Oligoproline helix as a scaffold for potent, selective and structurally defined dimeric ligands for the LHR

Introduction

The luteinizing hormone receptor (LHR)\(^1\) and follicle-stimulating hormone receptor (FSHR)\(^2\) play an important role in human reproduction. They belong to the family of G-protein coupled receptors (GPCRs) that are characterized by a seven helical transmembrane region. The LHR and FSHR are activated by binding of the endogenous glycoprotein ligand to the large N-terminal leucine rich repeat (LRR).\(^3\)\(^4\) Upon activation by LH in male Leydig cells, LHR induces testosterone production. In females, LH is primarily responsible for ovulation induction and FSH induces follicular growth in the ovaries. In males, FSH is involved in spermatogenesis.

Recent developments in fertility treatment led to the discovery of several low molecular weight (LMW) agonists for the LH receptor of which some also activate the FSH receptor.\(^5\)\(^6\)\(^7\)\(^8\) Chapter 5 described that increased selectivity for the LHR can be realized by dimerization of a known LMW LHR agonist (LHA). The dimeric ligands were based on both flexible polyethylene glycol spacers and a more rigid benzene substituted core. Since the spatial orientation of such spacer systems is difficult to establish, it was not possible to explain the observed selectivity increase by speculation.
on the interpharmacophoric distance or binding mode of the dimeric ligands to the receptors. It was reasoned that scaffolds based on a defined tertiary structure may help in elucidating how dimeric ligands act on GPCRs. Oligoprolines (OP), composed of at least three proline residues, adopt well-defined helical structures. Proline helices are common in biological systems and play a part in many protein structures and protein-protein interactions. In (bio)physical chemistry, oligoproline helices are widely used as molecular rulers to calibrate distances in resonance energy transfer experiments or as backbones to obtain amphiphilic molecules after decoration with specific side-chains. Remarkably, only a few reports applied the proline helices as a scaffold or spacer system for interconnecting two or more biologically active molecules. This chapter describes the use of the proline helix as a well defined, water soluble scaffold in the preparation of dimeric ligands for the LHR.

**Results and discussion**

The first objective was to prepare a set of oligoprolines containing several 4-azidoproline (Azp) residues for ensuing installation of the LHR agonist, by means of a Huisgen [2+3]-cycloaddition reaction. The OPs were designed to vary in length, the position and number of the Azp-residues incorporated. Both (4R)- and (4S)-azidoproline (Azp) were prepared as described. The helices were constructed by solid phase peptide synthesis (SPPS) as depicted in Scheme 1. The OPs were subsequently cleaved from the resin and subjected to a Huisgen [2+3]-cycloaddition with the LHR agonist (LHA) that was prepared as described in chapter 5. A representative example of the synthesis of dimeric ligand \(13S-LHA_2\) is depicted in Scheme 2. The reactions ran to completion in three hours at 60°C with one equivalent of copper sulfate, five equivalents of sodium ascorbate in a water/tert-butanol/acetonitrile mixture (Scheme 2). The end products and substitution patterns are listed in Table 1.

The compounds were assayed for their potency to activate both the LHR and FSHR. As is shown in Table 1, all compounds are potent LHR agonists, whereas their FHSR agonistic potency is at most rather modest. Some general trends are observed within the series. For example, compounds that are substituted with two LHAs are 2-5 times more potent on the LHR than the compounds with only one LHA. The most potent LHR agonist, \(19R-LHA_3\) incorporating three LHAs, is at least 7 times more potent than the compounds with one LHA and about four times more potent on the LHR than those with two LHAs. Compounds \(20LHA_4\), containing four LHAs, are less potent than \(19-LHA_3\). A similar increase in potency is also observed for the FSHR which increases with the number of LHA ligands. For this receptor, the most potent compounds are \(19LHA_3\), as was also observed for the LHR.
Dimeric and oligomeric LHR ligands

Scheme 1. A. Representative example for the preparation of ligands containing LHR agonist (LHA) by the copper catalyzed [2+3]-Huisgen cycloaddition. ○ represents proline; ● represents LHA functionalized proline. B. synthetic scheme of OP scaffolds. Reagents and conditions: i. 20% piperidine/DMF, 0.5h; ii. Fmoc-Pro-OH or Fmoc-Azp-OH, HCTU, DiPEA, NMP, 3 h; iii. Ac2O, DiPEA, DMF, 2 h; iv. 95% TFA/H2O, 1h; v. Sodium ascorbate (5 eq), CuSO4 (1 eq), tBuOH/CH3CN/H2O. C. CD-spectra of compound 13S-LHA2. The intensity in ellipticity observed for 13S-LHA2 (4E-5 M in 10% iPrOH/phosphate buffer pH 7.2) shows a minimum at 205 nm and maximum at 228 nm.

There are some small differences observed in the bioactivity between the R-series and the S-series (compare Table 1 left panel with Table 1 right panel). The mono-substituted compounds bearing the 4R substituted proline are slightly more potent on the LHR and the FSHR when compared to the compounds with 4S substituted prolines. This is especially true when the ligand is attached close to the N- or C-terminus of the helix (that is for compounds 1-LHA and 4-LHA). The potencies for the compounds that contain two agonists are in the same order of magnitude for both the LHR and the FSHR and do not differ dramatically between the R and S-series. Compounds 14R-LHA2 and 14S-LHA2 are the least potent for both the receptors compared to the other dimeric ligands. For these compounds, both the LHA ligands are attached in the middle of the helix. Compounds with more than two ligands (that is, 19-LHA3 and 20-LHA4) have similar potency between the R- and S-series for the LHR. For the FSHR, the compounds with R-substituted ligands are slightly more potent than the compounds in where the ligands are S-substituted.
<table>
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<th>EC_{50} (nM)</th>
<th>Compound</th>
<th>EC_{50} (nM)</th>
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Table 1. Mean agonistic potency (EC_{50}) and selectivity for the LHR and FSHR. All compounds are full agonists on the LHR and partial agonists on the FSHR. The mean EC_{50} are calculated from the -log EC_{50} values from two or three independent experiments performed in duplicate. The SD of pEC_{50} is generally lower than 0.2. • represents proline; ● represents LHA functionalized proline. Selectivity observed for the LHR (EC_{50} FSHR/EC_{50} LHR).

The observed differences in bioactivity within the (R)- and (S)-substituted prolines may be attributed to the relative stability of the proline helix. In literature, two types of polyproline helices are distinguished that differ in the configuration of the proline interconnecting amide bonds. PP type I (PPI) is defined as the structure resulting from cis interconnecting amide bonds. These α-helical structures are characterized by 3.3 proline residues per turn with a rise of 1.9 Å per residue. PP type II helices (PPII) assemble from all trans amide bonds, are more common and feature three proline residues in one turn. The PPII helix is less dense than PPI and the rise of one residue is approximately 3.1 Å. Wennemers et al. demonstrated that functionalization of proline with an (4R)-azide stabilizes the trans-amide isomer by n→π* interaction of the carbonyl.
functions while (4S)-azidoproline (Azp) directs the equilibrium towards the cis-isomer. This effect was also observed when other electron-withdrawing substituents, such as fluorine, were incorporated at the 4-position (Figure 1).

The helical distribution of the compounds can be evidenced by circular dichroism (CD) experiments. PPI type helices are characterized by a minimum at 230 nm and a maximum at 212 nm in their CD spectra, while PPII type helix has maximum at 225 nm and a minimum at 207 nm. All compounds, both the ligand-functionalized derivatives from Table 1 and their azidoproline containing precursors, were evaluated by circular dichroism experiments. In all cases a spectrum indicative for PP type II helix was observed, independent of the spacer length and substitution pattern when measured in a 10% iPrOH/phosphate buffer (minimum at 205 nm, maximum at 228 nm). It is generally observed that compounds with more stable PP type II helices have more intense ellipticities at 205 nm than compounds that equilibrate faster with PP type I configuration. Compounds 5-13 all possess polyproline helices of similar lengths. Within these series, a more intense ellipticity of the (4R)-azidoproline containing helices was observed, compared to the corresponding (4S)-Azp containing helices (Figure 2, left). This is in agreement with the results described by Wennemers et. al. The reverse trend is observed when the helices are substituted with a HLA ligand. Here, the (4R)-LHAs have less intense ellipticities than the (4S)-LHAs (Figure 3, right). This may indicate that the triazole substituted proline may destabilize the PPII conformation.
Previous studies towards the bioactivity of dimeric LHA ligands described in Chapter 5, indicated that, upon dimerization, improved selectivity towards the LHR was observed compared to the monomeric ligands. This was either a result of reduced potency or a reduced efficacy on the FSHR upon dimerization of the LHA ligands. In the here presented series, all ligands are full LHR agonists and partial FSHR agonists ($E_{\text{max}}$ between 40 and 72). Only compound $20S\text{-LHA}_4$, with four LHA ligands, shows an increase in selectivity compared to the other compounds. The reduced FSHR efficacy of dimeric compounds in comparison with the monomeric compounds was not observed when the oligoproline spacer was used.

**Conclusion**

In summary, this chapter describes the use of a polyproline type helix as a scaffold for interconnecting multiple LHAs. For all synthesized compounds a typical PP type II helix was evidenced by circular dichroism indicating that decoration of the helix with large LHR agonists did not affect the helical conformation. LMW LHAs not only activate the LHR but generally also trigger the FSHR. Pharmacological evaluation revealed two interesting features of the oligomerization of LHR agonists with the use of this scaffold. 1) A significant increase in potency on the LHR that is related to the increase in LHA functionalized prolines on the helix. 2) An increase in selectivity for the LHR compared to FSHR for compound $20S\text{-LHA}_4$, that holds four LHA ligands. These features indicate that oligoproline is a suitable scaffold for the development of dimeric or oligomeric ligands as probes to study the dimeric ligand effect to G-protein coupled receptors in more detail.
Experimental procedures

Measurement of CRE-induced luciferase activity

Materials. Recombinant human LH (recLH) and human recombinant FSH (recFSH) were synthesized at Schering-Plough Research Institute, Oss, The Netherlands. Luclite® was obtained from Packard. All cell culture supplies were obtained from Gibco/BRL unless indicated otherwise. The human LH receptor cDNA30 and human FSH receptor cDNA31 were kindly provided by Dr. A.J.W. Hsueh, Stanford University.

Luciferase assay. Chinese Hamster Ovary (CHO)-K1 cells stably expressing the CRE-luciferase reporter with the human LH receptor or human FSH receptor were grown to 80-90% confluency in Dulbecco’s MEM/Nutrient Mix F12 containing 5% bovine calf serum and supplemented with penicillin G (80 units/mL) and streptomycin (0.08 mg/mL) in 5% CO2 at 37 °C. Cells were harvested using cell dissociation solution (Sigma). Aliquots of the cells were cryopreserved in DMSO without a loss of functional activity on LH receptor or FSH receptor.32 On the day of the experiment, cells were thawed, washed with assay medium (Dulbecco’s MEM/Nutrient Mix F12 supplemented with 1 mg/L bovine insulin (Sigma), 5 mg/L apo-transferrin (Sigma), penicillin G (80 units/mL) and streptomycin (0.08 mg/mL)) and then resuspended in assay medium. The compounds were tested at 10 concentrations ranging from final concentrations of 10 μM to 0.316 nM with half log intervals. In the agonistic assays, 10 μL of assay medium containing test compound and 3% DMSO, 10 μL of assay medium containing 3% DMSO with recLH (final concentration of 1 nM) or recFSH (final concentration of 586 pM) or 10 μL of assay medium containing 3% DMSO alone were added to the wells of a 384-well white culture plate followed by the addition of 10 μL of assay medium. Then, 10 μL of cell suspension containing 7,500 cells was added to the wells. The final concentration of DMSO was 1%. After incubation for 4 h in a humidified atmosphere in 5% CO2 at 37 °C, plates were allowed to adjust to room temperature for 1 h. Then, 15 μL of LucLite solution (Packard) was added to the incubation mixture. Following 60 min at room temperature in the dark, luciferase activity was measured in a Packard Topcount Microplate Scintillation and Luminescence Counter. Agonistic effects of the compounds were determined as percentage of the (maximal) effect induced by 1 nM recLH or 586 pM recFSH. The EC50 values (concentration of the test compound that elicits half-maximal (50%) luciferase stimulation compared to the compound’s maximally attainable effect, respectively) and the efficacy values (maximal effect as percentage of the effect of recLH or recFSH) of the test compounds were determined using the software program MathIQ (version 2.0, ID Business Solutions Limited).

Chemical procedures

NMR spectra were recorded on a 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. Where indicated, NMR peak assignments were made using COSY, NOESY (τ mix = 1 sec) and HMBC experiments. For LC-MS analysis, a HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C8 column (4.6 mmD x 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 semi-preparative C8 column (5 μm C8, 10Å, 150 x 21.2 mm) was used. The applied buffers were A: H2O + ammonium acetate (20 mM) 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 dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). CD spectra were recorded using a spectral...
bandwidth of 1 nm, at 25 °C with a response time of 2 s. The spectra are the result of 2-3 accumulations. A peptide solution was measured in a concentration of 4E-5 M in 10% i-PrOH/phosphate buffer (10 mM, pH 7.2) in a quartz cell of 2 mm. CD data is given as mean residual molar ellipticities (θ in deg cm² dmol⁻¹). All samples were equilibrated at least 12 h before measurement.

**General procedure for SPPS of azido-containing polyprolines.**
Rink amide MBHA resin (loading 0.64 mmol/g, 78 mg, 0.05 mmol) was preswollen in DMF for 30 min, drained and the Fmoc protecting group removed with 20% piperidine in NMP. After shaking for 30 min, the resin was drained, washed with NMP (3×), DCM (5×) and NMP (3×). Subsequently, Fmoc-Pro-OH or Fmoc-(R/S)Azp-OH (3 eq) and HCTU (3 eq) are dissolved in NMP followed by DiPEA (9 eq). After standing for 5 min the mixture was added to the amino-functionalized resin (preswollen in NMP). After shaking for 3 h, the resin was drained, washed with NMP (3×), DCM (5×) and NMP (3×). Acetylation was accomplished by adding Ac2O (5 eq) and DiPEA (5 eq) in DMF to the resin and shaken for 2 h. The resin was drained, washed with NMP (3×), DCM (5×) and NMP (3×). All couplings were monitored by the qualitative Chloranil test. The polyproline peptide was cleaved from the resin by stirring in 95% TFA/H₂O (2 mL) for 2 h. The solution was then titrated in 40 mL of Et₂O and centrifuged. The solvent was decanted and the polyproline peptide dissolved in H₂O and purified by preparative HPLC (0 to 20 % B) to yield the compounds as white solids.

**Monomeric azidoproline helix 1R-Azp.** Yield after RP-HPLC purification: 16.0 mg (12.6 μmol, 25%). LC-MS analysis: tᵢ 5.07 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) δ 4.79 – 4.72 (m, 1H, H6), 4.54 – 4.48 (m, 1H, Hγ-Azp), 4.42 (dd, J = 5.5, 8.4, 1H, Hα-Pro-NH2), 3.94 – 3.82 (m, 12H, H6-Azp, H6), 3.74 – 3.59 (m, 12H, H6-Azp, H6), 2.57 (ddd, J = 1.5, 7.6, 11.4, 1H, Hβ-Azp), 2.44 – 2.30 (m, 11H, Hβ), 2.15 (s, 3H, CH3), 2.23 – 2.03 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 2.01 – 1.92 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C₆₂H₆₈N₁₆O₁₃ + H⁺: 1265.67895, obsd 1265.67892.

**Monomeric azidoproline helix 2R-Azp.** Yield after RP-HPLC purification: 37.8 mg (30.0 μmol, 60%). LC-MS analysis: tᵢ 5.05 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) δ 4.78 – 4.70 (m, 11H, H6), 4.59 – 4.52 (m, 1H, Hγ-Azp), 4.41 (dd, J = 5.5, 8.4, 1H, Hα-Pro-NH2), 3.99 (d, J = 12.2, 1H, H6-Azp), 3.93 – 3.80 (m, 11H, H6), 3.74 – 3.52 (m, 12H, H6-Azp, H6), 2.53 (ddd, J = 2.5, 8.3, 12.2, 1H, Hβ-Azp), 2.44 – 2.29 (m, 11H, Hβ), 2.13 (s, 3H, CH3), 2.18 – 2.01 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 2.02 – 1.87 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C₆₂H₆₈N₁₆O₁₃ + H⁺: 1265.67895, obsd 1265.67897.

**Monomeric azidoproline helix 3R-Azp.** Yield after RP-HPLC purification: 43.8 mg (34.6 μmol, 69%). LC-MS analysis: tᵢ 5.00 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) δ 4.88 – 4.69 (m, 11H, H6), 4.59 – 4.51 (m, 1H, Hγ-Azp), 4.41 (dd, J = 5.3, 8.4, 1H, Hα-Pro-NH2), 3.99 (d, J = 11.6, 1H, H6-Azp), 3.94 – 3.81 (m, 11H, H6), 3.74 – 3.65 (m, 12H, H6-Azp, H6), 2.53 (ddd, J = 1.5, 7.2, 10.2, 1H, Hβ-Azp), 2.45 – 2.28 (m, 11H, Hβ), 2.13 (s, 3H, CH3), 2.12 – 2.01 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 2.01 – 1.86 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C₆₂H₆₈N₁₆O₁₃ + H⁺: 1265.67895, obsd 1265.67870.

**Monomeric azidoproline helix 4R-Azp.** Yield after RP-HPLC purification: 8.7 mg (6.9 μmol, 14%). LC-MS analysis: tᵢ 4.94 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) δ 4.91 – 4.68 (m, 11H, H6), 4.60 – 4.52 (m, 1H, Hγ-Azp), 4.41 (dd, J = 5.4, 8.4, 1H, Hα-Pro-NH2), 3.99 (d, J = 11.1, 1H, H6-Azp), 3.95 – 3.80 (m, 11H, H6), 3.73 – 3.52 (m, 12H, H6-Azp), 2.54 (ddd, J = 3.1, 7.7, 10.4, 1H, Hβ-Azp), 2.46 – 2.26 (m, 11H, Hβ), 2.13 (s, 3H, CH3), 2.19 – 2.02 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 2.02 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C₆₂H₆₈N₁₆O₁₃ + H⁺: 1265.67895, obsd 1265.67898.
Dimeric azidoproline helix 5R-Azp. Yield after RP-HPLC purification: 33.2 mg (25.5 μmol, 51%). LC-MS analysis: tR 5.56 min (gradient 10 to 50% B). ESI-MS m/z: 1306.8 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.82 (t, J = 8.1, 1H, Ha-Azp), 4.75 (t, J = 8.0, 1H, Ha-Azp), 4.72 – 4.66 (m, 9H, Ha), 4.45 (dd, J = 3.0, 5.2, 7.5, 1H, Hy-Azp), 4.46 (dd, J = 2.9, 5.1, 7.8, 1H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 4.02 (d, J = 12.3, 1H, H6-Azp), 3.86 – 3.76 (m, 12H, Hδ), 3.71 (d, J = 12.1, 1H, H6'), 3.65 – 3.56 (m, 10H, H6'), 2.51 – 2.44 (m, 2H, Hβ-Azp), 2.36 – 2.26 (m, 9H, Hβ), 2.13 – 2.07 (m, 2H, Hβ'-Azp), 2.09 (s, 3H, CH3), 2.05 – 1.98 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.92 – 1.85 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H87N19O13 + H+: 1306.68035, obsd 1306.68115.

Dimeric azidoproline helix 6R-Azp. Yield after RP-HPLC purification: 31.5 mg (24.1 μmol, 48%). LC-MS analysis: tR 5.48 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 8.0, 1H, Ha-Azp), 4.74 – 4.67 (m, 10H, Ha), 4.49 (dd, J = 2.8, 5.1, 7.6, 1H, Hy-Azp), 4.46 (dd, J = 2.8, 5.2, 7.8, 1H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 3.93 (d, J = 12.3, 1H, H6-Azp), 3.86 – 3.78 (m, 12H, Hδ), 3.72 (d, J = 12.1, 1H, H6'), 3.66 – 3.57 (m, 10H, H6'), 2.54 – 2.47 (m, 2H, Hβ-Azp), 2.36 – 2.25 (m, 9H, Hβ), 2.15 – 2.09 (m, 2H, Hβ'-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.99 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.95 – 1.86 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H87N19O13 + H+: 1306.68035, obsd 1306.68115.

Dimeric azidoproline helix 7R-Azp. Yield after RP-HPLC purification: 37.5 mg (28.7 μmol, 57%). LC-MS analysis: tR 5.70 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 7.6, 1H, Ha-Azp), 4.73 – 4.66 (m, 10H, Ha), 4.49 (dd, J = 3.0, 5.3, 7.6, 1H, Hy-Azp), 4.44 (dd, J = 2.9, 5.3, 7.9, 1H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 3.93 (d, J = 11.4, 1H, H6-Azp), 3.87 – 3.76 (m, 12H, Hδ), 3.71 (d, J = 11.8, 1H, H6'), 3.66 – 3.56 (m, 10H, H6'), 2.53 – 2.45 (m, 2H, Hβ-Azp), 2.36 – 2.24 (m, 9H, Hβ), 2.16 – 2.06 (m, 2H, Hβ'-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.97 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.94 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H87N19O13 + H+: 1306.68035, obsd 1306.68164.

Dimeric azidoproline helix 8R-Azp. Yield after RP-HPLC purification: 18.8 mg (14.4 μmol, 29%). LC-MS analysis: tR 5.61 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 8.0, 1H, Ha-Azp), 4.75 – 4.68 (m, 10H, Ha), 4.50 (dd, J = 3.5, 5.8, 7.7, 1H, Hy-Azp), 4.46 (dd, J = 3.2, 5.3, 7.7, 1H, Hy-Azp), 4.36 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 3.94 (d, J = 11.0, 1H, H6-Azp), 3.88 – 3.78 (m, 12H, Hδ), 3.72 (d, J = 11.6, 1H, H6'), 3.67 – 3.57 (m, 10H, H6'), 2.55 – 2.46 (m, 2H, Hβ-Azp), 2.37 – 2.26 (m, 9H, Hβ), 2.16 – 2.08 (m, 2H, Hβ'-Azp), 2.10 (s, 3H, CH3), 2.06 – 1.96 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.95 – 1.86 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H87N19O13 + H+: 1306.68035, obsd 1306.68152.

Dimeric azidoproline helix 9R-Azp. Yield after RP-HPLC purification: 30.4 mg (23.3 μmol, 47%). LC-MS analysis: tR 5.43 min (gradient 10 to 50% B). ESI-MS m/z: 1306.9 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 8.3, 1H, Ha-Azp), 4.74 – 4.67 (m, 10H, Ha), 4.49 (dd, J = 3.0, 5.3, 7.9, 1H, Hy-Azp), 4.46 (dd, J = 3.2, 5.3, 7.8, 1H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 3.93 (d, J = 12.3, 1H, H6-Azp), 3.87 – 3.77 (m, 12H, Hδ), 3.71 (d, J = 11.4, 1H, H6'), 3.67 – 3.56 (m, 10H, H6'), 2.54 – 2.45 (m, 2H, Hβ-Azp), 2.37 – 2.25 (m, 9H, Hβ), 2.15 – 2.07 (m, 2H, Hβ'-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.96 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.95 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H87N19O13 + H+: 1306.68035, obsd 1306.68127.

Dimeric azidoproline helix 10R-Azp. Yield after RP-HPLC purification: 26.2 mg (20.1 μmol, 40%). LC-MS analysis: tR 5.59 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 8.0, 1H, Ha-Azp), 4.74 – 4.68 (m, 10H, Ha), 4.50 (dd, J = 3.0, 5.3, 7.8, 1H, Hy-Azp), 4.46 (dd, J = 3.1, 5.3, 8.0, 1H, Hy-Azp), 4.36 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH2), 3.94 (d, J = 12.1, 1H, H6-Azp), 3.87 – 3.78 (m, 12H,
Dimeric azidoprine helix 11R-Azp. Yield after RP-HPLC purification: 46.4 mg (35.6 μmol, 71%). LC-MS analysis: t1 5.59 min (gradient 10 to 50% B). ESI-MS m/z: 1306.8 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 3.0, 5.2, 7.7, 1H, Hy-Azp), 4.46 (dd, J = 2.7, 5.3, 7.9, 1H, Hy-Azp), 4.36 (dd, J = 5.5, 8.4, 1H, Hy-Pro-NH2), 3.94 (d, J = 12.1, 1H, H6-Azp), 3.87 – 3.78 (m, 12H, H6), 3.72 (d, J = 12.1, 1H, H6), 3.66 – 3.57 (m, 10H, H6), 2.54 – 2.46 (m, 2H, Hβ-Azp), 2.37 – 2.26 (m, 9H, Hβ), 2.16 – 2.08 (m, 2H, Hβ'-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.99 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.94 – 1.85 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calc for C60H89N20O13 + H+: 1306.68035, obsd 1306.68140.

Dimeric azidoprine helix 12R-Azp. Yield after RP-HPLC purification: 29.0 mg (22.2 μmol, 44%). LC-MS analysis: t1 5.45 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 3.0, 5.2, 7.7, 1H, Hy-Azp), 4.46 (dd, J = 2.7, 5.3, 7.9, 1H, Hy-Azp), 4.34 (dd, J = 5.5, 8.4, 1H, Hy-Pro-NH2), 3.92 (d, J = 12.1, 1H, H6-Azp), 3.87 – 3.75 (m, 12H, H6), 3.70 (d, J = 12.1, 1H, H6'), 3.65 – 3.54 (m, 10H, H6'), 2.53 – 2.44 (m, 2H, Hβ-Azp), 2.36 – 2.24 (m, 9H, Hβ), 2.14 – 2.05 (m, 2H, Hβ'-Azp), 2.08 (s, 3H, CH3), 2.05 – 1.95 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.94 – 1.83 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calc for C60H89N20O13 + H+: 1306.68035, obsd 1306.68091.

Dimeric azidoprine helix 13R-Azp. Yield after RP-HPLC purification: 46.9 mg (35.9 μmol, 72%). LC-MS analysis: t1 5.52 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 3.0, 5.2, 7.7, 1H, Hy-Azp), 4.46 (dd, J = 2.7, 5.3, 7.9, 1H, Hy-Azp), 4.34 (dd, J = 5.5, 8.4, 1H, Hy-Pro-NH2), 3.92 (d, J = 12.1, 1H, H6-Azp), 3.87 – 3.75 (m, 12H, H6), 3.70 (d, J = 12.1, 1H, H6'), 3.65 – 3.54 (m, 10H, H6'), 2.53 – 2.44 (m, 2H, Hβ-Azp), 2.36 – 2.24 (m, 9H, Hβ), 2.14 – 2.05 (m, 2H, Hβ'-Azp), 2.08 (s, 3H, CH3), 2.05 – 1.95 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.91 – 1.81 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calc for C60H89N20O13 + H+: 1306.68035, obsd 1306.68176.

Dimeric azidoprine helix 14R-Azp. Yield after RP-HPLC purification: 33.0 mg (25.3 μmol, 51%). LC-MS analysis: t1 5.64 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.74 – 4.65 (m, 11H, Hy-Azp), 4.51 – 4.47 (m, 2H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Hy-Pro-NH2), 3.93 (d, J = 12.2, 1H, H6-Azp), 3.87 – 3.77 (m, 12H, H6), 3.67 – 3.57 (m, 11H, H6'), 2.48 (dd, J = 2.3, 7.7, 11.0, 2H, Hβ-Azp), 2.38 – 2.25 (m, 9H, Hβ), 2.14 – 2.07 (m, 2H, Hβ'-Azp), 2.08 (s, 3H, CH3), 2.06 – 1.96 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.95 – 1.83 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calc for C60H89N20O13 + H+: 1306.68035, obsd 1306.68115.

Dimeric azidoprine helix 15R-Azp. Yield after RP-HPLC purification: 36.6 mg (28.0 μmol, 56%). LC-MS analysis: t1 5.52 min (gradient 10 to 50% B). ESI-MS m/z: 1306.8 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.72 – 4.66 (m, 11H, Hy), 4.51 – 4.47 (m, 2H, Hy-Azp), 4.34 (dd, J = 5.3, 8.4, 1H, Hy-Pro-NH2), 3.93 (d, J = 10.5, 1H, H6-Azp), 3.88 – 3.77 (m, 12H, H6), 3.65 – 3.57 (m, 11H, H6'), 2.51 – 2.45 (m, 2H, Hβ-Azp), 2.37 – 2.25 (m, 9H, Hβ), 2.13 – 2.06 (m, 2H, Hβ'-Azp), 2.08 (s, 3H, CH3), 2.06 – 1.97 (m, 21H, 1 × Hβ-Pro-NH2, Hy), 1.95 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calc for C60H89N20O13 + H+: 1306.68035, obsd 1306.68176.

Dimeric azidoprine helix 16R-Azp. Yield after RP-HPLC purification: 16.8 mg (10.5 μmol, 21%). LC-MS analysis: t1 5.65 min (gradient 10 to 50% B). ESI-MS m/z: 1598.6 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.90 –
4.69 (m, 14H, Ha), 4.61 – 4.54 (m, 1H, Hy-Azp), 4.54 – 4.48 (m, 1H, Hy-Azp), 4.42 (dd, J = 5.3, 8.3, 1H, Ha-Pro-NH3), 4.02 (d, J = 11.3, 1H, H8-Azp), 3.96 – 3.82 (m, 14H, H5-Azp, H6), 3.76 – 3.58 (m, 15H, H7, H8), 2.61 -2.50 (m, 2H, Hβ-Azp), 2.48 – 2.25 (m, 13H, Hβ), 2.16 (s, 3H, CH3), 2.23 – 2.02 (m, 29H, 1 × Hβ-Azp, 1 × Hβ-Pro-NH3, Hy), 2.02 – 1.83 (m, 12H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C77H108N22O16 + H+: 1597.83864, obsd 1597.83828.

Dimeric azidoproline helix 17R-Azp. Yield after RP-HPLC purification: 61.4 mg (32.5 µmol, 65%). LC-MS analysis: tR 5.77 min (gradient 10 to 50% B). ESI-MS m/z: 1888.8 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.74 – 4.61 (m, 17H, H302), 4.53 – 4.48 (m, 1H, Hy-Azp), 4.48 – 4.41 (m, 1H, Hy-Azp), 4.35 (dd, J = 5.3, 8.3, 1H, Ha-Pro-NH3), 3.90 – 3.76 (m, 17H, H5-Azp, H6), 3.70 – 3.50 (m, 18H, H303'), 2.57 -2.44 (m, 2H, H451-Azp), 2.41 – 2.16 (m, 16H, H451), 2.08 (s, 3H, CH3), 2.15 – 1.96 (m, 35H, 1 × H451-Azp, 1 × H451-Pro-NH2, H452), 1.96 – 1.80 (m, 15H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C92H129N25O19 + H+: 1888.99693, obsd 1888.99568.

Dimeric azidoproline helix 18R-Azp. Yield after RP-HPLC purification: 58.8 mg (27.0 µmol, 54%). LC-MS analysis: tR 5.86 min (gradient 10 to 50% B). ESI-MS m/z: 1091.7 [M + 2H]2+. 1H NMR (400 MHz, D2O) δ 4.74 – 4.61 (m, 20H, H302), 4.53 – 4.47 (m, 1H, Hy-Azp), 4.47 – 4.41 (m, 1H, Hy-Azp), 4.34 (dd, J = 5.6, 8.3, 1H, Ha-Pro-NH3), 3.93 (d, J = 11.9, 1H, H5-Azp), 3.89 – 3.74 (m, 20H, H303-Azp, H303), 3.69 – 3.46 (m, 21H, H303'), 2.55 -2.41 (m, 2H, H451-Azp), 2.41 – 2.19 (m, 19H, H451), 2.08 (s, 3H, CH3), 2.17 – 1.95 (m, 41H, 1 × H451-Azp, 1 × H451-Pro-NH2, H452), 1.94 – 1.78 (m, 18H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C107H150N28O22 + 2H+: 1090.58125, obsd 1090.58260.

Trimeric azidoproline helix 19R-Azp. Yield after RP-HPLC purification: 34.0 mg (25.2 µmol, 50%). LC-MS analysis: tR 6.24 min (gradient 10 to 50% B). ESI-MS m/z: 1347.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 (t, J = 8.1, 1H, H302-Azp), 4.73 – 4.67 (m, 10H, H302), 4.51 – 4.47 (m, 1H, Hy-Azp), 4.45 (ddd, J = 2.8, 5.3, 7.8, 1H, Hy-Azp), 4.35 (dd, J = 5.5, 8.6, 1H, Ha-Pro-NH3), 3.93 (d, J = 12.2, 2H, H5-Azp), 3.87 – 3.78 (m, 12H, H6), 3.72 (d, J = 11.9, 1H, H5-Azp), 3.66 – 3.57 (m, 9H, H5'), 2.54 – 2.46 (m, 3H, H451-Azp), 2.37 – 2.25 (m, 8H, H451), 2.16 – 2.07 (m, 3H, Hβ-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.98 (m, 19H, 1 × Hβ-Pro-NH2, H452), 1.95 – 1.84 (m, 9H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C62H86N22O13 + H+: 1347.68175, obsd 1347.68274.

Tetrameric azidoproline helix 20R-Azp. Yield after RP-HPLC purification: 14.3 mg (10.3 µmol, 21%). LC-MS analysis: tR 6.72 min (gradient 10 to 50% B). ESI-MS m/z: 1388.8 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.88 – 4.70 (m, 11H, Ha), 4.57 – 4.51 (m, 3H, Hy-Azp), 4.51 – 4.46 (m, 1H, Hy-Azp), 4.40 (dd, J = 5.5, 8.4, 1H, Ha-Pro-NH3), 3.98 (d, J = 11.4, 3H, H5-Azp), 3.94 – 3.79 (m, 9H, H5-Azp, H6), 3.72 (d, J = 11.9, 1H, H5-Azp), 3.66 – 3.57 (m, 9H, H5'), 2.54 – 2.46 (m, 3H, H451-Azp), 2.37 – 2.25 (m, 8H, H451), 2.16 – 2.07 (m, 3H, Hβ-Azp), 2.09 (s, 3H, CH3), 2.06 – 1.98 (m, 19H, 1 × Hβ-Pro-NH3, H452), 2.01 – 1.84 (m, 7H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C62H85N25O13 + H+: 1388.68314, obsd 1388.68314.

Monomeric azidoproline helix 1S-Azp. Yield after RP-HPLC purification: 50.0 mg (39.5 µmol, 79%). LC-MS analysis: tR 6.47 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.82 – 4.67 (m, 11H, Ha), 4.46 (dt, J = 5.2, 10.6, 1H, Hy-Azp), 4.38 (dd, J = 5.6, 8.7, 1H, Ha-Pro-NH3), 3.97 (dd, J = 6.3, 11.2, 1H, H5-Azp), 3.89 – 3.77 (m, 11H, H6), 3.70 – 3.53 (m, 12H, H5, H5-Azp), 2.73 (ddd, J = 6.2, 9.2, 13.2, 1H, H451-Azp), 2.41 – 2.24 (m, 11H, Hβ), 2.10 (s, 3H, CH3), 2.09 – 1.98 (m, 24H, 1 × Hβ-Azp, 1 × Hβ-Pro-NH3, H452), 1.98 – 1.84 (m, 10H, 1 × Hβ-Pro-NH3, Hβ). HRMS m/z calcd for C62H86N16O13 + H+: 1265.67881.
Monomeric azidoproline helix 2S-Azp. Yield after RP-HPLC purification: 22.9 mg (18.0 μmol, 36%). LC-MS analysis: tR 4.83 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.82 – 4.68 (m, 11H, Hα), 4.46 (dt, J = 5.9, 12.0, 1H, Hγ-Azp), 4.40 (dd, J = 5.4, 8.5, 1H, Hα-Pro-NH2), 4.19 (dd, J = 6.6, 10.9, 1H, Hβ-Azp), 3.92 – 3.79 (m, 11H, Hβ'), 3.73 – 3.60 (m, 11H, Hβ'), 3.56 (dd, J = 5.7, 10.7, 1H, Hβ'-Azp), 2.76 (ddd, J = 6.8, 8.6, 14.0, 1H, Hβ'-Azp), 2.44 – 2.27 (m, 11H, Hβ), 2.12 (s, 3H, CH3), 2.11 – 2.00 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 1.99 – 1.87 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C62H68N16O13 + H+: 1265.67895, obsd 1265.67888.

Monomeric azidoproline helix 3S-Azp. Yield after RP-HPLC purification: 23.6 mg (18.6 μmol, 37%). LC-MS analysis: tR 4.79 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.76 – 4.66 (m, 11H, Hα), 4.46 (dt, J = 6.0, 11.9, 1H, Hγ-Azp), 4.39 (dd, J = 5.4, 8.5, 1H, Hα-Pro-NH2), 4.19 (dd, J = 6.4, 11.0, 1H, Hβ-Azp), 3.90 – 3.79 (m, 11H, Hβ), 3.72 – 3.59 (m, 11H, Hβ'), 3.57 (dd, J = 5.7, 10.9, 1H, Hβ'-Azp), 2.75 (ddd, J = 6.3, 8.7, 14.0, 1H, Hβ'-Azp), 2.42 – 2.27 (m, 11H, Hβ), 2.12 (s, 3H, CH3), 2.10 – 1.99 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 1.99 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C6aH68NaO13 + H+: 1265.67895, obsd 1265.67897.

Monomeric azidoproline helix 4S-Azp. Yield after RP-HPLC purification: 22.3 mg (17.6 μmol, 35%). LC-MS analysis: tR 4.80 min (gradient 10 to 50% B). ESI-MS m/z: 1265.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.93 – 4.87 (m, 11H, Hα), 4.46 (dt, J = 6.5, 12.6, 1H, Hγ-Azp), 4.30 (dd, J = 5.3, 8.5, 1H, Hα-Pro-NH2), 4.22 (dd, J = 6.5, 10.9, 1H, Hβ-Azp), 3.93 – 3.81 (m, 11H, Hβ), 3.74 – 3.60 (m, 11H, Hβ'), 3.56 (dd, J = 5.5, 10.6, 1H, Hβ'-Azp), 2.78 (ddd, J = 6.6, 8.9, 13.5, 1H, Hβ'-Azp), 2.46 – 2.26 (m, 11H, Hβ), 2.13 (s, 3H, CH3), 2.11 – 1.01 (m, 24H, 1 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 2.01 – 1.84 (m, 10H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C6aH68NaO13 + H+: 1265.67895, obsd 1265.67961.

Dimeric azidoproline helix 5S-Azp. Yield after RP-HPLC purification: 31.8 mg (24.1 μmol, 48%). LC-MS analysis: tR 5.23 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.76 – 4.68 (m, 11H, Hα), 4.45 – 4.37 (m, 2H, Hγ-Azp), 4.36 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH2), 4.34 (dd, J = 6.6, 10.9, 1H, Hβ-Azp), 3.95 (ddd, J = 6.1, 11.2, 1H, Hβ-Azp), 3.87 – 3.77 (m, 10H, Hβ), 3.68 – 3.53 (m, 11H, Hβ'), 3.49 (ddd, J = 6.1, 10.9, 1H, Hβ'-Azp), 2.77 – 2.67 (m, 2H, Hβ'-Azp), 2.37 – 2.27 (m, 10H, Hβ), 2.09 (s, 3H, CH3), 2.08 – 1.98 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 1.97 – 1.85 (m, 9H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C6aH68N6O13 + H+: 1306.68035, obsd 1306.68140.

Dimeric azidoproline helix 6S-Azp. Yield after RP-HPLC purification: 37.5 mg (28.7 μmol, 57%). LC-MS analysis: tR 5.06 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.76 (dd, J = 3.5, 9.0, 2H, Hα-Azp), 4.74 – 4.67 (m, 9H, Hα), 4.46 – 4.38 (m, 2H, Hγ-Azp), 4.36 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH2), 4.18 (dd, J = 6.6, 11.0, 1H, Hβ-Azp), 3.94 (ddd, J = 6.2, 11.3, 1H, Hβ-Azp), 3.87 – 3.74 (m, 10H, Hβ), 3.68 – 3.50 (m, 12H, Hβ', Hβ'-Azp), 2.76 – 2.67 (m, 2H, Hβ'-Azp), 2.38 – 2.26 (m, 10H, Hβ), 2.09 (s, 3H, CH3), 2.07 – 1.97 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hγ), 1.97 – 1.84 (m, 9H, 1 × Hβ'-Pro-NH2, Hβ). HRMS m/z calcd for C6aH68N6O13 + H+: 1306.68035, obsd 1306.68188.

Dimeric azidoproline helix 7S-Azp. Yield after RP-HPLC purification: 19.4 mg (14.8 μmol, 30%). LC-MS analysis: tR 5.14 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]+. 1H NMR (400 MHz, D2O) δ 4.77 – 4.66 (m, 11H, Hα), 4.74 – 4.67 (m, 9H, Hα), 4.46 – 4.38 (m, 2H, Hγ-Azp), 4.39 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH2), 4.19 (dd, J = 6.4, 11.0, 1H, Hβ-Azp), 3.98 (ddd, J = 6.3, 11.3, 1H, Hβ-Azp), 3.90 – 3.77 (m, 10H, Hβ), 3.71 – 3.53 (m, 12H, Hβ', Hβ'-Azp), 2.79 – 2.70 (m, 2H, Hβ-Azp), 2.41 – 2.28 (m, 10H, Hβ), 2.10 (s, 3H, CH3), 2.10 –
Dimeric and oligomeric LHR ligands

2.00 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 2.00 – 1.86 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68140.

**Dimeric azidoproline helix 8S-Azp₂.** Yield after RP-HPLC purification: 4.9 mg (3.8 μmol, 8%). LC-MS analysis: tᵣ 5.21 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.77 – 4.66 (m, 11H, Hα), 4.44 (tt, J = 6.1, 12.0, 2H, Hγ-Azp), 4.39 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH₂), 4.19 (dd, J = 6.4, 11.0, 1H, Hβ-Azp), 3.98 (dd, J = 6.3, 11.3, 1H, Hβ-Azp), 3.90 – 3.77 (m, 10H, Hδ), 3.71 – 3.53 (m, 12H, Hδ', Hδ'-Azp), 2.80 – 2.70 (m, 2H, Hβ-Azp), 2.41 – 2.28 (m, 10H, Hβ), 2.12 (s, 3H, CH₃), 2.10 – 2.00 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 2.00 – 1.86 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68164.

**Dimeric azidoproline helix 9S-Azp₂.** Yield after RP-HPLC purification: 20.2 mg (15.5 μmol, 31%). LC-MS analysis: tᵣ 5.12 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.79 – 4.66 (m, 11H, Hα), 4.43 (ddd, J = 6.1, 12.0, 18.1, 2H, Hγ-Azp), 4.38 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH₂), 4.17 (dd, J = 6.5, 11.0, 1H, Hβ-Azp), 3.97 (dd, J = 6.3, 11.2, 1H, Hβ-Azp), 3.89 – 3.76 (m, 10H, Hδ), 3.69 – 3.48 (m, 12H, Hδ', Hδ'-Azp), 2.78 – 2.69 (m, 2H, Hβ-Azp), 2.41 – 2.27 (m, 10H, Hβ), 2.10 (s, 3H, CH₃), 2.09 – 1.99 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 1.98 – 1.83 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68127.

**Dimeric azidoproline helix 10S-Azp₂.** Yield after RP-HPLC purification: 26.2 mg (20.1 μmol, 40%). LC-MS analysis: tᵣ 5.12 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.79 – 4.64 (m, 11H, Hα), 4.42 (ddd, J = 6.3, 12.1, 18.1, 2H, Hγ-Azp), 4.37 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH₂), 4.16 (dd, J = 6.6, 11.3, 1H, Hβ-Azp), 3.96 (dd, J = 6.3, 11.2, 1H, Hβ-Azp), 3.87 – 3.75 (m, 10H, Hδ), 3.68 – 3.50 (m, 12H, Hδ', Hδ'-Azp), 2.77 – 2.68 (m, 2H, Hβ-Azp), 2.42 – 2.26 (m, 10H, Hβ), 2.10 (s, 3H, CH₃), 2.08 – 1.99 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 1.98 – 1.83 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68188.

**Dimeric azidoproline helix 11S-Azp₂.** Yield after RP-HPLC purification: 26.4 mg (20.2 μmol, 40%). LC-MS analysis: tᵣ 5.18 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.79 – 4.65 (m, 11H, Hα), 4.43 (tt, J = 5.4, 8.6, 2H, Hγ-Azp), 4.37 (dd, J = 5.4, 8.6, 1H, Hα-Pro-NH₂), 4.17 (dd, J = 6.7, 11.4, 1H, Hβ-Azp), 3.96 (dd, J = 6.4, 11.2, 1H, Hβ-Azp), 3.89 – 3.75 (m, 10H, Hδ), 3.70 – 3.44 (m, 12H, Hδ', Hδ'-Azp), 2.78 – 2.68 (m, 2H, Hβ-Azp), 2.45 – 2.24 (m, 10H, Hβ), 2.10 (s, 3H, CH₃), 2.09 – 1.98 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 1.98 – 1.84 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68140.

**Dimeric azidoproline helix 12S-Azp₂.** Yield after RP-HPLC purification: 17.8 mg (13.6 μmol, 27%). LC-MS analysis: tᵣ 5.14 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.80 – 4.67 (m, 11H, Hα), 4.44 (ddd, J = 6.1, 12.2, 18.0, 2H, Hγ-Azp), 4.38 (dd, J = 5.4, 8.5, 1H, Hα-Pro-NH₂), 4.18 (dd, J = 6.6, 11.1, 1H, Hβ-Azp), 3.98 (dd, J = 6.3, 11.3, 1H, Hβ-Azp), 3.90 – 3.76 (m, 10H, Hδ), 3.70 – 3.52 (m, 12H, Hδ', Hδ'-Azp), 2.74 (tdd, J = 4.0, 6.3, 9.3, 2H, Hβ-Azp), 2.39 – 2.27 (m, 10H, Hβ), 2.11 (s, 3H, CH₃), 2.10 – 2.00 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₂, Hγ), 1.99 – 1.86 (m, 9H, 1 × Hβ'-Pro-NH₂, Hβ). HRMS m/z caleed for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68140.

**Dimeric azidoproline helix 13S-Azp₂.** Yield after RP-HPLC purification: 42.3 mg (32.4 μmol, 65%). LC-MS analysis: tᵣ 5.05 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. ¹H NMR (400 MHz, D₂O) δ 4.75 –
4.67 (m, 11H, Ho), 4.44 (dt, J = 6.3, 12.8, 2H, Hy-Azp), 4.39 (dd, J = 5.2, 8.6, 1H, Ha-Pro-NH₂), 4.19 (dd, J = 6.6, 11.0, 1H, Hβ-Azp), 3.97 (dd, J = 6.3, 11.3, 1H, Hδ-Azp), 3.80 – 3.75 (m, 10H, Hδ), 3.71 – 3.46 (m, 12H, Hα', Hδ'-Azp), 2.74 (ddt, J = 6.6, 8.9, 13.6, 2H, Hβ-Azp), 2.42 – 2.25 (m, 10H, Hβ), 2.11 (s, 3H, CH₃), 2.09 – 1.99 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₃, Hy), 1.99 – 1.85 (m, 9H, 1 × Hβ'-Pro-NH₃, Hβ). HRMS m/z calcd for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68127.

Dimeric azidoproline helix 14S-Azp₂. Yield after RP-HPLC purification: 21.2 mg (16.2 µmol, 32%). LC-MS analysis: tR 5.21 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) 4.80 – 4.65 (m, 11H, Ho), 4.44 (ddd, J = 6.0, 11.8, 18.2, 2H, Hy-Azp), 4.38 (dd, J = 5.4, 8.5, 1H, Ha-Pro-NH₂), 4.17 (dt, J = 6.7, 11.4, 2H, Hδ-Azp), 3.89 – 3.76 (m, 10H, Hδ), 3.70 – 3.58 (m, 10H, Hδ'), 3.54 (ddd, J = 3.1, 5.0, 10.5, 2H, Hδ'-Azp), 2.78 – 2.69 (m, 2H, Hβ-Azp), 2.41 – 2.27 (m, 10H, Hβ), 2.10 (s, 3H, CH₃), 2.09 – 1.98 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₃, Hy), 1.98 – 1.85 (m, 9H, 1 × Hβ'-Pro-NH₃, Hβ). HRMS m/z calcd for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68127.

Dimeric azidoproline helix 15S-Azp₂. Yield after RP-HPLC purification: 40.6 mg (31.1 µmol, 62%). LC-MS analysis: tR 5.14 min (gradient 10 to 50% B). ESI-MS m/z: 1306.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) 4.78 – 4.62 (m, 11H, Ho), 4.43 (ddd, J = 5.8, 11.4, 17.1, 2H, Hy-Azp), 4.37 (dd, J = 5.3, 8.7, 1H, Ha-Pro-NH₂), 4.16 (ddd, J = 6.5, 11.1, 14.1, 2H, Hδ-Azp), 3.86 – 3.75 (m, 10H, Hδ), 3.68 – 3.48 (m, 12H, Hδ', Hδ'-Azp), 2.72 (ddd, J = 6.2, 9.0, 13.4, 2H, Hβ-Azp), 2.36 – 2.25 (m, 10H, Hβ), 2.08 (s, 3H, CH₃), 2.07 – 1.97 (m, 23H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₃, Hy), 1.97 – 1.84 (m, 9H, 1 × Hβ'-Pro-NH₃, Hβ). HRMS m/z calcd for C₆₂H₈₇N₁₉O₁₃ + H⁺: 1306.68035, obsd 1306.68176.

Dimeric azidoproline helix 16S-Azp₂. Yield after RP-HPLC purification: 35.3 mg (22.1 µmol, 44%). LC-MS analysis: tR 5.32 min (gradient 10 to 50% B). ESI-MS m/z: 1597.6 [M + H]⁺. 1H NMR (400 MHz, D₂O) 4.82 – 4.67 (m, 14H, Ho), 4.40 (dt, J = 6.6, 12.9, 2H, Hy-Azp), 4.35 (dd, J = 5.3, 8.9, 1H, Ha-Pro-NH₂), 4.15 (dd, J = 6.4, 10.5, 1H, Hδ-Azp), 3.92 (dd, J = 6.3, 11.3, 1H, Hδ-Azp), 3.89 – 3.78 (m, 13H, Hδ), 3.69 – 3.57 (m, 14H, Hδ'), 3.54 (dd, J = 5.7, 10.5, 1H, Hδ'-Azp), 2.74 (ddd, J = 6.7, 8.8, 13.6, 2H, Hβ-Azp), 2.40 – 2.25 (m, 12H, Hβ), 2.11 (s, 3H, CH₃), 2.10 – 1.99 (m, 29H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₃, Hy), 1.98 – 1.85 (m, 13H, 1 × Hβ'-Pro-NH₃, Hβ). HRMS m/z calcd for C₇₇H₁₀₈N₂₂O₁₆ + H⁺: 1597.83864, obsd 1597.83844.

Dimeric azidoproline helix 17S-Azp₂. Yield after RP-HPLC purification: 57.9 mg (30.7 µmol, 61%). LC-MS analysis: tR 5.44 min (gradient 10 to 50% B). ESI-MS m/z: 1888.7 [M + H]⁺. 1H NMR (400 MHz, D₂O) 4.83 – 4.62 (m, 17H, Ho), 4.40 (dt, J = 6.6, 12.8, 2H, Hy-Azp), 4.35 (dd, J = 5.3, 8.8, 1H, Ha-Pro-NH₂), 4.15 (dd, J = 6.4, 10.5, 1H, Hδ-Azp), 3.92 (dd, J = 6.2, 11.2, 1H, Hδ-Azp), 3.87 – 3.74 (m, 13H, Hδ), 3.69 – 3.57 (m, 14H, Hδ'), 3.54 (dd, J = 5.7, 10.5, 1H, Hδ'-Azp), 2.74 (ddd, J = 6.7, 8.8, 13.6, 2H, Hβ-Azp), 2.40 – 2.25 (m, 12H, Hβ), 2.11 (s, 3H, CH₃), 2.06 – 1.94 (m, 35H, 2 × Hβ'-Azp, 1 × Hβ-Pro-NH₃, Hy), 1.94 – 1.81 (m, 15H, 1 × Hβ'-Pro-NH₃, Hβ). HRMS m/z calcd for C₉₂H₁₂₉N₂₅O₁₉ + H⁺: 1888.99693, obsd 1888.99924.
Trimeric azidoproline helix 19S-Azp. Yield after RP-HPLC purification: 17.5 mg (13.0 μmol, 26%). LC-MS analysis: \( t_R \) 5.57 min (gradient 10 to 50% B). ESI-MS \( m/z \) 1347.7 [M + H]^+. H NMR (400 MHz, D_2O) \( δ \): 4.78 – 4.66 (m, 11H, H2), 4.47 – 4.41 (m, 3H, H2-Azp), 4.38 (dd, \( J \) = 5.4, 8.5, 1H, H2-Pro-NH2), 4.17 (ddd, \( J \) = 3.1, 5.6, 11.3, 2H, Hβ-Azp), 3.97 (dd, \( J \) = 6.2, 11.3, 1H, Hβ-Azp), 3.90 – 3.76 (m, 9H, Hβ), 3.70 – 3.51 (m, 12H, Hβ, Hβ'-Azp), 2.78 – 2.69 (m, 3H, Hβ-Azp), 2.39 – 2.27 (m, 9H, Hβ), 2.11 (s, 3H, CH3), 2.10 – 1.99 (m, 21H, 3 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hβ), 1.99 – 1.86 (m, 8H, 1 × Hβ'-Pro-NH2, Hβ). HRMS \( m/z \) calcd for C_{62}H_{86}N_{22}O_{13} + H^+: 1347.68175, obsd 1347.68274.

Tetrameric azidoproline helix 20S-Azp. Yield after RP-HPLC purification: 13.7 mg (9.9 μmol, 20%). LC-MS analysis: \( t_R \) 5.98 min (gradient 10 to 50% B). ESI-MS \( m/z \) 1388.8 [M + H]^+. H NMR (400 MHz, D_2O) \( δ \): 4.84 – 4.63 (m, 11H, H2), 4.50 – 4.36 (m, 5H, Hβ-Azp, H2-Pro-NH2), 4.25 – 4.13 (m, 3H, Hβ-Azp), 3.97 (dd, \( J \) = 6.3, 11.2, 1H, Hβ-Azp), 3.92 – 3.76 (m, 8H, Hβ), 3.72 – 3.46 (m, 12H, Hβ), 2.81 – 2.67 (m, 4H, Hβ-Azp), 2.43 – 2.25 (m, 8H, Hβ), 2.12 (s, 3H, CH3), 2.10 – 2.00 (m, 21H, 4 × Hβ'-Azp, 1 × Hβ-Pro-NH2, Hβ), 2.00 – 1.87 (m, 7H, 1 × Hβ'-Pro-NH2, Hβ). HRMS \( m/z \) calcd for C_{62}H_{85}N_{25}O_{13} + H^+: 1388.68314, obsd 1388.68298.

General procedure for the functionalization of azidoproline peptides with LHA.
To a solution of the desired azidoproline peptide (5.0 μmol) and LHA (1.2 eq. per azide, 6 μmol, 2.9 mg) in a mixture of degassed tBuOH/MeCN/H_2O (2/2/1; v/v/v, 500 μL) were added sodium ascorbate (2.5 eq. per azide, 50 μL of a 0.25M solution in H_2O) and CuSO_4 (0.5 eq. per azide, 25 μL of a 0.1M solution in H_2O). The reaction mixture was stirred and heated at 60 °C for 3 h. The mixture was evaporated, redissolved H_2O/CH_3CN (1/1; v/v, 1 mL) and filtrated. The crude products were analyzed by LC-MS and purified by semi-preparative RP-HPLC (linear gradient of 5.0 CV; 40 to 80% B). Evaporation and lyophilization of the combined fractions from Dioxane/H_2O (1/1; v/v) furnished the ligands as yellow amorphous powders.

Monomeric ligand 1R-LHA. Yield after RP-HPLC purification: 2.0 mg (1.1 μmol, 21%). LC-MS analysis: \( t_R \) 6.58 min (gradient 10 to 90% B). ESI-MS \( m/z \) 1747.6 [M + H]^+. HRMS \( m/z \) calcd for C_{85}H_{114}N_{22}O_{15}S_2 + H^+: 1747.83482, obsd 1747.83601.

Monomeric ligand 2R-LHA. Yield after RP-HPLC purification: 1.6 mg (0.9 μmol, 17%). LC-MS analysis: \( t_R \) 6.54 min (gradient 10 to 90% B). ESI-MS \( m/z \) 1747.6 [M + H]^+. HRMS \( m/z \) calcd for C_{85}H_{114}N_{22}O_{15}S_2 + H^+: 1747.83482, obsd 1747.83581.

Monomeric ligand 3R-LHA. Yield after RP-HPLC purification: 1.1 mg (0.6 μmol, 12%). LC-MS analysis: \( t_R \) 6.40 min (gradient 10 to 90% B). ESI-MS \( m/z \) 1747.6 [M + H]^+. HRMS \( m/z \) calcd for C_{85}H_{114}N_{22}O_{15}S_2 + H^+: 1747.83482, obsd 1747.83570.

Monomeric ligand 4R-LHA. Yield after RP-HPLC purification: 4.1 mg (2.2 μmol, 44%). LC-MS analysis: \( t_R \) 6.50 min (gradient 10 to 90% B). ESI-MS \( m/z \) 1747.7 [M + H]^+. HRMS \( m/z \) calcd for C_{85}H_{114}N_{22}O_{15}S_2 + H^+: 1747.83482, obsd 1747.83561.

Dimeric ligand 5R-LHA. Yield after RP-HPLC purification: 2.2 mg (1.0 μmol, 19%). LC-MS analysis: \( t_R \) 8.24 min (gradient 10 to 90% B). ESI-MS \( m/z \) 1136.4 [M + 2H]^2+. HRMS \( m/z \) calcd for C_{108}H_{139}N_{31}O_{17}S_4 + 2H^+: 1135.99968, obsd 1136.00061.
Dimeric ligand 6R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.7 mg (1.2 μmol, 24%). LC-MS analysis: t<sub>R</sub> 8.20 min (gradient 10 to 90% B). ESI-MS m/z: 1136.5 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00098.

Dimeric ligand 7R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.1 mg (0.9 μmol, 19%). LC-MS analysis: t<sub>R</sub> 8.17 min (gradient 10 to 90% B). ESI-MS m/z: 1136.5 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00012.

Dimeric ligand 8R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.4 mg (1.1 μmol, 21%). LC-MS analysis: t<sub>R</sub> 8.06 min (gradient 10 to 90% B). ESI-MS m/z: 1136.1 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00024.

Dimeric ligand 9R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.3 mg (1.0 μmol, 20%). LC-MS analysis: t<sub>R</sub> 7.98 min (gradient 10 to 90% B). ESI-MS m/z: 1136.1 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1135.99927.

Dimeric ligand 10R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.5 mg (1.1 μmol, 22%). LC-MS analysis: t<sub>R</sub> 7.94 min (gradient 10 to 90% B). ESI-MS m/z: 1136.4 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00110.

Dimeric ligand 11R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.3 mg (1.0 μmol, 20%). LC-MS analysis: t<sub>R</sub> 7.98 min (gradient 10 to 90% B). ESI-MS m/z: 1136.4 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00037.

Dimeric ligand 12R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 1.8 mg (0.8 μmol, 16%). LC-MS analysis: t<sub>R</sub> 7.89 min (gradient 10 to 90% B). ESI-MS m/z: 1135.9 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00171.

Dimeric ligand 13R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.8 mg (1.2 μmol, 25%). LC-MS analysis: t<sub>R</sub> 7.94 min (gradient 10 to 90% B). ESI-MS m/z: 1135.7 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00159.

Dimeric ligand 14R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 2.2 mg (1.0 μmol, 19%). LC-MS analysis: t<sub>R</sub> 8.11 min (gradient 10 to 90% B). ESI-MS m/z: 1136.5 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00110.

Dimeric ligand 15R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 3.0 mg (1.3 μmol, 26%). LC-MS analysis: t<sub>R</sub> 8.03 min (gradient 10 to 90% B). ESI-MS m/z: 1136.7 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>108</sub>H<sub>139</sub>N<sub>31</sub>O<sub>17</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1135.99968, obsd 1136.00110.

Dimeric ligand 16R-LHA<sub>2</sub>. Yield after RP-HPLC purification: 1.9 mg (0.7 μmol, 14%). LC-MS analysis: t<sub>R</sub> 7.76 min (gradient 10 to 90% B). ESI-MS m/z: 1281.5 [M + 2H]<sup>2+</sup>. HRMS m/z calcd for C<sub>128</sub>H<sub>160</sub>N<sub>34</sub>O<sub>20</sub>S<sub>4</sub> + 2H<sup>+</sup>: 1281.57882, obsd 1281.57994.
Dimeric ligand 17R-LHA2. Yield after RP-HPLC purification: 3.6 mg (1.2 μmol, 24%). LC-MS analysis: *t* < 7.56 min (gradient 10 to 90% B). ESI-MS *m/z*: 1427.1 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₉₈H₁₈₅N₃₇O₂₃S₄ + 2H⁺: 1427.15797, obsd 1427.15894.

Dimeric ligand 18R-LHA2. Yield after RP-HPLC purification: 2.8 mg (0.8 μmol, 17%). LC-MS analysis: *t* < 7.43 min (gradient 10 to 90% B). ESI-MS *m/z*: 1572.7 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₅₃H₂₀₂N₄₀O₂₆S₄ + 2H⁺: 1572.73712, obsd 1572.73759.

Trimeric ligand 19R-LHA3. Yield after RP-HPLC purification: 2.7 mg (1.0 μmol, 19%). LC-MS analysis: *t* < 9.51 min (gradient 10 to 90% B). ESI-MS *m/z*: 1397.9 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₃₁H₁₆₄N₄₀O₁₉S₆ + 2H⁺: 1397.57831, obsd 1397.57825.

Tetrameric ligand 20R-LHA4. Yield after RP-HPLC purification: 1.4 mg (0.4 μmol, 7%). LC-MS analysis: *t* < 10.91 min (gradient 10 to 90% B). ESI-MS *m/z*: 1659.6 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₅₄H₁₈₉N₄₉O₂₁S₈ + 2H⁺: 1659.15694, obsd 1659.15793.

Monomeric ligand 1S-LHA. Yield after RP-HPLC purification: 2.2 mg (1.1 μmol, 24%). LC-MS analysis: *t* < 6.48 min (gradient 10 to 90% B). ESI-MS *m/z*: 1747.7 [M + H]²⁺. HRMS *m/z* calcd for C₈₅H₁₁₄N₂₂O₁₅S₂ + H⁺: 1747.83482, obsd 1747.83534.

Monomeric ligand 2S-LHA. Yield after RP-HPLC purification: 1.9 mg (1.0 μmol, 20%). LC-MS analysis: *t* < 6.39 min (gradient 10 to 90% B). ESI-MS *m/z*: 1747.7 [M + H]²⁺. HRMS *m/z* calcd for C₈₅H₁₁₄N₂₂O₁₅S₂ + H⁺: 1747.83482, obsd 1747.83455.

Monomeric ligand 3S-LHA. Yield after RP-HPLC purification: 1.9 mg (1.0 μmol, 20%). LC-MS analysis: *t* < 6.32 min (gradient 10 to 90% B). ESI-MS *m/z*: 1747.5 [M + H]²⁺. HRMS *m/z* calcd for C₈₅H₁₁₄N₂₂O₁₅S₂ + H⁺: 1747.83482, obsd 1747.83542.

Monomeric ligand 4S-LHA. Yield after RP-HPLC purification: 1.5 mg (0.8 μmol, 16%). LC-MS analysis: *t* < 6.39 min (gradient 10 to 90% B). ESI-MS *m/z*: 1747.6 [M + H]²⁺. HRMS *m/z* calcd for C₈₅H₁₁₄N₂₂O₁₅S₂ + H⁺: 1747.83482, obsd 1747.83532.

Dimeric ligand 5S-LHA2. Yield after RP-HPLC purification: 2.9 mg (1.3 μmol, 26%). LC-MS analysis: *t* < 8.01 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.3 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₀₈H₁₃₉N₃₁O₁₇S₄ + 2H⁺: 1135.99968, obsd 1135.99915.

Dimeric ligand 6S-LHA2. Yield after RP-HPLC purification: 4.7 mg (2.1 μmol, 41%). LC-MS analysis: *t* < 7.97 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.5 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₀₈H₁₃₉N₃₁O₁₇S₄ + 2H⁺: 1135.99968, obsd 1136.00085.

Dimeric ligand 7S-LHA2. Yield after RP-HPLC purification: 3.5 mg (1.5 μmol, 31%). LC-MS analysis: *t* < 8.08 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.5 [M + 2H]²⁺. HRMS *m/z* calcd for C₁₀₈H₁₃₉N₃₁O₁₇S₄ + 2H⁺: 1135.99968, obsd 1136.00012.
Chapter 7

**Dimeric ligand 8S-LHA**

Yield after RP-HPLC purification: 1.0 mg (0.4 μmol, 9%). LC-MS analysis: *t* 7.93 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.0 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00085.

**Dimeric ligand 9S-LHA**

Yield after RP-HPLC purification: 4.4 mg (1.9 μmol, 39%). LC-MS analysis: *t* 7.86 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.4 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00064.

**Dimeric ligand 10S-LHA**

Yield after RP-HPLC purification: 4.1 mg (1.8 μmol, 36%). LC-MS analysis: *t* 7.92 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.3 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1135.99890.

**Dimeric ligand 11S-LHA**

Yield after RP-HPLC purification: 3.4 mg (1.5 μmol, 30%). LC-MS analysis: *t* 7.95 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.1 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00049.

**Dimeric ligand 12S-LHA**

Yield after RP-HPLC purification: 3.5 mg (1.5 μmol, 31%). LC-MS analysis: *t* 7.91 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.3 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1135.99976.

**Dimeric ligand 13S-LHA**

Yield after RP-HPLC purification: 3.5 mg (1.5 μmol, 31%). LC-MS analysis: *t* 8.00 min (gradient 10 to 90% B). ESI-MS *m/z*: 1137.3 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00000.

**Dimeric ligand 14S-LHA**

Yield after RP-HPLC purification: 3.7 mg (1.6 μmol, 33%). LC-MS analysis: *t* 7.97 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.2 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00073.

**Dimeric ligand 15S-LHA**

Yield after RP-HPLC purification: 3.2 mg (1.4 μmol, 28%). LC-MS analysis: *t* 8.07 min (gradient 10 to 90% B). ESI-MS *m/z*: 1136.5 [M + 2H]^2+. HRMS *m/z* calcd for C_{108}H_{139}N_{31}O_{17}S_{4} + 2H^+: 1135.99968, obsd 1136.00000.

**Dimeric ligand 16S-LHA**

Yield after RP-HPLC purification: 2.9 mg (0.7 μmol, 20%). LC-MS analysis: *t* 7.77 min (gradient 10 to 90% B). ESI-MS *m/z*: 1281.5 [M + 2H]^2+. HRMS *m/z* calcd for C_{123}H_{160}N_{34}O_{20}S_{4} + 2H^+: 1281.57882, obsd 1281.57958.

**Dimeric ligand 17S-LHA**

Yield after RP-HPLC purification: 1.3 mg (0.4 μmol, 9%). LC-MS analysis: *t* 7.61 min (gradient 10 to 90% B). ESI-MS *m/z*: 1426.9 [M + 2H]^2+. HRMS *m/z* calcd for C_{138}H_{181}N_{37}O_{23}S_{4} + 2H^+: 1427.15797, obsd 1427.15883.

**Dimeric ligand 18S-LHA**

Yield after RP-HPLC purification: 2.9 mg (0.9 μmol, 17%). LC-MS analysis: *t* 7.46 min (gradient 10 to 90% B). ESI-MS *m/z*: 1572.5 [M + 2H]^2+. HRMS *m/z* calcd for C_{153}H_{202}N_{40}O_{26}S_{4} + 2H^+: 1572.73712, obsd 1572.73805.
Dimeric and oligomeric LHR ligands

Trimeric ligand 19S-LHA3. Yield after RP-HPLC purification: 2.7 mg (1.0 μmol, 19%). LC-MS analysis: tR 9.50 min (gradient 10 to 90% B). ESI-MS m/z: 1397.9 [M + 2H]2+. HRMS m/z calcd for C131H164N40O19S6 + 2H+: 1397.57831, obsd 1397.57898.

Tetrameric ligand 20S-LHA4. Yield after RP-HPLC purification: 1.5 mg (0.4 μmol, 8%). LC-MS analysis: tR 10.9 min (gradient 10 to 90% B). ESI-MS m/z: 1659.6 [M + 2H]2+. HRMS m/z calcd for C154H189N49O21S8 + 2H+: 1659.15694, obsd 1659.15774.

References and notes


28. To further evaluate the stability of the helices, the ellipticity was measured at different temperatures for some selected compounds (R/S-1, R/S-7 and R/S-19 decorated with azides or LHAs). The azidoproline containing peptides adopted a PP type II helix and only 14-18% loss in ellipticity was observed at a temperature of 80 °C. The stability also seems independent on the (4R)- or (4S)-Azp incorporated. The ligand-containing helices also adopt a PP type II helix and only the thrice substituted polyproline 19R-LHA or 19S-LHA shows some destabilization of the helix at 80 °C (loss of >50% of ellipticity).

29. Recent studies showed that prolyl isomerisation to cis amide bond are likely on extended OPs that could lead to an undefined structure. For the azidoproline containing compounds described in this chapter such effect could not be established by 1H NMR experiments as described in: Best, R. B.; Merchant, K. A.; Gopich, I. V.; Schuler, B.; Bax, A.; Eaton, W. A. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 18964-18969.


