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Synthesis and evaluation of homodimeric GnRHR antagonists having a rigid bis-propargylated benzene core

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Abstract—The fact that GPCRs might function in a dimeric fashion is currently well accepted. For GnRHR, a GPCR that regulates gonadotropin release, there is evidence that the receptor also functions as a dimer. We here describe the design and synthesis of a set of dimeric GnRHR antagonists in order to understand the interaction of dimeric ligands to the receptor and to address the question whether GnRHR dimerisation is a prerequisite for signalling. Biological evaluation of the compounds shows no discrimination between monomeric and dimeric ligands in respect to binding affinities, however, the dimeric ligands appear to have different functional properties.

1. Introduction

The Gonadotropin Releasing Hormone Receptor (GnRHR) belongs to the family of membrane bound G-Protein Coupled Receptors (GPCRs). The GnRHR is located in anterior pituitary cells and plays an important role in the reproductive system. Stimulation of the receptor with GnRH, a decapeptidic agonist, initiates the release of the gonadotropins Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). FSH and LH in turn induce follicle stimulation and ovulation in females and stimulate steroidogenesis in both males and females. GnRHR antagonists have found widespread use in Controlled Ovarian Stimulation (COS) protocols for IVF treatment. By inhibiting the gonadal axis in males, GnRHR antagonists suppress androgen production, rationalizing their therapeutic use in, for instance, prostate cancer.

Several research reports point towards the existence of GnRHR dimers as the active species involved in GnRHR signaling.[1] GPCR dimerisation and/or oligomerisation[2] is a well-recognized phenomenon which may be capitalized upon in the development of more active or specific (ant)agonists.[3,4] However, to date there are no dimeric GnRHR ligands known, with the exception of the dimeric antibody complexed peptide agonist with which the possible relevance of GnRHR dimerisation was demonstrated for the first time.[5] With the aim to develop effective dimeric GnRHR (ant)agonists as research tools to investigate GnRHR signaling, we recently reported our results in the preparation and evaluation of a library of homodimeric compounds (A, Fig. 1) based on the imidazopyrimidinone GnRHR antagonist **1**.[6] The library was constructed by modification of antagonist **1**[7] with an acetylene function and connecting modified ligand **2** to a set of bis-azide functionalized hydrophilic polyethylene glycol spacers by a 1,3-dipolar cycloaddition. As reference compounds, a set of monomeric ligands were prepared in which the bis-azide polyethylene glycol

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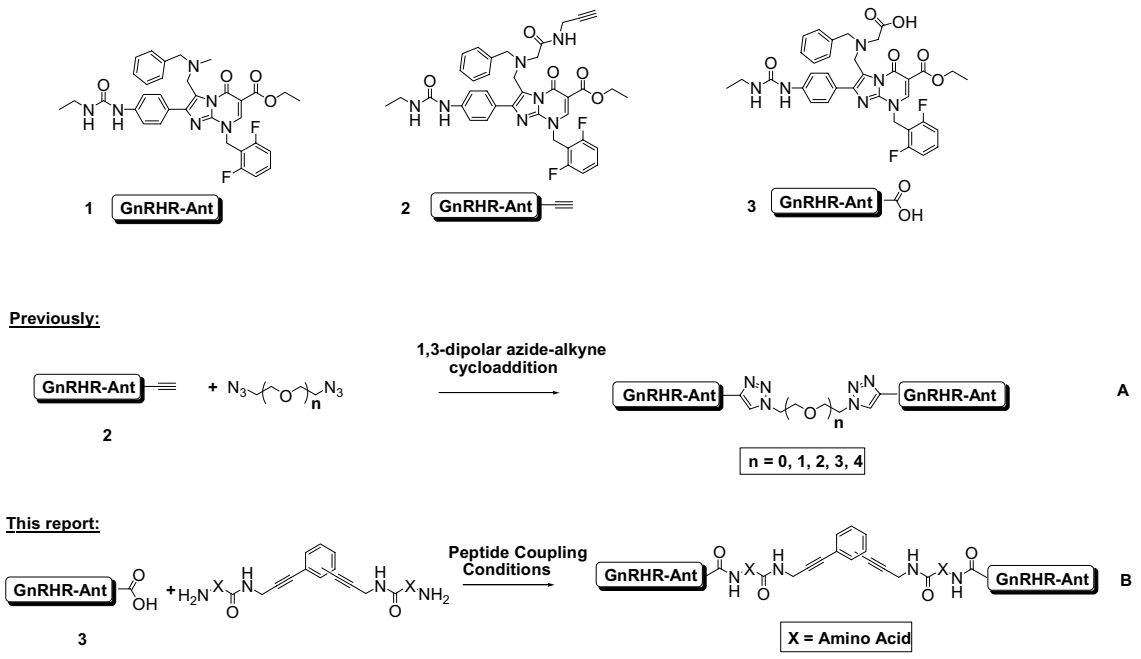
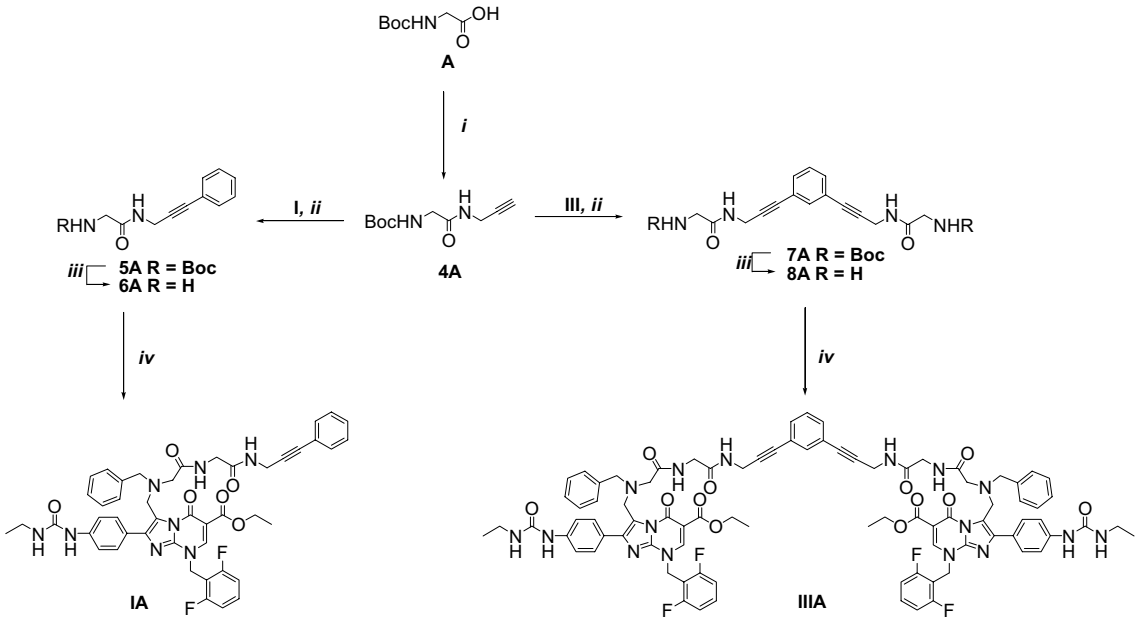


Figure 1. Overview of previously reported library (A) and current library (B) of dimeric GnRHR antagonists.

spacers were substituted on one site with the acetylene functionalized ligand. Our library of compounds did not contain a dimeric species with either a significantly enhanced GnRHR binding affinity or a significantly enhanced functional (ant)agonistic activity. Although one might conclude from these results that GnRHR dimerisation is not a prerequisite for signaling, at least in our system, we deem it more likely that we have not hit upon the correct dimeric ligand design. Many permutations are possible, and one specific alteration in the design that we address here is the replacement of the flexible, hydrophilic polyethylene glycol linker system by the rigid, hydrophobic benzene-based scaffolds as in B depicted in Figure 1. We thought it of interest to investigate if the thus imposed spatial constraint in the orientation of the pharmacophores exerts an effect on the binding and functional activity of the ligands.

2. Synthesis

The strategy for the preparation of our second-generation library is outlined in Scheme 1. *N*-Boc-glycine (**A**) was coupled to propargyl amine to afford acetylene **4A**



Scheme 1. Representative route of synthesis. Reagents and conditions: (i) Isobutyl chloroformate, NMM, propargylamine, DCM, −20 °C to rt 18 h, 76%. (ii) CuI, $Pd(PPh_3)_4$, pyrrolidine, DMF, 18 h, 66% for **5A**, 99% for **7A**. (iii) TFA/DCM; 1:1; v/v, 1% TIS, 18 h, HPLC purification, then (iv) pharmacophore **3**, BOP, DiPEA, DMF, 18 h, HPLC purification.

in 76% yield. Sonogashira cross coupling (CuI, $Pd(PPh_3)_4$, pyrrolidine and DMF) of **4A** to phenyl iodide (**I**) gave compound **5A** in 66% yield. After Boc removal (TFA/DCM and 1% TIS) and HPLC purification of the resulting ammonium salt compound **IA** was prepared by condensation with pharmacophore **3** (prepared as described in Ref. 6 under the agency of BOP and DiPEA in DMF. The HPLC purification of **6A** proved necessary, since direct condensation of crude **6A** after Boc removal proceeded sluggishly and in low yield. Reaction of **4A** with 1,3-diiodobenzene (**III**) gave bis-propargyl benzene **7A**. Now, Boc-removal, HPLC purification and condensation with 2 equivalents of carboxylate **3** gave bifunctional ligand **IIIA**. In this fashion, a 4×7 compound library was assembled, using the four iodo benzene derivatives **I**–**IV** and the seven amino acids **A**–**G** (Fig. 2), leading to seven mono-functional compounds (**IA**–**G**), seven *ortho*-disubstituted benzene derivatives (**IIA**–**G**), seven *meta*-disubstituted benzene derivatives (**IIIA**–**G**) and seven *para*-disubstituted benzene derivatives (**IVA**–**G**). All synthesis routes proceeded in an efficiency compared to that of the examples outlined in Scheme 1 (see Section 4 for details). All target compounds are depicted in Figure 3.

2.1. Biological evaluation

All newly synthesized compounds were tested on their ability to bind to the GnRH receptor and to antagonize GnRH-mediated signaling. The results are summarized in Tables 1 and 2. The binding affinity of the compounds was measured by monitoring their ability to displace the radioactive GnRHR agonist [^{125}I]triptorelin from plasma membranes of GnRHR-expressing Chinese Hamster Ovary (CHO) cells. As it is evident from Table 1 most monomeric compounds bind to GnRHR with higher affinity than their corresponding dimeric compounds. An exception is dimeric ligand **IVB** for which a 3-fold increase was observed compared to the monomeric counterpart **IB**.

In general, the compounds with alanine (**B**) or valine (**C**) moieties possess lower binding affinity compared to the other compounds. Also, the *ortho*- and *meta*-substituted aromatic scaffolds (that is, series **II** and **III**, respectively) show slightly reduced affinity compared to the para-substituted scaffolds (series **IV**). Exceptions are dimeric ligands with spacer **G**, which show comparable binding affinities in all cases, but reduced affinities compared to the monomeric ligand **IG**.

For the functional assay we made use of CHO cells stably transfected with the GnRHR and equipped with the NFAT luciferase reporter gene. These cells were stimulated with a submaximal (EC_{80}) concentration of the agonist GnRH in the presence of several concentrations of the test compounds. Antagonistic activity was detected as a decrease of the luminescence signal upon addition of the luciferase substrate. The IC_{50} values of the mono functionalized compounds **IA**–**G** and the antagonistic effects at 3 μM and 10 μM of all compounds (**IA**–**G**, **IIA**–**G**, **IIIA**–**G** and **IVA**–**G**) are listed in Table 2.

The ability of the monomeric ligands to antagonize GnRHR signaling, expressed as IC_{50} values, are in good agreement with the binding affinity (K_i) observed in the radioligand displacement assay (Table 1). However, compounds **IB** and **IC**, the linker system of which is derived from the amino acids alanine (**B**) or valine (**C**) show a reduced effect (E_{max}) compared to the other monomeric ligands. Most of the bifunctional ligands show a concentration dependent inhibition of GnRH-induced luminescence. However, full inhibition at the highest concentration tested (10 μM) was never observed, suggesting that the potency of the dimeric ligands is relatively modest. Six compounds (that is compounds **IID**, **E** and **G**, **IIIG** and **IVD** and **E**) show over 50% antagonism at 3 μM. These compounds all hold longer spacers (that is amino-butanoate **D**, -hexanoate **E** and -pipecolate **G**).

The literature on the antibody-mediated dimerisation of GnRHR antagonistic peptides, with agonists as a result,[8] led us to investigate whether our bifunctional molecules possess agonistic activity. Additional assays performed with all compounds in an agonistic set-up, that is, when tested alone in the luciferase reporter gene assay, did not provide any actives (data not shown).

The discrepancy in binding and functional properties of the dimeric compounds prompted us to perform addi-

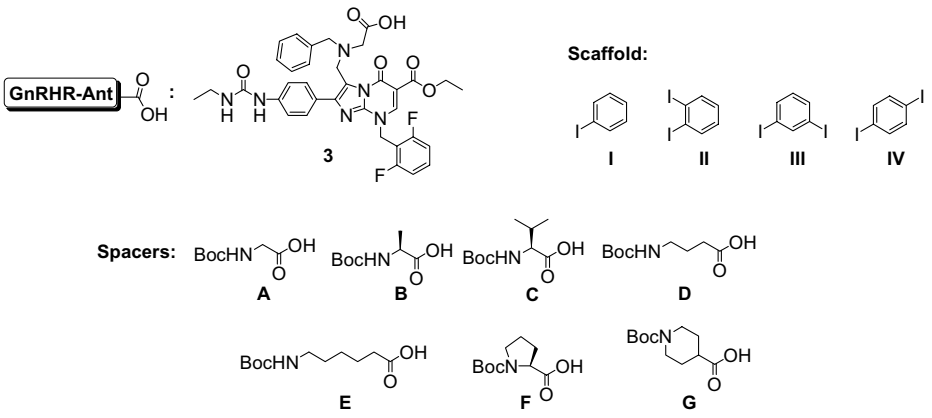


Figure 2. Aryl iodides and amino acids employed in the construction of the library.

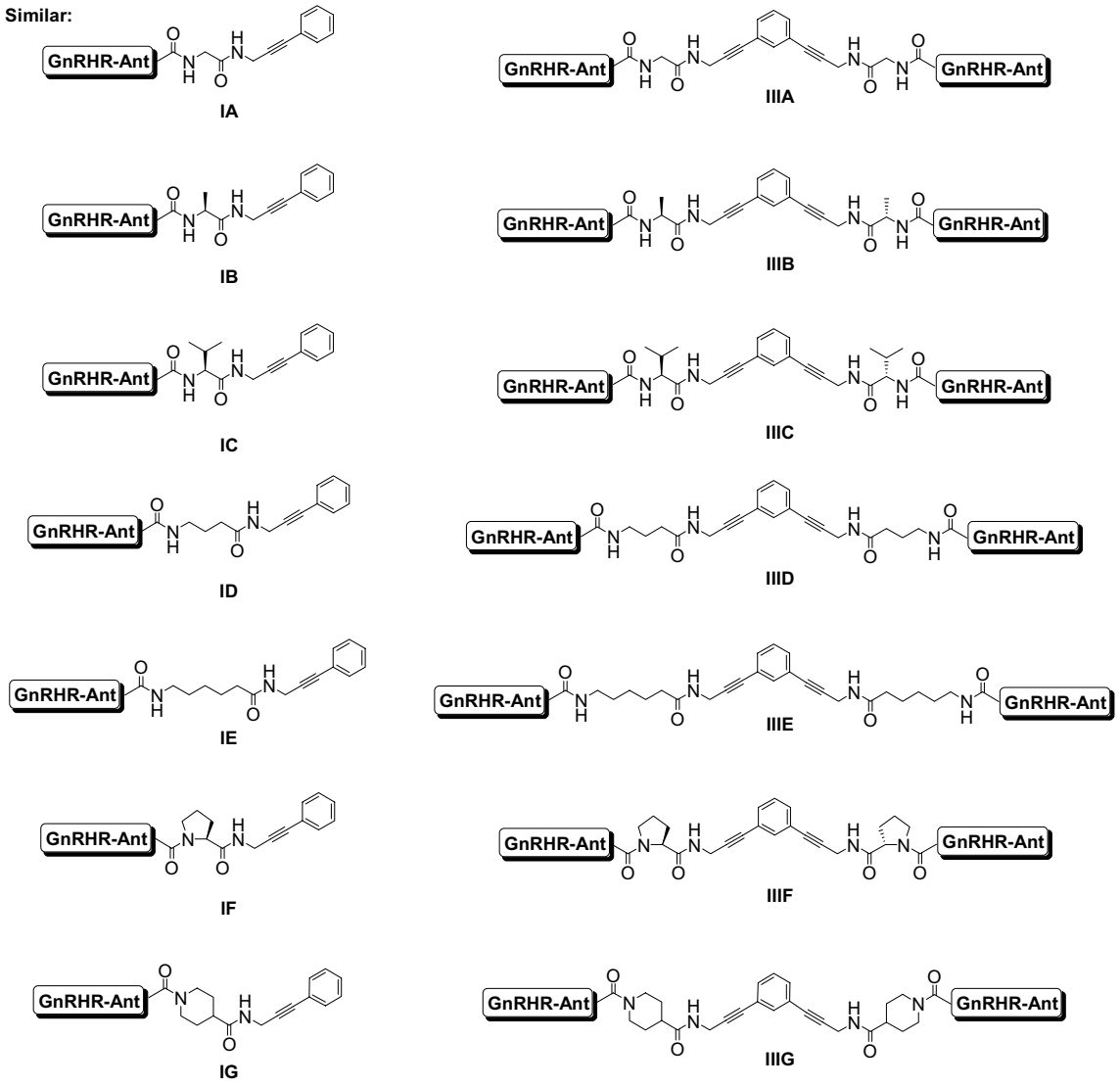


Figure 3. Structure of monovalent ligands **IA–G** and dimeric ligands **IIIA–G**. Structure of dimeric ligands **IIA–G** and **IVA–G**.

tional experiments. We examined the effects of two of our ligands on an entire concentration–effect curve of the peptide agonist triptorelin. Thus we recorded triptorelin curves in the absence and in the presence of two concentrations (2 and 10 μM) of selected compounds **ID** and **IVD** (Fig. 4).

As is evident from the curves shown in Figure 4, both monomeric- and dimeric-ligand **ID** and **IVD** show a rightward shift of the dose–response curve, a clear indication of the antagonism of the peptide agonist triptorelin. Compound **IVD** shows less antagonistic potency against triptorelin compared to the monomer, which is in agreement with the outcome of the functional assay as depicted in Table 2. However, the maximal efficacy (E_{max}) of triptorelin is also decreased, to the same amount in the presence of both monomeric ligand **ID** and dimeric ligand **IVD**. This result might indicate that our compounds also possess non-competitive characteristics, such as binding to an allosteric binding site that may be close to, or partially overlapping with the peptide binding site. In order to exclude the possibility that the reduced efficacy of our compounds is due to cytotoxicity we determined cell viability by a trypan blue exclusion experiment. We found that cell viability always exceeded 95% after 4 h of incubation in the absence (control) or presence of 10 μM of a relevant subset of our compounds (see Section 4).

3. Discussion

In our study, dimeric ligands derived from ligand **I** show interesting biological properties compared to the monomeric counterparts. While the binding properties of the dimeric ligands and monomeric ligands were in the same range, some different functional properties were observed for the dimeric ligands. For example, dimeric ligand **IVD** and monomeric ligand **ID** share similar binding affinities in the displacement assay, whereas a decrease in antagonistic potency for dimeric ligand **IVD** is observed. A similar trend was observed for

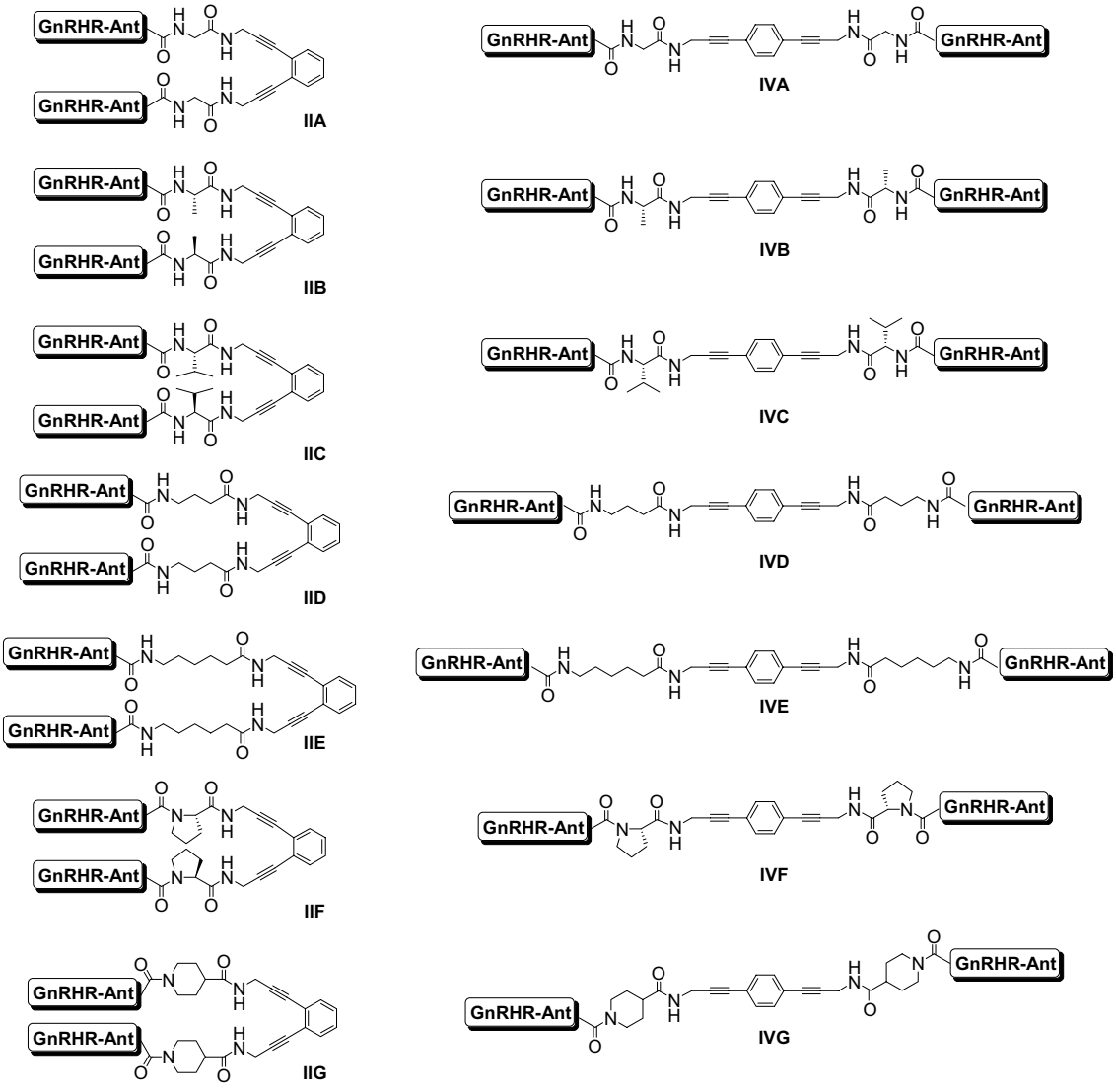


Fig. 3 (*continued*).

Table 1. Binding affinities (K_i values) of monomeric GnRHR antagonists **IA–IG** and dimeric GnRHR antagonists **IIA–G**, **IIIA–G**, and **IVA–G**

	K_i (μM) ± SEM			
	1 0.004 ± 0.0006			
	I	**II**	**III**	**IV**
A	0.17 ± 0.01	1.0 ± 0.26	1.1 ± 0.27	0.53 ± 0.26
B	1.6 ± 0.23	3.0 ± 1.7	5.3 ± 0.26	0.48 ± 0.04
C	0.77 ± 0.08	3.3 ± 0.46	2.2 ± 0.37	0.72 ± 0.05
D	0.58 ± 0.003	0.84 ± 0.003	0.82 ± 0.008	0.60 ± 0.04
E	0.38 ± 0.12	0.70 ± 0.05	0.93 ± 0.01	0.62 ± 0.009
F	0.26 ± 0.06	0.85 ± 0.04	0.93 ± 0.09	0.74 ± 0.11
G	0.14 ± 0.05	0.50 ± 0.23	0.40 ± 0.02	0.51 ± 0.002

dimeric ligand **IVB**, which show a 3-fold increase in binding affinities compared to monomeric ligand **IB** and a reduced antagonistic potency in the functional assay.

From the functional assay, a general correlation is observed between spacer length and potency of the dimeric ligands. For example, compounds **IID**, **E**, **G**, **IIIG**, **IVD** and **E**, all bearing longer spacers compared to the other compounds, are more potent than the other dimeric compounds. This observation suggests that dimeric ligands in this series with a larger interpharmacophoric distance will exhibit enhanced antagonistic properties.

Additional assays to support evidence for an allosteric interaction of the dimeric ligands to the GnRH receptor show that addition of dimeric ligand **IVD** to the peptide agonist triptorelin reduced both potency and efficacy of the peptide (Fig. 4). However, the monomeric counterpart **ID** affected the dose-response curve to the same extent. Recently, a distinct, that is, non-GnRH peptide, binding site was reported for a different set of heterocyclic GnRHR antagonists.[9] It is conceivable that our compounds bind to the receptor in a similar fashion as the reported non-peptidic antagonists.

The fact that dimeric ligands derived from antagonist **1** do not show enhanced pharmacological properties com-

Table 2. Antagonistic activities (IC_{50}) of monomeric GnRHR antagonist (**IA–G**) and % inhibition of dimeric GnRHR antagonist **IIA–G**, **IIIA–G**, and **IVA–G** at 3 and 10 μM concentration

	IC_{50} (μM) ± SEM								
	1 0.076 ± 0.027								
	I			II		III		IV	
	IC_{50} (μM) ± SEM	% *E* at 3 μM	% *E* at 10 μM	% *E* at 3 μM	% *E* at 10 μM	% *E* at 3 μM	% *E* at 10 μM	% *E* at 3 μM	% *E* at 10 μM
A	0.46 ± 0.009	92 ± 4	95 ± 3	37 ± 11	73 ± 12	20 ± 9	9 ± 9	34 ± 9	59 ± 6
B	1.1 ± 0.07	63 ± 5	61 ± 2	13 ± 2	57 ± 7	18 ± 17	14 ± 8	42 ± 7	52 ± 3
C	0.52 ± 0.09	64 ± 9	60 ± 4	0 ± 12	43 ± 13	28 ± 17	43 ± 15	31 ± 0	34 ± 6
D	0.68 ± 0.15	97 ± 5	96 ± 4	73 ± 8	96 ± 3	40 ± 1	57 ± 3	68 ± 15	85 ± 12
E	0.44 ± 0.07	97 ± 1	99 ± 1	52 ± 12	95 ± 2	44 ± 16	58 ± 13	54 ± 3	67 ± 5
F	0.56 ± 0.03	87 ± 5	89 ± 6	21 ± 7	42 ± 14	31 ± 7	67 ± 8	46 ± 9	65 ± 12
G	0.46 ± 0.01	83 ± 4	82 ± 2	58 ± 6	70 ± 2	68 ± 13	86 ± 12	35 ± 5	48 ± 5

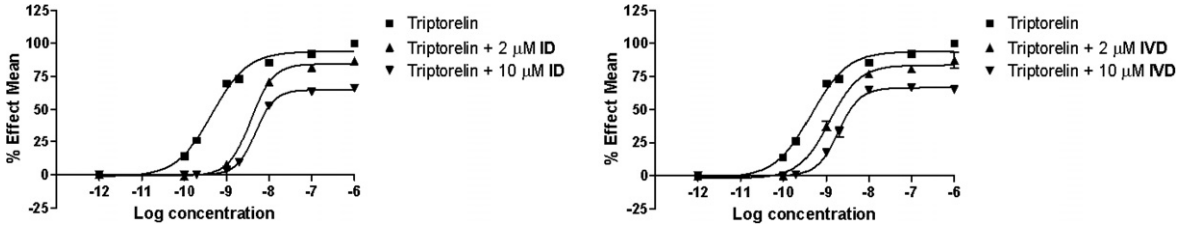


Figure 4. Curve signatures of peptide-agonist triptorelin in the absence and presence of 2 and 10 μM of monomeric ligand **ID** (left) and dimeric ligand **IVD** (right).

pared to the monomeric counterparts do not allow us to distinguish between different modes of binding of bivalent ligands to the GnRHR. Recently, in a similar study targeting the serotonin 5-HT_4 receptor dimer specifically with bivalent ligands which are constructed in a similar fashion as the library described in this report, no discrepancy was observed in monomeric- and dimeric-ligands in respect to binding properties. In the functional assay, most dimeric ligands lost the agonistic character of the monomeric reference compound.[10] Yet, the authors show a conformational change of 5-HT_4R dimers with bioluminescence resonance energy transfer (BRET) when subjecting the dimeric partial agonist to the receptor thus suggesting a simultaneous interaction of the two pharmacophores of the bivalent ligands to the receptor dimer. The dimeric ligands we present here are highly reminiscent to those reported in the 5-HT_4 study when considering the nature and size of the linker systems. It is therefore not unlikely that our dimeric compounds behave in a similar fashion, that is, simultaneous binding to a GnRHR dimer but without a pharmacological effect. However, further studies are required to establish the validity of this hypothesis.

In conclusion, we have developed a strategy for the preparation of a set of dimeric ligands containing hydrophobic, rigid linker systems to target the GnRH receptor. The results described in this paper concerning binding and functional properties do not provide us further information to establish the interaction of dimeric ligands to GnRHR. Combination of the binding and functional antagonistic properties and the reduction of the maximal effect of the peptide agonist triptorelin in the presence of our compounds might indicate an different mode of binding of the dimeric ligands compared to the monomeric counterparts. To establish whether dimerisation of GnRH receptor is a prerequisite for signaling or ligand binding, more research is needed. Fortunately, our synthetic strategy is flexible with respect to the nature of the linker system and the pharmacophore. Furthermore, the diiodobenzene scaffold can be easily modified to, for instance triiodobenzene or other oligo-iodoaryl systems, allowing the preparation of tri- and tetravalent compounds. Future work along these lines combined with the development of a new library based on a homo- and hetero-dimeric ligands containing a different antagonist are now conducted in our laboratory.

4. Experimental

4.1. GnRHR luciferase reporter gene assay

Chinese Hamster Ovary, CHO-K1, cells with stable expression of the human Gonadotropin Releasing Hormone Receptor (GnRH-R) and Nuclear Factor Activated T-cell luciferase reporter gene were grown to 80–90% confluence in culture medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% w/v fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin and 400 μg/ml geniticin. On the day of the assay, cells were washed twice with phosphate buffered saline and then harvested with cell dissociation solution. Cells were resuspended in assay medium consisting of DMEM supplemented with 1 mg/L insulin and 5 mg/L apo-transferrin and 3% v/v DMSO. Then, 10 μl cell suspension containing 7500 cells

was added to each well of a 384-well white culture plate. Thereafter, 10 μl of test compound was added at 10 concentrations ranging from final concentration of 10 μM to 0.3 nM with half log intervals. Compounds were allowed to preincubate with cells for 30 min followed by addition of 10 μl agonist GnRH at a final concentration of 3 nM which produces approximately 80% of the maximal effect (EC80) when given alone. After 4 h stimulation, 15 μl of luclite® was added to each well for detection of luciferase protein and plates were left at room temperature for 1 h in the dark. Finally, the luminescence signal was quantified on the TopCount® Microplate Scintillation and Luminescence Counter.

4.2. Radioligand binding assays

Ganirelix was provided by Organon (Oss, The Netherlands). [^{125}I]Triptorelin (specific activity 2200 Ci mmol^{-1}) was purchased from Perkin Elmer Life Sciences B.V. (Groningen, The Netherlands). CHO cells stably expressing the human GnRH receptor were kindly provided by Organon (Oss, The Netherlands). All other chemicals and cell culture materials were obtained from standard commercial sources.

CHO (Chinese hamster ovary) cells expressing the wild-type human GnRH receptor were grown in Ham's F12 medium containing 10% bovine calf serum, streptomycin (100 μg mL^{-1}), penicillin (100 IU mL^{-1}) and G418 (0.4 mg mL^{-1}) at 37 °C in 5% CO_2. The cells were subcultured twice weekly at a ratio of 1:20. For membrane preparation the cells were subcultured 1:10 and transferred to large 14-cm diameter plates. For membrane preparation the cells were detached from the plates by scraping them into 5 mL PBS, collected and centrifuged at 1400*g* (3000 rpm) for 5 min. Pellets derived from 30 plates were pooled and resuspended in 30 mL of ice-cold 50 mM Tris–HCl buffer supplemented with 2 mM $MgCl_2$, pH 7.4. An UltraThurrax was used to homogenize the cell suspension. Membranes and the cytosolic fraction were separated by centrifugation at 100,000*g* (31,000 rpm) at 4 °C for 20 min. The pellet was resuspended in 10 mL of the Tris buffer and the homogenization and centrifugation steps were repeated. Tris buffer (10 mL) was used to resuspend the pellet and the membranes were stored in 500 μL aliquots at −80 °C. Membrane protein concentrations were measured using the BCA (bicinchoninic acid) method.[11]

On the day of the assay membrane aliquots containing 20 μg protein were incubated in a total volume of 100 μL assay buffer (50 mM Tris–HCl, pH 7.4, supplemented with 2 mM $MgCl_2$ and 0.1% BSA) at 22 °C for 45 min. Displacement experiments were performed using five concentrations of competing ligand in the presence of 30,000 cpm [^{125}I]triptorelin. Non-specific binding was determined in the presence of 1 μM Ganirelix and represented approximately 20% of the total binding. Total binding was determined in the presence of buffer and was set at 100% in all experiments, whereas non-specific binding was set at 0%. Incubations were terminated by dilution with ice-cold Tris–HCl buffer. Separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B filters presoaked with 0.25% PEI for 1 h using a Brandel harvester. Filters were subsequently washed three times with ice-cold wash buffer (50 mM Tris–HCl, pH 7.4, supplemented with 2 mM $MgCl_2$ and 0.05% BSA). Filter-bound radioactivity was determined in a γ-counter.

All data were analyzed using the non-linear regression curve-fitting program GraphPad Prism v. 4 (GraphPad Software Inc., San Diego, CA, USA). Inhibitory binding constants (K_i values) were derived from the IC_{50} values according to $K_i = IC_{50}/(1 + [C]/K_d)$ where [C] is the concentration of the radioligand and K_d its dissociation constant.[12] The K_d value (1.1 nM) of [^{125}I]triptorelin was obtained by computer analysis of saturation curves (data not shown). All values obtained are means of at least two independent experiments performed in duplicate.

4.3. Cytotoxicity

CHOhGnRH_luc cells were seeded on 5-cm diameter plates in assay medium in the absence (control) or presence of 10 μM of test compounds. As relevant compounds in this toxicity assay we selected the following: **ID–IVD**, **IVA** and **IVE**. The cells were incubated for 4 h at 37 °C. Thereafter the cells were harvested using 0.5 ml trypsol and resuspended in 2 ml of PBS. Subsequently the number of viable cells was determined by trypan blue exclusion, where a trypan blue solution (0.8% (w/v) in PBS) was added to an equal amount of cell suspension. The proportion of live cells was determined by counting in a hemocytometer.

4.4. Chemical procedures

Reactions were executed at ambient temperatures unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis using silica gel coated plates (0.2 mm thickness) and detection by 254 nm UV-light or by either spraying with a solution of $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4 \times 2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C. Column chromatography was performed on silica gel (40–63 μm). NMR spectra were recorded on a 200/50.1 MHz, 300/75.1 MHz, 400/100 MHz, 500/125 MHz or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. Coupling constants (J) are given in Hz. All presented ^{13}C-APT spectra are proton decoupled. For LC/MS analysis, a HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C_{18} column (4.6 mmD × 250 mmL, 5 μ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq TFA and coupled to a mass instrument with an electronspray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C_{18} column (5 μm C_{18}, 10 Å, 150 × 21.2 mm) was used. The applied buffers were A: 5% MeCN/H_2O + 0.1% TFA and B: MeCN. High resolution mass spectra were recorded on a mass

spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10%, capillary temperature 275 °C) with resolution $R = 100{,}000$ at m/z 400. The high resolution mass spectrometer was calibrated prior to measurements with a calibration solution (caffeine, MRFA, Ultramark 1621).

4.4.1. General procedure for coupling of amino acids with propargylamine (4A–4G). Isobutyl chloroformate (1.43 mL, 11 mmol) was added to a cooled (−20 °C) solution of amino acid (10 mmol) and *N*-methylmorpholine (1.52 mL, 14 mmol) in DCM (50 mL). After stirring for 1 h, propargylamine (0.96 mL, 14 mmol) was added. The reaction mixture was warmed to room temperature over a period of 2 h and subsequently stirred for 16 h. The mixture was successively washed with 1 M HCl (50 mL) and 10% aq $NaHCO_3$ (50 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in EtOAc and triturated with petroleum ether to afford titled product as white crystals.

4.4.1.1. *N*-α-*tert*-Boc-glycine propargylamide (4A). Yield: 1.60 g (7.55 mmol, 76%). $R_f = 0.60$ (EtOAc). ESI-MS (m/z) calcd for $C_{10}H_{16}N_2O_3 + H^+$: 213.1, obsd 213.0. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.59 (br t, 1H), 5.25 (br t, 1H), 4.07 (dd, 2H, $J = 5.5$ Hz, $J = 2.6$ Hz), 3.82 (d, 2H, $J = 5.8$ Hz), 2.24 (t, 1H, $J = 2.6$ Hz), 1.46 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.2, 156.0, 89.8, 80.4, 71.7 (5× C), 29.1 (CH_2), 28.3 (3× CH_3).

4.4.1.2. *N*-α-*tert*-Boc-alanine propargylamide (4B). Yield: 1.94 g (8.55 mmol, 86%). $R_f = 0.55$ (MeOH/DCM, 1:19, v/v). ESI-MS (m/z) calcd for $C_{11}H_{18}N_2O_3 + H^+$: 227.1, obsd 227.0. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.59 (br t), 5.00 (br d, 1H), 4.28–4.12 (m, 1H) 4.05 (dd, 2H, $J = 5.5$ Hz, $J = 2.6$ Hz), 2.22 (t, 1H, $J = 2.6$ Hz), 1.45 (s, 9H), 1.35 (d, 3H, $J = 6.9$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 155.5, 80.2, 79.3, 71.5 (5× C), 49.9 (CH), 29.1 (CH_2), 28.3 (3× CH_3), 18.3 (CH_3).

4.4.1.3. *N*-α-*tert*-Boc-valine propargylamide (4C). Yield: 2.52 g (9.92 mmol, 99%). $R_f = 0.65$ (MeOH/DCM, 1:9, v/v). ESI-MS (m/z) calcd for $C_{13}H_{22}N_2O_3 + H^+$: 255.2, obsd 255.1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.40 (br t, 1H), 5.04 (br d, 1H), 4.05 (dd, 2H, $J = 5.5$ Hz, $J = 2.2$ Hz), 3.92 (dd, 1H, $J = 8.9$ Hz, $J = 6.4$ Hz), 2.22 (t, 1H, $J = 2.6$ Hz), 2.15 (m, 1H), 1.45 (s, 9H), 0.97 (d, 3H, $J = 6.6$ Hz), 0.93 (d, 3H, $J = 7.0$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.6, 155.9, 79.9, 79.3, 71.4 (5× C), 59.8, 31.0 (2× CH), 28.9 (CH_2), 28.3 (3× CH_3), 17.9, 19.1 (2× CH_3).

4.4.1.4. *N*-γ-*tert*-Boc-γ-amino butaric acid-propargylamide (4D). Yield: 1.68 g (7.00 mmol, 70%). $R_f = 0.50$ (MeOH/DCM, 1:19, v/v). ESI-MS (m/z) calcd for $C_{12}H_{20}N_2O_3 + H^+$: 241.2, obsd 240.9. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.39 (br t, 1H), 4.73 (br t, 1H), 4.05 (dd, 2H, $J = 5.1$ Hz, $J = 2.9$ Hz), 3.18 (q, 2H, $J = 6.6$ Hz), 2.27–2.21 (m, 3H), 1.88–1.75 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.2, 156.7, 89.3, 79.3, 70.9 (5× C), 39.4, 32.9 28.6, (3× CH_2), 28.1 (3× CH_3), 25.8 (CH_2).

4.4.1.5. *N*-ε-*tert*-Boc-ε-amino hexanoic acid-propargylamide (4E). Yield: 2.41 g (8.99 mmol, 90%). $R_f = 0.60$ (MeOH/DCM, 1:9, v/v). ESI-MS (m/z) calcd for $C_{14}H_{24}N_2O_3 + H^+$: 269.2, obsd 269.1. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.77 (br t, 1H), 4.56 (br t, 1H), 4.05 (dd, 2H, $J = 5.5$ Hz, $J = 2.6$ Hz), 3.11 (q, 2H, $J = 6.6$ Hz), 2.24–2.17 (m, 3H), 1.44 (s, 9H), 1.74–1.34 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$/CD_3OD): δ 173.3, 156.3, 79.5, 79.2, 71.0 (5× C), 39.9, 35.7, 29.3, 28.7 (4× CH_2), 28.2 (3× CH_3), 26.0, 24.9 (2× CH_2).

4.4.1.6. *N*-α-*tert*-Boc-proline propargylamide (4F). Yield: 1.71 g (6.79 mmol, 68%). $R_f = 0.55$ (MeOH/DCM, 1:9, v/v). ESI-MS (m/z) calcd for $C_{13}H_{20}N_2O_3 + H^+$: 253.2, obsd 253.0. ^{1}H NMR mixture of rotamers (200 MHz, $CDCl_3$): δ 7.35 (br s, 1H), 6.25 (br s, 1H), 4.26 (br s, 1H), 4.02 (br s, 2H), 3.35 (br s, 2H), 2.23 (s, 1H), 2.13 (br s, 2H), 1.88 (br s, 2H), 1.46 (br s, 9H). ^{13}C NMR of rotamers (50 MHz, $CDCl_3$): δ 171.9, 80.3, 79.3, 71.2 (4× C), 46.8, 28.7 (2× CH_2), 28.3 (3× CH_3), 24.2, 23.6 (2× CH_2).

4.4.1.7. *N*-δ-*tert*-Boc-4-amino cyclohexanoic acid-α-propargylamide (4G). Yield: 0.97 g (3.65 mmol, 37%). $R_f = 0.60$ (MeOH/DCM, 1:9, v/v). ESI-MS (m/z) calcd for $C_{14}H_{22}N_2O_3 + H^+$: 267.2, obsd 267.0. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.65 (s, 1H), 4.25–4.02 (m, 2H), 4.05 (dd, 2H, $J = 5.1$ Hz, $J = 2.6$ Hz), 2.74 (br t, 2H, $J = 11.0$ Hz), 2.24 (t, 1H, $J = 2.6$ Hz), 2.33–2.18 (m, 1H), 1.85–1.73 (m, 2H), 1.73–1.58 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.1, 154.5, 79.5, 71.3 (4× C), 43.0 (CH_2), 42.6 (CH), 28.9, 28.3 (2x CH_2), 28.3 (3× CH_3).

4.4.2. General procedure for coupling of propargylamide functionalized amino acids with iodobenzene (affording 5A–5G). In separate flasks, a solution of the propargyl functionalized amino acid (**4A**–**G**, 1.0 mmol), iodobenzene (1.5 mmol, 111μL) in pyrrolidine/DMF (1/4: v/v), a solution of CuI (0.1 mmol, 19.1 mg) in DMF (2 mL) and a solution of $Pd(PPh_3)_4$ (0.05 mmol, 57.8 mg) in DMF (4 mL) were flushed with argon for 1 h in an ultrasonic bath. To the alkyne solution were added subsequently the CuI and the $Pd(PPh_3)_4$ solutions and the mixture were stirred for 18 h under inert atmosphere. The volatiles were removed and the crude product dissolved in MeOH/DCM (1/9, v/v, 50 mL) and washed with water (3× 50 mL) and 10% aq $NaHCO_3$ (50 mL). The organic layer was dried with Na_2SO_4 and concentrated. The crude material was purified by automated silica gel column chromatography (35–65% 1:10; MeOH/DCM; v/v in petroleum ether).

4.4.2.1. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-glycine (5A). Yield: 189 mg (0.66 mmol, 66%). $R_f = 0.65$ (EtOAc). ESI-MS (m/z) calcd for $C_{16}H_{20}N_2O_3 + H^+$: 288.1, obsd 289.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.28 (m, 3H), 7.17 (br t, 1H), 5.69 (s, 1H), 4.28 (d, 2H, $J = 4.5$ Hz), 3.88 (s, 2H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.5, 156.1 (2× C), 131.5, 128.1 (2× CH), 122.3 (C), 84.4, 83.1 (2× C), 80.1 (C), 43.9, 29.7 (2× CH_2), 28.0 (3× CH_3).

4.4.2.2. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-alanine (5B). Yield: 298 mg (0.99 mmol, 99%). $R_f = 0.70$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{17}H_{22}N_2O_3 + H^+$: 302.2, obsd 303.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.27 (m, 3H), 7.09 (s, 1H), 5.42 (d, 1H, $J = 7.0$ Hz), 4.30 (br s, 1H), 4.26 (d, 2H, $J = 4.5$ Hz), 1.40 (s, 9H), 1.37 (d, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.5, 155.5 (2x C), 131.5, 128.1 (2× CH), 122.5, 84.6, 83.1, 79.9 (4× C), 49.8 (CH), 29.7 (CH_2), 28.2 (3× CH_3), 18.3 (CH_3).

4.4.2.3. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-valine (5C). Yield: 322 mg (0.98 mmol, 98%). $R_f = 0.80$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{19}H_{26}N_2O_3 + H^+$: 330.2, obsd 331.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (s, 1H), 7.37 (m, 2H), 7.27 (m, 3H), 5.64 (d, 1H, $J = 9.5$ Hz), 4.34 (dd, 1H, $J = 17.5$ Hz, $J = 5.5$ Hz), 4.13 (d, 2H, $J = 4.5$ Hz), 2.10–2.06 (m, 1H), 1.42 (s, 9H), 0.99 (d, 3H, $J = 7.0$ Hz), 0.96 (d, 3H, $J = 7.0$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8 (C), 155.9 (C), 131.5, 128.0, (2× CH), 122.5, 84.7, 82.8, 79.5 (4× C), 59.6, 31.2 (2× CH), 29.4 (CH_2), 28.2 (3× CH_3), 19.0, 18.1 (2× CH_3).

4.4.2.4. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-amino butaric acid (5D). Yield: 205 mg (0.65 mmol, 65%). $R_f = 0.55$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{18}H_{24}N_2O_3 + H^+$: 316.2, obsd 217.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41 (m, 2H), 7.29 (m, 3H), 7.23 (s, 1H), 5.25 (s, 1H), 4.23 (s, 2H), 3.13 (t, 2H, $J = 4.0$ Hz), 2.25 (t, 2H, $J = 7.0$ Hz), 1.83–1.78 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.9, 155.6 (2× C), 131.5, 128.2 (2× CH), 122.5, 84.7, 82.9, 79.3 (4× C), 39.5, 29.6, 25.9, 33.1 (4× CH_2), 28.2 (3× CH_3).

4.4.2.5. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-amino hexanoic acid (5E). Yield: 332 mg (0.97 mmol, 97%). $R_f = 0.70$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{20}H_{28}N_2O_3 + H^+$: 344.2, obsd 343.1. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (m, 2H), 7.28 (m, 3H), 7.15 (s, 1H), 5.12 (s, 1H), 4.23 (d, 2H, $J = 4.0$ Hz), 3.06 (t, 2H, $J = 4.0$ Hz), 2.22 (t, 2H, $J = 7.5$ Hz), 1.65 (m, 2H), 1.49 (m, 2H), 1.43 (s, 9H), 1.32 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.3, 155.3 (2× C), 131.4, 128.0 (2× CH), 122.4, 84.7, 82.7, 79.0 (4× C), 39.9, 35.7, 29.5, 29.2 (4× CH_2), 28.1 (3× CH_3), 25.9, 24.9 (2x CH_2).

4.4.2.6. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-proline (5F). Yield: 302 mg (0.92 mmol, 92%). $R_f = 0.55$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{19}H_{24}N_2O_3 + H^+$: 328.2, obsd 329.1. ^{1}H NMR of rotamers (500 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.27 (m, 3H), 6.63 (br s, 1H), 4.35 (br d, 1H), 4.24 (br s, 2H), 3.40 (br d, 2H), 2.25 (br dd, 2H), 1.84 (br d, 2H), 1.45 (s, 9H). ^{13}C NMR of rotamers (125 MHz, $CDCl_3$): δ 172.2, 155.3 (2× C), 131.4, 128.0 (2× CH), 122.3, 84.7, 82.9, 80.2 (4× C), 60.8 (CH), 46.8, 29.5 (2× CH_2), 28.1 (3× CH_3), 24.4, 23.1 (2× CH_2).

4.4.2.7. *N*-α-*tert*-Boc-(3-phenylprop-2-yn-1-amide)-amino cyclohexanoic acid (5G). Yield: 323 mg (0.94 mmol, 94%). $R_f = 0.85$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{20}H_{26}N_2O_3 + H^+$: 342.2, obsd 343.0. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (m, 2H), 7.28 (m, 3H), 6.43 (s, 1H), 4.26 (d, 2H, $J = 5.0$ Hz), 4.12 (br s, 2H), 2.72 (br s, 2H), 2.30 (m, 1H), 1.80 (m, 2H), 1.65 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.1, 154.5 (2× C) 131.5, 128.1 (2× CH), 122.3, 84.7, 83.1, 79.5 (4× C), 42.9 (CH_2), 42.8 (CH), 29.8, 28.3 (2× CH_2), 27.4 (3× CH_3).

4.4.3. General procedure for coupling of propargylic spacers 4A–G with 1,3-diiodobenzene (affording 7A–G), 1,2-diiodobenzene (affording 9A–G) and 1,4-diiodobenzene (affording 11A–G). In separate flasks, a solution of the propargyl functionalized amino acid (**4A**–**G**, 0.90 mmol), the desired diiodobenzene (0.30 mmol, 91.1 mg) and pyrrolidine (1.80 mmol, 147 μL) in DMF (3 mL), a solution of CuI (0.06 mmol, 11.5 mg) in DMF (1 mL) and a solution of $Pd(PPh_3)_4$ (0.03 mmol, 34.7 mg) in DMF (1 mL) were was flushed with argon for 1 h in an ultrasonic bath. To the alkyne solution were added subsequently the CuI and the $Pd(PPh_3)_4$ solutions and the mixture were stirred for 18 h under argon atmosphere. The volatiles were removed and the crude product dissolved in MeOH/DCM (1/9, v/v, 50 mL) and washed with water (3× 10 mL) and 10% aq $NaHCO_3$ (10 mL). The organic layer was dried with Na_2SO_4 and concentrated. The crude material was purified by automated silica gel column chromatography (35–65% 1:10; MeOH/DCM; v/v in petroleum ether).

4.4.3.1. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-yn-1-amide)-glycine (7A). Yield: 148 mg (0.30 mmol, 99%). $R_f = 0.50$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{26}H_{34}N_4O_6$, $+ H^+$: 499.3, obsd 499.5. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45–7.16 (m, 4H), 7.07 (br t, 2H), 5.57 (br t, 2H), 4.25 (d, 4H, $J = 5.1$ Hz), 3.86 (d, 4H, $J = 5.1$ Hz), 1.44 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 156.2 (2× C), 131.5, 127.9 (2x CH), 125.4, 88.9, 81.2, 79.9 (4× C), 44.0, 29.6 (2× CH_2), 28.2 (3× CH_3).

4.4.3.2. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-alanine (7B). Yield: 128 mg (0.24 mmol, 81%). $R_f = 0.70$ (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{28}H_{38}N_4O_6 + H^+$: 527.4, obsd 527.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (br s, 2H), 7.65–7.20 (m, 4H), 5.61 (br d, 2H), 4.41–4.29 (m, 2H), 4.24 (d, 4H, $J = 4.4$ Hz), 1.43 (s, 18H), 1.37 (d, 6H, $J = 6.9$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 155.5 (2× C), 131.9, 128.4 (2× CH), 122.7, 85.4, 82.0, 79.8 (4× C), 49.8 (CH), 29.7 (CH_2), 28.2 (3× CH_3), 18.4 (CH_3).

4.4.3.3. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-valine (7C). Yield: 179 mg (0.30 mmol, 99%). $R_f = 0.83$ (1:10; MeOH/DCM; v/v). ESI-MS (*m*/*z*) calcd for $C_{32}H_{46}N_4O_6 + Na^+$: 605.3, obsd 605.6. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.18 (m, 4H), 7.02 (br s, 2H), 5.36 (br d, 2H), 4.24 (d, 4H, $J = 4.4$ Hz), 4.06–3.98 (m, 2H), 2.18–2.06 (m, 2H), 1.43 (s, 18H), 0.99–0.94 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 156.0 (2× C), 134.8, 131.3, 128.2 (3× CH), 122.9, 85.3, 82.4, 79.8 (4× C), 59.9 (CH), 31.0 (CH_3), 29.7 (CH_2), 28.3 (3× CH_3), 19.2 (CH).

4.4.3.4. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-amino butaric acid (7D). Yield: 165 mg

(0.30 mmol, 99%). R_f = 0.30 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{30}H_{42}N_4O_6 + H^+$: 555.9, obsd 555.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69–7.21 (m, 4H), 6.77 (br s, 2H), 4.84 (br s, 2H), 4.25 (d, 4H, J = 5.2 Hz), 3.21–3.09 (m, 4H), 2.35–2.20 (m, 4H), 1.88–1.75 (m, 4H), 1.44 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 156.4 (C), 134.7, 131.8, 128.4 (3× CH), 122.8, 85.6, 81.9, 79.1 (4× C), 39.7, 33.2, 29.7 (CH_2), 28.3 (3× CH_3), 26.1 (CH_2).

4.4.3.5. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-amino hexanoic acid (7E). Yield: 179 mg (0.29 mmol, 98%). R_f = 0.45 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{34}H_{50}N_4O_2 + H^+$: 611.9, obsd 611.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.19 (m, 4H), 7.01 (t, 2H, J = 5.2 Hz), 4.89 (br t, 2H), 4.24 (d, 4H, J = 5.2 Hz), 3.12–3.02 (m, 4H), 2.28–2.19 (m, 6H), 1.70–1.58 (m, 6H), 1.52–1.40 (m, 22H), 1.38–1.27 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 156.0 (2× C), 134.7, 131.3, 128.3 (3x CH), 122.8, 85.9, 81.9, 78.9 (4× C), 40.2, 36.0, 29.6 (3× CH_2), 28.3 (3× CH_3), 26.2, 25.1 (2× CH_2).

4.4.3.6. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-proline (7F). Yield: 172 mg (0.30 mmol, 99%). R_f = 0.45 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{32}H_{42}N_4O_6 + H^+$: 579.4, obsd 579.2. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.15 (m, 4H), 6.43 (br s, 1H), 4.19 (br m, 6H), 3.42 (br m, 4H), 2.15 (br m, 4H), 1.83 (br m, 4H), 1.41 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.8, 155.8 (2× C), 134.6, 131.4, 128.1 (3× CH), 122.7, 85.4, 82.1, 80.5 (4× C), 60.5 (CH), 46.7, 30.5, 29.3 (3× CH_2), 28.2 (3× CH_3), 24.0 (CH_2).

4.4.3.7. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid (7G). Yield: 133 mg (0.22 mmol, 73%). R_f = 0.55 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{34}H_{46}N_4O_6 + H^+$: 607.5, obsd 607.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (s, 1H), 7.31–7.20 (m, 3H), 6.87 (t, 2H, J = 2.4 Hz), 4.21 (d, 4H, J = 5.2 Hz), 4.20–4.02 (br m, 4H), 2.80–2.65 (br m, 4H), 2.40–2.30 (m, 2H), 1.88–1.73 (m, 4H), 1.72–1.58 (m, 4H), 1.41 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 154.6 (2× C), 143.8, 131.4, 128.3 (3× CH), 122.8, 85.7, 82.1, 79.6 (4× C), 43.1, 42.8, 29.7, 28.4 (4× CH_2), 28.3 (3× CH_3).

4.4.3.8. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-glycine (9A). Yield: 148 mg (0.30 mmol, 99%). R_f = 0.40 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{26}H_{34}N_4O_6 + H^+$: 499.4, obsd 499.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (br s, 2H), 7.37–7.34 (m, 2H), 7.24–7.20 (m, 2H), 5.97 (br t, 2H), 4.29 (d, 4H, J = 5.4 Hz), 3.88 (d, 4H, J = 5.2 Hz), 1.44 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 156.2 (2× C), 131.5, 127.9 (2× CH), 125.4, 88.9, 81.2, 79.9 (4× C), 44.0, 29.8 (2× CH_2), 28.2 (3× CH_3).

4.4.3.9. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-alanine (9B). Yield: 157 mg (0.3 mmol, 99%). R_f = 0.75 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{28}H_{38}N_4O_6 + H^+$: 527.4, obsd 527.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (br s, 2H), 7.39–7.34 (m, 2H), 7.24–7.20 (m, 2H), 5.67 (d, 2H, J = 7.3 Hz), 4.37 (d, 4H, J = 5.1 Hz), 4.30–4.00 (m, 2H), 1.41 (s, 18H), 1.37 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 155.7 (2× C), 131.6, 127.9 (2× CH), 125.6, 88.9, 81.3, 79.9 (4× C), 49.9 (CH), 29.9 (CH_2), 28.2 (3× CH_3), 18.4 (CH_3).

4.4.3.10. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-valine (9C). Yield: 164 mg (0.28 mmol, 94%). R_f = 0.75 (1:1; Tol/EtOAc; v/v). ESI-MS (*m*/*z*) calcd for $C_{32}H_{46}N_4O_6 + H^+$: 583.3, obsd 583.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (br s, 2H), 7.37–7.34 (m, 2H), 7.22–7.20 (m, 2H), 5.64 (br s, 2H), 4.48 (dd, 2H, J = 17.6 Hz, J = 5.8 Hz), 4.13–4.02 (m, 4H), 2.09–2.01 (m, 2H), 1.41 (s, 18H), 0.93 (d, 12H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 156.1 (2× C), 131.5, 127.7 (2× CH), 125.5, 88.9, 80.9, 79.5 (4× C), 60.0, 31.0 (2× CH), 29.6 (CH_2), 28.2 (3× CH_3), 19.2, 18.4 (2× CH_3).

4.4.3.11. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-amino butaric acid (9D). Yield: 118 mg (0.21 mmol, 71%). R_f = 0.35 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{30}H_{42}N_4O_6 + H^+$: 555.9, obsd 555.4. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.69 (s, 2 H), 7.40–7.20 (m, 4H), 5.18 (s, 2H), 4.27 (d, 4H, J = 5.5 Hz), 3.18–3.12 (m, 4H), 2.38–2.24 (m, 4H), 1.90–1.74 (m, 4H), 1.43 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.1, 156.4 (2× C), 131.5, 127.9 (2× CH), 125.5, 89.3, 81.0, 79.0 (4× C), 39.6, 33.2, 29.8 (3× CH_2), 28.3 (3× CH_3), 26.2 (CH_2).

4.4.3.12. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-amino hexanoic acid (9E). Yield: 181 mg (0.30 mmol, 99%). R_f = 0.43 (1:10; MeOH/DCM; v/v). ESI-MS (*m*/*z*) calcd for $C_{34}H_{50}N_4O_2 + H^+$: 611.9, obsd 611.4. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.73 (br t, 2H), 7.40–7.20 (m, 4H), 5.21 (br t, 2H), 4.04 (d, 4H, J = 5.1 Hz), 3.06 (t, 4H, J = 5.5 Hz), 2.30–2.15 (m, 4H), 1.72–1.60 (m, 4H), 1.43 (s, 18H), 1.43–1.23 (m, 8H). ^{13}C NMR (50 MHz, $CDCl_3$/CD_3OD): δ 173.6, 156.3 (2× C), 131.5, 127.8 (2 × CH), 125.3, 89.0, 80.8, 79.0 (4× C), 39.9, 35.6, 29.4, 29.2 (4× CH_2), 28.1 (3× CH_3), 25.9, 25.0 (2× CH_2).

4.4.3.13. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-proline (9F). Yield: 170 mg (0.29 mmol, 98%). R_f = 0.35 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{32}H_{42}N_4O_6 + H^+$: 579.4, obsd 579.4. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.39–7.20 (m, 4H), 7.05 (br s, 2H), 6.50 (br s, 2H), 4.35–4.20 (br m, 4H), 4.20–4.05 (br m, 2H), 3.56–3.30 (br m, 4H), 2.40–2.05 (br m, 4H), 2.05–1.82 (br m, 4H), 1.42 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.5, 131.6 (2× CH), 125.4, 89.0, 80.5, 80.3 (4× C), 60.0 (CH), 46.9, 29.7, 29.3 (3× CH_2), 28.2 (3× CH_3), 23.9 (CH_2).

4.4.3.14. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,2-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid (9G). Yield: 144 mg (0.24 mmol, 79%). R_f = 0.70 (EtOAc). ESI-MS (*m*/*z*) calcd for $C_{34}H_{46}N_4O_6 + H^+$: 607.5, obsd 607.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.62 (m, 2H), 7.50–7.46 (m, 2H), 4.25 (d, 4H, J = 5.4 Hz), 4.23–4.03 (m, 4H), 2.85–2.62 (m, 4H), 2.55–2.37 (m, 2H), 1.85–1.61 (m, 8H), 1.45 (s, 18H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 174.7, 154.5 (2× C), 131.9, 128.3 (2× CH), 125.6, 89.5, 80.9, 79.3 (4× C), 43.1 (CH_2), 40.6 (CH), 29.6 (CH_2), 28.2 (3× CH_3), 27.7 (CH_2).

4.4.3.15. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,4-phenylene)diprop-2-yn-1-amide)-glycine (11A). Yield: 148 mg (0.30 mmol, 99%). $R_f = 0.70$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{26}H_{34}N_4O_6$, + H^+: 499.3, obsd 499.5. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (s, 4H), 7.09 (br t, 2H), 5.71 (br t, 2H), 4.24 (d, 4H, $J = 5.2$ Hz), 3.85 (s, 4H), 1.44 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 156.2 (2× C), 131.5 (4× CH), 122.5, 86.5, 82.6, 80.2 (4× C), 44.2, 29.7 (2× CH_2), 28.3 (3× CH_3).

4.4.3.16. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,4-phenylene)diprop-2-yn-1-amide)-alanine (11B). Yield: 91.5 mg (0.17 mmol, 58%). $R_f = 0.70$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{28}H_{38}N_4O_6 + H^+$: 527.4, obsd 527.2. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (s, 2H), 7.29 (s, 4H), 5.58 (br d, 2H), 4.31–4.15 (m, 6H), 1.43 (s, 18H), 1.38 (d, 6H, $J = 7.2$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 155.6 (2× C), 131.5 (4× CH), 122.5, 86.5, 82.7, 80.2 (4× C), 49.9 (CH), 29.8 (CH_2), 28.2 (3× CH_3), 18.2 (CH_3).

4.4.3.17. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,4-phenylene)diprop-2-yn-1-amide)-valine (11C). Yield: 140 mg (0.23 mmol, 77%). $R_f = 0.75$ (1:10; MeOH/DCM; v/v). ESI-MS (*m/z*) calcd for $C_{32}H_{46}N_4O_6 + Na^+$: 605.3, obsd 605.6. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (s, 4H), 6.70 (s, 2H), 5.26 (br d, 2H), 4.32–4.19 (m, 4H), 4.04–3.96 (m, 2H), 2.20–2.02 (m, 2H), 1.44 (s, 18H), 1.01–0.90 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 156.0 (2× C), 131.5 (4× CH), 122.5, 86.4, 82.8, 79.9 (4× C), 59.9 (CH), 30.9 (CH_3), 29.8 (CH_2), 28.2 (3× CH_3), 19.2 (CH).

4.4.3.18. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,4-phenylene)-diprop-2-yn-1-amide)-amino butaric acid (11D). Yield: 135 mg (0.23 mmol, 78%). $R_f = 0.45$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{30}H_{42}N_4O_6 + Na^+$: 577.8, obsd 577.4. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.38 (s, 4H), 6.80 (br s, 2H), 4.10 (d, 4H, $J = 5.2$ Hz), 2.93–2.89 (m, 4H), 2.12–2.05 (m, 4H), 1.66–1.53 (m, 4H), 1.36 (s, 18H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.9, 156.8 (2× C), 132.8 (4× CH), 123.6, 90.4, 82.2, 78.7 (4× C), 56.1, 33.8, 29.8 (3× CH_2), 29.5 (3× CH_3), 26.9 (CH_2).

4.4.3.19. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)-diprop-2-yn-1-amide)-amino hexanoic acid (11E). Yield: 64 mg (0.11, 35%). $R_f = 0.40$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{34}H_{50}N_4O_2 + H^+$: 611.8, obsd 611.3. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30 (t, 2H, $J = 5.2$ Hz), 7.37 (s, 4H), 5.75 (t, 2H, $J = 2.4$ Hz), 4.09 (t, 4H, $J = 2.8$ Hz), 2.86 (q, 4H, $J = 6.8$ Hz), 2.07 (t, 4H, $J = 7.6$ Hz), 1.51–1.43 (m, 4H,), 1.35 (s, 18H, *t*Bu Boc), 1.36–1.31 (m, 4H), 1.23–1.16 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.9, 155.5 (2× C), 131.5 (4× CH), 122.2, 89.2, 80.9, 77.2 (4× C), 35.0, 29.2, 28.5 (3× CH_2), 28.2 (3x CH_3), 25.9, 24.8 (2× CH_2).

4.4.3.20. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)-diprop-2-yn-1-amide)-proline (11F). Yield: 172 mg (0.30 mmol, 99%). $R_f = 0.40$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{32}H_{42}N_4O_6 + H^+$: 579.4, obsd 579.1. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (t, 2H, $J = 2.8$ Hz), 7.35 (m, 4H), 4.12 (d, 4H, $J = 5.6$ Hz), 4.05–4.02 (m, 2H), 3.43–3.31 (m, 4H), 3.30–3.20 (m, 2H), 2.17–2.01 (m, 2H), 1.85–1.68 (m, 4H), 1.30 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.4, 153.2 (2× C), 131.5 (4× CH), 122.3, 90.2, 80.8, 78.4 (4× C), 59.7 (CH), 46.4, 30.3, 28.4 (3x CH_2), 27.9 (3× CH_3), 23.6 (CH_2).

4.4.3.21. *N,N′*-α,α′-di-*tert*-Boc-(3,3′(1,3-phenylene)-diprop-2-yn-1-amide)-amino cyclohexanoic acid (11G). Yield: 120 mg (0.20 mmol, 66%). $R_f = 0.45$ (EtOAc). ESI-MS (*m/z*) calcd for $C_{34}H_{46}N_4O_6 + H^+$: 607.5, obsd 607.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 (s, 4H), 6.19 (t, 2H, $J = 4.9$ Hz), 4.24 (d, 4H, $J = 5.1$ Hz), 4.13–4.08 (m, 4H), 2.70 (t, 4H, $J = 12.0$ Hz), 2.31–2.22 (m, 2H), 1.81–1.77 (m, 4H), 1.69–1.55 (m, 4H), 1.42 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 154.5 (2× C), 131.5 (4× CH), 122.5, 86.7, 82.7, 79.6 (4× C), 43.0, 42.9, 29.8, 28.4 (4× CH_2), 28.3 (3× CH_3).

4.4.4. General procedure for coupling of pharmacophore 3 with monomeric spacers 5A–G affording IA–G. Boc protected compounds **5A**–**G** were subjected to a solution of 1:1 DCM/TFA v/v + 1% TIS for 18 h. The volatiles were evaporated and the compounds were purified with semi-preperative HPLC system (0 to 40% B). Accordingly, 20 μmol of amine (**6A**–**G**) was dissolved in 100 μL of DMF and added to a solution containing pharmacophore **3**[6,7] (30 μmol, 13.4 mg), BOP (39 μmol, 17.6 mg) and DiPEA (120 μmol, 20.4 μL) in 300 μL DMF. The reaction mixture was stirred at rt for 16 h and diluted with a mixture of DCM and MeOH (9:1; v/v, 20 mL). The organic layer was successively washed with water (3× 10 mL), 10% aq $NaHCO_3$ (3× 10 mL) and brine (1× 20 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified on a semi-preperative HPLC system (40 to 60% B) and lyopholized to obtain the title compounds as amorphous solids.

4.4.4.1. Compound IA. Yield after RP-HPLC purification: 3.0 mg (3.6 μmol, 12%). LC/MS analysis: R_t 7.00 min (gradient 30–90% B) and R_t 3.75 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{46}H_{44}F_2N_8O_6 + H^+$: 842.9, obsd 843.5. ^{1}H NMR (600 MHz, DMSO): δ 8.82 (s, 1 H), 8.54 (s, 1H), 8.36 (t, 1H, $J = 5.5$ Hz), 7.93 (t, 1H, $J = 5.7$ Hz), 7.61 (d, 2H, $J = 8.4$ Hz), 7.47–7.33 (m, 9H), 7.21–7.06 (m, 6H), 6.15 (t, 1H, $J = 5.5$ Hz), 5.54 (s, 2H), 5.31 (t, 1H, $J = 5.2$ Hz), 4.26 (m, 4H), 4.10 (d, 2H, $J = 5.4$ Hz), 3.63–3.61 (m, 4H), 3.32–3.30 (m, 2H), 3.13–3.09 (m, 2H), 1.30 (t, 3H, $J = 7.1$ Hz), 1.08 (t, 3H, $J = 7.2$ Hz). HRMS *m/z* calcd for $C_{46}H_{44}F_2N_8O_6 + H^+$: 843.34246, obsd 843.34254.

4.4.4.2. Compound IB. Yield after RP-HPLC purification: 4.3 mg (5.0 μmol, 17%). LC/MS analysis: R_t 7.37 min (gradient 30–90% B) and R_t 4.23 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{47}H_{46}F_2N_8O_6 + H^+$: 856.9, obsd 857.5. ^{1}H NMR (600 MHz, DMSO): δ 8.82 (s, 1H), 8.53 (s, 1H), 8.37 (t, 1H, $J = 5.3$ Hz), 7.86 (d, 1H, $J = 7.8$ Hz), 7.57 (d, 2H, $J = 8.4$ Hz), 7.48–7.42 (m, 4H), 7.35–7.30 (m, 5H), 7.28–7.05 (m, 6H), 6.68 (br s, 1H), 5.54 (s, 2H), 5.31 (t, 1H, $J = 5.1$ Hz), 4.36 (d, 1H, $J = 13.2$ Hz), 4.29–4.22 (m, 4H), 4.08 (d, 2H, $J = 1.9$ Hz), 3.68–3.53 (m, 4H), 3.12–3.06 (m, 2H), 1.33 (t, 3H, $J = 7.1$ Hz), 1.25–1.20 (m, 3H), 1.03 (t, 3H,

J = 7.2 Hz). HRMS m/z calcd for $C_{47}H_{46}F_2N_8O_6 + H^+$: 857.35811, obsd 857.35817.

4.4.4.3. Compound IC. Yield after RP-HPLC purification: 5.1 mg (5.8 μmol, 19%). LC/MS analysis: R_t 8.02 min (gradient 30–90% B) and R_t 4.82 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{49}H_{50}F_2N_8O_6 + H^+$: 885.0, obsd 885.6. ^{1}H NMR (600 MHz, DMSO): δ 8.80 (s, 1H), 8.66 (s, 1H), 8.45 (t, 1H, J = 5.4 Hz), 7.88 (m, 1H), 7.60 (d, 2H, J = 9.0 Hz), 7.46–7.07 (m, 15H), 6.27 (s, 1 H), 5.54 (s, 2H), 5.31 (t, 1H, J = 4.9 Hz), 4.38 (d, 1H, J = 13.2 Hz), 4.28–4.20 (m, 3H), 4.14–4.03 (m, 4H), 3.69–3.58 (m, 4H), 3.20 (d, 2H, J = 16.2 Hz), 3.11–3.08 (m, 2H), 1.85–1.83 (m, 1H), 1.30 (t, 3H, J = 7.1 Hz), 1.05 (t, 3H, J = 7.2 Hz), 0.72 (d, 3H, J = 6.6 Hz), 0.65 (d, 3H, J = 6.6 Hz). HRMS m/z calcd for $C_{49}H_{50}F_2N_8O_6 + H^+$: 885.38941, obsd 885.38886.

4.4.4.4. Compound ID. Yield after RP-HPLC purification: 5.8 mg (6.7 μmol, 22%). LC/MS analysis: R_t 7.15 min (gradient 30–90% B) and R_t 3.71 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{48}H_{48}F_2N_8O_6$, + H^+: 871.0, obsd 871.7. ^{1}H NMR (600 MHz, DMSO): δ 8.79 (s, 1H), 8.84 (s, 1H), 8.27 (t, 1H, J = 3.6 Hz), 7.64 (m, 1H, J = 3.6 Hz), 7.59 (d, 2H, J = 8.4 Hz), 7.45 (d, 2H, J = 8.4 Hz), 7.39–7.28 (m, 7H), 7.19–7.07 (m, 6H), 6.49 (br s, 1H), 5.53 (s, 2H), 5.31 (t, 1H, J = 4.8 Hz), 4.32–4.26 (m, 4H), 4.10–4.07 (m, 2H), 3.54 (s, 2H), 3.12–3.00 (m, 4H), 2.90 (q, 2H, J = 6.0 Hz), 2.13–2.05 (m, 2H), 1.52–1.49 (m, 2H), 1.31 (t, 3H, J = 7.2 Hz), 1.05 (t, 3H, J = 7.2 Hz). HRMS m/z calcd for $C_{48}H_{48}F_2N_8O_6$, + H^+: 871.37376, obsd 871.37354.

4.4.4.5. Compound IE. Yield after RP-HPLC purification: 5.3 mg (5.9 μmol, 20%). LC/MS analysis: R_t 7.52 min (gradient 30–90% B) and R_t 4.42 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{50}H_{52}F_2N_8O_6 + H^+$: 899.0, obsd 899.8. ^{1}H NMR (600 MHz, DMSO): δ 8.80 (s, 1H), 8.55 (s, 1H), 8.27 (t, 1H, J = 3.6 Hz), 6.85 (br s, 1H), 7.59 (d, 2H, J = 8.4 Hz), 7.44 (d, 2H, J = 8.4 Hz), 7.39–7.32 (m, 7H), 7.18–7.06 (m, 6H), 6.15 (t, 1H, J = 6.0 Hz), 5.53 (s, 2H), 5.31 (t, 1H, J = 5.4 Hz), 4.33 (s, 2H), 4.28 (q, 2H, J = 7.2 Hz), 4.09–4.07 (m, 2H), 3.54 (s, 2H), 3.13–3.00 (m, 4H), 2.79 (q, 2H, J = 6.6 Hz), 2.09–2.02 (m, 2H), 1.50–1.39 (m, 2H), 1.30–1.13 (m, 7H), 1.06 (t, 3H, J = 7.2 Hz). HRMS m/z calcd for $C_{50}H_{52}F_2N_8O_6 + H^+$: 899.40506, obsd 899.40559.

4.4.4.6. Compound IF. Yield after RP-HPLC purification: 7.03 mg (8.0 μmol, 27%). LC/MS analysis: R_t 7.56 min (gradient 30–90% B) and R_t 4.21 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{49}H_{48}F_2N_8O_6 + H^+$: 883.0, obsd 883.4. ^{1}H NMR (600 MHz, DMSO): δ 8.81 (s, 1H), 8.64 (s, 1H), 8.39 (t, 1H, J = 3.6 Hz), 7.60 (d, 2H, J = 9.0 Hz), 7.45–7.03 (m, 15H), 6.30 (t, 1H, J = 6.0 Hz), 5.56 (s, 2H), 5.31 (t, 1H, J = 4.8 Hz), 4.38 (br d, 1H), 4.30–4.25 (m, 2H), 4.12–4.04 (m, 4H), 3.70–3.59 (m, 4H), 3.12–3.06 (m, 4H), 1.90–1.85 (m, 1H), 1.72–1.65 (m, 1H), 1.62–1.52 (m, 2H), 1.29 (t, 3H, J = 7.2 Hz), 1.04 (t, 3H, J = 7.2 Hz). HRMS m/z calcd for $C_{49}H_{48}F_2N_8O_6 + H^+$: 883.37376, obsd 883.37334.

4.4.4.7. Compound IG. Yield after RP-HPLC purification: 2.0 mg (2.2 μmol, 7%). LC/MS analysis: R_t 7.41 min (gradient 30–90% B) and R_t 4.27 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{50}H_{50}F_2N_8O_6 + H^+$: 897.0, obsd 897.7. ^{1}H NMR (600 MHz, DMSO): δ 8.84 (s, 1H), 8.51 (s, 1H), 8.27 (t, 1H, J = 3.6 Hz), 7.59 (d, 2H, J = 9.0 Hz), 7.43–7.35 (m, 8H), 7.21–7.12 (m, 7H), 6.15 (t, 1H, J = 3.6 Hz), 5.55 (s, 2H), 5.31 (t, 1H, J = 5.0 Hz), 4.38–4.26 (m, 4H), 4.12–3.98 (m, 3H), 3.62–3.55 (m, 3H), 3.30–3.23 (m, 1H), 3.14–3.08 (m, 3H), 2.52–2.49 (m, 1H), 2.23–2.20 (m, 2H), 1.55–1.52 (m, 1H), 1.30 (t, 3H, J = 7.2 Hz), 1.29–1.17 (m, 3H), 1.05 (t, 3H, J = 7.2 Hz). HRMS m/z calcd for $C_{50}H_{50}F_2N_8O_6 + H^+$: 897.38941, obsd 897.38932.

4.4.5. General procedure for coupling of pharmacophore 3 with dimeric spacers 7A–G, 9A–G or 11A–G affording IIIA–G, IIA–G or IVA–G. Boc protected compounds **7A–G**, **9A–G** or **11A–G** were subjected to a solution of 1:1 DCM/TFA v/v + 1% TIS for 18 h. The volatiles were evaporated and the compounds were purified with semi-preparative HPLC system (0–30% B). Accordingly, 15 μmol of amine (**8A–G**, **10A–G** or **12A–G**) was dissolved in 100 μL of DMF and added to a solution containing pharmacophore **3**[6,7] (33 μmol, 14.8 mg), BOP (39 μmol, 17.6 mg) and DiPEA (120 μmol, 20.4 μL) in 300 μL DMF. The reaction mixture was stirred at rt for 16 h and diluted with a mixture of DCM and MeOH (9:1; v/v, 20 mL). The organic layer was successively washed with water (3× 10 mL), 10% aq $NaHCO_3$ (3× 10 mL) and brine (1× 20 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude product was purified on a semi-preparative HPLC system (40–60% B) and lyopholized to obtain the title compounds as amorphous solids.

4.4.5.1. Compound IIA. Yield after RP-HPLC purification: 1.83 mg (1.1 μmol, 8%). LC/MS analysis: R_t 8.43 min (gradient 30–90% B) and R_t 5.74 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{86}H_{82}F_4N_{16}O_{12} + H^+$: 1607.9, obsd 1607.8. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 2H), 8.56 (s, 2H), 8.36 (br t, 2H), 7.92 (br t, 2H), 7.63–7.55 (m, 4H), 7.52–7.28 (m, 12H), 7.21–7.02 (m, 12H), 6.17 (s, 2), 5.53 (s, 4H), 5.31 (t, 2H, J = 4.8 Hz), 4.32–4.22 (m, 8H), 4.18–4.12 (m, 4H), 3.59–3.56 (m, 8H), 3.18–3.08 (m, 8H), 1.29 (t, 6H, J = 7.0 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{86}H_{82}F_4N_{16}O_{12} + 2H^+$: 804.31899, obsd 804.31909.

4.4.5.2. Compound IIB. Yield after RP-HPLC purification: 2.09 mg (1.3 μmol, 9%). LC/MS analysis: R_t 8.80 min (gradient 30–90% B) and R_t 6.25 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{88}H_{86}F_4N_{16}O_{12} + H^+$: 1635.8, obsd 1636.9. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 2H), 8.55 (s, 2H), 8.32 (br s, 2H), 7.86 (br s, 2H), 7.62–7.28 (m, 16H), 7.19–7.02 (m, 12H), 6.16 (br s, 2H), 5.53 (s, 4H), 5.31 (t, 2H, J = 4.8 Hz), 4.36–4.30 (m, 2H), 4.28–4.22 (m, 8H), 4.18–4.07 (m, 4H), 3.66–3.55 (m, 4H), 3.18–3.05 (m, 8H), 1.32–1.27 (m, 12H), 1.05 (m, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{88}H_{86}F_4N_{16}O_{12} + 2H^+$: 816.33464, obsd 818.33529.

4.4.5.3. Compound IIC. Yield after RP-HPLC purification: 1.50 mg (0.9 μmol, 6%). LC/MS analysis: R_t 9.54 min (gradient 30–90% B) and R_t 7.38 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{92}H_{94}F_4N_{16}O_{12} + H^+$: 1691.9, obsd 1693.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (s, 2H), 8.66 (s, 2H), 8.45 (br s, 2H), 7.78 (br s, 2H), 7.60 (d, 4H, *J* = 9.0 Hz), 7.53–7.05 (m, 24H), 6.12 (s, 2H), 5.54 (s, 4H), 5.31 (t, 2H, *J* = 5.4 Hz), 4.38 (br d, 2H), 4.31–4.02 (m, 12), 3.68–3.55 (m, 4H), 3.17–3.05 (m, 8H), 1.89–1.82 (m, 2H), 1.28 (t, 6H, *J* = 7.2 Hz), 0.97 (t, 6H, *J* = 7.2 Hz), 0.73 (br d, 6H), 0.65 (br d, 6H). HRMS *m/z* calcd for $C_{92}H_{94}F_4N_{16}O_{12} + 2H^+$: 846.36594, obsd 846.36597.

4.4.5.4. Compound IID. Yield after RP-HPLC purification: 1.26 mg (0.8 μmol, 5%). LC/MS analysis: R_t 8.57 min (gradient 30–90% B) and R_t 5.88 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{90}H_{90}F_4N_{16}O_{12} + H^+$: 1663.8, obsd 1663.9. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 2H), 8.52 (s, 2H), 8.27 (br t, 2H), 7.68–7.58 (m, 6H, 7.48–7.38 (m, 6H), 7.35–7.30 (m, 2H), 7.20–7.12 (m, 10H), 7.08–7.01 (m, 6H), 6.15 (br t, 2H), 5.52 (s, 4H), 5.31 (t, 2H, *J* = 5.4 Hz), 4.32–4.26 (m, 7H), 4.15–4.10 (m, 5H), 3.56–3.49 (m, 4H), 3.15–3.02 (m, 8H), 2.93–2.88 (m, 4H), 1.97–1.90 (m, 4H), 1.57–1.50 (m, 4H), 1.28 (t, 6H, *J* = 7.8 Hz), 1.05 (t, 6H, *J* = 7.2 Hz). HRMS *m/z* calcd for $C_{90}H_{90}F_4N_{16}O_{12} + 2H^+$: 832.35029, obsd 832.35010.

4.4.5.5. Compound IIE. Yield after RP-HPLC purification: 1.42 mg (0.8 μmol, 6%). LC/MS analysis: R_t 8.89 min (gradient 30–90% B) and R_t 6.48 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{94}H_{98}F_4N_{16}O_{12} + H^+$: 1719.9, obsd 1721.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (s, 2H), 8.55 (s, 2H), 8.30 (br s, 2H), 7.80 (br s, 2H), 7.59 (br d, 4H), 7.50–7.48 (m, 8H), 7.37–7.30 (m, 2H), 7.38–7.03 (m, 14H), 6.15 (br t, 2H), 5.53 (s, 4H), 5.31 (t, 2H, *J* = 5.4 Hz), 4.37–4.22 (m, 8H), 4.16–4.11 (m, 4H), 3.60–3.49 (br s, 4H), 3.15–2.97 (m, 8H), 2.82–2.73 (m, 4H), 2.12–2.05 (m, 4H), 1.48–1.40 (m, 4H), 1.30–1.14 (m, 14H), 1.05 (t, 6H, *J* = 6.6 Hz). HRMS *m/z* calcd for $C_{94}H_{98}F_4N_{16}O_{12} + 2H^+$: 860.38159, obsd 860.38187.

4.4.5.6. Compound IIF. Yield after RP-HPLC purification: 1.55 mg (0.9 μmol, 6%). LC/MS analysis: R_t 9.76 min (gradient 30–90% B) and R_t 7.51 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{92}H_{90}F_4N_{16}O_{12} + H^+$: 1687.8, obsd 1687.4. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.78 (s, 2H), 8.52 (s, 2H), 8.28 (t, 2H), 7.62 (d, 1H, *J* = 8.4 Hz), 7.57 (d, 4H, *J* = 8.4 Hz), 7.48–7.39 (m, 6H), 7.35 (d, 4H, *J* = 8.4 Hz), 7.30–7.19 (m, 6H), 7.18–7.05 (m, 4H), 7.03–6.95 (m, 3H), 6.15 (t, 2H, *J* = 5.4 Hz), 5.53 (s, 4H), 5.31 (t, 2H), 4.35–4.28 (m. 2H), 4.24 (q, 4H, *J* = 6.6 Hz), 4.15–4.02 (m, 5H), 3.67–3.40 (m, 8H), 3.15–3.05 (m, 4H), 2.99 (s, 4H), 1.92–1.80 (m, 2H), 1.72–1.63 (m, 2H), 1.62–1.42 (m, 4H), 1.28 (m, 6H, *J* = 6.6 Hz), 1.04 (t, 6H, *J* = 7.2 Hz). HRMS *m/z* calcd for $C_{92}H_{90}F_4N_{16}O_{12} + 2H^+$: 844.35029, obsd 844.34997.

4.4.5.7. Compound IIG. Yield after RP-HPLC purification: 2.87 mg (1.7 μmol, 11%). LC/MS analysis: R_t 9.72 min (gradient 30–90% B) and R_t 7.56 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{94}H_{94}F_4N_{16}O_{12} + H^+$: 1715.9, obsd 1716.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 2H), 8.49 (s, 2H), 8.27 (t, 2H, *J* = 3.6 Hz), 7.59 (d, 4H, *J* = 9.0 Hz), 7.50–7.39 (m, 4H), 7.38–7.32 (m, 6H), 7.20–7.07 (m, 14H), 6.13 (t, 2H, *J* = 5.4 Hz), 5.55 (s, 4H), 4.34–4.24 (m, 8H), 4.13–4.08 (m, 4H), 4.03 (br d, 2H), 3.66–3.58 (m, 6H), 3.28–3.25 (m, 1H), 3.17–3.07 (m, 3H), 2.56–2.50 (m, 2H), 2.28–2.17 (m, 4H), 2.53 (br d, 2H), 1.29 (t, 6H, *J* = 7.2 Hz), 1.28–1.20 (m, 6H), 1.05 (t, 6H, *J* = 7.2 Hz). HRMS *m/z* calcd for $C_{94}H_{94}F_4N_{16}O_{12} + 2H^+$: 858.36594, obsd 858.36542.

4.4.5.8. Compound IIIA. Yield after RP-HPLC purification: 3.32 mg (2.1 μmol, 14%). LC/MS analysis: R_t 8.02 min (gradient 30–90% B) and R_t 4.90 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{86}H_{82}F_4N_{16}O_{12} + H^+$: 1607.7, obsd 1607.8. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 2H), 8.56 (s, 2H), 8.36 (br t, 2H), 7.97–7.90 (m, 2H), 7.61 (d, 4H, *J* = 8.4 Hz), 7.46–7.32 (m, 12H), 7.20–7.15 (m, 12H), 6.17 (br t, 2H), 5.53 (s, 4H), 5.31 (t, 2H, *J* = 3.6 Hz), 4.27–4.25 (m, 8H), 4.17–4.08 (m, 4H), 3.66–3.58 (m, 8H), 3.18–3.04 (m, 8H), 1.30 (t, 6H, *J* = 7.2 Hz), 1.05 (t, 6H, *J* = 7.1 Hz). HRMS *m/z* calcd for $C_{86}H_{82}F_4N_{16}O_{12} + 2H^+$: 804.31899, obsd 804.31900.

4.4.5.9. Compound IIIB. Yield after RP-HPLC purification: 1.13 mg (0.7 μmol, 5%). LC/MS analysis: R_t 8.62 min (gradient 30–90% B) and R_t 5.71 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{88}H_{86}F_4N_{16}O_{12} + H^+$: 1635.8, obsd 1636.9. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.84 (s, 2H), 8.51 (s, 2H), 8.29 (t, 2H, *J* = 4.8 Hz), 7.59 (d, 4H, *J* = 9.0 Hz), 7.43–7.35 (m, 12H), 7.21–7.12 (m, 12H), 6.15 (t, 2H, *J* = 4.8 Hz), 5.55 (s, 4H,), 5.31 (t, 2H, *J* = 5.10 Hz), 4.40–4.25 (m, 10H), 4.12–3.99 (d, 3H), 3.68–3.55 (m, 4H), 3.30–3.20 (m, 4H), 3.16–3.07 (m, 4H), 1.32–28 (m, 12H), 1.05 (t, 6H, *J* = 7.2 Hz). HRMS *m/z* calcd for $C_{88}H_{86}F_4N_{16}O_{12} + 2H^+$: 818.33464, obsd 818.33554.

4.4.5.10. Compound IIIC. Yield after RP-HPLC purification: 3.56 mg (2.1 μmol, 14%). LC/MS analysis: R_t 9.61 min (gradient 30–90% B) and R_t 7.04 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{92}H_{94}F_4N_{16}O_{12} + H^+$: 1691.9, obsd 1693.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.82 (s, 2H), 8.55 (s, 2H), 8.49 (br t, 2H), 7.75 (d, 2H, *J* = 9.0 Hz), 7.60 (d, 4H, *J* = 9.0 Hz), 7.48–7.40 (m, 4H), 7.39 (d, 4H, *J* = 9.0 Hz), 7.36–7.07 (m, 16H), 6.14 (br t, 2H), 5.54 (s, 4H), 5.31 (t, 2H, *J* = 3.6 Hz), 4.37 (br d, 2H), 4.30–4.05 (m, 8H), 3.62 (dd, 4H, *J* = 63.6 Hz, *J* = 19.8 Hz), 3.20 (d, 4H, *J* = 16.8 Hz), 3.15–3.04 (m, 8H), 1.87–1.78 (m, 2H), 1.29 (t, 6H, *J* = 7.1 Hz), 1.04 (t, 6H, *J* = 7.1 Hz), 0.71 (d, 6H, *J* = 6.6 Hz), 0.63 (d, 6H, *J* = 6.6 Hz). HRMS *m/z* calcd for $C_{92}H_{94}F_4N_{16}O_{12} + 2H^+$: 846.36594, obsd 846.36585.

4.4.5.11. Compound IIID. Yield after RP-HPLC purification: 3.35 mg (2.0 μmol, 14%). LC/MS analysis: R_t 8.08 min (gradient 30–90% B) and R_t 5.01 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{90}H_{90}F_4N_{16}O_{12} + H^+$: 1663.8, obsd 1665.0. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 2H), 8.57 (s, 2H), 8.29 (br t, 2H), 7.67 (br t, 2H), 7.60 (d, 4H, *J* = 9.0 Hz), 7.50–7.30 (m,

16H), 7.25–7.05 (m, 12H), 6.19 (br t, 2H), 5.53 (s, 4H), 5.31 (br t, 2H), 4.36–4.28 (m, 7H), 4.16–4.04 (m, 5H), 3.60–3.49 (m, 4H), 3.15–3.05 (m, 8H), 2.92–2.76 (m. 4), 2.18–2.12 (m, 4H), 1.56–1.48 (m, 4H), 1.30 (t, 6H, J = 7.08 Hz), 1.04 (t, 6H, J = 7.14 Hz). HRMS m/z calcd for $C_{90}H_{90}F_4N_{16}O_{12} + 2H^+$: 832.35029, obsd 832.34990.

4.4.5.12. Compound IIIE. Yield after RP-HPLC purification: 2.53 mg (1.5 μmol, 10%). LC/MS analysis: R_t 8.61 min (gradient 30–90% B) and R_t 5.78 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{94}H_{98}F_4N_{16}O_{12} + H^+$: 1719.9, obsd 1720.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (s, 2 H), 8.54 (s, 2H), 8.30 (br t, 2H), 8.26 (br t, 2H), 7.58 (d, 4H, J = 8.4 Hz), 7.50–7.28 (m, 12H), 7.22–7.20 (m, 16H), 6.16 (br t, 2H), 5.53 (s, 4H), 5.31 (br t, 2H), 4.33 (s, 4H), 4.27 (q, 4H, J = 6.6 Hz), 4.12–4.00 (m, 4H), 3.58–3.50 (m, 4H), 3.15–2.98 (m, 8H), 2.82–2.75 (m, 4H), 2.25–1.94 (m, 4H), 1.52–1.38 (m, 4H), 1.30 (t, 6H, J = 6.6 Hz), 1.22–1.12 (m, 8H), 1.05 (t, 6H, J = 7.1 Hz). HRMS m/z calcd for $C_{94}H_{98}F_4N_{16}O_{12} + 2H^+$: 860.38159, obsd 860.38145.

4.4.5.13. Compound IIIF. Yield after RP-HPLC purification: 1.59 mg (0.9 μmol, 6%). LC/MS analysis: R_t 8.93 min (gradient 30–90% B) and R_t 6.43 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{92}H_{90}F_4N_{16}O_{12} + H^+$: 1687.8, obsd 1689.0. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.82 (s, 2H), 8.60 (s, 2H), 8.35 (br s, 2H), 7.90 (m, 2H), 7.60 (d, 4H, J = 9.0 Hz), 7.49–7.06 (m, 24H), 6.21 (br t, 2H), 5.56 (s, 4H), 5.31 (t, 2H, J = 4.8 Hz), 4.38 (br d, 2H), 4.34–4.22 (m, 4H), 4.12–4.00 (m, 5H), 3.69–3.60 (m, 4H), 3.56 (s, 4H), 3.16–3.05 (m, 4H), 1.95–1.52 (br m, 8H), 1.28 (t, 6H, J = 7.2 Hz), 1.04 (t, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{92}H_{90}F_4N_{16}O_{12} + 2H^+$: 844.35029, obsd 844.35087.

4.4.5.14. Compound IIIG. Yield after RP-HPLC purification: 2.57 mg (1.5 μmol, 10%). LC/MS analysis: R_t 8.50 min (gradient 30–90% B) and R_t 6.03 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{94}H_{94}F_4N_{16}O_{12} + H^+$: 1715.9, obsd 1717.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.82 (s, 2H), 8.69 (s, 2H), 8.26 (s, 2H), 7.59 (d, 4H, J = 8.4 Hz), 7.51–7.35 (m, 12H), 7.25–7.06 (m, 12H), 6.15 (t, 2H, J = 5.4 Hz), 5.55 (s, 4H), 5.31 (br t, 2H), 4.37–4.22 (m, 8H), 4.15–3.97 (m, 6H), 3.67–3.56 (m, 6H), 3.28–3.23 (m, 2H), 2.58–2.50 (m, 2H), 2.29–2.12 (m, 4H), 1.78–1.70 (m, 2H), 1.58–1.50 (m, 2H), 1.31–1.13 (m, 8H), 1.05 (t, 6H, J = 6.6 Hz). HRMS m/z calcd for $C_{94}H_{94}F_4N_{16}O_{12} + 2H^+$: 858.36594, obsd 858.36643.

4.4.5.15. Compound IVA. Yield after RP-HPLC purification: 2.06 mg (1.3 μmol, 9%). LC/MS analysis: R_t 7.94 min (gradient 30–90% B) and R_t 5.08 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{86}H_{82}F_4N_{16}O_{12} + H^+$: 1607.7, obsd 1608.0. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 2H), 8.52 (s, 2H), 8.32 (br t, 2H), 7.91 (br t, 2H), 7.61 (d, 8H, J = 8.4 Hz), 7.48–7.32 (m, 12H), 7.22–7.05 (m, 12H), 6.14 (br t, 2H), 5.54 (s, 4H), 5.32–4.23 (m, 6H), 4.18–4.05 (m, 8H), 3.63–3.58 (m, 8H), 3.18–3.06 (m, 8H), 1.30 (t, 6H, J = 7.2 Hz), 1.05 (t, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{86}H_{82}F_4N_{16}O_{12} + 2H^+$: 804.31899, obsd 804.31867.

4.4.5.16. Compound IVB. Yield after RP-HPLC purification: 2.01 mg (1.2 μmol, 8%). LC/MS analysis: R_t 8.65 min (gradient 30–90% B) and R_t 5.82 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{88}H_{86}F_4N_{16}O_{12} + H^+$: 1635.8, obsd 1637.2. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 2H), 8.52 (s, 2H), 8.31 (t, 2H, J = 3.6 Hz), 7.60 (d, 4H, J = 9.0 Hz), 7.50–7.38 (m, 12H), 7.20–7.05 (m, 12H), 6.13 (t, 2HJ = 4.2 Hz), 5.53 (s, 4H), 5.31 (t, 2H, J = 3.6 Hz), 4.37–4.24 (m, 2H), 4.18–4.06 (m, 8H), 4.08 (d, 4H), 3.69–3.55 (m, 4H), 3.17–3.06 (m, 8H), 1.32–1.27 (m, 12H), 1.05 (t, 6H, J = 7.1 Hz). HRMS m/z calcd for $C_{88}H_{86}F_4N_{16}O_{12} + 2H^+$: 818.33464, obsd 818.33469.

4.4.5.17. Compound IVC. Yield after RP-HPLC purification: 1.31 mg (0.8 μmol, 5%). LC/MS analysis: R_t 9.77 min (gradient 30–90% B) and R_t 7.36 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{92}H_{94}F_4N_{16}O_{12} + H^+$: 1691.9, obsd 1693.3. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (s, 2H), 8.52 (s, 2H), 8.48–8.42 (m, 2H), 7.72 (d, 2H, J = 9.0 Hz), 7.60 (d, 4H, J = 9.0 Hz), 7.48–7.02 (m, 24H), 6.11 (t, 2H, J = 4.2 Hz), 5.54 (s, 4H), 5.31 (t, 2H, J = 3.6 Hz), 4.38 (br d, 2H), 4.30–4.06 (m, 12H), 3.60 (dd, 4H, J = 63.6 Hz, J = 19.8 Hz), 3.22–3.05 (m, 8H), 1.85–1.78 (m, 2H), 1.29 (t, 6H, J = 6.6 Hz), 1.04 (t, 6H, J = 7.2 Hz), 0.71 (d, 6H, J = 6.6 Hz), 0.63 (d, 6H, J = 6.6 Hz). HRMS m/z calcd for $C_{92}H_{94}F_4N_{16}O_{12} + 2H^+$: 846.36594, obsd 846.36576.

4.4.5.18. Compound IVD. Yield after RP-HPLC purification: 2.44 mg (1.5 μmol, 10%). LC/MS analysis: R_t 7.95 min (gradient 30–90% B) and R_t 4.92 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{90}H_{90}F_4N_{16}O_{12} + H^+$: 1663.8, obsd 1665.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.79 (s, 2H), 8.54 (s, 2H), 8.26 (t, 2H, J = 5.4 Hz), 7.64 (t, 2H, J = 5.4 Hz), 7.60 (d, 4H, J = 9.0 Hz), 7.49–7.39 (m, 14H), 7.20–6.97 (m, 10H), 6.17 (t, 2H, J = 5.4 Hz), 5.53 (s, 4H), 5.31 (br t, 2H), 4.32–4.25 (m, 8H), 4.15–4.05 (m, 6H), 3.53 (s, 4H), 3.13–3.02 (m, 8H), 2.90–2.86 (m. 4H), 2.03–1.98 (m, 4H), 1.56–1.49 (m, 4H), 1.30 (t, 6H, J = 7.2 Hz), 1.04 (t, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{90}H_{90}F_4N_{16}O_{12} + 2H^+$: 832.35029, obsd 832.35036.

4.4.5.19. Compound IVE. Yield after RP-HPLC purification: 2.04 mg (1.2 μmol, 8%). LC/MS analysis: R_t 9.25 min (gradient 30–90% B) and R_t 6.76 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{94}H_{98}F_4N_{16}O_{12} + H^+$: 1719.9, obsd 1720.1. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.80 (s, 2H), 8.54 (s, 2H), 8.27 (br t, 4H), 7.58 (d, 4H, J = 9.0 Hz), 7.55–7.28 (m, 12H), 7.21–7.05 (m, 12H), 6.15 (t, 2H), 5.54 (s, 4H), 5.33 (s, 4H), 4.28 (q, 4H, J = 6.6 Hz), 4.08 (d, 4H, J = 4.8 Hz), 3.56–3.52 (m, 8H), 3.14–3.09 (m, 4H), 3.00 (s, 4H), 2.71–2.65 (m, 4H), 2.04 (t, 4H, J = 9.0 Hz), 1.43–1.38 (m, 4H), 1.31 (t, 6H, J = 7.2 Hz), 1.26–1.15 (m, 8H), 1.05 (t, 6H, J = 7.2 Hz). HRMS m/z calcd for $C_{94}H_{98}F_4N_{16}O_{12} + 2H^+$: 871.37256, obsd 871.37218.

4.4.5.20. Compound IVF. Yield after RP-HPLC purification: 2.60 mg (1.5 μmol, 10%). LC/MS analysis: R_t 9.14 min (gradient 30–90% B) and R_t 6.39 min (gradient 50–90% B). ESI-MS (m/z) calcd for $C_{92}H_{90}F_4N_{16}$

$O_{12} + H^+$: 1687.8, obsd 1688.9. ^1H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 2H), 8.51 (s, 2H), 8.27 (br s, 2H), 7.60 (d, 4H, $J = 9.0$ Hz), 7.49–7.08 (m, 20H), 6.16 (br s, 2H), 5.56 (s, 4H), 5.31 (t, 2H), 4.39–4.32 (m, 2H), 4.30–4.23 (m, 4H), 4.18–4.00 (m, 5H), 3.70–3.58 (m, 4H), 3.56 (s, 4H), 3.15–3.05 (m, 4H), 2.05–1.96 (m, 2H), 1.95–1.80 (m, 2H), 1.72–1.68 (m, 2H), 1.66–1.50 (m, 2H), 1.29 (t, 6H, $J = 7.2$ Hz), 1.04 (t, 6H, $J = 7.2$ Hz). HRMS *m/z* calcd for $C_{92}H_{90}F_4N_{16}O_{12} + 2H^+$: 844.35029, obsd 844.35040.

4.4.5.21. Compound IVG. Yield after RP-HPLC purification: 2.04 mg (1.2 μmol, 8%). LC/MS analysis: R_t 9.09 min (gradient 30–90% B) and R_t 6.61 min (gradient 50–90% B). ESI-MS (*m/z*) calcd for $C_{94}H_{94}F_4N_{16}O_{12} + H^+$: 1715.9, obsd 1716.2. ^1H NMR (600 MHz, DMSO-d_6): δ 8.82 (s, 2H), 8.50 (s, 2H), 8.26 (s, 2H), 7.59 (d, 4H, $J = 7.8$ Hz), 7.57–7.32 (m, 12H), 7.31–7.06 (m, 12H) 6.15 (s, 2H), 5.55 (s, 4H), 5.31 (t, 2H), 4.51–4.23 (m, 8H), 4.18–4.09 (m, 6H), 3.62–3.58 (m, 6H), 3.28–3.20 (m, 2H), 3.18–3.08 (m, 8H), 2.60–2.52 (m, 2H), 2.30–2.12 (m, 4H), 1.65–1.50 (m, 4H), 1.49–1.39 (m, 2H), 1.38–1.23 (m, 8H), 1.05 (t, 6H, $J = 7.1$ Hz). HRMS *m/z* calcd for $C_{94}H_{94}F_4N_{16}O_{12} + 2H^+$: 858.36594, obsd 858.36579.

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References and notes

1. (a) Cornea, A.; Janovick, J. A.; Maya-Núñez, G.; Conn, P. M. *J. Biol. Chem.* **2001**, *276*, 2153–2158; (b) Horvat, R. D.; Roess, D. A.; Nelson, S. E.; Barisas, B. G.; Clay, C. M. *Mol. Endo.* **2001**, *15*, 695–703.
2. (a) Bulenger, S.; Marullo, S.; Bouvier, M. *Trends Pharmacol. Sci.* **2005**, *26*, 131–137; (b) Maggio, R.; Novi, F.; Scarselli, M.; Corsini, G. U. *FEBS J.* **2005**, *272*, 2939–2946; (c) Milligan, G. *Mol. Pharm.* **2004**, *66*, 1–7; (d) Terrillon, S.; Bouvier, M. *EMBO Rep.* **2004**, *5*, 30–34; (e) Angers, S.; Salahpour, A.; Bouvier, M. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 409–435; (f) George, S. R.; O'Dowd, B.; Lee, S. P. *Nat. Rev. Drug Discov.* **2002**, *1*, 808–820.
3. (a) Zhang, A.; Liu, Z.; Kan, Y. *Curr. Top. Med. Chem.* **2007**, *7*, 343–345; (b) Messer, W. S., Jr. *Curr. Pharm. Des.* **2004**, *10*, 2015–2020; (c) Halazy, S. *Exp. Opin. Ther. Patents* **1999**, *9*, 431–446; (d) Carrithers, M. D.; Lerner, M. R. *Chem. Biol.* **1996**, *3*, 537–542.
4. Muscarinic receptor: (a) Fejada, F. R.; Nagy, P. I.; Xu, M.; Wu, C.; Katz, T.; Dorsey, J.; Rieman, M.; Lawlor, E.; Warrier, M.; Messer, W. S., Jr. *J. Med. Chem.* **2006**, *49*, 7518–7531; Opioid receptor (b) Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. *J. Med. Chem.* **2006**, *49*, 256–262; (c) Daniels, D. J.; Lenard, N. R.; Etienne, C. L.; Law, P.-Y.; Roerig, S. C.; Portoghese, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 19208–19213; (d) Neumeyer, J. L.; Zhang, A.; Xiong, W.; Gu, X.-H.; Hilbert, J. E.; Knapp, B. I.; Negus, S. S.; Mello, N. K.; Bidlack, J. M. *J. Med. Chem.* **2003**, *46*, 5162–5170, Serotonine receptor; (e) see Ref. 10.
5. (a) Janovich, J. A.; Conn, P. M. *Endocrinology* **1996**, *137*, 3602–3605; (b) Hazum, E.; Keinan, D. *Biochem. Biophys. Res. Comm.* **1985**, *133*, 449–456; (c) Conn, P. M.; Rogers, D. C.; Stewart, J. M.; Niedel, J.; Sheffield, T. *Nature* **1982**, *296*, 653–655.
6. Bonger, K. M.; Van den Berg, R. J. B. H. N.; Heitman, L. H.; IJzerman, A. P.; Oosterom, J.; Timmers, C. M.; Overkleeft, H. S.; Van der Marel, G. A. *Bioorg. Med. Chem.* **2007**, *15*, 4841–4856.
7. (a) Sasaki, S.; Imaeda, T.; Hayase, Y.; Shimizu, Y.; Kasai, S.; Cho, N.; Harada, M.; Suzuki, N.; Furuya, S.; Fujino, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2073–2077; (b) Wilcoxen, K. M.; Zhu, Y.-F.; Connors, P. J.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers, R. S.; Chen, C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2179–2183; (c) Furuya, S.; Imaeda, T.; Sasaki, S. WO 99/33831, **1999**.
8. Conn, P. M.; Rogers, D. C.; Stewart, J. M.; Niedel, J.; Sheffield, T. *Nature* **1982**, *296*, 653–655.
9. (a) Betz, S. F.; Reinhart, G. J.; Lio, F. M.; Chen, C.; Struthers, R. S. *J. Med. Chem.* **2006**, *49*, 637–647; (b) Betz, S. F.; Lio, F. M.; Gao, Y.; Reinhart, G. J.; Guo, Z.; Mesleh, M. F.; Zhu, Y.-F.; Struthers, R. S. *J. Med. Chem.* **2006**, *49*, 6170–6176.
10. Russo, O.; Berthouze, M.; Giner, M.; Soulier, J.-L.; Rivail, L.; Sicsic, S.; Lezoualc'h, F.; Jockers, R.; Berque-Bestel, I. *J. Med. Chem.* **2007**, *50*, 4482–4492.
11. Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, G. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. *Anal. Biochem.* **1985**, *150*, 76–85.
12. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.