Chapter 3

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

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. GPCR dimerization and/or oligomerization is a well-recognized phenomenon which may be capitalized upon in the development of more active or specific (ant)agonists. However, to date there are no dimeric GnRHR ligands known, with the exception of the dimeric
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antibody complexed peptide agonist with which the possible relevance of GnRHR dimerization was demonstrated for the first time.\textsuperscript{18,19} The results in the preparation and evaluation of a library of homodimeric compounds (A, Figure 1) based on the imidazopyrimidinone GnRHR antagonist 1 were described in Chapter 2.\textsuperscript{20} The library was constructed by modification of antagonist 1 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 spacers were substituted on one site with the acetylene functionalized ligand. The 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 dimerization is not a prerequisite for signaling, at least in the Chapter 2 described system, it was considered more likely that the correct dimeric ligand design was not met. Many permutations are possible, and one specific alteration in the design that is addressed 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. This Chapter addresses the question whether the imposed spatial constraint in the orientation of the pharmacophores exerts an effect on the binding and functional activity of the ligands.

Chapter 2:

\[
\text{GnRHR-Ant}_1 + N_3 \xrightarrow{2+3\text{-dipolar azide-alkyne cycloaddition}} N_3 \xrightarrow{\text{peptide coupling conditions}} N_3
\]

This Chapter:

\[
\text{GnRHR-Ant}_3 + H_{AA}N \xrightarrow{\text{peptide coupling}} N_{AA}H \xrightarrow{\text{peptide coupling conditions}} N_{AA}H
\]

**Figure 1.** Overview of the in Chapter 2 described library (A) and current library (B) of dimeric GnRHR antagonists.
Results and discussion

The strategy for the preparation of the second-generation library is outlined in Scheme 1. N-Boc-glycine (A) was coupled to propargyl amine to afford acetylene 4A in 76% yield. Sonogashira cross coupling (CuI, Pd(PPh$_3$)$_4$, pyrrolidine and DMF) of 4A to phenyl iodide (I) gave compound [0,1]-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 of [0,1]-6A with pharmacophore 3 (prepared as described in reference 20) under the agency of BOP and DiPEA in DMF. The HPLC purification of [0,1]-6A proved necessary, since direct condensation of crude [0,1]-6A after Boc removal with 3 proceeded sluggishly and in low yield. Reaction of 4A with 1,3-diiodobenzene (III) gave bis-propargyl benzene [1,3]-5A. 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 AG (Figure 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). Synthesis of all members in the library proceeded in an efficiency compared to that of the examples outlined in scheme 1 (see the experimental section for details). All target compounds are depicted in Figure 3.

Scheme 1. Representative route for the synthesis of monomeric and dimeric GnRHR ligands. 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 [0,1]-5A, 99% for [1,3]-5A; iii. TFA/DCM; 1/1; v/v, 1% TIS, 18 h, HPLC purification; iv. pharmacophore 3, BOP, DiPEA, DMF, 18 h, HPLC purification.
Figure 2. Aryl iodides and amino acids employed in the construction of the library.

Figure 3. Structures of monovalent ligands IA-G and dimeric ligands IIIA-G.
All synthesized compounds were tested for 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 \(^{125}\text{I}\)triptorelin from plasma membranes of GnRHR-expressing Chinese Hamster Ovary (CHO) cells. As 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 (P<0.05). In general, the compounds with alanine (B) or valine (C) spacers 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.

Figure 3 continued. Structures of dimeric ligands IIA-G, and IVA-G.

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For the functional assay, CHO cells stably transfected with the GnRHR and equipped with the NFAT luciferase reporter gene was used. These cells were stimulated with a submaximal (EC₈₀) 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 IC₅₀ values of the mono functionalized compounds IA-G and the antagonistic effects at 3.16 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 IC₅₀ values, is in good agreement with the binding affinity (Kᵢ) 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 3.16 µM. These compounds all hold longer spacers (that is amino butanoate D, -hexanoate E and pipelicolic acid G).
Table 2. Antagonistic activities (IC50) of monomeric GnRHR antagonist \( \text{I} \) and \( \text{IA-G} \) and % inhibition of dimeric GnRHR antagonists \( \text{IIA-G}, \text{IIIA-G} \) and \( \text{IVA-G} \) at 3.16 and 10 \( \mu \text{g} \) concentration. \( ^c \)CHO cells that stably express the GnRHR were stimulated with a submaximal (EC80) concentration of GnRH and were incubated with increasing concentrations of the compounds. The IC50 value is the concentration of compound needed to inhibit the agonistic response by 50%. The mean IC50 are calculated from the -log IC50 values from two or three independent experiments performed in duplicate. The SD of pIC50 is generally lower than 0.2.

The literature on the antibody-mediated dimerization of GnRHR antagonistic peptides, with agonists as a result,\(^{18} \) was an incentive to investigate whether the dimeric 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 invited some additional experiments. The effects of two of the ligands were examined on an entire concentration-effect curve of the peptide agonist triptorelin. Thus, triptorelin curves in the absence and in the presence of two concentrations (2 and 10 \( \mu \text{M} \)) of selected compounds \( \text{ID} \) and \( \text{IVD} \) was recorded (Figure 4).

![Fig4](image-url)
As is evident from the curves shown in Figure 4, both monomeric- and dimeric ligand \textbf{ID} and \textbf{IVD} show a rightward shift of the dose-response curve, a clear indication of the antagonism of the peptide agonist triptorelin. Compound \textbf{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 ($E_{\text{max}}$) of triptorelin is also decreased, to the same amount in the presence of both monomeric ligand \textbf{ID} and dimeric ligand \textbf{IVD}. This result might indicate that the 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 the compounds is due to cytotoxicity the cell viability by a trypan blue exclusion experiment was determined. It was found that cell viability always exceeded 95 % after 4 h of incubation in the absence (control) or presence of 10 \(\mu\)M of a relevant subset of the compounds (see experimental part).

In this study, dimeric ligands derived from ligand 1 show interesting biological properties compared to the monomeric counterparts. While the binding affinities of the dimeric ligands and monomeric ligands were in the same order of magnitude, some different functional properties were observed for the dimeric ligands. For example, dimeric ligand \textbf{IVD} and monomeric ligand \textbf{ID} share similar binding affinities in the displacement assay, whereas a decrease in antagonistic potency for dimeric ligand \textbf{IVD} is observed. A similar trend was observed for dimeric ligand \textbf{IVB}, which show a 3-fold increase in binding affinities compared to monomeric ligand \textbf{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 \textbf{IID}, \textbf{IIE}, \textbf{IIIG}, \textbf{IIIG}, \textbf{IVD} and \textbf{IVE}, 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 \textbf{IVD} to the peptide agonist triptorelin reduced both potency and efficacy of the peptide (Figure 4). However, the monomeric counterpart \textbf{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.\textsuperscript{24-26} It is conceivable that the compounds bind to the receptor in a similar fashion as the reported non-peptidic antagonists. The dimeric ligands derived from antagonist 1 do not show enhanced pharmacological properties compared to the monomeric counterparts. Therefore, this study does not give new insights into different modes of binding of bivalent ligands to the GnRHR. Recently, in a similar study targeting the serotonin 5-HT\textsubscript{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 bivalent compounds lost the agonistic character of the monomeric reference compound.\textsuperscript{27} Yet, the authors show a conformational change of 5-HT\textsubscript{4}R 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 presented here are highly reminiscent to those reported in the 5-HT4 study when considering the nature and size of the linker systems. It is therefore not unlikely that the here described 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.

Conclusion

In conclusion, a strategy for the preparation of a set of dimeric ligands containing hydrophobic, rigid linker systems to target the GnRH receptor was developed. The results described in this Chapter concerning binding and functional properties do not provide 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 the compounds might indicate a different mode of binding of the dimeric ligands compared to the monomeric counterparts. To establish whether dimerization of GnRH receptor is a prerequisite for signaling or ligand binding, more research is needed. The next Chapter describes the development of a library based on a homo- and heterodimeric ligands containing a different antagonist.

Experimental Procedures

GnRHR Luciferase reporter gene assay

Chinese Hamster Ovary, CHO-K1, cells with stable expression of the human Gonadotropin-Releasing Hormone Receptor (GnRHR) 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 units/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 7,500 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.
Radioligand Binding Assay.
Ganirelix was provided by Schering-Plough research institute (Oss, The Netherlands). [125I]Triptorelin (specific activity 2200 Ci mmol⁻¹) was purchased from Perkin Elmer Life Sciences B.V. (Groningen, The Netherlands). CHO-K1 cells stably expressing the human GnRH receptor were provided by Schering-Plough research institute (Oss, The Netherlands). All other chemicals and cell culture materials were obtained from standard commercial sources.

CHO (Chinese hamster ovary) -K1 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% CO₂. 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 MgCl₂, 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-HCl buffer and the homogenization and centrifugation steps were repeated. Tris-HCl 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.²⁸

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 MgCl₂ 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 [125I]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 pre-soaked 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 MgCl₂ and 0.05% BSA). Filter-bound radioactivity was determined in a γ-counter.

All data was analyzed using the non-linear regression curve-fitting program GraphPad Prism v. 4 (GraphPad Software Inc, San Diego, CA, U.S.A.). Inhibitory binding constants (Ki values) were derived from the IC₅₀ 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.²⁹ The $K_d$ value (0.35 nM) of [125I]Triptorelin was obtained by computer analysis of saturation curves.³⁰ All values obtained are means of at least two independent experiments performed in duplicate.

Cytotoxicity
CHO-GnRH_luc cells were seeded on 5-cm diameter plates in assay medium in the absence (control) or presence of 10 μM of test compounds. Compounds ID-IVD, IVA and IVE were selected as relevant compounds in this toxicity assay. 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.
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 (NH₄)₆Mo₇O₂₄ × 4H₂O (25 g/L) or (NH₄)₄Ce(SO₄)₄ × 2H₂O (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 MHz, 300/75 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 ¹³C-APT spectra are proton decoupled. For LC-MS analysis, a HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C₈ column (4.6 mmOD × 250 mmL, 5µ particle size) in combination with buffers A: H₂O, B: CH₃CN and C: 1% aq. TFA and coupled to a mass instrument with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a preparative C₁₈ column (5 µm C₁₈, 10Å, 150 × 21.2 mm) was used. The applied buffers were A: H₂O + 0.1% TFA and B: CH₃CN. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and diocetylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

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 1M HCl (50 mL) and 10% aqueous NaHCO₃ (50 mL). The organic layer was dried (Na₂SO₄), filtered and concentrated. The residue was dissolved in EtOAc and triturated with petroleum ether to afford titled product as white crystals.

*N-α-tert-Boc-glycine propargylamide (4A)*. Yield: 1.60 g (7.55 mmol, 76%). Rₚ = 0.60 (EtOAc). ESI-MS m/z: 213.0 [M + H]⁺. ¹H NMR (200 MHz, CDCl₃) δ 6.59 (br t, 1H), 5.25 (br t, 1H), 4.07 (dd, 2H, J = 5.5, J = 2.6), 3.82 (d, 2H, J = 5.8), 2.24 (t, 1H, J = 2.6), 1.46 (s, 9H). ¹³C NMR (50 MHz, CDCl₃) δ 169.2, 156.0, 89.8, 80.4, 71.7 (5 × C), 29.1 (CH₂), 28.3 (3 × CH₃).

*N-α-tert-Boc-alanine propargylamide (4B)*. Yield: 1.94 g (8.55 mmol, 86%). Rₚ = 0.55 (5% MeOH in DCM). ESI-MS m/z: 227.0 [M + H]⁺. ¹H NMR (200 MHz, CDCl₃) δ 6.59 (br t, 1H), 5.00 (br d, 1H), 4.28 – 4.12 (m, 1H) 4.05 (dd, 2H, J = 5.5, J = 2.6), 2.22 (t, 1H, J = 2.6), 1.45 (s, 9H), 1.35 (d, 3H, J = 6.9). ¹³C NMR (50 MHz, CDCl₃) δ 172.4, 155.5, 80.2, 79.3, 71.5 (5 × C), 49.9 (CH), 29.1 (CH₂), 28.3 (3 × CH₃), 18.3 (CH₃).

*N-α-tert-Boc-valine propargylamide (4C)*. Yield: 2.52 g (9.92 mmol, 99%). Rₚ = 0.65 (5% MeOH in DCM). ESI-MS m/z: 255.1 [M + H]⁺. ¹H NMR (200 MHz, CDCl₃) δ 6.40 (br t, 1H), 5.04 (br d, 1H), 4.05 (dd, 2H, J = 5.5, J = 2.2), 3.92 (dd, 1H, J = 8.9, J = 6.4), 2.22 (t, 1H, J = 2.6), 2.15 (m, 1H), 1.45 (s, 9H), 0.97 (d, 3H, J = 6.6), 0.93 (d, 3H, J = 7.0). ¹³C NMR (50 MHz, CDCl₃) δ 171.6, 155.9, 79.9, 79.3, 71.4 (5 × C), 59.8, 31.0 (2 × CH), 28.9 (CH₂), 28.3 (3 × CH₃), 17.9, 19.1 (2 × CH₃).
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**N-γ-tert-Boc-γ-amino butaric acid-propargylamide (4D).** Yield: 1.68 g (7.00 mmol, 70%). Rf = 0.50 (5% MeOH in DCM). ESI-MS m/z: 240.9 [M + H]+. 1H NMR (200 MHz, CDCl3) δ 6.39 (br t, 1H), 4.73 (br t, 1H), 4.05 (dd, 2H, J = 5.1, J = 2.9), 3.18 (q, 2H, J = 6.6), 2.27 – 2.21 (m, 3H), 1.88 – 1.75 (m, 2H), 1.44 (s, 9H). 13C NMR (50 MHz, CDCl3) δ 173.2, 156.7, 89.3, 79.3, 70.9 (5 × C), 39.4, 32.9, 28.6, (3 × CH2), 28.1 (3 × CH3), 25.8 (CH3).

**N-e-tert-Boc-e- amino hexanoic acid-propargylamide (4E).** Yield: 2.41 g (8.99 mmol, 90%). Rf = 0.60 (5% MeOH in DCM). ESI-MS m/z: 269.1 [M + H]+. 1H NMR (200 MHz, CDCl3) δ 5.77 (br t, 1H), 4.56 (br t, 1H), 4.05 (dd, 2H, J = 5.5, J = 2.6), 3.11 (q, 2H, J = 6.6), 2.24 – 2.17 (m, 3H), 1.44 (s, 9H), 1.74 – 1.34 (m, 6H). 13C NMR (50 MHz, CDCl3/CD3OD) δ 173.3, 156.3, 79.5, 79.2, 71.0 (5 × C), 39.9, 35.7, 29.3, 28.7 (4 × CH2), 28.2 (3 × CH3), 26.0, 24.9 (2 × CH2).

**N-α-tert-Boc-proline propargylamide (4F).** Yield: 1.71 g (6.79 mmol, 68%). Rf = 0.55 (5% MeOH in DCM). ESI-MS m/z: 253.0 [M + H]+. 1H NMR mixture of rotamers (200 MHz, CDCl3) 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). 13C NMR of rotamers (50 MHz, CDCl3) δ 171.9, 80.3, 79.3, 71.2 (4 × C), 46.8, 28.7 (2 × CH2), 28.3 (3 × CH3), 24.2, 23.6 (2 × CH2).

**General procedure for coupling of propargylamide functionalized amino acids with iodo benzene (affording [0,1]-5A-5G).**

In separate flasks, a solution of the propargyl functionalized amino acid (4A-G, 1.0 mmol), iodo benzene (1.5 mmol, 111/g L) 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(PPh3)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(PPh3)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% aqueous NaHCO3 (50 mL). The organic layer was dried with Na2SO4, filtrated and concentrated. The crude material was purified by automated silica gel column chromatography (35 to 65% MeOH/DCM (1/10) in Petroleum ether).

**N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-glycine ([0,1]-5A).** Yield: 189 mg (0.66 mmol, 66%). Rf = 0.65 (EtOAc). ESI-MS m/z: 289.0 [M + H]+. 1H NMR (500 MHz, CDCl3) 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), 3.88 (s, 2H), 1.43 (s, 9H). 13C NMR (125 MHz, CDCl3) δ 174.1, 156.5, 79.5, 71.3 (4 × C), 43.0 (CH3), 42.6 (CH), 28.9, 28.3 (2 × CH2), 28.3 (3 × CH3).

**N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-alanine ([0,1]-5B).** Yield: 298 mg (0.99 mmol, 99%). Rf = 0.70 (EtOAc). ESI-MS m/z: 303.0 [M + H]+. 1H NMR (500 MHz, CDCl3) δ 7.39 (m, 2H), 7.27 (m, 3H), 7.09 (s, 1H), 5.42 (d, 1H, J = 7.0), 4.39 (br s, 1H), 4.26 (d, 2H, J = 4.5), 1.40 (s, 9H), 1.37 (d, 3H). 13C NMR (125 MHz, CDCl3) δ 172.5, 155.5 (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 × CH3), 28.0 (3 × CH3).
N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-valine ([0,1]-5C). Yield: 322 mg (0.98 mmol, 98%). \( R_l = 0.80 \) (EtOAc). ESI-MS m/z: 331.0 [M + H]+. \( ^1\)H NMR (500 MHz, CDCl3) \( \delta \) 7.56 (s, 1H), 7.37 (m, 2H), 7.27 (m, 3H), 5.64 (d, 1H, \( J = 9.5 \)), 4.34 (dd, 1H, \( J = 17.5 \), \( J = 5.5 \)), 4.13 (d, 2H, \( J = 4.5 \)), 2.10 – 2.06 (m, 1H), 1.42 (s, 9H), 0.99 (d, 3H, \( J = 7.0 \)), 0.96 (d, 3H, \( J = 7.0 \)). \( ^{13}\)C NMR (125 MHz, CDCl3) \( \delta \) 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 (CH2), 28.2 (3 × CH3), 19.0, 18.1 (2 × CH3).

N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-amino butaric acid ([0,1]-5D). Yield: 205 mg (0.65 mmol, 65%). \( R_f = 0.55 \) (EtOAc). ESI-MS m/z: 317.0 [M + H]+. \( ^1\)H NMR (500 MHz, CDCl3) \( \delta \) 7.41 (m, 2H), 7.29 (m, 3H), 7.23 (s, 1H), 5.25 (s, 1H), 4.23 (s, 2H), 3.13 (t, 2H, \( J = 4.0 \)), 2.25 (t, 2H, \( J = 7.0 \)), 1.83 – 1.78 (m, 2H), 1.43 (s, 9H).

N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-amino hexanoic acid ([0,1]-5E). Yield: 332 mg (0.97 mmol, 97%). \( R_f = 0.70 \) (EtOAc). ESI-MS m/z: 343.1 [M + H]+. \( ^1\)H NMR (500 MHz, CDCl3) \( \delta \) 7.38 (m, 2H), 7.28 (m, 3H), 7.15 (s, 1H), 5.12 (s, 1H), 4.23 (d, 2H, \( J = 4.0 \)), 3.06 (t, 2H, \( J = 4.0 \)), 2.22 (t, 2H, \( J = 7.5 \)), 1.65 (m, 2H), 1.49 (m, 2H), 1.43 (s, 9H), 1.32 (m, 2H). \( ^{13}\)C NMR (125 MHz, CDCl3) \( \delta \) 173.3, 155.3 (2 × C), 131.4, 128.0 (2 × CH), 122.4, 84.7, 82.7, 79.0 (4 × C), 39.9, 29.5, 25.9, 24.9 (2 × CH2).

N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-proline ([0,1]-5F). Yield: 302 mg (0.92 mmol, 92%). \( R_f = 0.55 \) (EtOAc). ESI-MS m/z: 329.1 [M + H]+. \( ^1\)H NMR of rotamers (500 MHz, CDCl3) \( \delta \) 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, \( J = 5.0 \)), 2.25 (br dd, 2H), 1.84 (br d, 2H), 1.45 (s, 9H), 1.32 (m, 2H). \( ^{13}\)C NMR of rotamers (125 MHz, CDCl3) \( \delta \) 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 × CH2), 28.1 (3 × CH3), 24.4, 23.1 (2 × CH3).

N-α-tert-Boc-(3-phenylprop-2-yn-1-amide)-amino cyclohexanoic acid ([0,1]-5G). Yield: 323 mg (0.94 mmol, 94%). \( R_f = 0.85 \) (EtOAc). ESI-MS m/z: 343.0 [M + H]+. \( ^1\)H NMR (500 MHz, CDCl3) \( \delta \) 7.39 (m, 2H), 6.43 (s, 1H), 4.26 (d, 2H, \( J = 5.0 \)), 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, CDCl3) \( \delta \) 174.1, 154.5 (2 × C) 131.4, 128.1 (2 × CH), 122.3, 84.7, 83.1, 79.4 (4 × C), 42.9 (CH2), 42.8 (CH), 29.8, 28.3 (2 × CH2), 27.4 (3 × CH3).

General procedure for coupling of propargylic spacers 4A-G with 1,2-diiodobenzene (affording [1,2]-5A-G), 1,3-diiodobenzene (affording [1,3]-5A-G) and 1,4-diiodobenzene (affording [1,4]-5A-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 g) in DMF (3 mL), a solution of CuI (0.06 mmol, 11.5 mg) in DMF (1 mL) and a solution of Pd(PPh3)4 (0.03 mmol, 34.7 mg) in DMF (1 mL) were flushed with argon for 1 h in an ultrasonic bath. To the alkyne solution were added subsequently the CuI and the Pd(PPh3)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% aqueous NaHCO3 (10 mL). The organic layer was dried with Na2SO4 and concentrated. The crude material was purified by automated silica gel column chromatography (35 to 65% MeOH/DCM (1/10) in Petroleum ether).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,2-phenylene)diprop-2-yn-1-amide)-glycine ([1,2]-5A). Yield: 148 mg (0.90 mmol, 99%). \( R_l = 0.40 \) (EtOAc). ESI-MS m/z: 443.9 [M + H]+. \( ^1\)H NMR (400 MHz, CDCl3) \( \delta \) 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, \( J = 5.4 \)), 3.88 (d, 4H, \( J = 5.2 \)), 1.44 (s, 18H).
$\text{C NMR (100 MHz, CDCl}_3\rangle \delta 170.0, 156.2 (2 \times C), 131.5, 127.9 (2 \times CH), 125.4, 88.9, 81.2, 79.9 (4 \times C), 44.0, 29.8 (2 \times CH_2), 28.2 (3 \times CH_3)$.

$\text{N',N'-Di-tert-Boc-(3,3'(1,2-phenylene)diprop-2-yn-1-amide)-alanine ([1,2]-5B). Yield: 157 mg (0.3 mmol, 99%).}$

$\text{RF = 0.75 (EtOAc). ESI-MS m/z: 527.4 [M + H]^+}$. $\text{1H NMR (400 MHz, CDCl}_3\rangle \delta 7.45 (\text{br s}, 2H), 7.39 – 7.34 (m, 2H), 5.67 (d, 2H, J = 7.3), 4.37 (d, 4H, J = 5.1), 4.30 – 4.00 (m, 2H), 1.41 (s, 18H), 1.37 (t, 6H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,2-phenylene)diprop-2-yn-1-amide)-valine ([1,2]-5C). Yield: 164 mg (0.28 mmol, 94%).}$

$\text{RF = 0.75 (50% EtOAc in toluene). ESI-MS m/z: 583.4 [M + H]^+}$. $\text{1H NMR (400 MHz, CDCl}_3\rangle \delta 7.75 (\text{br s}, 2H), 7.37 – 7.34 (m, 2H), 7.22 – 7.20 (m, 2H), 4.48 (dd, 2H, J = 17.6, J = 5.8), 4.13 – 4.02 (m, 4H), 2.09 – 2.01 (m, 2H), 1.41 (s, 18H), 0.93 (d, 12H, J = 6.8) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,2-phenylene)diprop-2-yn-1-amide)-amino butaric acid ([1,2]-5D). Yield: 118 mg (0.21 mmol, 71%).}$

$\text{RF = 0.35 (EtOAc). ESI-MS m/z: 555.4 [M + H]^+}$. $\text{1H NMR (200 MHz, CDCl}_3\rangle \delta 7.69 (s, 2H), 7.40 – 7.20 (m, 4H), 5.18 (s, 2H), 4.27 (d, 4H, J = 5.5), 3.06 (t, 4H, J = 5.5), 2.30 – 2.15 (m, 4H), 1.72 – 1.60 (m, 4H), 1.43 (t, 4H, J = 7.3) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,2-phenylene)diprop-2-yn-1-amide)-amino hexanoic acid ([1,2]-5E). Yield: 181 mg (0.30 mmol, 99%).}$

$\text{RF = 0.43 (10% MeOH in DCM). ESI-MS m/z: 611.4 [M + H]^+}$. $\text{1H NMR (200 MHz, CDCl}_3\rangle \delta 7.73 (\text{br t}, 2H), 7.40 – 7.20 (m, 4H), 5.21 (t, 2H, J = 5.2), 4.04 (d, 4H, J = 5.1), 3.06 (t, 4H, J = 5.5), 2.30 – 2.15 (m, 4H), 1.72 – 1.60 (m, 4H), 1.43 (t, 4H, J = 7.3), 1.43 – 1.23 (m, 4H), 0.93 (d, 12H, J = 6.8) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-glycine ([1,3]-5A). Yield: 148 mg (0.29 mmol, 98%).}$

$\text{RF = 0.50 (EtOAc). ESI-MS m/z: 579.4 [M + H]^+}$. $\text{1H NMR (200 MHz, CDCl}_3\rangle \delta 7.45 – 7.16 (m, 4H), 7.05 (br s, 2H), 6.50 (br s, 2H), 4.35 – 4.20 (m, 4H), 4.20 – 4.05 (m, 2H), 3.56 – 3.30 (m, 4H), 2.40 – 2.05 (m, 4H), 2.05 – 1.82 (m, 4H), 1.44 (s, 18H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid ([1,3]-5G). Yield: 144 mg (0.24 mmol, 79%).}$

$\text{RF = 0.70 (EtOAc). ESI-MS m/z: 607.3 [M + H]^+}$. $\text{1H NMR (300 MHz, CDCl}_3\rangle \delta 7.67 – 7.62 (m, 2H), 7.50 – 7.46 (m, 2H), 4.25 (d, 4H, J = 5.4), 4.23 – 4.03 (m, 4H), 2.85 – 2.62 (m, 4H), 2.55 – 2.37 (m, 2H), 1.85 – 1.61 (m, 4H), 1.45 (s, 18H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-glycine ([1,3]-5A). Yield: 148 mg (0.30 mmol, 99%).}$

$\text{RF = 0.70 (EtOAc). ESI-MS m/z: 599.5 [M + H]^+}$. $\text{1H NMR (200 MHz, CDCl}_3\rangle \delta 7.45 – 7.16 (m, 4H), 7.07 (br t, 2H), 5.57 (br t, 2H), 4.25 (d, 4H, J = 5.1), 3.86 (d, 4H, J = 5.1), 1.44 (s, 18H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid ([1,3]-5G). Yield: 144 mg (0.24 mmol, 79%).}$

$\text{RF = 0.70 (EtOAc). ESI-MS m/z: 607.3 [M + H]^+}$. $\text{1H NMR (300 MHz, CDCl}_3\rangle \delta 7.67 – 7.62 (m, 2H), 7.50 – 7.46 (m, 2H), 4.25 (d, 4H, J = 5.4), 4.23 – 4.03 (m, 4H), 2.85 – 2.62 (m, 4H), 2.55 – 2.37 (m, 2H), 1.85 – 1.61 (m, 4H), 1.45 (s, 18H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-glycine ([1,3]-5A). Yield: 148 mg (0.30 mmol, 99%).}$

$\text{RF = 0.70 (EtOAc). ESI-MS m/z: 599.5 [M + H]^+}$. $\text{1H NMR (200 MHz, CDCl}_3\rangle \delta 7.45 – 7.16 (m, 4H), 7.07 (br t, 2H), 5.57 (br t, 2H), 4.25 (d, 4H, J = 5.1), 3.86 (d, 4H, J = 5.1), 1.44 (s, 18H) \text{.}$

$\text{N',N'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid ([1,3]-5G). Yield: 144 mg (0.24 mmol, 79%).}$
N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-alanine ([1,3]-5B). Yield: 128 mg (0.24 mmol, 81%). Rf = 0.70 (EtOAc). ESI-MS m/z: 527.4 [M + H]+. 1H NMR (400 MHz, CDCl3) 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), 1.43 (s, 18H), 1.37 (d, 6H, J = 6.9). 13C NMR (100 MHz, CDCl3) 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 (CH2), 28.2 (3 × CH3), 18.4 (CH3).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-valine ([1,3]-5C). Yield: 179 mg (0.30 mmol, 99%). Rf = 0.83 (10% MeOH in DCM). ESI-MS m/z: 605.6 [M + H]+. 1H 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), 4.06 – 3.98 (m, 2H), 2.18 – 2.06 (m, 2H), 1.43 (s, 18H), 0.99 – 0.94 (m, 12H). 13C 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 (CH3), 29.7 (CH2), 28.3 (3 × CH3), 19.2 (CH).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino butaric acid ([1,3]-5D). Yield: 165 mg (0.30 mmol, 99%). Rf = 0.30 (EtOAc). ESI-MS m/z: 555.3 [M + H]+. 1H 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), 3.12 – 3.09 (m, 4H), 2.35 – 2.20 (m, 4H), 1.88 – 1.75 (m, 4H), 1.44 (s, 18H). 13C NMR (100 MHz, CDCl3) 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 (CH2), 28.3 (3 × CH3), 26.1 (CH3).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino hexanoic acid ([1,3]-5E). Yield: 179 mg (0.29 mmol, 98%). Rf = 0.45 (EtOAc). ESI-MS m/z: 611.4 [M + H]+. 1H NMR (400 MHz, CDCl 3) δ 7.42 – 7.19 (m, 4H), 7.01 (t, 2H, J = 5.2), 4.89 (br t, 2H), 4.24 (d, 4H, J = 5.2), 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 – 1.58 (m, 4H), 1.52 (s, 18H). 13C NMR (75 MHz, CDCl3) δ 172.7, 156.0 (2 × C), 134.7, 131.3, 128.3 (3 × CH), 122.8, 85.9, 81.9, 78.9 (4 × C), 40.2, 36.0, 29.6 (3 × CH3), 28.3 (3 × CH3), 26.2, 25.1 (2 × CH2).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-proline ([1,3]-5F). Yield: 172 mg (0.29 mmol, 98%). Rf = 0.45 (EtOAc). ESI-MS m/z: 595.5 [M + H]+. 1H NMR (300 MHz, CDCl 3) δ 7.37 – 7.15 (m, 4H), 6.43 (br s, 1H), 4.19 (m, 6H), 3.42 (m, 4H), 2.15 (m, 4H), 1.83 (m, 4H), 1.41 (s, 9H). 13C NMR (75 MHz, CDCl3) 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 × CH3), 28.2 (3 × CH3), 24.0 (CH2).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,4-phenylene)diprop-2-yn-1-amide)-glycine ([1,4]-5A). Yield: 148 mg (0.30 mmol, 99%). Rf = 0.70 (EtOAc). ESI-MS m/z: 499.5 [M + H]+. 1H NMR (400 MHz, CDCl3) δ 7.31 – 7.20 (m, 3H), 6.87 (t, 2H, J = 2.4), 4.21 (d, 4H, J = 5.2), 4.20 – 4.02 (m, 4H), 2.80 – 2.65 (m, 4H), 2.40 – 2.30 (m, 2H), 1.88 – 1.73 (m, 4H), 1.72 – 1.58 (m, 4H), 1.41 (s, 18H). 13C NMR (75 MHz, CDCl3) 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 × CH3), 28.3 (3 × CH3).
N,N'-α,α'-Di-tert-Boc-(3,3'(1,4-phenylene) diprop-2-yn-1-amide)-alanine ([1,4]-5B). Yield: 91.5 mg (0.17 mmol, 58%). Rf = 0.70 (EtOAc). ESI-MS m/z: 527.2 [M + H]+. 1H NMR (300 MHz, CDCl3) 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). 13C NMR (100 MHz, CDCl3) 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 (CH2), 28.2 (3 × CH3), 18.2 (CH3).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,4-phenylene)diprop-2-yn-1-amide)-valine ([1,4]-5C). Yield: 140 mg (0.23 mmol, 77%). Rf = 0.75 (10% MeOH in DCM). ESI-MS m/z: 605.6 [M + H]+. 1H NMR (400 MHz, CDCl3) 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). 13C NMR (100 MHz, CDCl3) 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 (CH3), 29.8 (CH2), 28.2 (3 × CH3), 19.2 (CH).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,4-phenylene)diprop-2-yn-1-amide)-amino butaric acid ([1,4]-5D). Yield: 135 mg (0.23 mmol, 78%). Rf = 0.45 (EtOAc). ESI-MS m/z: 577.4 [M + Na]+. 1H NMR (400 MHz, DMSO-d6) δ 7.38 (s, 4H), 6.80 (br s, 2H), 4.10 (d, 4H, J = 5.2), 2.93 – 2.89 (m, 4H), 2.12 – 2.05 (m, 4H), 1.66 – 1.53 (m, 4H), 1.36 (s, 18H). 13C NMR (150 MHz, CDCl3) 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 × CH2), 29.5 (3 × CH3), 26.9 (CH2).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino hexanoic acid ([1,4]-5E). Yield: 64 mg (0.11 mmol, 35%). Rf = 0.40 (EtOAc). ESI-MS m/z: 611.3 [M + H]+. 1H NMR (400 MHz, DMSO-d6) δ 8.30 (t, 2H, J = 5.2), 7.37 (s, 4H), 5.75 (t, 2H, J = 2.4), 4.09 (t, 4H, J = 2.8), 2.86 (q, 4H, J = 5.2), 2.12 – 2.05 (m, 2H), 1.51 – 1.43 (m, 4H), 1.36 – 1.31 (m, 4H), 1.35 (s, 18H), 1.36 – 1.31 (m, 2H), 1.23 – 1.16 (m, 4H). 13C NMR (150 MHz, DMSO-d6) 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 × CH2), 28.2 (3 × CH3), 25.9, 24.8 (2 × CH2).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-proline ([1,4]-5F). Yield: 172 mg (0.30 mmol, 99%). Rf = 0.40 (EtOAc). ESI-MS m/z: 579.1 [M + H]+. 1H NMR (400 MHz, DMSO-d6) δ 8.42 (t, 2H, J = 2.8), 7.35 (m, 4H), 4.12 (d, 4H, J = 5.6), 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), 1.36 – 1.31 (m, 4H), 1.35 (s, 18H), 1.23 – 1.16 (m, 4H). 13C NMR (100 MHz, DMSO-d6) 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 (3 × CH2), 27.9 (3 × CH3), 23.6 (CH2).

N,N'-α,α'-Di-tert-Boc-(3,3'(1,3-phenylene)diprop-2-yn-1-amide)-amino cyclohexanoic acid ([1,4]-5G). Yield: 120 mg (0.20 mmol, 66%). Rf = 0.45 (EtOAc). ESI-MS m/z: 607.5 [M + H]+. 1H NMR (300 MHz, CDCl3) δ 7.28 (s, 4H), 6.19 (t, 2H, J = 4.9), 4.24 (d, 4H, J = 5.1), 4.13 – 4.08 (m, 4H), 2.70 (t, 4H, J = 12.0), 2.31 – 2.22 (m, 2H), 1.81 – 1.77 (m, 4H), 1.69 – 1.55 (m, 4H), 1.42 (s, 18H). 13C NMR (75 MHz, CDCl3) 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 × CH2), 28.3 (3 × CH3).

General procedure for coupling of pharmacophore 3 with monomeric spacers [0,1]-5A-G affording IA-G.

Boc protected compounds [0,1]-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-preparative HPLC system (0 to 40% B). Accordingly, 20 µmol of amine ([0,1]-6A-G) was dissolved in 100 µL of DMF and added to a solution containing pharmacophore 3 (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% aqueous NaHCO3 (3 × 10 mL) and brine (1
x 20 mL). The organic layer was dried (NaSO₄), filtered and concentrated. The crude product was purified on a semi-preparative HPLC system (40 to 60% B) and lyophilized to obtain the title compounds as amorphous solids.

**Monomeric ligand IA.** Yield after RP-HPLC purification: 3.0 mg (3.6 µmol, 12%). LC-MS analysis: tᵣ 7.00 min (gradient 30 to 90% B) and tᵣ 3.75 min (gradient 50 to 90% B). ESI-MS m/z: 843.5 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) δ 8.82 (s, 1H), 8.54 (s, 1H), 8.36 (t, 1H, J = 5.5), 7.93 (t, 1H, J = 5.7), 7.61 (d, 2H, J = 8.4), 7.47 – 7.33 (m, 9H), 7.21 – 7.06 (m, 6H), 6.15 (t, 1H, J = 5.5), 5.54 (s, 2H), 5.31 (t, 1H, J = 5.2), 4.26 (m, 4H), 4.10 (d, 2H, J = 5.4), 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), 1.08 (t, 3H, J = 7.2). HRMS m/z calecd for C₉H₄F₂N₆O₆ + H+: 843.34246, obsd 843.34254.

**Monomeric ligand IB.** Yield after RP-HPLC purification: 4.3 mg (5.0 µmol, 17%). LC-MS analysis: tᵣ 7.37 min (gradient 30 to 90% B) and tᵣ 4.23 min (gradient 50 to 90% B). ESI-MS m/z: 856.9 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) δ 8.82 (s, 1H), 8.53 (s, 1H), 8.37 (t, 1H, J = 5.3), 7.86 (d, 1H, J = 7.8), 7.57 (d, 2H, J = 8.4), 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), 4.36 (d, 1H, J = 13.2), 4.29 – 4.22 (m, 4H), 4.08 (d, 2H, J = 1.9), 3.68 – 3.53 (m, 4H), 3.12 – 3.06 (m, 2H), 1.33 (t, 3H, J = 7.1), 1.25 – 1.20 (m, 3H), 1.03 (t, 3H, J = 7.2). HRMS m/z calecd for C₉H₇F₂N₆O₆ + H+: 857.35811, obsd 857.35817.

**Monomeric ligand IC.** Yield after RP-HPLC purification: 5.1 mg (5.8 µmol, 19%). LC-MS analysis: tᵣ 8.02 min (gradient 30 to 90% B) and tᵣ 4.82 min (gradient 50 to 90% B). ESI-MS m/z: 885.6 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) 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), 7.46 – 7.07 (m, 15H), 6.27 (s, 1H), 5.54 (s, 2H), 5.31 (t, 1H, J = 4.9), 4.38 (d, 1H, J = 13.2), 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), 3.11 – 3.08 (m, 2H), 1.85 – 1.83 (m, 1H), 1.30 (t, 3H, J = 7.1), 1.05 (t, 3H, J = 7.2), 0.72 (d, 3H, J = 6.6), 0.65 (d, 3H, J = 6.6). HRMS m/z calecd for C₉H₈F₂N₆O₆ + H+: 885.38941, obsd 885.38886.

**Monomeric ligand ID.** Yield after RP-HPLC purification: 5.8 mg (6.7 µmol, 22%). LC-MS analysis: tᵣ 7.15 min (gradient 30 to 90% B) and tᵣ 3.71 min (gradient 50 to 90% B). ESI-MS m/z: 871.7 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) δ 7.89 (s, 1H), 8.84 (s, 1H), 8.27 (t, 1H, J = 3.6), 7.64 (m, 1H, J = 3.6), 7.59 (d, 2H, J = 8.4), 7.45 (d, 2H, J = 8.4), 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), 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), 2.13 – 2.05 (m, 2H), 1.52 – 1.49 (m, 2H), 1.31 (t, 3H, J = 7.2), 1.05 (t, 3H, J = 7.2). HRMS m/z calecd for C₉H₈F₂N₆O₆ + H+: 871.37376, obsd 871.37354.

**Monomeric ligand IE.** Yield after RP-HPLC purification: 5.3 mg (5.9 µmol, 20%). LC-MS analysis: tᵣ 7.52 min (gradient 30 to 90% B) and tᵣ 4.42 min (gradient 50 to 90% B). ESI-MS m/z: 899.0 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) δ 8.80 (s, 1H), 8.55 (s, 1H), 8.27 (t, 1H, J = 3.6), 6.85 (br s, 1H), 7.59 (d, 2H, J = 8.4), 7.44 (d, 2H, J = 8.4), 7.39 – 7.32 (m, 7H), 7.18 – 7.06 (m, 6H), 6.15 (t, 1H, J = 6.0), 5.53 (s, 2H), 5.31 (t, 1H, J = 5.4), 4.33 (s, 2H), 4.28 (q, 2H, J = 7.2), 4.09 – 4.07 (m, 2H), 3.54 (s, 2H), 3.13 – 3.00 (m, 4H), 2.79 (q, 2H, J = 6.0), 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). HRMS m/z calecd for C₉H₈F₂N₆O₆ + H+: 899.40506, obsd 899.40559.

**Monomeric ligand IF.** Yield after RP-HPLC purification: 7.03 mg (8.0 µmol, 27%). LC-MS analysis: tᵣ 7.56 min (gradient 30 to 90% B) and tᵣ 4.21 min (gradient 50 to 90% B). ESI-MS m/z: 883.4 [M + H]+. ¹H NMR (600 MHz, DMSO-d₆) δ 8.81 (s, 1H), 8.64 (s, 1H), 8.39 (t, 1H, J = 3.6), 7.60 (d, 2H, J = 9.0), 7.45 – 7.03 (m, 15H), 6.30 (t, 1H, J = 6.0), 5.56 (s, 2H), 5.31 (t, 1H, J = 4.8), 4.38 (br d, 1H), 4.30 – 4.25 (m, 2H), 4.12 – 4.04 (m, 4H), 3.70 – 3.59
Monomeric ligand IG. Yield after RP-HPLC purification: 2.0 mg (2.2 μmol, 7%). LC-MS analysis: \( t_R \) 7.41 min (gradient 30 to 90% B) and \( t_R \) 4.27 min (gradient 50 to 90% B). ESI-MS m/z: 897.7 [M + H]+. 1H NMR (600 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 8.84 (s, 1H), 8.56 (s, 1H), 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), 6.19 (s, 2H), 5.35 (s, 4H), 5.31 (t, 2H, \( J = 4.8 \)), 4.36 – 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, \( J = 7.0 \)), 1.05 (t, 6H, \( J = 7.2 \)). HRMS m/z calcd for C_{86}H_{82}F_4N_{16}O_{12} + 2H+: 804.31899, obsd 804.31909.

Dimeric ligand IIA. Yield after RP-HPLC purification: 1.83 mg (1.1 μmol, 8%). LC-MS analysis: \( t_R \) 8.43 min (gradient 30 to 90% B) and \( t_R \) 5.74 min (gradient 50 – 90% B). ESI-MS m/z: 1607.8 [M + H]+. 1H NMR (600 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 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, \( J = 4.8 \)), 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 (t, 6H, \( J = 7.2 \)). HRMS m/z calcd for C_{88}H_{86}F_4N_{16}O_{12} + 2H+: 816.33464, obsd 818.33529.

Dimeric ligand IIB. Yield after RP-HPLC purification: 2.09 mg (1.3 μmol, 9%). LC-MS analysis: \( t_R \) 8.80 min (gradient 30 to 90% B) and \( t_R \) 6.25 min (gradient 50 to 90% B). ESI-MS m/z: 1636.9 [M + H]+. 1H NMR (600 MHz, DMSO-\( \text{d}_6 \)) \( \delta \) 8.80 (s, 2H), 8.66 (s, 2H), 8.45 (br s, 2H), 7.78 (br s, 2H), 7.60 (d, 4H, \( J = 9.0 \)), 7.53 – 7.05 (m, 24H), 6.12 (s, 2H), 5.54 (s, 4H), 5.31 (t, 2H, \( J = 5.4 \)), 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, \( J = 7.2 \)), 0.97 (t, 6H, \( J = 7.2 \)), 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.
**Dimeric ligand IID.** Yield after RP-HPLC purification: 1.26 mg (0.8 µmol, 5%). LC-MS analysis: 7.55 min (gradient 30 to 90% B) and 7.68 min (gradient 50 to 90% B). ESI-MS m/z: 1663.9 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 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), 1.05 (t, 6H, J = 7.2). HRMS m/z calcd for C50H48F2N16O12 + 2H+: 832.35029, obsd 832.35010.

**Dimeric ligand III.** Yield after RP-HPLC purification: 1.42 mg (0.8 µmol, 6%). LC-MS analysis: 7.89 min (gradient 30 to 90% B) and 7.48 min (gradient 50 to 90% B). ESI-MS m/z: 1721.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 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). HRMS m/z calcd for C36H36F4N8O12 + 2H+: 860.38159, obsd 860.38187.

**Dimeric ligand IIIA.** Yield after RP-HPLC purification: 1.55 mg (0.9 µmol, 6%). LC-MS analysis: 8.97 min (gradient 30 to 90% B) and 7.51 min (gradient 50 to 90% B). ESI-MS m/z: 1687.4 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.78 (s, 2H), 8.52 (s, 2H), 8.28 (t, 2H), 7.62 (d, 1H, J = 8.4), 7.57 (d, 4H, J = 8.4), 7.48 – 7.39 (m, 6H), 7.35 (d, 4H, J = 8.4), 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), 5.53 (s, 4H), 5.31 (t, 2H), 4.35 – 4.28 (m, 2H), 4.24 (q, 4H, J = 6.6), 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), 1.04 (t, 6H, J = 7.2). HRMS m/z calcd for C52H50F4N16O12 + 2H+: 844.34997, obsd 844.34997.

**Dimeric ligand IIG.** Yield after RP-HPLC purification: 2.87 mg (1.7 µmol, 11%). LC-MS analysis: 9.72 min (gradient 30 to 90% B) and 7.56 min (gradient 50 to 90% B). ESI-MS m/z: 1716.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.81 (s, 2H), 8.49 (s, 2H), 8.27 (t, 2H, J = 3.6), 7.59 (d, 4H, J = 9.0), 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), 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), 1.28 – 1.20 (m, 6H), 1.05 (t, 6H, J = 7.2). HRMS m/z calcd for C42H38F4N10O12 + 2H+: 858.36594, obsd 858.36542.

**Dimeric ligand IIIIA.** Yield after RP-HPLC purification: 3.32 mg (2.1 µmol, 14%). LC-MS analysis: 8.02 min (gradient 30 to 90% B) and 4.90 min (gradient 50 to 90% B). ESI-MS m/z: 1607.8 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 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), 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), 1.05 (t, 6H, J = 7.1). HRMS m/z calcd for C36H36F4N10O12 + 2H+: 804.31899, obsd 804.31900.

**Dimeric ligand IIIB.** Yield after RP-HPLC purification: 1.13 mg (0.7 µmol, 5%). LC-MS analysis: 8.62 min (gradient 30 to 90% B) and 5.71 min (gradient 50 to 90% B). ESI-MS m/z: 1636.9 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.84 (s, 2H), 8.51 (s, 2H), 8.29 (t, 2H, J = 4.8), 7.59 (d, 4H, J = 9.0), 7.43 – 7.35 (m, 12H), 7.21 – 7.12 (m, 12H), 6.15 (t, 2H, J = 4.8), 5.55 (s, 4H), 5.31 (t, 2H, J = 5.10), 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). HRMS m/z calcd for C38H38F4N10O12 + 2H+: 818.33464, obsd 818.33554.
Dimeric ligand IIIC. Yield after RP-HPLC purification: 3.56 mg (2.1 μmol, 14%). LC-MS analysis: tR 9.61 min (gradient 30 to 90% B) and tR 7.04 min (gradient 50 to 90% B). ESI-MS m/z: 1693.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.82 (s, 2H), 8.55 (s, 2H), 8.49 (br t, 2H), 7.75 (d, 2H, J = 9.0), 7.60 (d, 4H, J = 9.0), 7.48 – 7.40 (m, 4H), 7.39 (d, 4H, J = 9.0), 7.36 – 7.07 (m, 16H), 6.14 (br t, 2H), 5.54 (s, 4H), 5.31 (t, 2H, J = 3.6); 4.37 (br d, 2H), 4.30 – 4.05 (m, 8H), 3.62 (dd, 4H, J = 6.6, J = 19.8), 3.20 (d, 4H, J = 16.8), 3.15 – 3.04 (m, 8H), 1.87 – 1.78 (m, 2H), 1.29 (t, 6H, J = 7.1), 1.04 (t, 6H, J = 7.1), 0.71 (d, 6H, J = 6.6), 0.63 (d, 6H, J = 6.6). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 846.36594, obsd 846.36585.

Dimeric ligand IIID. Yield after RP-HPLC purification: 3.35 mg (2.0 μmol, 14%). LC-MS analysis: tR 8.08 min (gradient 30 to 90% B) and tR 5.01 min (gradient 50 to 90% B). ESI-MS m/z: 1665.0 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 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), 1.04 (t, 6H, J = 7.14). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 832.35029, obsd 832.34990.

Dimeric ligand IIIE. Yield after RP-HPLC purification: 2.53 mg (1.5 μmol, 10%). LC-MS analysis: tR 8.61 min (gradient 30 to 90% B) and tR 5.78 min (gradient 50 to 90% B). ESI-MS m/z: 1720.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.80 (s, 2H), 8.54 (s, 2H), 8.30 (br t, 2H), 8.26 (br t, 2H), 7.58 (d, 4H, J = 8.4), 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 (q, 4H, J = 6.6), 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), 1.22 – 1.12 (m, 8H), 1.05 (t, 6H, J = 7.1). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 860.38159, obsd 860.38145.

Dimeric ligand IIIF. Yield after RP-HPLC purification: 1.59 mg (0.9 μmol, 6%). LC-MS analysis: tR 8.93 min (gradient 30 to 90% B) and tR 6.43 min (gradient 50 to 90% B). ESI-MS m/z: 1689.0 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 7.49 – 7.06 (m, 24H), 6.21 (br t, 2H), 5.56 (s, 4H), 5.31 (t, 2H, J = 4.8), 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 (m, 8H), 1.28 (t, 6H, J = 7.2), 1.04 (t, 6H, J = 7.2). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 844.35029, obsd 844.35087.

Dimeric ligand IIIG. Yield after RP-HPLC purification: 2.57 mg (1.5 μmol, 10%). LC-MS analysis: tR 8.50 min (gradient 30 to 90% B) and tR 6.03 min (gradient 50 to 90% B). ESI-MS m/z: 1717.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.82 (s, 2H), 8.69 (s, 2H), 8.26 (s, 2H), 7.59 (d, 4H, J = 8.4), 7.51 – 7.35 (m, 12H), 7.25 – 7.06 (m, 12H), 6.15 (t, 2H, J = 5.4), 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). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 858.36594, obsd 858.36643.

Dimeric ligand IVA. Yield after RP-HPLC purification: 2.06 mg (1.3 μmol, 9%). LC-MS analysis: tR 7.94 min (gradient 30 to 90% B) and tR 5.08 min (gradient 50 to 90% B). ESI-MS m/z: 1608.0 [M + H]+. 1H NMR (600 MHz, DMSO-d6) 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), 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), 1.05 (t, 6H, J = 7.2). HRMS m/z calcd for C90H86F4N16O12 + 2H+: 804.31899, obsd 804.31867.
Dimeric ligand IVB. Yield after RP-HPLC purification: 2.01 mg (1.2 µmol, 8%). LC-MS analysis: tR 8.65 min (gradient 30 to 90% B) and tR 5.82 min (gradient 50 to 90% B). ESI-MS m/z: 1637.2 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.79 (s, 2H), 8.52 (s, 2H), 8.31 (t, 2H, J = 3.6), 7.60 (d, 4H, J = 9.0), 7.50 – 7.38 (m, 12H), 7.20 – 7.05 (m, 12H), 6.13 (t, 2H, J = 4.2), 5.53 (s, 4H), 5.31 (t, 2H, J = 3.6), 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). HRMS m/z calcld for C88H80F4N16O12 + 2H+: 818.33464, obsd 818.33469.

Dimeric ligand IVC. Yield after RP-HPLC purification: 1.31 mg (0.8 µmol, 5%). LC-MS analysis: tR 9.77 min (gradient 30 to 90% B) and tR 7.36 min (gradient 50 to 90% B). ESI-MS m/z: 1693.3 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.80 (s, 2H), 8.52 (s, 2H), 8.48 – 8.42 (m, 2H), 7.72 (d, 2H, J = 9.0), 7.60 (d, 4H, J = 9.0), 7.48 – 7.02 (m, 24H), 6.11 (t, 2H, J = 4.2), 5.54 (s, 4H), 5.31 (t, 2H, J = 3.6), 4.38 (br d, 2H), 4.30 – 4.06 (m, 12H), 3.60 (dd, 4H, J = 63.6, J = 19.8), 3.22 – 3.05 (m, 8H), 1.85 – 1.78 (m, 2H), 1.29 (t, 6H, J = 6.6), 1.04 (t, 6H, J = 7.2), 0.71 (d, 6H, J = 6.6), 0.63 (d, 6H, J = 6.6). HRMS m/z calcld for C92H92F4N16O12 + 2H+: 846.36594, obsd 846.36576.

Dimeric ligand IVD. Yield after RP-HPLC purification: 2.44 mg (1.5 µmol, 10%). LC-MS analysis: tR 7.95 min (gradient 30 to 90% B) and tR 4.92 min (gradient 50 to 90% B). ESI-MS m/z: 1665.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.79 (s, 2H), 8.54 (s, 2H), 8.26 (t, 2H, J = 5.4), 7.64 (t, 2H, J = 5.4), 7.60 (d, 4H, J = 9.0), 7.49 – 7.39 (m, 14H), 7.20 – 6.97 (m, 10H), 6.17 (t, 2H, J = 5.4), 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), 1.04 (t, 6H, J = 7.2). HRMS m/z calcld for C90H90F4N16O12 + 2H+: 832.35029, obsd 832.35036.

Dimeric ligand IV E. Yield after RP-HPLC purification: 2.04 mg (1.2 µmol, 8%). LC-MS analysis: tR 9.25 min (gradient 30 to 90% B) and tR 6.76 min (gradient 50 to 90% B). ESI-MS m/z: 1720.1 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.80 (s, 2H), 8.54 (s, 2H), 8.27 (br t, 4H), 7.58 (d, 4H, J = 9.0), 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), 4.08 (d, 4H, J = 4.8), 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), 1.43 – 1.38 (m, 4H), 1.31 (t, 6H, J = 7.2), 1.26 – 1.15 (m, 8H), 1.05 (t, 6H, J = 7.2). HRMS m/z calcld for C92H92F4N16O12 + 2H+: 871.37218.

Dimeric ligand IVF. Yield after RP-HPLC purification: 2.60 mg (1.5 µmol, 10%). LC-MS analysis: tR 9.14 min (gradient 30 to 90% B) and tR 6.39 min (gradient 50 to 90% B). ESI-MS m/z: 1688.9 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.81 (s, 2H), 8.51 (s, 2H), 8.27 (br s, 2H), 7.60 (d, 4H, J = 9.0), 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), 1.04 (t, 6H, J = 7.2). HRMS m/z calcld for C90H92F4N16O12 + 2H+: 844.35040.

Dimeric ligand IVG. Yield after RP-HPLC purification: 2.04 mg (1.2 µmol, 8%). LC-MS analysis: tR 9.09 min (gradient 30 to 90% B) and tR 6.61 min (gradient 50 to 90% B). ESI-MS m/z: 1716.2 [M + H]+. 1H NMR (600 MHz, DMSO-d6) δ 8.82 (s, 2H), 8.50 (s, 2H), 8.26 (s, 2H), 7.59 (d, 4H, J = 7.8), 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). HRMS m/z calcld for C92H94F4N16O12 + 2H+: 858.36594.


References and notes


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