m, 8H). ^{13}C RMN (50 MHz, CD_3OD): δ 175.9, 166.5, 162.0, 152.5, 134.1, 110.3, 108.0, 98.8, 62.6, 58.0, 56.3, 55.1, 37.9, 36.9, 34.2, 32.7, 29.3, 26.6. ESI m/z 835.3 ($M + H^+$). Anal. ($C_{41}H_{60}Cl_2N_6O_8 \cdot 2H_2O$), C, H, N.

Dimer 13d. Method described for **4a**, from **7c**[21] (2 eq), dodecanedioic acid (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). EtOAc/MeOH/NH_4OH 20% 88:10:2; Rf (EtOAc/MeOH/NH_4OH 20% 88:10:2) 0.42; 46% yield; white foam; ^{1}H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.19 (m, 4H), 3.04 (m, 4H), 2.78 (t, *J* = 5.7 Hz, 4H), 2.17 (m, 8H), 1.84–1.53 (m, 8H), 1.53–1.19 (m, 22H). ^{13}C RMN (50 MHz, CD_3OD): δ 176.0, 166.4, 162.0, 151.5, 133.9, 110.3, 108.4, 98.7, 62.7, 58.0, 56.2, 55.1, 38.8, 37.8, 37.1, 34.1, 32.7, 30.6, 30.5, 28.6 ,27.0. ESI m/z 905.5 ($M + H^+$). Anal. ($C_{46}H_{70}Cl_2N_6O_8 \cdot 2H_2O$), C, H, N.

2-{4-[({5-[(*tert*-Butoxycarbonyl)amino]pentyl}amino)carbonyl]piperidino}ethyl 4-amino-5-chloro-2-methoxybenzoate (14). Same procedure as described for **4a** from **3** and *tert*-Butyl *N*-(6-aminohexyl)carbamate. CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.56; 46% yield; white foam; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 6.28 (s,1H), 5.55 (bm, 1H), 4.53 (bs, 1H), 4.46 (bs, 2H), 4.36 (t, *J* = 5.9 Hz, 2H), 3.83 (s, 3H), 3.23 (m, 2H), 3.09 (m, 2H), 3.02 (m, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.09 (m, 2H), 2.02 (m, 1H), 1.90-1.60 (m, 4H), 1.58-1.21 (m, 15H). Anal. ($C_{26}H_{41}ClN_4O_6 \cdot 0.5H_2O$), C, H, N.

2-(4-{[(5-Aminopentyl)amino]carbonyl}piperidino)ethyl 4-amino-5-chloro-2-methoxybenzoate (15). Same procedure as described for **9a.**Very hygroscopic beige foam; 82% yield; ^{1}H NMR (200 MHz): δ. 7.78 (s, 1H), 6.27 (s,1H), 5.60 (bt, 1H), 4.49 (bs, 2H), 4.33 (t, *J* = 5.9 Hz, 2H), 3.82 (s, 3H), 3.22 (m, 2H), 3.01 (m, 2H), 2.71 (t, *J* = 5.9 Hz, 2H), 2.66 (t, *J* = 6.7 Hz, 2H), 2.20-1.95 (m, 3H), 1.89-1.64 (m, 4H), 1.57-1.22 (m, 6H), 0.88 (bs, 2H). Anal. ($C_{21}H_{33}ClN_4O_4 \cdot 2HCl \cdot 1.5H_2O$), C, H, N.

Dimer 16a. Method described for **4a**, from **3** (2 eq), ethylenediamine (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2) 0.06; 13% yield; beige foam; ^1H NMR (400 MHz, CD_3OD): δ 7.75 (s, 2H), 6.45 (s, 2H), 4.35 (t, *J* = 5.6 Hz, 4H), 3.79 (s, 6H), 3.27 (m, 4H) 3.09 (m, 4H), 2.77 (t, *J* = 5.6 Hz, 4H), 2.28-2.11 (m, 6H), 1.85-1.69 (m, 8H). ^{13}C NMR (100 MHz, CD_3OD): δ 177.0, 165.1, 160.6, 150.3, 132.7, 108.3, 106.2, 97.5, 61.6, 56.8, 55.2, 53.4, 42.5, 39.0, 28.3. ESI m/z 7.59.4 ($M + Na^+$). Anal. ($C_{34}H_{46}Cl_2N_6O_8 \cdot 3H_2O$), C, H, N.

Dimer 16b. Method described for **4a**, from **3** (2 eq), 1,3-diaminopropane (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.52; 57% yield; white foam; ^1H NMR (200 MHz, CD_3OD): δ 7.77 (s, 2H), 6.49 (s, 2H), 4.37 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.21 (t, *J* = 6.7 Hz, 4H), 3.10 (m, 4H), 2.78 (t, *J* = 5.7 Hz, 4H), 2.31-2.10 (m, 6H), 1.81 (m, 8H), 1.68 (q, *J* = 6.7 Hz, 2H). ^{13}C NMR (50 MHz, CD3OD): δ 178.7, 167.3, 162.8, 152.3, 135.0, 111.2, 109.3, 99.6, 63.6, 58.7, 55.1, 55.3, 44.7, 38.4, 31.1, 30.5. ESI m/z 751.2 ($M + H^+$), 773.3 ($M + Na^+$). Anal. ($C_{35}H_{48}Cl_2N_6O_8 \cdot H_2O$), C, H, N.

Dimer 16c. Method described for **4a**, from **3** (1 eq), **15** (2 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 80:20; R*f* (CH_2Cl_2/MeOH 80:20) 0.4; 31% yield; white foam; ^1H NMR (200 MHz, CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.40 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.16 (m , 8H), 2.85 (t, *J* = 5.7 Hz, 4H), 2.40-2.20 (m, 6H), 1.89-1.47 (m, 8H), 1.52 (m, 4H), 1.34 (m, 2H) . ^{13}C NMR (50 MHz, CD3OD): δ 178.4, 167.3, 162.9, 152.4, 135.0, 111.2, 109.3, 99.6, 63.3, 58.7, 57.2, 55.2, 44.4, 41.0, 30.9, 30.3, 26.0. ESI m/z 779.2 ($M + H^+$), 801.3 ($M + Na^+$). Anal. ($C_{37}H_{52}Cl_2N_6O_8 \cdot 2.5\ H_2O$), C, H, N.

Dimer 16d. Method described for **4a**, from **17** (2 eq), 1,10-diaminodecane (1 eq), EDC (2.95 eq), HOBt (2.95 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2; R*f* (CH_2Cl_2/MeOH/NH_4OH 20% 88:10:2) 0.31; 64% yield; beige foam; ^1H NMR (200 MHz,

CD_3OD): δ 7.78 (s, 2H), 6.49 (s, 2H), 4.39 (t, *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.17 (m , 8H), 2.85 (t, *J* = 5.7 Hz, 4H), 2.40-2.15 (m, 6H), 1.90-1.70 (m, 8H), 1.52 (m, 4H), 1.33 (bs, 12H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 174.9, 164.6, 160.6, 150.8, 133.3, 108.8, 107.8, 98.7 , 62.1, 57.1, 56.5, 54.0, 42.5, 39.2, 30.0, 29.8, 29.6, 29.3, 27.2. ESI m/z 849.3 (M + H^+), 871.4 (M + Na^+). Anal. ($C_{42}H_{62}Cl_2N_6O_8$·1.5 H_2O), C, H, N.

Dimer 18a. Method described for **4a,** from **17**[26] (2 eq), pimelic acid (1 eq), EDC (2.6 eq), HOBt (2.6 eq), and NEt_3 (6 eq). Acetone then CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 90:10) 0.1; 91% yield; resinous amber solid; ^{1}H NMR (200 MHz, CD_3OD): δ 7.77 (s, 2H), 6.49 (s, 2H), 4.39 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.60 (m , 8H), 2.80 (t , *J* = 5.7 Hz, 4H), 2.60 (s, 8H), 2.38 (t, *J* = 7.34 Hz, 4H), 1.75-1.52 (m, 4H), 1.41 (m, 2H). ^{13}C NMR (50 MHz, CD_3OD): δ 171.4, 164.7, 160.6, 150.8, 133.3, 108.8, 107.9, 98.7, 62.3, 56.9, 56.5, 54.1, 53.6, 45.9, 41.9, 34.1, 33.0, 29.4, 25.3. ESI m/z 751.4 (M + H^+), 773.3 (M + Na^+). Anal. ($C_{35}H_{48}Cl_2N_6O_8$), C, H, N.

Dimer 18b. Method described for **4a**, from **17**[26] (2 eq), dodecanedioic acid (1 eq), EDC (2.6 eq), HOBt (2.6 eq), and NEt_3 (6 eq). CH_2Cl_2/*i*Pr_2OH 80:20 then CH_2Cl_2/MeOH 90:10; R*f* (CH_2Cl_2/MeOH 80:20) 0.74; 68% yield; resinous amber solid; ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.76 (s, 2H), 6.49 (s, 2H), 4.38 (t , *J* = 5.7 Hz, 4H), 3.83 (s, 6H), 3.60 (m , 8H), 2.82 (t , *J* = 5.7 Hz, 4H), 2.6 (s, 8H), 2.40 (t, *J* = 7.34 Hz, 4H), 1.6 (m, 4H), 1.33 (bs, 12H). ^{13}C NMR (50 MHz, CD_3OD): δ 171.5, 164.8, 160.7, 150.7, 133.3, 108.8 108.0, 98.7, 62.3, 57.0, 56.5, 54.1, 53.6, 46.0, 42.0, 33.1, 29.9, 29.8, 29.7, 25.8. ESI m/z 821.4 (M + H^+), 843.3 (M + Na^+). Anal. ($C_{40}H_{58}Cl_2N_6O_8$), C, H, N.

Ethyl 12-[1-benzylpiperidin-4-yl)amino]-12-oxododecanoate (19). Same procedure as described for **4a** from 4-amino-1-benzylpiperidine dihydrochloride and dodecanedioic acid monoethyl ester[22] as described for **4a**. CH_2Cl_2, then CH_2Cl_2/MeOH 97:3 and CH_2Cl_2/MeOH 95:5; R*f* (CH_2Cl_2/MeOH 95:5) 0.42; 63% yield; yellow solid; mp 76-78 °C;^{1}H NMR (200

MHz): δ 7.33-7.23 (m, 5H), 5.44-5.31 (bd, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.90-3.68 (m, 1H), 3.54 (s, 2H), 2.85 (m, 2H), 2.34-2.05 (m, 6H), 1.96-1.80 (m, 2H), 1.70-1.39 (m, 6H), 1.35-1.21 (m, 15H). ^{13}C NMR (50 MHz): δ 174.1, 172.6, 137.5, 129.5, 128.4, 127.4, 62.9, 60.3, 52.2, 46.2, 37.0, 34.5, 32.0, 29.5, 29.4, 29.3, 29.2, 25.9, 25.4, 25.1, 14.4.

2-[4-({12-[(1-Benzylpiperidin-4-yl)amino]-12oxododecanoyl}amino)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (20). To a solution of **19** (449 mg, 1.04 mmol, 1 eq) in 24 mL dioxane was added a solution of LiOH (87 mg, 2.09 mmol, 2 eq) in water (6 mL). The resulting mixture was stirred 7 h at room temperature, and the solvents were evaporated. The solid obtained was dissolved in 15 mL DMF, and **7a** (416 mg, 1.04 mmol, 1 eq), HOBt (142 mg, 1.04 mmol, 1 eq), NEt3 (725 µL, 5.20 mmol, 5 eq) and finaly EDC (200 mg, 1.04 mmol, 1 eq) were added. The reaction mixture was stirred at room temperature for 8 h, and the solvent removed under vacuum. The solid obtained was washed with saturated Na_2CO_3 (5 mL), and extracted with AcOEt (3 x 15 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (AcOEt/MeOH 90/10, then AcOEt/MeOH/NH_4OH 20% 88/10/2) to afford 277 mg (37%) of **20** as a white solid. R*f* (AcOEt/MeOH/NH_4OH 20% 88/10/2) 0.2. ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 7.35-7.16 (m, 5H), 6.27 (s, 1H), 5.32 (bd, *J* = 8.1 Hz, 2H), 4.45 (bs, 2H), 4.35 (t, *J* = 6.1 Hz, 2H), 3.48 (s, 2H), 3.87-3.68 (m, 5H), 3.02-2.66 (m, 6H), 2.34-2.01 (m, 8H), 1.98-1.74 (m, 4H), 1.68-1.53 (m, 4H), 1.53-1.34 (m, 4H), 1.32-1.19 (m, 12H). ^{13}C NMR (50 MHz): δ 172.6, 148.0, 138.6, 133.4, 129.2, 128.4, 127.1, 98.5, 63.2, 62.4, 56.8, 56.2, 52.8, 52.4, 46.5, 46.4, 37.1, 32.5, 29.5, 29.4, 25.9. ESI m/z 712.6 ($M + H^+$). Anal. ($C_{39}H_{58}ClN_5O_5 \cdot H_2O$), C, H, N.

2-(4-{[(3-{[(*tert*-Butoxycarbonyl)amino]methyl}benzyl)amino]carbonyl}piperidino)ethyl 4-amino-5-chloro-2-methoxybenzoate (21). Same procedure as described for **4a** from **3** and 3-(Boc-aminomethyl) -benzylamine. CH_2Cl_2/MeOH 95:5; R*f* (CH_2Cl_2/MeOH 95:5) 0.30; 57% yield; beige foam; ^{1}H NMR (200 MHz): δ 7.80 (s, 1H), 7.33-7.08 (m, 4H),6.28 (s,1H),

5.76 (bt, 1H), 4.83 (bd, 1H), 4.54-4.40 (m, 4H), 4.36 (t, *J* = 5.9 Hz, 2H), 4.29 (m, 2H), 3.83 (s, 3H), 3.03 (m, 2H), 2.73 (t, *J* = 5.9 Hz, 2H), 2.23-2.03 (m, 3H), 1.95-1.69 (m, 4H), 1.86 (s, 9H). Anal. ($C_{29}H_{39}ClN_4O_6$), C, H, N.

2-[4-({[3-(aminomethyl)benzyl]amino}carbonyl)piperidino]ethyl 4-amino-5-chloro-2-methoxybenzoate (22). Same procedure as described for **9a**. Very hygroscopic beige foam; 99% yield; ^{1}H NMR (200 MHz): δ**.** 7.78 (s, 1H), 7.30-7.05 (m, 4H), 6.26 (s,1H), 5.90 (bt, 1H), 4.50 (bs, 2H), 4.40 (d, *J* = 5.3 Hz, 2H), 4.33 (t, *J* = 5.9 Hz, 2H), 3.80 (d, *J* = 5.5 Hz, 2H), 3.81 (s, 3H), 3.01 (m, 2H), 2.70 (t, *J* = 5.9 Hz, 2H), 2.24-2.01 (m, 3H), 1.93-1.65 (m, 4H), 0.92 (bs, 2H). Anal. ($C_{24}H_{31}ClN_4O_4$·2HCl·3H_2O), C, H, N.

Dimer 23. Method described for **4a,** from **22** and **3.** CH_2Cl_2/MeOH 90:10, then CH_2Cl_2/MeOH/NH_4OH 90:8:2 ; R*f*(CH_2Cl_2/MeOH/NH_4OH 20% 90:8:2) 0.6 ; 29% yield ; beige foam ; ^{1}H NMR (200 MHz, CD_3 OD): δ 7.86 (s, 2H), 7.44-7.20 (m, 4H), 6.57 (s, 2H), 4.52-4.41 (m, 8H), 3.90 (s, 6H), 3.20 (m, 4H), 2.88 (t , *J* = 5.7 Hz, 4H), 2.47-2.20 (m, 6H), 2-1.82 (m, 8H). ^{13}C NMR (50 MHz, CD_3OD): δ 176.2, 166.7, 162.8,141.2, 135.3, 128.3, 111.8, 109.0, 100.1, 60.5, 58.1, 54.7, 54.4, 44.8, 41.7, 28, 4. ESI m/z 813.2 (M + H^+). Anal. ($C_{40}H_{50}Cl_2N_6O_8$·2HCl·7H_2O), C, H, N.

Dimer 24. Method described for **4a**, from **7a**[21] (2 eq), 3-[6-(3-hydroxy-3-oxopropyl)dibenzo[b,d]furan-4-yl]propanoic acid[23] (1 eq), EDC (2.95 eq), HOBt (2.95 eq) and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 90:10 ; R*f* (CH_2Cl_2/MeOH 90:10) 0.12 ; 62% yield ; white foam ; ^{1}H NMR (200 MHz, CD_3OD): δ 7.84 (m, 2H), 7.76 (s, 2H), 7.38-7.23 (m, 4H), 6.50 (s, 2H), 4.36 (t, *J* = 5.7 Hz, 4H), 3.80 (s, 6H), 3.70 (m, 2H), 3.33 (t, *J* = 7.1 Hz, 4H), 3.01 (m, 4H), 2.86 (t , *J* = 5.7 Hz, 4H), 2.72 (t, *J* = 7.1 Hz, 4H), 2.40 (m, 4H), 1.78 (m, 4H), 1.48 (m, 4H). ^{13}C NMR (50 MHz, CD_3OD): δ 175.3, 167.2, 156.5, 152.4, 135.0, 129.3, 125.0, 120.7, 111.2, 109.1, 99.6, 62.9, 58.3, 57.1, 54.3, 47.8, 38.1, 32.5, 28.1. ESI m/z 933.4 (M + H^+), 954.4 (M + Na^+). Anal. ($C_{48}H_{56}Cl_2N_6O_9$·2.5H_2O), C, H, N.

Methyl 11-({4-(benzylamino)-6-[(11-methoxy-11-oxoundecyl)amino]-1,3,5-triazin-2-yl}amino)undecanoate (25). A solution of N-benzyl-4,6 dichloro-1,3,5-triazin-2-amine[24] (1.5 g, 5.88 mmol, 1 eq) in anhydrous CH_3CN (30-40 mL) was treated with methyl 11-aminododecanoate[25] (2.4 eq, 14.1 mmol, 2.4 eq) and DIEA (4.56 g, 35.3 mmol, 6 eq). The resulting solution was heated to reflux for 24h and then cooled to room temperature. The reaction was poured into 5% aqueous citric acid (20 mL), extracted with AcOEt (3 x 50 mL). The organic layers were combined, washed with brine (50 mL) and dried ($MgSO_4$). The crude oil was chromatographed on silica gel (AcOEt/cyclohexane 50:50) to afford 2.1 g (58%) of **25** as a light yellow oil. R*f* (AcOEt/cyclohexane 50:50) 0.36. ^{1}H NMR (200 MHz): δ 7.34-7.15 (m, 5H), 5.19 (bs, 1H), 4.87 (bs, 2H), 4.49 (d, *J* = 5.8 Hz, 2H), 3.57 (s, 6H), 3.24 (m, 4H), 2.22 (t, *J* = 7.4 Hz, 4H), 1.71-1.35 (m, 8H),1.20 (bs, 24H). Anal. ($C_{34}H_{56}N_6O_4$), C, H, N.

11-({4-(benzylamino)-6-[(11-hydroxy-11-oxoundecyl)amino]-1,3,5-triazin-2-yl}amino)undecanoic acid (26). To a solution of **25** (1.9 g, 3.1 mmol) in dioxane (40 mL) was added dropwise NaOH (0.5 g in 10 mL water) The reaction mixture was stirred for 3h at room temperature, then heated under reflux for 1h. After cooling and half concentrated, the mixture was acidified to pH 3 with conc HCl and treated with 10% aqueous $NaHSO_4$. The precipitate formed was extracted with AcOEt, dried ($MgSO_4$) and concentrated to give 1.63 g (90%) of **26** as a beige solid. A sample was recrystallized from toluene/AcOEt to afford a white amorphous solid, mp 122-124 °C;. ^{1}H NMR (200 MHz, CD_3OD): δ 7.50-7.17 (m, 5H), 4.62 (s, 2H), 3.40 (t , *J* = 7.2 Hz, 4H), 2.29 (t , *J* = 7.2 Hz, 4H), 1.80-1.45 (m, 8H),1.25 (bs, 24H). Anal. ($C_{32}H_{52}N_6O_4 \cdot 2H_2O$), C, H, N.

Dimer 27. Method described for **4a,** from **7a**[21] (2 eq), diacid **26** (1 eq), EDC (2.6 eq), HOBt (3.4 eq), and NEt_3 (5 eq), except anhydrous DMF was used instead of CH_2Cl_2. CH_2Cl_2/MeOH 80:20; R*f* (CH_2Cl_2/MeOH 80:20) 0.40; 43% yield; beige foam; ^{1}H NMR (200 MHz): δ 7.78 (s, 2H), 7.35-7.15 (m, 5H), 6.28 (s, 2H), 6.24-5.70 (bs, 3H), 5.50 (bs, 2H), 4.57 (m, 6H), 4.41

(t , *J* = 5.7 Hz, 4H), 3.80 (m, 2H), 3.79 (s, 6H), 3.29 (m, 4H), 3.00 (m, 4H), 2.83 (t , *J* = 5.7 Hz, 4H), 2.34 (m, 4H), 2.12 (t , *J* = 7.2 Hz, 4H), 2.00-1.82 (m, 4H), 1.70-1.40 (m, 12H), 1.25 (m, 24H). ^{13}C NMR (50 MHz): δ 172.9, 164.8, 160.6, 148.4, 133.6, 128.8, 127.8, 127.5, 110.2, 109.6, 98.5, 62.2, 56.9, 56.3, 52.9, 46.3, 41.1, 37.2, 32.3, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 27.1, 26.0. ESI m/z 1203.45 (M + H^+), Anal. ($C_{62}H_{92}$ $Cl_2N_6O_4$·2.5H_2O), C, H, N.

4-[2-Benzyloxycarbonylamino-acetylamino)-methyl]-piperidine-1-carboxylic acid *tert*-butyl ester (28). Method described for **4a**, from Z-gly-OH (1 eq), EDC (1.5 eq), HOBt (1 eq), and NEt_3 (4.5 eq). CH_2Cl_2/MeOH 95:5; R*f* (CH_2Cl_2/MeOH 95:5) 0.7; mp 114 °C; 95% yield; beige solid; ^{1}H NMR (200 MHz,): δ 7.34 (s, 5H), 6.22 (bs, 1H), 5.48 (bt, 1H), 5.12 (s, 2H), 4.05 (m, 2H), 3.82 (d, *J* = 5.8 Hz, 2H), 3.13 (m, 2H), 2.64 (m, 2H); 1.60 (m, 3H), 1.44 (s, 9H),1.07 (m, 2H). Anal. ($C_{21}H_{31}N_3O_5$·0.25H_2O), C, H, N.

4-[(2-Amino-acetylamino)-methyl]-piperidine-1-carboxlic acid *tert*-butyl ester (29). A solution of **28** (2.5 g, 6.6 mmol) and Pd/C 10% (0.6 g) in MeOH (120 mL) was hydrogenated under atmospheric pressure at room temperature for 3h. The reaction mixture was filtered through celite and then the filtrate was concentrated in vacuo to give 1.45 g (89%) of a white solid which was used in next step without any further purification. mp 98-100 °C; ^{1}H NMR (200 MHz,): δ 7.45 (bt, 1H), 4.05 (m, 2H), 3.37 (s, 2H), 3.17 (m, 2H), 2.73 (m, 2H), 1.95 (bs, 2H), 1.63 (m, 3H), 1.44 (s, 9H), 1.10 (m, 2H). Anal. ($C_{13}H_{25}N_3O_5$·0.25H_2O), C, H, N.

4-({2-[Bis-(10-methoxycarbonyl-decyl-amino]-acetylamino}-methyl)-piperidine-1-carboxylic acid *tert*-butyl ester (30). To a stirred solution of **29** (1.45 g, 5.35 mmol, 1 eq) in 20 mL of anhydrous DMF, were added Cs_2CO_3 (4.19 g 12.86 mmol, 2.4 eq) and methyl 11-bromoundecanoate (3.59 g, 12.86 mmol, 2.4 eq). The mixture was stirred at room temperature for 48 h and then concentrated. The resulting residue was taken up by CH_2Cl_2 (40 mL), washed with brine (40 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated under vacuo. The crude residue obtained was first chromatographed on silica gel

(CH_2Cl_2/MeOH 90:10) to give a limpid yellow oil (R*f* 0.45) which was again chromatographed using (AcOEt/cyclohexane 60:30) to afford 1.25 g (35%) of a limpid oil. R*f* (AcOEt/cyclohexane 60:30) 0.51; ^{1}H NMR (200 MHz, DMSO-*d6*): δ 7.50 (bt, 1H), 3.94 (m, 2H), 3.33 (s, 6H), 3.04 (m, 2H), 2.95 (s, 2H), 2.69 (m, 2H), 2.42 (bt, 4H), 2.31 (t, *J* = 7.3 Hz, 4H), 1.56 (m, 3H), 1.42 (s, 9H), 1.28 (bs, 32H), 1.03 (m, 2H). Anal ($C_{37}H_{69}N_3O_7$ 0.5H_2O), C, H, N.

4-({2-[Bis-(10-{1-[2-(4-amino-5-chloro-2-methoxy-benzoyloxy)-ethyl]-piperidin-4-ylcarbamoyl}-decyl)-amino]-acetylamino}-methyl)-piperidine-1-carboxylic acid *tert*-butyl ester (31). To a solution of **30** (1.25 g, 1.87 mmol) in THF (20 mL) was added at room temperature aqueous lithium hydroxide (0.19 g in 10 mL water) dropwise. This solution was stirred for 7 h and evaporated to give 1.37 g of a white solid. 0.34 g of this solid was dissolved in anhydrous DMF (30-40 mL) and treated at 0 °C sequentially with HOBt (155 mg, 1.17 mmol, 1.17 eq), EDC (0.22 g, 1.15 mmol, 1.15 eq), NEt_3 (0.25 g, 2.5 mmol, 2.5 eq) then **7a**[21] (0.4 g, 1.0 mmol, 1 eq) was added. The mixture was allowed to warm to room temperature, stirred for 24 h and evaporated. The crude product was taken up with CH_2Cl_2 (40 mL), and successively washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated. The crude product was chromatographed on silica gel (CH_2Cl_2/*i*PrOH 90:10) then (CH_2Cl_2/MeOH 90:10) to yield 0.16 g (24%) of a white foam. R*f* (CH_2Cl_2/MeOH 90:10) 0.43; ^{1}H NMR (400 MHz): δ 7.81 (s, 2H), 7.50 (bt, 1H), 6.30 (s, 2H), 5.54 (bd , 2H), 4.54 (bs, 4H), 4.37 (t, *J* = 5.9 Hz, 4H), 4.10 (bs, 2H), 3.84 (s, 6H), 3.81 (m, 2H), 3.13 (bs, 2H), 3.03 (s, 2H), 2.96 (m, 4H), 2.76 (t, *J* = 5.9 Hz, 4H), 2.68 (m, 4H), 2.44 (t, *J* = 7.4 Hz, 4H), 2.28 (m, 4H), 2.15 (t, *J* = 7.3 Hz, 4H), 1.93 (m, 4H), 1.64 (m, 11H), 1.46 (m, 13H), 1.28 (bs, 24H), 1.14 (m, 2H). C^{13} NMR (100 MHz): δ 172.5, 172.1, 167.7, 160.3, 147.9, 133.3, 109.9, 109.6, 98.2, 62.0, 56.6, 56.0, 55.5, 52.6, 46.1,

36.9, 32.2, 29.3, 28.4, 27.4, 25.8. . ESI m/z 1258.5 (M + H^+), 1280.3 (M + Na^+). Anal. ($C_{65}H_{105}Cl_2N_9O_{11}$ 1.5H_2O), C, H, N.

4-({2-[Bis-(10-{1-[2-(4-amino-5-chloro-2-methoxy-benzoyloxy)-ethyl]-piperidin-4-yl-carbamoyl}-decyl)-amino]-acetylamino}-methyl)-piperidine (32). Method described for **9a**. Very hygroscopic white foam; 99% yield; ^{1}H NMR (400 MHz): δ 7.80 (s, 2H), 7.50 (bt, 1H), 6.30 (s, 2H), 5.67 (bd , *J* = 8 Hz, 2H), 4.59 (bs, 4H), 4.35 (t, *J* = 5.9 Hz, 4H), 3.83 (s, 6H), 3.78 (m, 2H), 3.15 (m, 4H) 3.01 (s, 2H), 2.93 (m, 4H), 2.73 (t, *J* = 5.9 Hz, 4H), 2.63 (bt, 2H), 2.43 (t, *J* = 7.4 Hz, 4H), 2.24 (m, 4H), 2.14 (t, *J* = 7.6 Hz, 4H), 1.92 (m, 4H), 1.72 (m, 2H), 1.61 (m, 6H), 1.45 (m, 8H), 1.28 (bs, 26H). C^{13} NMR (100 MHz): δ 172.5, 172.1, 164.5 160.2, 147.9, 133.3, 109.9, 109.5, 98.2, 62.1, 58.7, 56.7, 56.0, 55.5, 52.7, 46.2, 45.6, 44.6, 38.9, 36.2, 32.3, 30.2, 29.50, 29.44, 29.35, 29.27, 29.18, 27.5, 27.4, 25.8. ESI m/z 1158.6 (M + H). Anal (C_{60} $H_{97}Cl_2N_9O_9$ 2H_2O), C, H, N.

Biology

Membrane Preparation and Radioligand Binding Assays

Briefly, C6 glial cells stably transfected with h5-$HT_{4(e)}$ receptors, grown to confluence were incubated with serum-free medium for 4 h, washed twice with phosphate-buffered-saline (PBS) and centrifuged at 300g for 5 min. The pellet was used immediately or stored at –80°C. The pellet was resuspended in 10 volumes of ice-cold HEPES buffer (50 mM, pH 7.4) and centrifuged at 40000g for 20 min at 4°C. The resulting pellet was resuspended in 15 volumes of HEPES buffer (50 mM, pH 7.4). The protein concentration was determined by the method of Bradford using bovine serum albumin as the standard.

Radioligand binding studies were performed in 500 µL of HEPES buffer (50 mM, pH 7.4), 20 µL of the studied ligand (7 concentrations), 20 µL of [^{3}H]GR113808 at a concentration of 0.2 nM and 50 µL of membranes preparation (100-200 µg of protein). Non-specific binding was

determined with 10 μM GR113808. Tubes were incubated at 25°C for 30 min, and the reaction was terminated by filtration through Whatman GF/B Filter paper using the Brandel 48R cell harvester. Filters were presoaked in a 0.1% solution of polyethylenimine. Filters were subsequently washed with ice-cold buffer (50 mM Tris-HCl, pH 7.4) and placed overnight in 4 mL of ready-safe scintillation cocktail. Radioactivity was measured using a Beckman model LS 6500C liquid scintillation counter. Binding data (Ki) were analyzed by computer-assisted nonlinear regression analysis (Prism, Graphpad Software, San Diego, CA). The data are the results of two or three determinations in triplicate.

Measurement of cAMP

C6 glial cells stably transfected with human 5-$HT_{4(e)}$ receptors were grown to confluence and incubated with serum-free medium for 4 h before the beginning of the assay. Then the cells were preincubated for 15 min with serum-free medium supplemented with 5 mM theophylline and 10 μM pargyline. 5-HT (1 μM) and/or compounds were added and incubated for an additional 15 min at 37 °C in 5% CO_2. The reaction was stopped by aspiration of the medium and addition of 50 μL of ice-cold perchloric acid (20%). After a 30 min period, neutralization buffer was added (HEPES 25 mM, KOH 2N) and supernatant was extracted after centrifugation at 2000g for 5 min, cAMP was quantified using radioimmunoassay kit (cAMP competitive radioimmunoassay, Beckman, France). The 5-HT concentration-effect curve was calculated using seven concentrations (10^{-10}-10^{-5}) alone or in the presence of compounds. The ligand concentration-effect curves were calculated using seven concentrations (10^{-10}-10^{-5}).

References

(1) Bulenger, S. M., S.; Bouvier, M. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends in pharmacological sciences* **2005**, *in press*.

(2) Breitwieser, G. G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. *Circ Res.* **2004**, *94*, 17-27.

(3) Bai, M. Dimerization of G-protein-coupled receptors: roles in signal transduction. *cell. signal.* **2004**, *16*, 175-186.

(4) Milligan, G. Oligomerization of G-protein-coupled receptors. *J. Cell. Sci.* **2001**, *114*, 1265-1271.

(5) Hansen, J. L. Sheikh, S. P. Functional consequences of 7TM receptor dimerization. *Eur. J. of Pharm. Sci.* **2004**, *23*, 301-317.

(6) Berthouze, M. Ayoub, M.; Russo, O.; Rivail, L.; Sicsic, S.; Fischmeister, R.; Berque-Bestel, I.; Jockers, R.; Lezoualc'h, F. Constitutive dimerization of human serotonin 5-HT_4 receptors in living cells. *Febs letter* **2005**, in press.

(7) Langlois, M.; Fischmeister, R. 5-HT_4 Receptor Ligands: Applications and New Prospects. *J. Med. Chem.* **2003**, *46*, 319-344.

(8) Robert, S. J.; Zugaza, J. L.; Fischmeister, R.; Gardier, A. M.; Lezoualc'h, F. The human serotonin 5-HT_4 receptor regulates secretion of non-amyloidogenic precursor protein. *J. Biol. Chem.* **2001**, *276*, 44881-44888.

(9) Maillet, M.; Robert, S.J.; Lezoualc'h, F. New insights into serotonin 5-HT_4 receptors: a novel therapeutic target for Alzheimer's disease? *Curr. Alzheimer Res.* **2004**, *1*, 79-86.

(10) Portoghese, P. S. 2000 Alfred Burger Award Address in Medicinal Chemistry. From Models to Molecules: Opioid Receptor Dimers, Bivalent Ligands, and Selective Opioid Receptor Probes. *J. Med. Chem.* **2001**, *44*, 3758-3758.

(11) Bhushan, R. G.; Sharma, S. K.; Xie, Z.; Daniels, D. J.; Portoghese, P. S. A bivalent ligand (KDN-21) reveals spinal delta and kappa opioid receptors are organized as heterodimers that give rise to delta(1) and kappa(2) phenotypes. Selective targeting of delta-kappa heterodimers. *J. Med. Chem.* **2004**, *47*, 2969-2972.

(12) Rivail, L.; Giner, M.; Gastineau, M.; Berthouze, M.; Soulier, J.L.; Fischmeister, R.; Lezoualc'h, F.; Maigret, B.; Sicsic, S. and Berque-Bestel, I. First exploration of the hydrophobic pocket essential for the binding of bulky ligands on human 5-HT_4 receptors. *Br. J. Pharmacol.* **2004**, *143*, 361-370.

(13) Vakser, I. A. Protein docking for low-resolution structures. *Protein Eng.* **1995**, *8*, 371-377.

(14) Vakser, I. A. Evaluation of GRAMM low-resolution docking methodology on the hemagglutinin-antibody complex. *Proteins* **1997**, *Suppl 1*, 226-230.

(15) Vakser, I. A.; Jiang, S. Strategies for modeling the interactions of transmembrane helices of G protein-coupled receptors by geometric complementarity using the GRAMM computer algorithm. *Methods Enzymol* **2002**, *343*, 313-328.

(16) Guo, W.; Shi, L.; Javitch, J.A. The fourth transmembrane segments forms the interface oh the dopamine D2 receptor homodimer. *J. Biol. Chem.* **2003**, *278*, 4385-4388.

(17) Klco, J. M.; Lassere, T. B.; Baranski, T. J. C5a receptor oligomerization. I. Disulfide trapping reveals oligomers and potential contact surfaces in a G protein-coupled receptor. *J Biol Chem* **2003**, *278*, 35345-35353.

(18) Fotiadis, D.; Liang, Y.; Filipek, S.; Sapertstein, D.A.; Engel, A.; Palczewski, K. The G protein-coupled receptor rhodopsin in the native membrane. *FEBS Lett.* **2004**, *564*, 281-288.

(19) Overton, M. C.; Chinault, S.L.; Blumer, K.J. Oligomerization, biogenesis, and signaling is promoted by a glycophorin A-like dimerization motif in transmembrane domain 1 of a yeast G protein-coupled receptor. *J. Biol. Chem.* **2003**, *278*, 49369-49377.

(20) Yang, D.; Soulier, J. L.; Sicsic, S.; Mathe-Allainmat, M.; Bremont, B. et al. New esters of 4-amino-5-chloro-2-methoxybenzoic acid as potent agonists and antagonists for 5-HT_4 receptors. *J. Med. Chem.* **1997**, *40*, 608-621.

(21) Berque-Bestel, I.; Soulier, J. L.; Giner, M.; Rivail, L.; Langlois, M. et al. Synthesis and characterization of the first fluorescent antagonists for human 5-HT_4 receptors. *J. Med. Chem.* **2003**, *46*, 2606-2620.

(22) Roy, B. C.; Hormas, D.; Mallik, S. Synthesis of new, pyrene-containing metal, chelating lipids and sensing of cupric ions. *Org. Lett.* **2003**, *5*, 11-14.

(23) Diaz, H. K., J.W. The synthesis of dibenzofuran based diacics and aminoacids designed to nucleate parallel and antiparallel beta-sheat formation. *Tetrahedron Lett.* **1991**, *32*, 5725-5728.

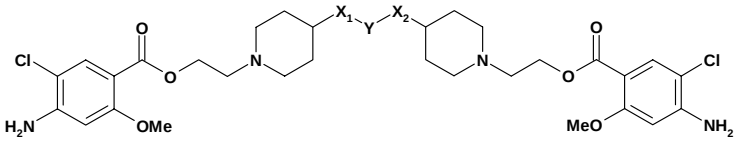
(24) Rathod, K. T.; Patel, P.M.; Patel, S.K.; Patel, K.C. Synthesis and physico chemical properties based on s-triazine. *Ultra Science* **1999**, *11*, 36-41.

(25) Leydet, A.; Barragan, V.; Boyer, B.; Montero, J. L.; Roque, J. P. et al. Polyanion inhibitors of human immunodeficiency virus and other viruses. 5. Telomerized anionic surfactants derived from amino acids. *J. Med. Chem.* **1997**, *40*, 342-349.

(26) Curtet, S.; Soulier, J.L.; Zahradnik, I.; Giner, M.; Berque-Bestel, I.; Mialet, J.; Lezoualc'h, F.; Donzeau-Gouge P.; Sicsic, S.; Fischmeister, R; Langlois, M. New

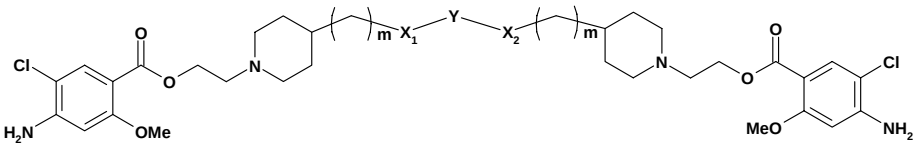
arylpiperazine derivates as antagonits of the human cloned 5-HT_4 receptor isoforms. *J. Med. Chem.* **2000**, *43*, 3761-3769.

(27) Kawanishi, Y.; Ishihara, S.; Takahashi, K.; Tsushima, T.; Hagishita, S.; Ishikawa, M.; Ishihara, Y. Synthesis and biological evaluation of a new reversely linked type of dual histamine H2 and gastrin receptor antagonist. *Chem. Pharm. Bull.* **1997**, *45(1)*, 116-124.



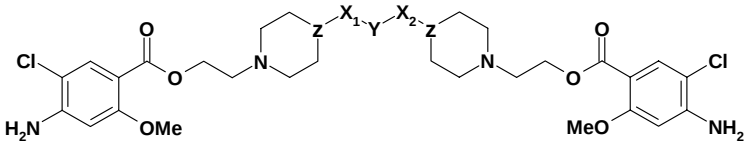
N°	X_1	Y	X_2	Spacer Size (Atom number)	Ki (nM)	cAMP (%)
ML10302					5 ± 2.5	45
6a	CONH	$(CH_2)_2$-O-$(CH_2)_2$	NHCO	9	19 ± 9.4	0
6b	CONH	$(CH_2)_2$-O-$(CH_2)_2$-O-$(CH_2)_2$	NHCO	12	3 ± 2.6	3
6c	CONH	$CH_2(CH_2$-CH_2-O$)_3$-CH_2	NHCO	15	6 ± 1.8	0
10a	NHCO	$(CH_2)_2$	NHCO	6	24 ± 11.6	10
10b	NHCO	$(CH_2)_3$	NHCO	7	31 ± 6.9	42
10c	NHCO	$(CH_2)_5$	NHCO	9	7 ± 3.2	46
10d	NHCO	$(CH_2)_{10}$	NHCO	14	20 ± 12.7	5
11a	NHCO	$(CH_2)_2$	CONH	6	21 ± 1,4	47
11b	NHCO	$(CH_2)_3$	CONH	7	5 ± 0,8	32
11c	NHCO	$(CH_2)_5$	CONH	9	9 ± 3,1	26
11d	NHCO	$(CH_2)_{10}$	CONH	14	16 ± 0,5	20
16a	CONH	$(CH_2)_2$	NHCO	6	37 ± 12.6	3
16b	CONH	$(CH_2)_3$	NHCO	7	13 ± 4.1	13
16c	CONH	$(CH_2)_5$	NHCO	9	5 ± 3.5	5
16d	CONH	$(CH_2)_{10}$	NHCO	14	8 ± 1.7	8

Table 1



N°	m	X_1	Y	X_2	Spacer Size (Atom number)	Ki (nM)	cAMP (%)
11a	0	NHCO	$(CH_2)_2$	CONH	6	21 ± 1.4	47
11b	0	NHCO	$(CH_2)_3$	CONH	7	5 ± 2.8	32
11c	0	NHCO	$(CH_2)_5$	CONH	9	9 ± 3.1	26
11d	0	NHCO	$(CH_2)_{10}$	CONH	14	16 ± 9.3	20
12a	1	NHCO	$(CH_2)_2$	CONH	8	7 ± 2.3	16
12b	1	NHCO	$(CH_2)_3$	CONH	9	13 ± 6.5	20
12c	1	NHCO	$(CH_2)_5$	CONH	11	26 ± 15.7	20
12d	1	NHCO	$(CH_2)_{10}$	CONH	16	22 ± 13.1	21
13a	2	NHCO	$(CH_2)_2$	CONH	10	5 ± 2.4	15
13b	2	NHCO	$(CH_2)_3$	CONH	11	3 ± 3.2	24
13c	2	NHCO	$(CH_2)_5$	CONH	13	11 ± 3.4	31
13d	2	NHCO	$(CH_2)_{10}$	CONH	18	9 ± 4.0	6

Table 2



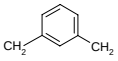
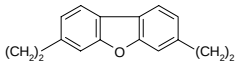
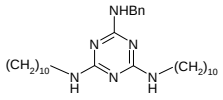
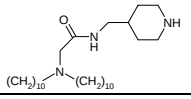
N°	Z	X_1	Y	X_2	Spacer Size (Atom number)	Ki (nM)	cAMP (%)
18a	N	CO	$(CH_2)_5$	CO	7	488 ± 245	nd
18b	N	CO	$(CH_2)_{10}$	CO	12	200 ± 60	nd
23	C	CONH		NHCO	9	5 ± 1.2	16
24	C	NHCO		CONH	15	47 ± 17.5	23
27	C	NHCO		CONH	31	113 ± 85	12
32	C	NHCO		CONH	25	50 ± 32	10

Table 3

Figure legends

Figure 1

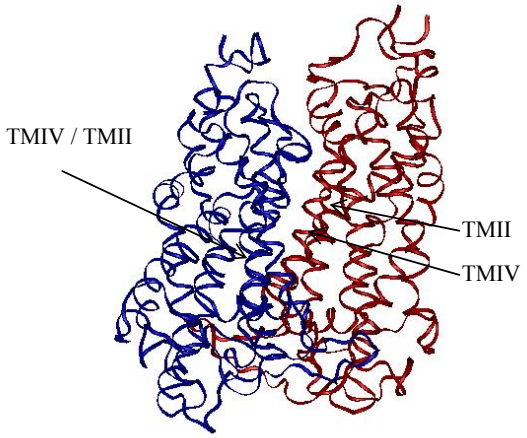
5-HT_4 receptor dimer models obtained by protein-protein docking experiments using the GRAMM software. **A:** dimer where dimer interfaces included helices II and IV. **B:** dimer where dimer interfaces included helices IV and VI.

Figure 2

Concentration-effect curves for 5-HT on adenylyl cyclase activity in C6 glial cells stably transfected with the h5-$HT_{4(e/g)}$ receptor isoform: ■, 5-HT alone; ▲, 5-HT + **10d** (200 nM).

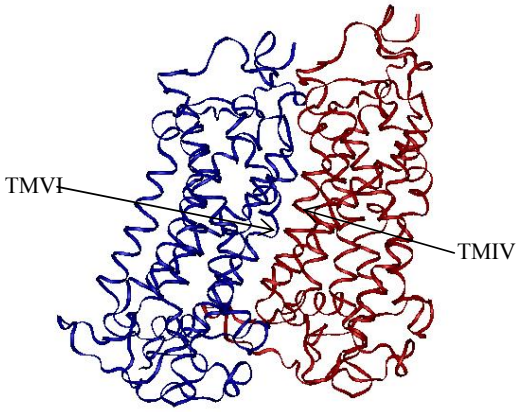
Figure 3

Concentration-effect curves for ligands on adenylyl cyclase activity. **A:** In C6 glial cells stably transfected with the h5-$HT_{4(e/g)}$ receptor isoform: ■, 5-HT; ▽, **ML10302**; ◆, **9d**; ▲, **20**; ▼, **10d**. **B:** In CHO cells stably transfected with the h5-$HT_{4(e/g)}$ receptor isoform: ■, 5-HT; ◆, **9d**; ▼, **10d**.



Dimer II-IV/II-IV

A



Dimer IV-VI/IV-VI

B

Figure 1

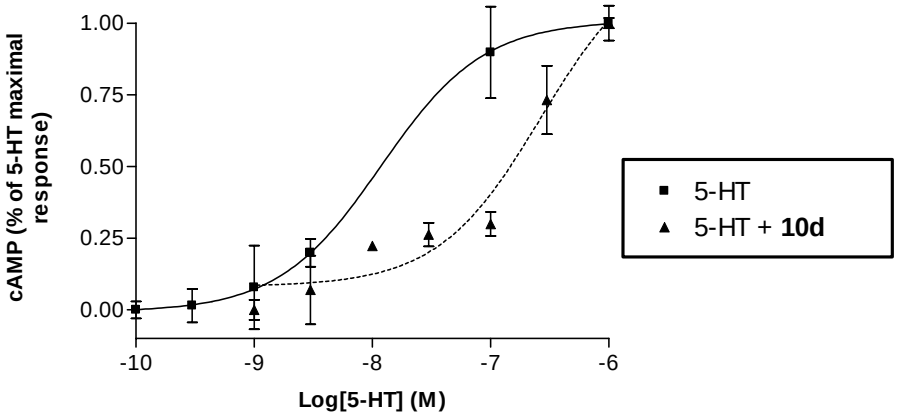
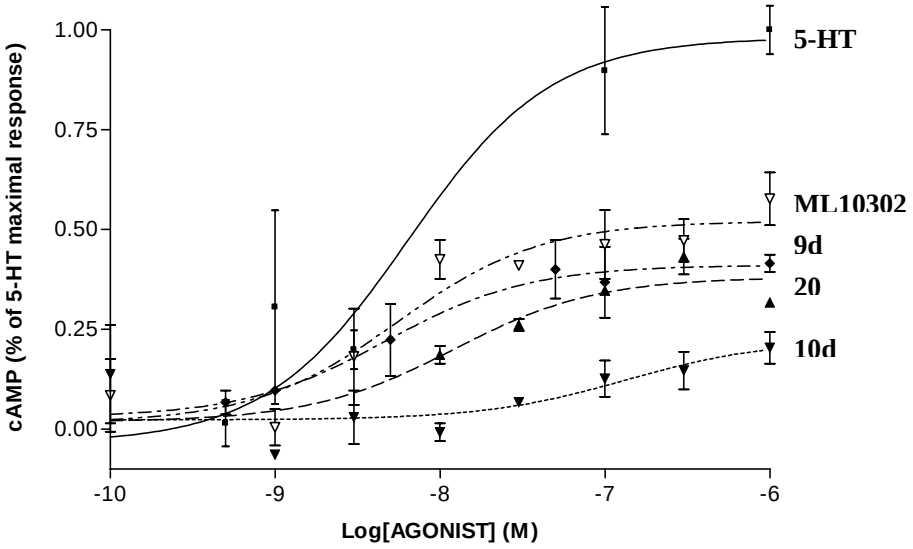
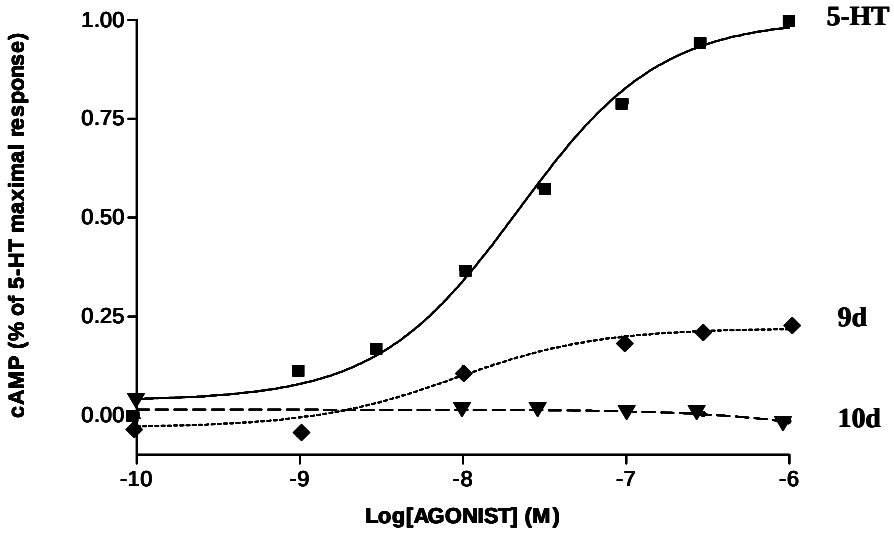

cAMP (% of 5-HT maximal response)
1.00
0.75
0.50
0.25
0.00
-10
-9
-8
-7
-6
Log[5-HT] (M)
5-HT
5-HT + 10d

Figure 2



A- C6 glial cells

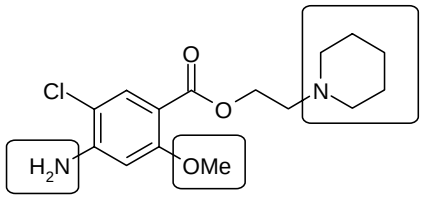


B- CHO Cells

Figure 3

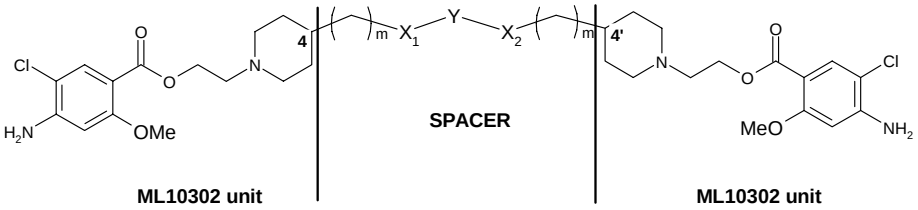
Scheme 1

Possible linking points of the spacer



ML10302

Scheme 2



ML10302 unit **SPACER** **ML10302 unit**

m = 0, 1

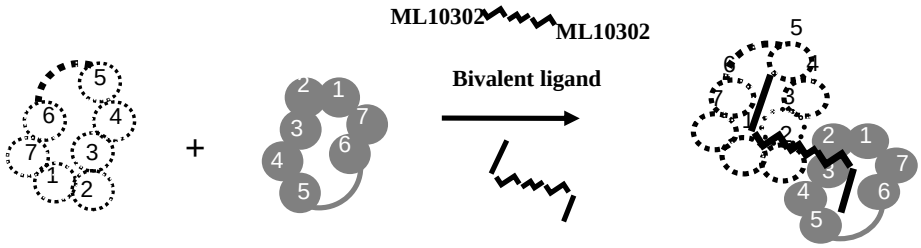
X_1 = X_2 = CONH, NHCO

Y =

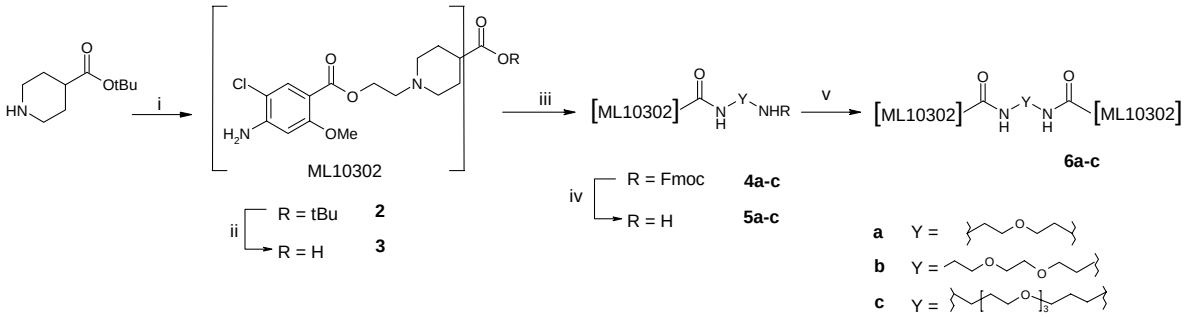
Series 1: Polar oxygenated spacers

Series 2: Hydrophobic spacers

Series3: central cyclic core alkyl spacers

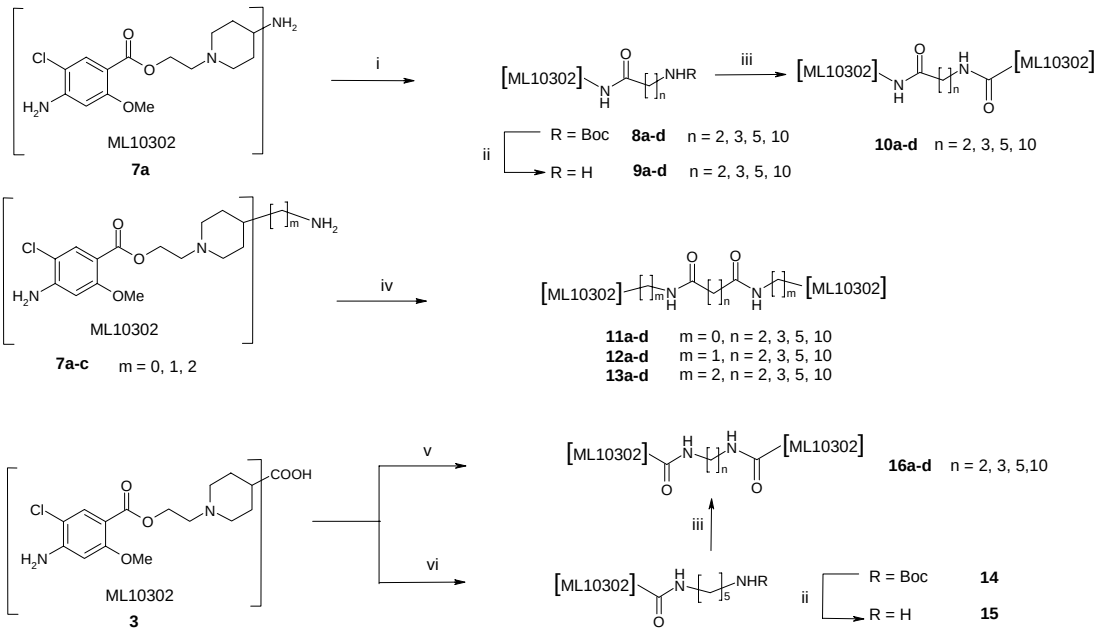


[a]**Scheme 3**



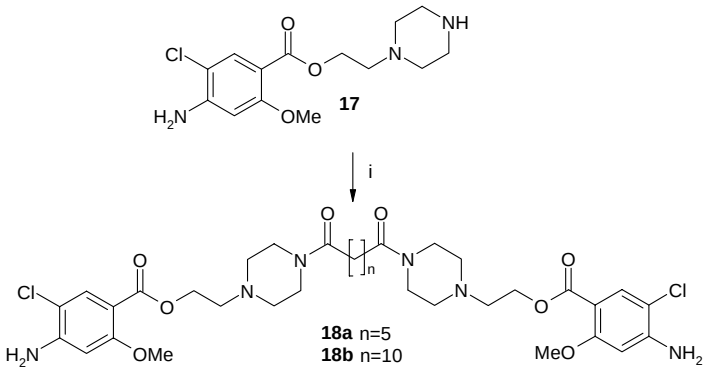
[a]Reagents and conditions: (i) 4-amino-5-chloro-2-methoxy-benzoic acid 2-bromo-ethyl ester,[20] DIEA, DMF, 40-50 °C, 24 h; (ii) CF_3COOH, CH_2Cl_2, rt, 24 h; (iii) EDC, HOBt, NEt_3, CH_2Cl_2, Fmoc-2-(2-aminoethoxy)-ethylamine hydrochloride, or Fmoc-1-amino-3,6-dioxa-8-octanamine hydrochloride, or Fmoc-1-amino-4,7,10-trioxa-13-tridecamine hydrochloride, 0 °C to rt, 24 h; (iv) 20 % piperidine, DMF, 10-15 min, 4N MeOH/HCl; (v) **3**, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h.

[a]Scheme 4



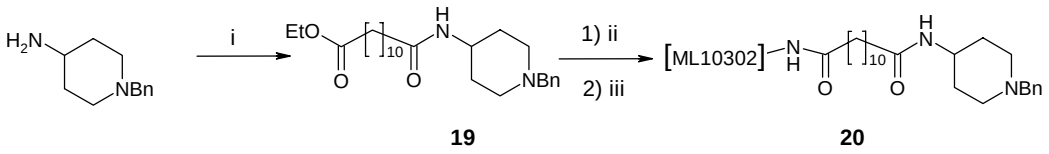
[a]Reagents and conditions: (i) $HOOC(CH_2)_nNHBoc$, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h; (ii) 4N MeOH/HCl, rt, 3-5 h; (iii) **3**, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h; (iv) $HOOC(CH_2)_nCOOH$, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h; (v) $NH_2(CH_2)_nNH_2$, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h; (vi) $NH_2(CH_2)_5NHBoc$, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h.

[a]**Scheme 5**



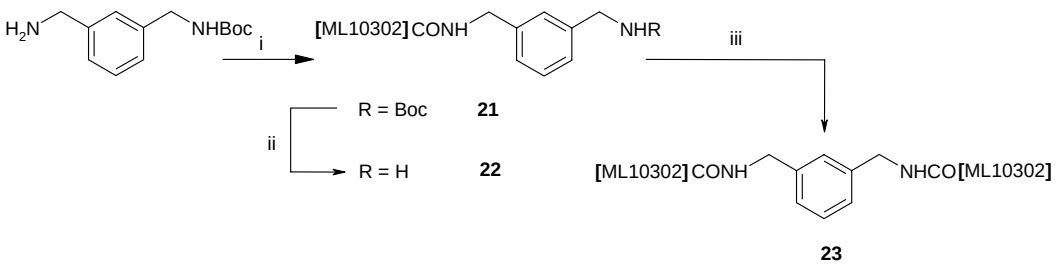
[a]Reagents and conditions: (i) $HOOC(CH)_nCOOH$, EDC, HOBt, NEt_3,CH_2Cl_2, 0 °C to rt, 24 h.

[a]**Scheme 6**



[a]Reagents and conditions: (i) $HOOC(CH_2)_{10}COOEt$,[22] EDC, HOBt, NEt_3, CH_2Cl_2, rt, 5 h; (ii) LiOH, H_2O / dioxane 20:80, rt, 7 h; (iii) **7a**,[21] EDC, HOBt, NEt_3, DMF, rt, 8 h.

[a]**Scheme 7**



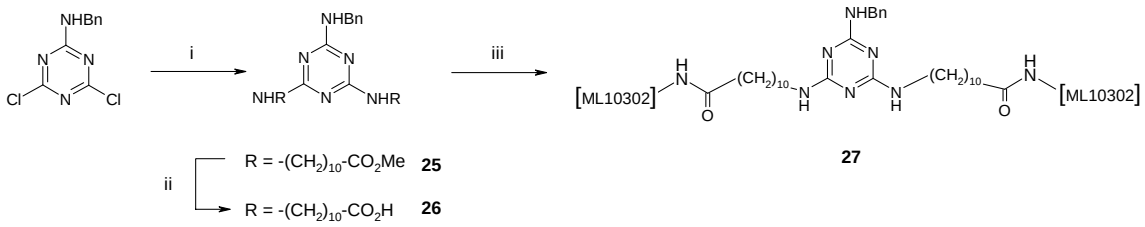
[a]Reagents and conditions: (i) **3**, EDC, HOBt, NEt3, CH_2Cl_2, 0 °C to rt, 24 h; (ii) 4N MeOH/HCl, 2 h; (iii) **3**, EDC, HOBt, NEt_3, CH_2Cl_2/DMF, 0 °C to rt, 24 h.

[a]**Scheme 8**

24

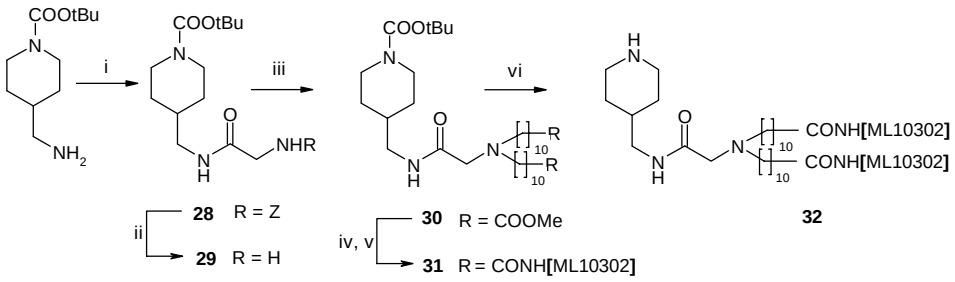
[a]Reagents and conditions: (i) **7a**,[21] EDC, HOBt, NEt_3, CH_2Cl_2/DMF, 0 °C to rt, 24 h.

[a]**Scheme 9**



[a]Reagents and conditions: (i) $NH_2(CH_2)_{10}COOMe$,[25] DIEA, CH_3CN, reflux, 24h; (ii) NaOH, H_2O/dioxane 8/2, rt, 3 h, then reflux 1 h; (iii) **7a**,[21] EDC, HOBt, NEt_3, DMF, 0 °C to rt, 24 h.

[a]**Scheme 10**



[a]Reagents and conditions: (i) Z-Gly-OH, EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C to rt, 24 h; (ii) Pd/C, H_2, MeOH, rt, 3 h; (iii) Br-$(CH_2)_{10}$-COOMe, Cs_2CO_3, DMF, rt, 48 h; (iv) LiOH, THF/H_2O, rt, 7 h; (v) EDC, HOBt, NEt_3, **7a**,[21] DMF, 0 °C to rt, 24 h; (vi) HCl/MeOH 4N, rt, 3 h.