

Dynamic Combinatorial Development of a Neutral Synthetic Receptor that Binds Sulfate with Nanomolar Affinity in Aqueous Solution

*Zaida Rodriguez-Docampo,[†] Eugenia Eugenieva-Ilieva,[‡] Carsten Reyheller,[‡] Ana M. Belenguer,[†]
Stefan Kubik^{*‡} and Sijbren Otto^{*§}*

[†]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom, [‡]Fachbereich Chemie - Organische Chemie, Technische Universität Kaiserslautern, Erwin-Schrödinger-Straße, D-67663 Kaiserslautern, Germany, and [§]Centre for Systems Chemistry, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.

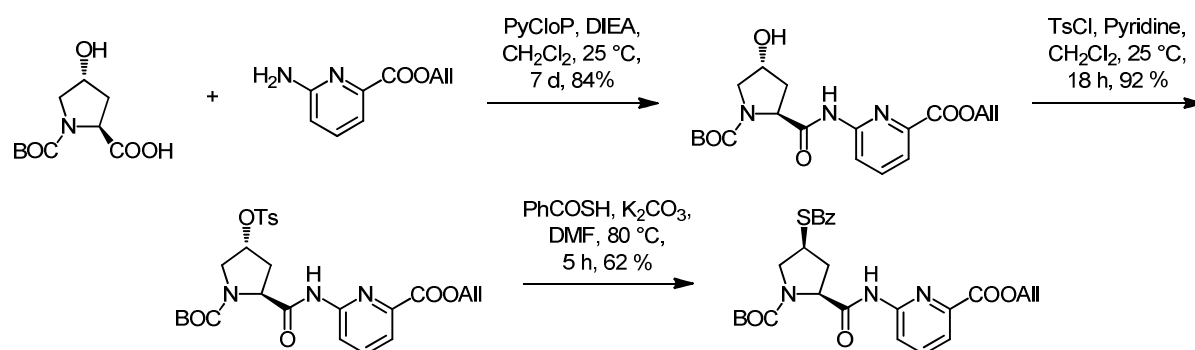
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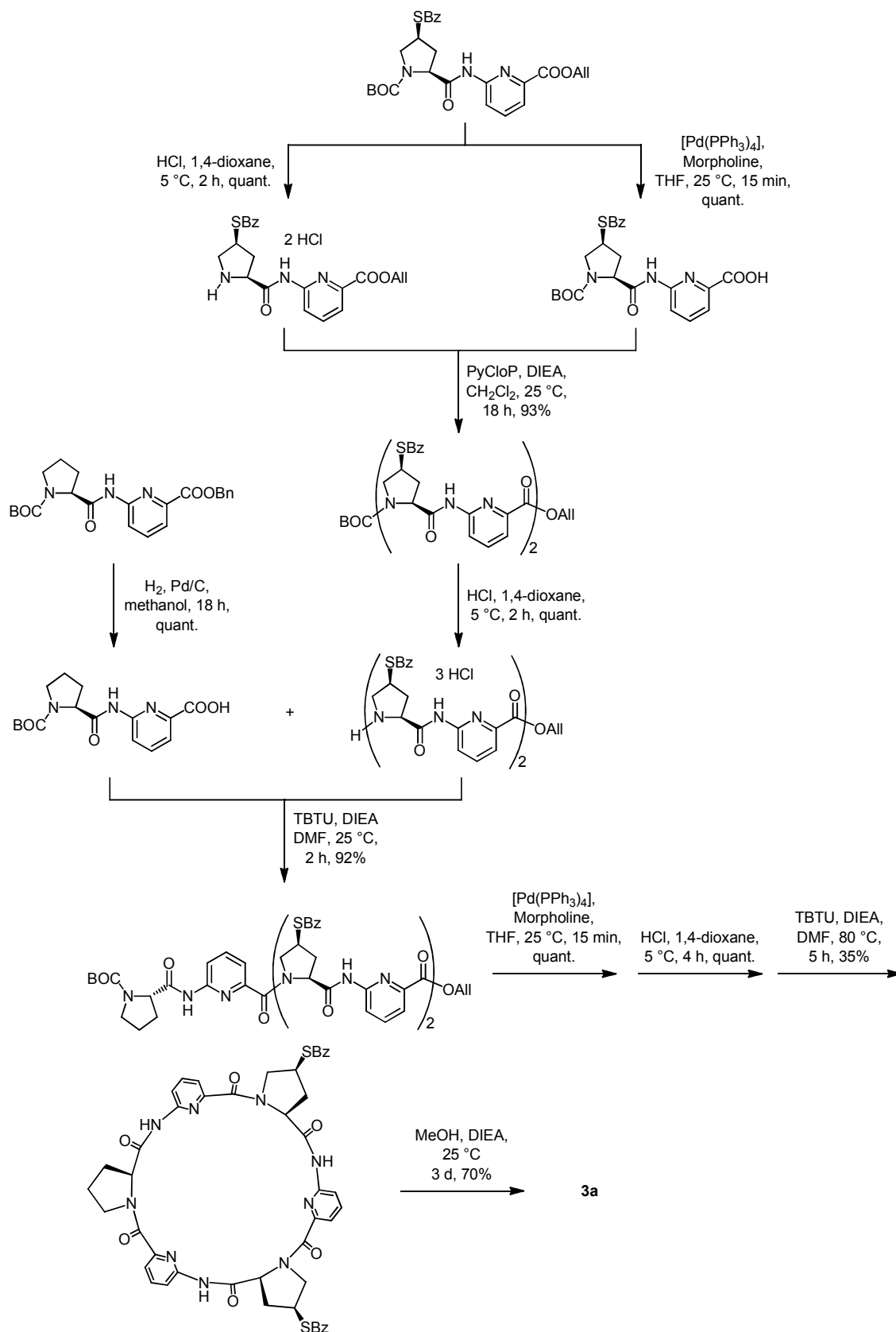
1. Synthesis of bis(cyclopeptide) **3a**

Bis(cyclopeptide) **3a** was prepared by deprotection of the corresponding benzoyl protected cyclopeptide dithiol. This peptide was assembled in solution from appropriately protected dipeptide building blocks according to the following procedure.

The functionalized dipeptide was prepared by coupling commercial BOC protected L-hydroxyproline and 6-aminopicolinic acid allyl ester by using PyCloP as the coupling reagent, followed by tosylation of the hydroxy group, and reaction with potassium thiobenzoate.



BOC deprotection of the functionalized dipeptide and coupling with one equivalent of the allyl deprotected derivative afforded the linear tetrapeptide, which was again BOC deprotected and coupled to the free acid of an unfunctionalized dipeptide, whose synthesis is described elsewhere.^{S1} The hexapeptide thus obtained was deprotected at both chain ends and cyclized under pseudo high-dilution conditions. Chromatographic purification afforded analytically pure product. This product was then deprotected at the two thiol groups by treatment with *N*-ethyl-diisopropylamine in methanol in the presence of oxygen. HPLC indicated that the product thus obtained mainly consisted in a mixture of two compounds (besides small amounts of monomeric and oligomeric material), both having the mass of bis(cyclopeptide) **3a**. These compounds most likely correspond to two configurational isomers of **3a**, a C-shaped and an S-shaped one. No purification of this material was necessary prior to setting up the dynamic libraries.



General Methods. Analyses were carried out as follows: melting points, Müller SPM-X 300; NMR, Bruker Avance 600, Bruker DPX 400; MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; chromatography, MERCK LiChroprep RP-8 (40-63 µm) prepacked column size B (310 x 25 mm). The following abbreviations are used: BOC, *tert*-butoxy carbonyl; DIEA, *N*-ethyl-diisopropylamine;

PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate; Pro, L-proline; Hyp, L-hydroxyproline, Tpro, 4*S*-Thio-L-proline, APA, 6-aminopicolinic acid.

Materials. All solvents were dried according to standard procedures prior to use. DMF p.A. was purchased from Fluka and used without further purification. PyCloP was prepared according to the literature procedures.^{S2} TBTU was purchased from Novabiochem.

General procedure for the cleavage of benzyl esters. The ester was dissolved in 1,4-dioxane/H₂O 9:1 (50 mL/mmol). After the addition of 10% Pd/C (100 mg) the resulting reaction mixture was subjected to hydrogenation at 1 atm overnight. Completion of the reaction was checked by TLC. The catalyst was then filtered off by passage through a layer of Celite and washed with dioxane/H₂O 9:1. The combined filtrate and washings were evaporated to dryness *in vacuo*, the residue was dissolved in CH₂Cl₂, and the solvent was evaporated again. The crude product was dried *in vacuo*, and used for the coupling reaction without further purification.

General procedure for the cleavage of allyl esters. The allyl ester was dissolved in dry THF (20 mL/mmol) under inert conditions. Tetrakis(triphenylphosphine)palladium(0) (5 mg) and freshly distilled morpholine (3 equiv) were added, and the reaction mixture was stirred at room temperature. Progress of the reaction was checked by TLC after 5 min. In case starting material was still detectable additional catalyst (5 mg) was added. Stirring was continued for another 30 min before the solvent was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (or in the case of linear hexapeptides dichloromethane). The organic layer was washed three times with 10% aqueous KHSO₄ and twice with water. The solvent was evaporated, and the residue dried *in vacuo*. It was used in the following step without further purification.

General procedure for the cleavage of *N*-tert-butoxycarbonyl groups. The carbamate was suspended in 1,4-dioxane (20 mL). This suspension was cooled in an ice bath and a 6 N solution of HCl in 1,4-dioxane (40 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0-5°C and then concentrated to dryness *in vacuo*.

Dipeptide BOC-Hyp-APA-OAll. 6-Aminopicolinic acid allyl ester (2.67 g, 15.0 mmol),^{S3} BOC-L-hydroxyproline (5.30 g, 22.5 mmol) and PyCloP (9.48 g, 22.5 mmol) were dissolved in CH₂Cl₂ (180 mL). At room temperature, DIEA (7.8 mL, 45.0 mmol) was added dropwise, and then the reaction mixture was stirred for 7 d. The solvent was subsequently evaporated *in vacuo*, and the product was isolated from the residue by chromatographic workup (hexane/ethyl acetate, 1:3). Yield 4.94 g (84%); ¹H-NMR (600 MHz, [D₆]DMSO, 100 °C) δ 1.34 (s, 9H; *t*BuCH₃), 1.97-2.03 (m, 1H, HypH(β)), 2.15-2.19 (m, 1H, HypH(β)), 3.31 (d, 1H, ²*J*(H,H) = 11.1 Hz, HypH(δ)), 3.50 (dd, 1H, ²*J*(H,H) = 11.8 Hz, ³*J*(H,H) = 4.7 Hz, HypH(δ)), 4.30-4.36 (m, 1H, HypH(γ)), 4.57 (t, 1H, ³*J*(H,H) = 7.6 Hz, HypH(α)), 4.71 (s, br, 1H, OH), 4.83 (d, 2H, ³*J*(H,H) = 4.1 Hz, AllCH₂), 5.29 (d, 1H, ³*J*(H,H) = 9.2 Hz, AllH_{cis}), 5.41 (d, 1H, ³*J*(H,H) = 17.2 Hz, AllH_{trans}), 6.01-6.08 (m, 1H, AllH_{vic}), 7.75 (d, 1H, ³*J*(H,H) = 7.6 Hz, APAH(3)), 7.95 (t, 1H, ³*J*(H,H) = 7.9 Hz, APAH(4)), 8.26 (d, 1H, ³*J*(H,H) = 8.3 Hz, APAH(5)), 10.46 (s, 1H, NH); MS (MALDI-TOF) *m/z* (%) 392.0 (100) [M+H]⁺, 414.0 (88) [M+Na]⁺, 430.0 (16) [M+K]⁺.

Dipeptide BOC-(Ts)Hyp-APA-OAll. BOC-Hyp-APA-OAll (4.69 g, 12.0 mmol) was dissolved in a mixture of dry CH₂Cl₂ (12 mL) and pyridine (12 mL). 4-Toluenesulfonyl chloride (11.4 g, 60 mmol) was added in one portion and the mixture was stirred overnight at room temperature. The solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. The resulting solution was washed three times with 10 % aqueous Na₂CO₃, three times with water, dried, and evaporated to dryness. The residue was purified chromatographically on silica (hexane/ethyl acetate, 1:1). Yield 5.86 g (92%); ¹H-NMR (600 MHz, [D₆]DMSO, 100 °C) δ 1.37 (s, 9H; *t*BuCH₃), 2.19-2.23 (m, 1H, HypH(β)), 2.29 (m, 4H, HypH(β) + TsCH₃), 3.54 (d, 1H, ²*J*(H,H) = 12.5 Hz, HypH(δ)), 3.83 (dd, 1H, ²*J*(H,H) = 12.5 Hz, ³*J*(H,H) = 4.3 Hz, HypH(δ)), 4.60-4.63 (m, 1H, HypH(α)), 4.85 (d, 2H, ³*J*(H,H) = 5.5 Hz, AllCH₂), 5.14-5.18 (m, 1H, HypH(γ)), 5.29 (d, 1H, ³*J*(H,H) = 10.4 Hz, AllH_{cis}), 5.42 (d, 1H, ³*J*(H,H) = 17.2 Hz, AllH_{trans}), 6.02-6.08 (m, 1H, AllH_{vic}), 7.49 (d, 2H, ³*J*(H,H) = 8.1 Hz, TsH(3)), 7.77 (d, 1H, ³*J*(H,H) = 7.5 Hz, APAH(3)), 7.80 (d, 2H, ³*J*(H,H) = 8.2 Hz, TsH(2)), 7.96 (t, 1H, ³*J*(H,H) = 7.9 Hz, APAH(4)), 8.24 (d, 1H, ³*J*(H,H) = 8.3 Hz, APAH(5)), 10.61 (s, 1H, NH); MS (MALDI-TOF) *m/z* (%) 546.1 (18) [M+H]⁺, 568.1 (100) [M+Na]⁺, 584.1 (50) [M+K]⁺.

Dipeptide BOC-(Bz)Tpro-APA-OAll. BOC-(Ts)Hyp-APA-OAll (5.46 g, 10 mmol) and K_2CO_3 (4.15 g, 30 mmol) were suspended in DMF (200 mL). Thiobenzoic acid (90%, 4.91 g, 32 mmol) was added, and the resulting mixture was heated to 80 °C for 5 h. Afterward, the solvent was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate. The resulting solution was washed three times with water, dried, and evaporated. The residue was purified chromatographically on silica (hexane/ethyl acetate, 1:1). Yield 3.17 g (62%); mp. 68-75 °C; 1H -NMR (600 MHz, $[D_6]DMSO$, 100 °C) δ 1.41 (s, 9H; *t*BuCH₃), 2.06-2.11 (m, 1H, TproH(β)), 2.92-2.97 (m, 1H, TproH(β)), 3.42-3.45 (m, 1H, TproH(γ)), 4.09-4.12 (m, 1H, TproH(δ)), 4.17-4.22 (m, 1H, TproH(δ)), 4.66-4.68 (d, 1H, $^3J(H,H) = 7.5$ Hz, TproH(α)), 4.88 (dt, 2H, $^3J(H,H) = 5.6$ Hz, $^4J(H,H) = 1.3$ Hz, AllCH₂), 5.34 (dd, 1H, $^3J(H,H) = 10.5$ Hz, $^4J(H,H) = 1.3$ Hz, AllH_{cis}), 5.46 (dd, 1H, $^3J(H,H) = 17.2$ Hz, $^4J(H,H) = 1.6$ Hz, AllH_{trans}), 6.09 (ddt, 1H, $^3J(H,H) = 17.0$ Hz, $^3J(H,H) = 11.0$ Hz, $^3J(H,H) = 5.7$ Hz, AllH_{vic}), 7.58 (t, 2H, $^3J(H,H) = 7.9$ Hz, BzH(3)), 7.72 (t, 1H, $^3J(H,H) = 7.4$ Hz, BzH(4)), 7.82 (d, 1H, $^3J(H,H) = 7.5$ Hz, APAH(3)), 7.92 (dd, 2H, $^3J(H,H) = 8.3$ Hz, $^4J(H,H) = 1.2$ Hz, BzH(2)), 8.02 (t, 1H, $^3J(H,H) = 7.9$ Hz, APAH(4)), 8.33 (d, 1H, $^3J(H,H) = 8.3$ Hz, APAH(5)), 10.64 (s, 1H, NH); 1H -NMR (151 MHz, $[D_6]DMSO$, 25 °C) δ 27.7 (*t*BuCH₃), 36.0 (TproC(β)), 38.5 (TproC(γ)), 52.0 (TproC(δ)), 59.4 (TproC(α)), 65.2 (AllC(1)), 79.2 (*t*BuC), 117.1 (AllC(3)), 118.0 (APAC(3)), 120.0 (APAC(5)), 126.5 (BzC(2)), 128.7 (BzC(3)), 132.1 (AllC(2)), 133.5 (BzC(4)), 136.1 (BzC(1)), 139.0 (APAC(4)), 145.9 (APAC(2)), 151.6 (APAC(6)), 163.7 (*t*BuCO), 171.2 (TproCO), 190.4 (BzCO); MS (MALDI-TOF) *m/z* (%) 512.2 (47) $[M+H]^+$, 534.2 (100) $[M+Na]^+$, 550.2 (74) $[M+K]^+$; elemental analysis calcd (%) for C₂₆H₃₉N₃O₆S: C 61.04, H 5.71, N 8.21; found C 60.83, H 5.66, N 8.08.

Tetrapeptide BOC-[(Bz)Tpro-APA]₂-OAll. Prior to coupling, the dipeptide **BOC-(Bz)Tpro-APA-OAll** (1.02 g, 2.0 mmol) was deprotected at the terminal amino group according to the general method for the cleavage of BOC groups and an equimolar amount was deprotected at the carboxyl group according to the general procedure for the cleavage of allyl esters. Both products as well as PyCloP (1.01 g, 2.4 mmol) were dissolved in CH₂Cl₂ (40 mL). At room temperature, DIEA (1.54 mL, 8.8 mmol) was added dropwise, and then the reaction mixture was stirred overnight. The solvent was subsequently evaporated *in vacuo*, and the product was isolated from the residue by

chromatographic workup (hexane/ethyl acetate, 1:2). Yield 1.61 g (93%); MS (MALDI-TOF) m/z (%) 865.2 (70) $[M+H]^+$, 887.2 (100) $[M+Na]^+$, 903.2 (92) $[M+K]^+$.

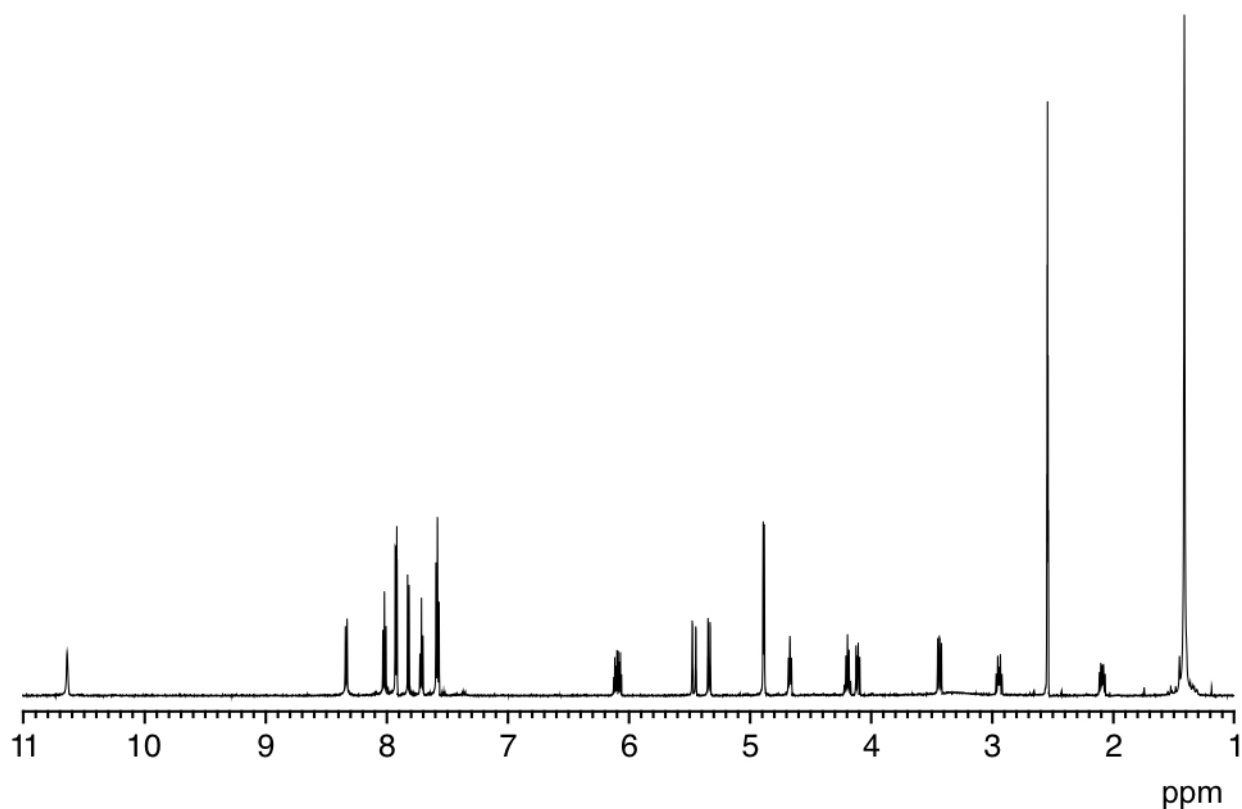
Hexapeptide BOC-Pro-APA-[(Bz)Tpro-APA]₂-OAll. Prior to coupling, the tetrapeptide **BOC-[(Bz)Tpro-APA]₂-OAll** (1.56 g, 1.8 mmol) was deprotected at the terminal amino group according to the general procedure for the cleavage of BOC groups, and an equimolar amount of the unfunctionalized dipeptide **BOC-Pro-APA-OBn**^{S1} (0.77 g, 1.8 mmol) was hydrogenated according to the general procedure for the cleavage of benzyl esters. Both compounds as well as TBTU (0.64 g, 2.0 mmol) were dissolved in DMF (50 mL). DIEA (1.64 mL, 9.4 mmol) was added dropwise at room temperature, and stirring continued for 2 h. The reaction mixture was then poured into water (300 mL) under stirring. The pH was adjusted to ca. 4 with 1 N HCl, and the solution was stirred for another 10 min. The precipitate was filtered off, washed with water, and dried. According to TLC, the product is usually obtained in high purity by this procedure and can be used for the following step without further purification. Yield 1.79 g (92%); MS (MALDI-TOF) m/z (%) 1104.5 (100) $[M+Na]^+$, 1120.5 (49) $[M+K]^+$.

Cyclopeptide *cyclo*{Pro-APA-[(Bz)Tpro-APA]₂. The linear hexapeptide **BOC-Pro-APA-[(Bz)Tpro-APA]₂-OAll** (1.77 g, 1.6 mmol) was deprotected at the carboxyl group according to the general method for the cleavage of allyl esters. Afterward, it was deprotected at the terminal amino group according to the general method for the cleavage of BOC groups. The product obtained was triturated with diethyl ether. The resulting completely deprotected peptide was dissolved in a mixture of degassed DMF (60 mL) and DIEA (1.68 mL, 9.6 mmol). This solution was added dropwise over the course of 4 h to a solution of TBTU (2.57 g, 8.0 mmol) and DIEA (0.66 mL, 3.8 mmol) in degassed DMF (320 mL) at 80 °C. If necessary, the pH of the reaction mixture was adjusted afterward to ca. 9 by adding more DIEA, and stirring was continued for 1 h at 80 °C. The solvent was then evaporated *in vacuo*, and the product was isolated from the residue by chromatographic workup. An initial purification step was carried out using a silica gel column (acetone). The material recovered was further purified on a RP-8 column. For this, it was dissolved in a small amount of DMF and applied to a column conditioned with 1,4-dioxane/H₂O, 1:10. The eluent composition was

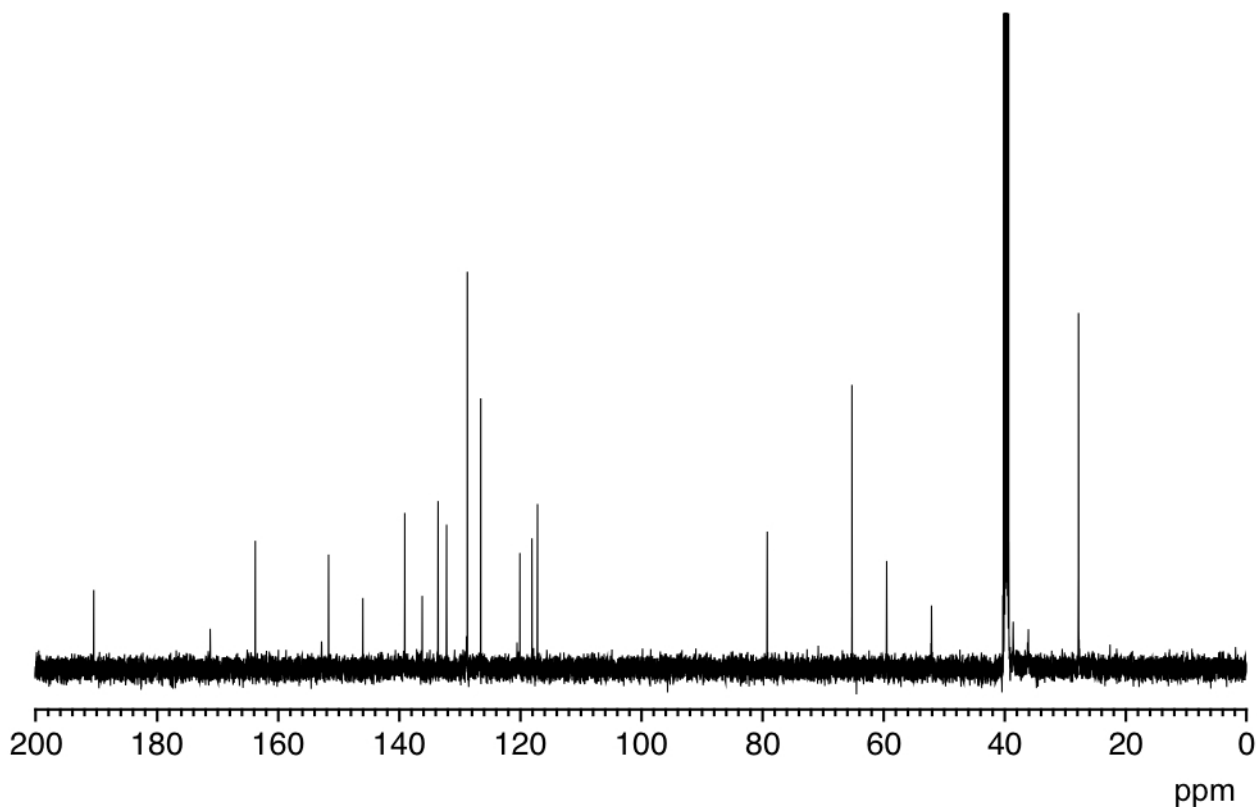
gradually changed until the pure product eluted (1,4-dioxane/H₂O, 2:1). In case the material thus obtained was still impure, it was once more chromatographed on silica (acetone). Yield 0.52 g (35%); mp. softening from 194 °C, > 230 °C (dec.); ¹H-NMR (600 MHz, [D₆]DMSO, 25°C) δ 1.80-1.93 (m, 2H, ProH(γ)), 2.03-2.11 (m, 1H, ProH(β)), 2.18-2.28 (m, 2H, ProH(β) + TproH(β)), 2.54-2.59 (m, 1H, TproH(β)), 3.21-3.31 (m, 2H, TproH(β)), 3.57-3.68 (m, 3H, ProH(δ) + TproH(γ)), 3.70-3.77 (m, 1H, ProH(δ)), 4.19-4.32 (m, 4H, TproH(δ)), 5.67-5.75 (m, 1H, ProH(α)), 5.85-5.99 (m, 2H, TproH(α)), 7.35 (d, 1H, ³J(H,H) = 8.2 Hz, APAH(3)), 7.40 (d, 1H, ³J(H,H) = 8.1 Hz, APAH(5)), 7.45 (d, 2H, ³J(H,H) = 7.5 Hz, APAH(3)), 7.52 (d, 2H, ³J(H,H) = 7.5 Hz, APAH(5)), 7.56-7.60 (m, 4H, BzH(4) + BzH(3)), 7.70-7.75 (m, 2H, BzH(3)), 7.75-7.80 (m, 3H, APAH(4)), 7.88-7.92 (m, 4H, BzH(2)), 9.95 (s, 1H, APANH), 10.06 (s, 2H, APANH); ¹³C-NMR (151 MHz, [D₆]DMSO, 25°C) δ 22.6 (ProC(γ)), 32.8 (ProC(β)), 38.0 (TproC(γ)), 38.3 + 38.4 (TproC(β)), 48.3 (ProC(δ)), 53.1 + 53.2 (TproC(δ)), 61.3 + 61.4 (TproC(α)), 61.7 (ProC(α)), 115.7 + 115.8 + 116.1 (APAC(3)), 119.9 (APAC(5)), 127.1 (BzC(2)), 129.4 (BzC(3)), 134.4 (BzC(4)), 136.2 (BzC(1)), 139.3 + 139.4 + 139.5 (APAC(4)), 148.8 + 148.9 + 149.0 (APAC(2)), 151.5 + 152.1 (APAC(6)), 166.0 + 166.1 (APACO), 170.3 + 170.4 (TproCO), 171.4 (ProCO), 191.0 (BzCO); MS (MALDI-TOF) *m/z* (%) 923.9 (42) [M+H]⁺, 945.9 (100) [M+Na]⁺, 961.9 (43) [M+K]⁺; elemental analysis calcd (%) for C₄₇H₄₁N₉O₈S₂·4H₂O: C 56.67, H 4.96, N 12.66; found C 56.90, H 4.68, N 12.40.

Bis(cyclopeptide) 3a. Cyclopeptide *cyclo*{Pro-APA-[(Bz)Tpro-APA]₂} (0.28 g, 0.3 mmol) was suspended in dry methanol (25 mL). After addition of DIEA (0.42 ml, 2.4 mmol), the reaction mixture was stirred under an oxygen atmosphere for 3 d. The solvent was evaporated *in vacuo* to a volume of 5 ml. This solution was poured under stirring into diethyl ether (100 mL). The precipitate was filtered off, washed with diethyl ether and dried. This material was pure enough for setting up the dynamic libraries. Yield 0.15 g (70%); mp. > 300 °C (dec.); no NMR spectroscopic characterization was possible because the product consisted in a mixture of isomers, presumably the C-shaped and the S-shaped configurational isomers of **3a**; MS (MALDI-TOF) *m/z* (%) 1427.1 (100) [M+H]⁺, 1449.1 (46) [M+Na]⁺, 1465.1 (14) [M+K]⁺.

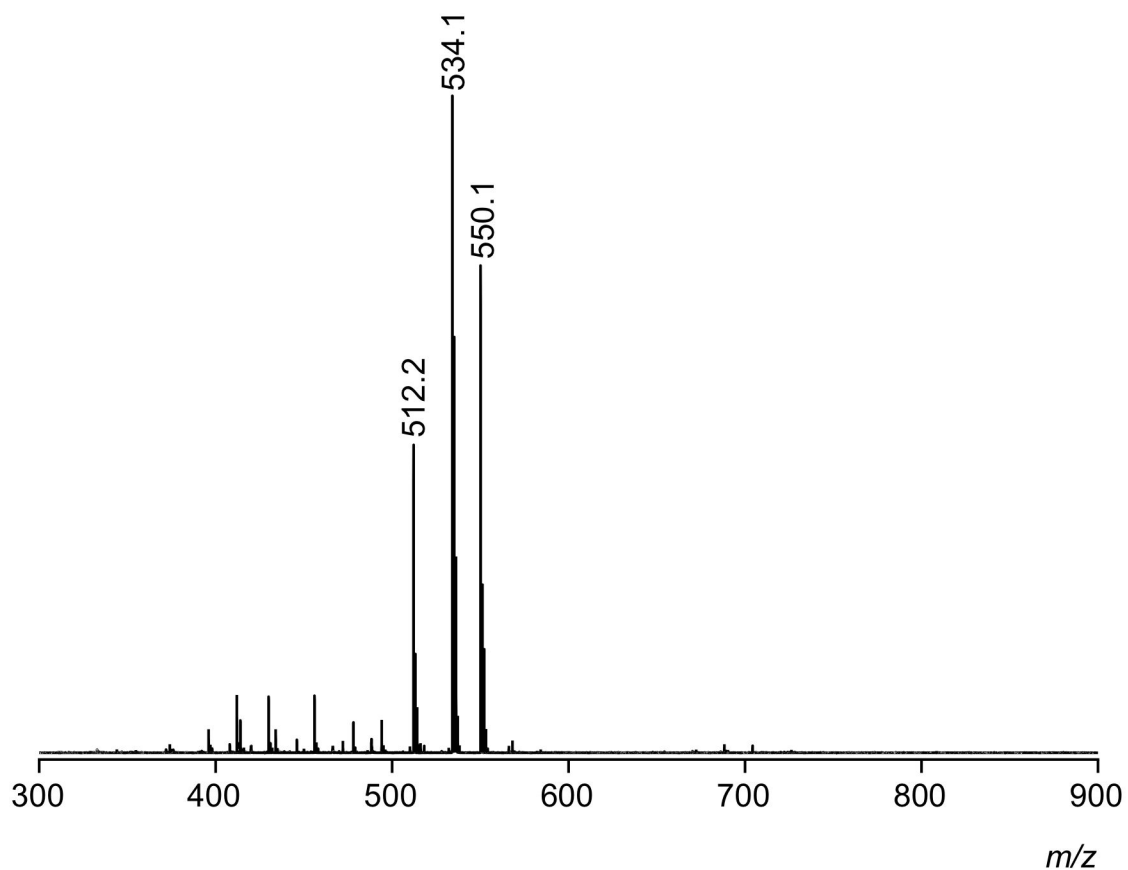
^1H NMR Spectrum: BOC-(Bz)Tpro-APA-OAll in $[\text{D}_6]\text{DMSO}$ (600 MHz, 100 °C, Bruker Avance 600).



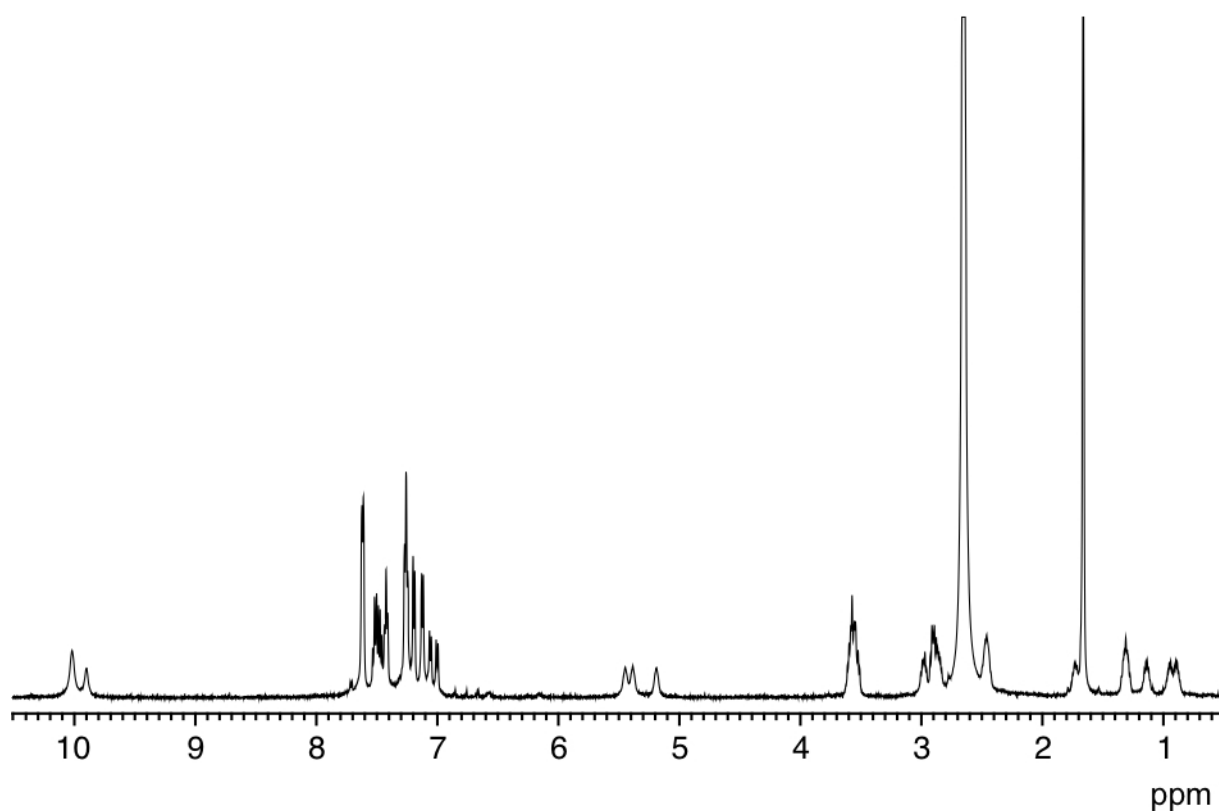
^{13}C NMR Spectrum: BOC-(Bz)Tpro-APA-OAll in $[\text{D}_6]\text{DMSO}$ (151 MHz, 25 °C, Bruker Avance 600).



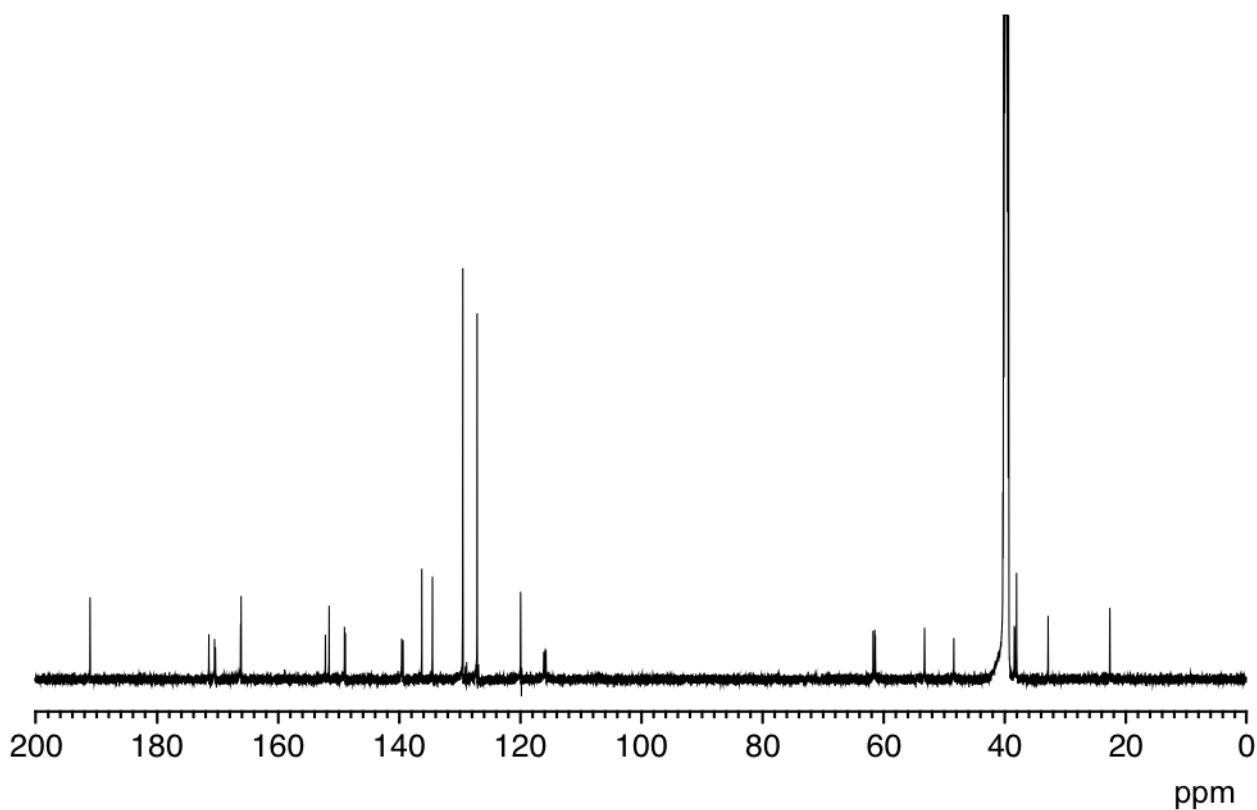
MALDI-TOF MS Spectrum: BOC-(Bz)Tpro-APA-OAlI (Bruker Ultraflex TOF/TOF).



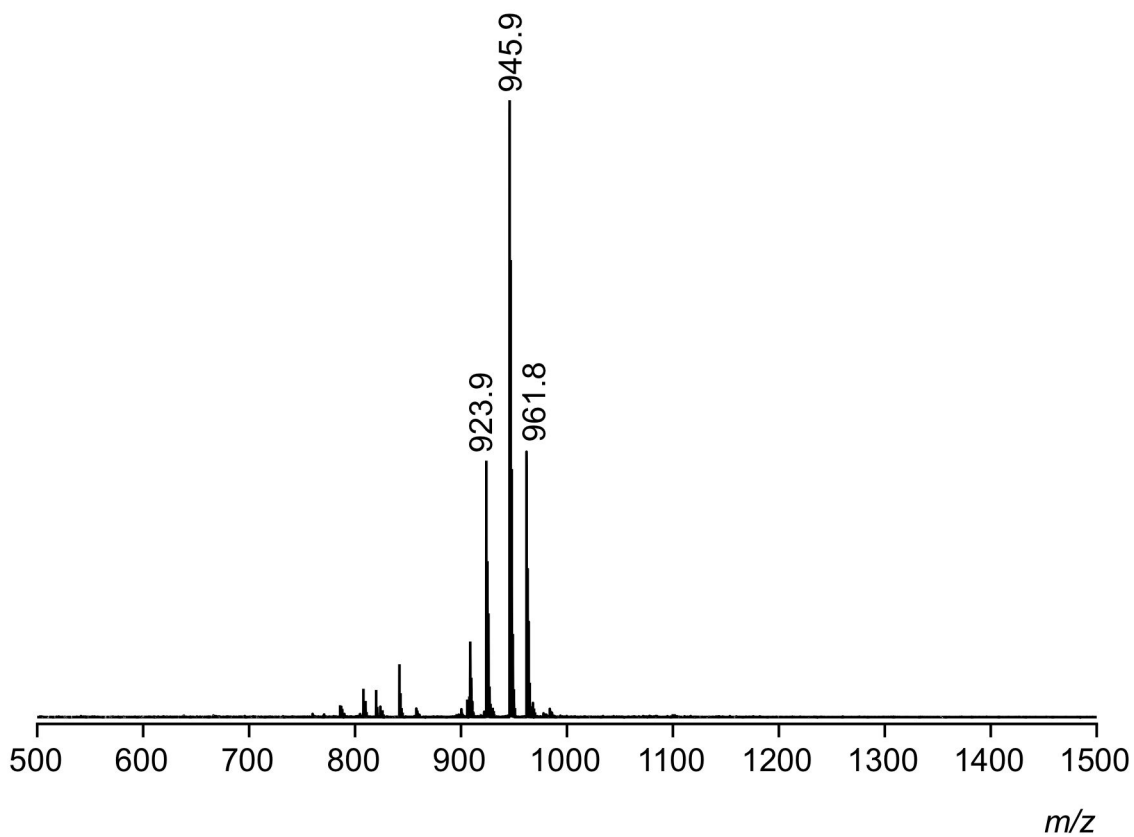
^1H NMR Spectrum: *cyclo*{Pro-APA-[(Bz)Tpro-APA] $_2$ } in $[\text{D}_6]$ DMSO (600 MHz, 100 °C, Bruker Avance 600).



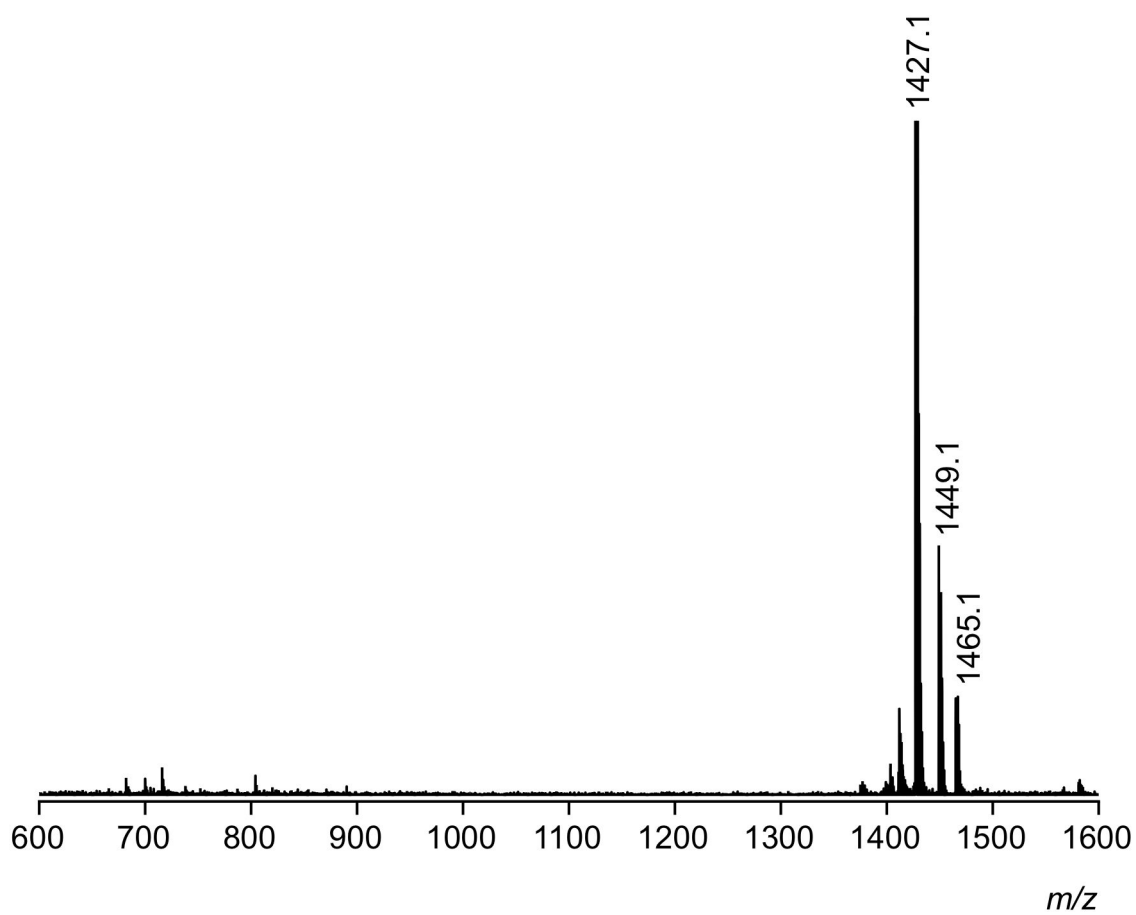
^{13}C NMR Spectrum: *cyclo*{Pro-APA-[(Bz)Tpro-APA]₂} in [D₆]DMSO (151 MHz, 25 °C, Bruker Avance 600).



MALDI-TOF MS Spectrum: *cyclo*{Pro-APA-[(Bz)Tpro-APA]₂} (Bruker Ultraflex TOF/TOF).



MALDI-TOF MS Spectrum: 3a (Bruker Ultraflex TOF/TOF).



2. Dynamic combinatorial libraries: preparation and analysis

All chemicals and solvents were purchased from Aldrich, TCI, Fluka, Fisher or Lancaster and used without further purification. Water was purified using a Millipore water purification system. Biscyclopeptide **2a** was prepared as described previously.^{S3}

2.1 Libraries

A stock solution (1.25 mM) of cyclopeptide **3a** in CH₃CN/H₂O (2:1 v/v) was prepared.

Spacers **c** (1,2-ethanedithiol) and **d** (1,3-benzenedithiol) were dissolved to an 8 mM concentration in CH₃CN. The templates (KBr, KI, Na₂SO₄, Na₂SeO₄) were dissolved to a concentration of 0.5 M in H₂O.

These stock solutions were pipetted in the appropriate proportions into 2 mL HPLC vials equipped with a micro stirrer bar, capped and allowed to equilibrate under air. The following table contains the proportions of each stock solution used to make the libraries:

Compound	Concentration in library	Stock solutions
Cyclopeptide 3a	0.66 mM	633.6 μL from 1.25 mM in 2:1 CH ₃ CN/H ₂ O
Spacer (c or d)	1.32 mM	198.0 μL from 8 mM in CH ₃ CN
H ₂ O pH 9		188.8 μL
CH ₃ CN		179.6 μL
Resulting solution		1200.0 μL 2:1 CH ₃ CN/H ₂ O

Aliquots of the resulting solutions (200 μL) were transferred to other HPLC vials equipped with a micro insert and the templates/anions (4 μL, 0.5 M in water, 10 mM in the library) were added and left for a period of 4-7 days at room temperature before HPLC analysis.

2.2 Chromatographic Analysis

Analytical HPLC was carried out using an Agilent 1100 series instrument coupled to a UV multi wavelength detector or a UV diode array detector. The column was a Zorbax Eclipse XDB-C8, 4.6×150mm with a particle size of 5 μm, heated to 45 °C. The eluent was a mixture of water

(Millipore purified) and acetonitrile (Fisher HPLC grade) with TFA (Aldrich). Data was processed using the Agilent ChemStations software.

HPLC parameters

Injection volume: 5 μ L

Flow rate: 1 mL/min

Mobile phase: Acetonitrile + 0.1% TFA; Water + 0.1% TFA.

Gradient:	Time (min)	% Acetonitrile
	0	25
	20	50
	25	60
	35	90
	40	97
	48	97

LC-MS was performed using an Agilent 1100 series HPLC equipped with a diode array UV detector and an Agilent XCT ion trap mass spectrometer. A 1:20 splitter (reducing the flow exiting the UV flowcell at 1 mL/min to around 50 μ L/min) was installed in front of the solvent entry to the electrospray ionization chamber to improved ionization efficiency. Solvents and TFA were acquired from Romil. The LC parameters were the same as described in the previous section. The data was processed using the LCMSD Trap Data Analysis software provided by Agilent.

MS Parameters

Mass range mode: Standard Enhanced

Ion polarity: Positive mode

Ion Source: ESI

Drying temperature: 350 $^{\circ}$ C

Nebuliser pressure: 20.00 psi

Drying gas flow: 5 L/min

HV capillary: 3500 V

Scan: 700-2200 m/z

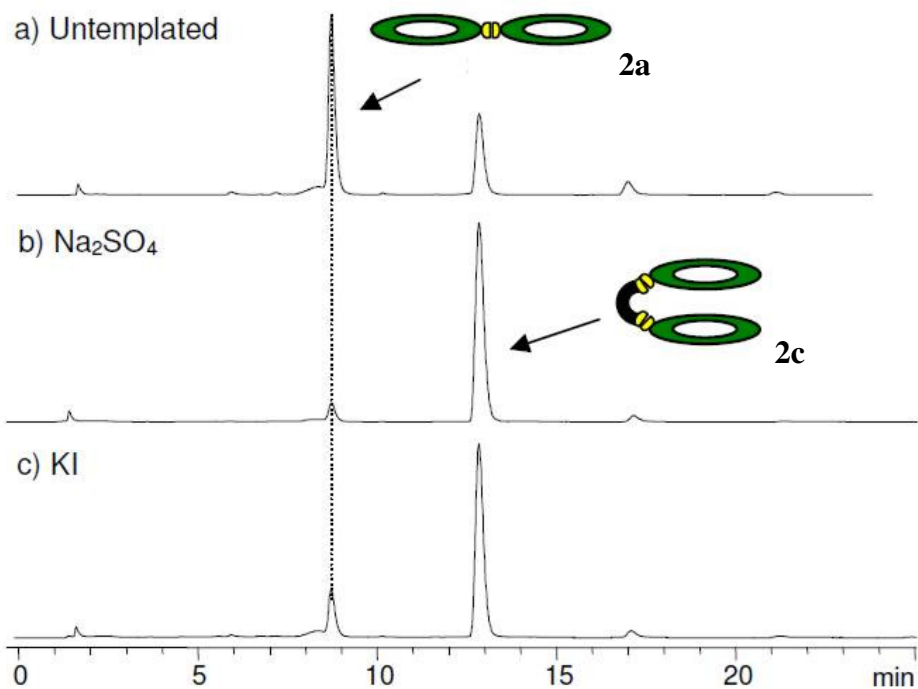
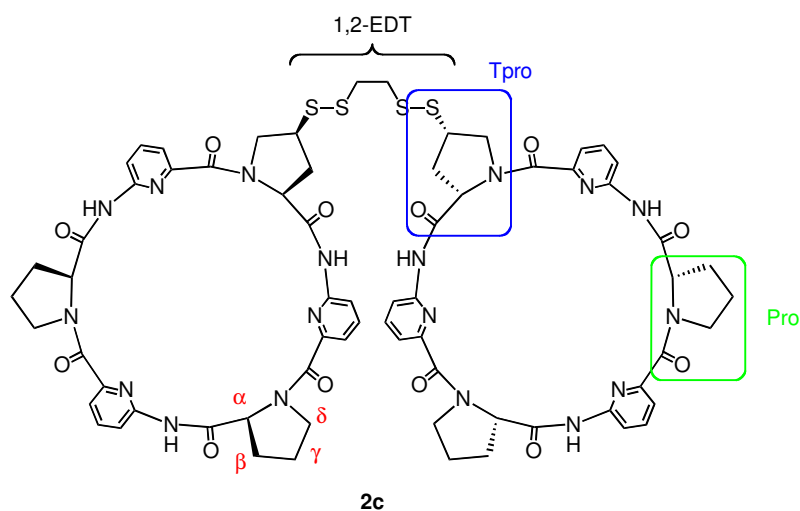


Figure S1: HPLC traces ($\lambda=290$ nm) of a library containing disulfide **2a** (1.33 mM) and spacer **c** (1.33 mM) in 41 mol% acetonitrile in water pH 9 recorded after 7 days of equilibration. (a) untemplated; (b) using Na₂SO₄ (10 mM) as a template, and (c) using KI (10 mM) as a template.

3. Isolation and characterization of the receptors

3.1 Receptor 2c



A solution containing cyclopeptide **2a** (20.50 mg, 1.33 mM, 1 equiv), 1,2-ethanedithiol (**c**) (1.12 μ L, 1.33 mM, 1 equiv), Na_2SO_4 (100 μ L of a 0.5 M solution in water, 5 mM, 3.76 equiv.), KOH (125 μ L of a 0.1 M solution in water), water (3.06 mL) and CH_3CN (6.66 mL) was stirred for 55 days. The solution was acidified with formic acid (400 μ L). BaCl_2 (150 μ L of a solution 0.5 M in water) was added inducing the precipitation of BaSO_4 . The library was stirred for 20 min and the precipitate allowed to settle for 30 min. The supernatant was filtered (regular cellulose, 0.45 μ m, 17 mm internal diameter). The solid was washed with a 2:1 mixture of CH_3CN and water and the resulting supernatants filtered. The combination of all the supernatants was reduced under vacuum until a cloudy solution was observed (~2 mL). DMSO was added until a homogeneous solution was obtained. The resulting solution was immediately purified by semi-preparative HPLC (column: Zorbax Eclipse XDB-C8, 9.4 \times 250 mm, 5 μ m, solvents: water + 0.1% TFA and CH_3CN + 0.1% TFA, elution: isocratic 40% CH_3CN in 15 min at 4 mL/min, retention time: 6.2-8.0 min). Fractions were concentrated and dried using a Genevac Evaporator (mode: HPLC fraction dry pure at 25 $^\circ\text{C}$) to give a white solid [8.85 mg, 46% yield]. $^1\text{H-NMR}$ (500 MHz, 8:2 $\text{CD}_3\text{OD}/\text{D}_2\text{O}$): δ 1.89-2.03 (m, 12H, ProH(γ) ProH(β)), 2.28 (m, 2H, ProH(β)), 2.58-2.73 (m, 6H, TproH(β) + ProH(β)), 2.80 (m, 2H, TproH(γ)), 2.97 (m, 4H, 1,2-EDT(CH_2)), 3.66 (m, 2H, ProH(δ)), 3.72-3.83 (m, 8H, TproH(δ) + ProH(δ)), 4.07 (dd, $^2J(\text{H,H}) = 12.4$ Hz, $^3J(\text{H,H}) = 7.2$ Hz, 2H, TproH(δ)), 5.70 (m, 4H, ProC(δ)), 5.89

(m, 2H, TproH(α)), 7.25 (d, $^3J(\text{H,H}) = 8.3$ Hz, 2H, APAH(3)), 7.36 (d, $^3J(\text{H,H}) = 8.3$ Hz, 2H, APAH(3)), 7.41 (d, $^3J(\text{H,H}) = 8.6$ Hz, 2H, APAH(3)), 7.60 (m, 6H, APAH(4)), 7.80 (m, 6H, APAH(5))

The UV trace, the base peak chromatogram and the ESI-MS spectrum corresponding to the LC-MS analysis of **2c** and the $^1\text{H-NMR}$ spectrum are shown in Figures S2 and S3, respectively.

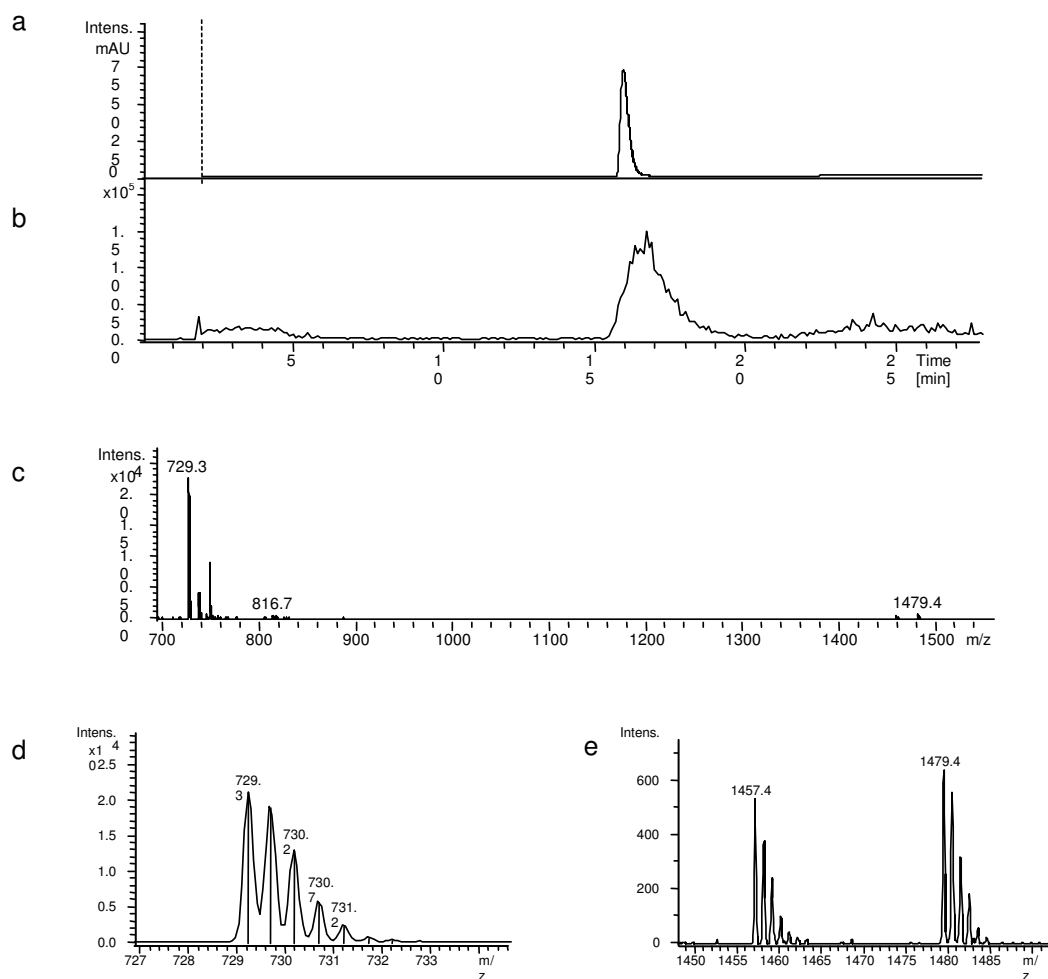


Figure S2: LC-MS analysis of the isolated receptor **2c**. (a) UV-chromatogram monitored at 250-270 nm. (b) Base-peak mass chromatogram. (c) Mass spectrum of the main peak in the chromatogram (positive ion mode). (d) Expansion of the mass spectrum showing the doubly-charged receptor ion (M+2H⁺). (e) Expansion of the mass spectrum showing the singly charged receptor ion (M+H⁺: obsd m/z = 1457.4; calcd 1457.42 and M+Na⁺: obsd m/z = 1479.4; calcd 1479.40).

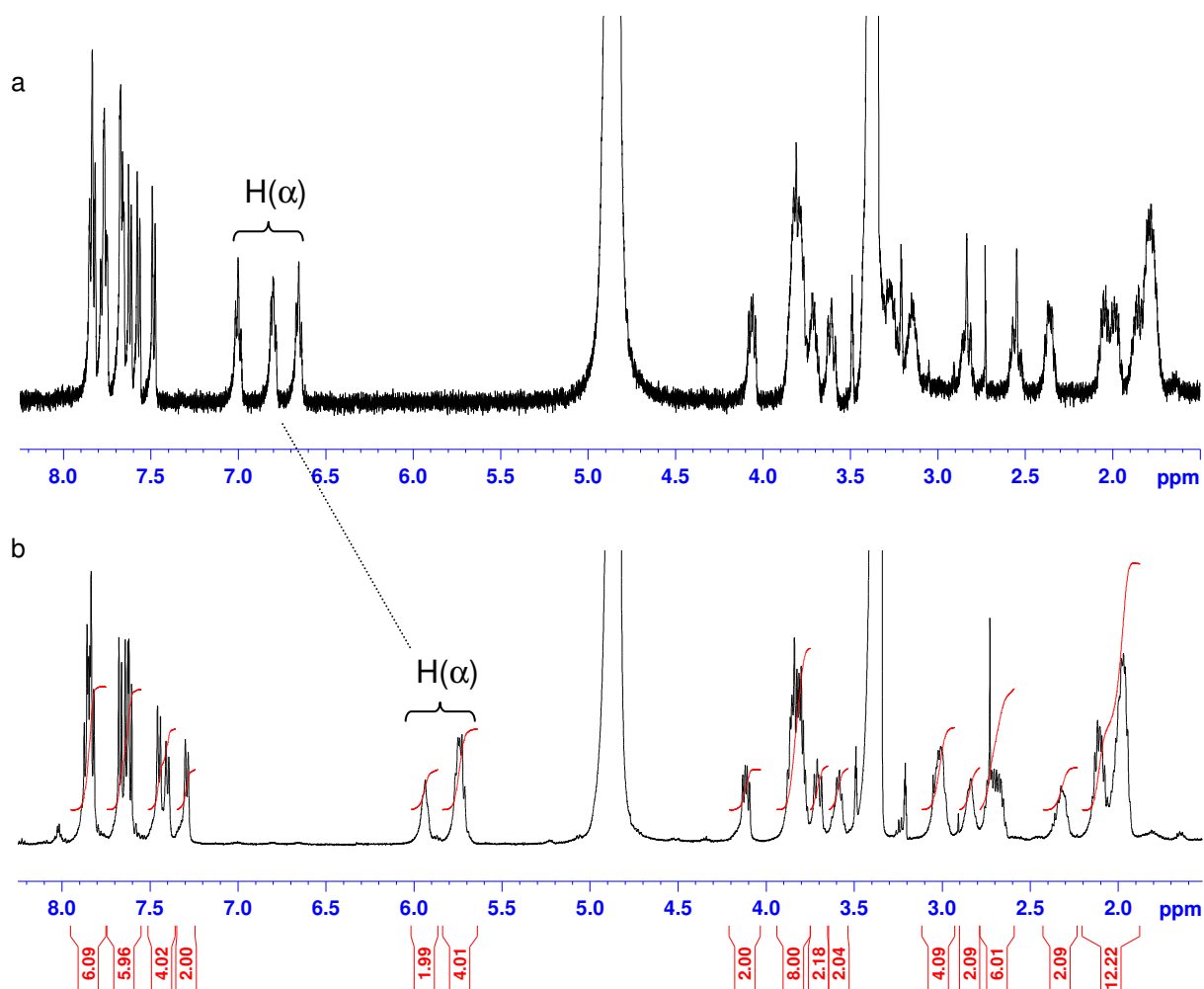
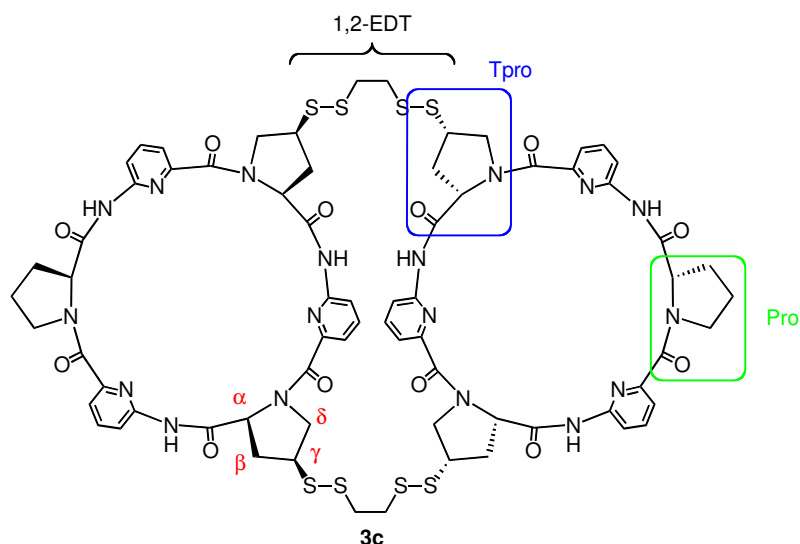


Figure S3: $^1\text{H-NMR}$ spectra of receptor **2c** in 8:2 $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (v/v) (a) as the sulfate complex, and (b) in the absence of sulfate. Poor phasing of the spectra caused by the large peaks of solvent makes reliable integration difficult. Note the pronounced downfield shifts of the three $\text{H}(\alpha)$ signals caused by the addition of sodium sulfate that are indicative of complex formation.

3.3 Synthesis of receptor **3c**



A solution containing cyclopeptide **3a** (21.33 mg, 0.66 mM, 1 equiv), 1,2-ethanedithiol **c** (2.26 μ L, 1.35 mM, 2.04 equiv), Na_2SO_4 (200 μ L of a 0.5 M solution in water, 5 mM, 7.6 equiv), KOH (170 μ L of a 0.1 M solution in water), water (6.30 mL) and CH_3CN (13.33 mL) was prepared and stirred for 30 days. The solution was acidified with formic acid (400 μ L). BaCl_2 (500 μ L of a solution of 0.25 M in water) was added, causing the precipitation of BaSO_4 . The suspension was stirred for 20 min and the precipitate allowed to settle for 30 min. The supernatant was filtered (regular cellulose, 0.45 μ m, 17 mm internal diameter). The solid was washed with a mixture of 2:1 CH_3CN and water the resulting supernatants filtered. The combination of all the supernatants was reduced under vacuum until a cloudy solution was observed (~3 mL). DMSO was added until a transparent solution was obtained. The resulting solution was immediately purified by semiprep HPLC (column: Zorbax Eclipse XDB-C8, 9.4 \times 250 mm, 5 μ m, solvents: water + 0.1% TFA and CH_3CN + 0.1% TFA, elution: 40 to 60% of CH_3CN in 15 min at 4 mL/min, retention time: 9.1-9.5 min). Fractions were concentrated and dried using a Genevac Evaporator (mode: HPLC fraction dry pure at 25 $^\circ\text{C}$) to give a white solid [9.43 mg, 45% yield].

The UV trace, the base peak chromatogram and the ESI-MS spectra corresponding to the LC-MS analysis of receptor **3c** are shown in Figure S4.

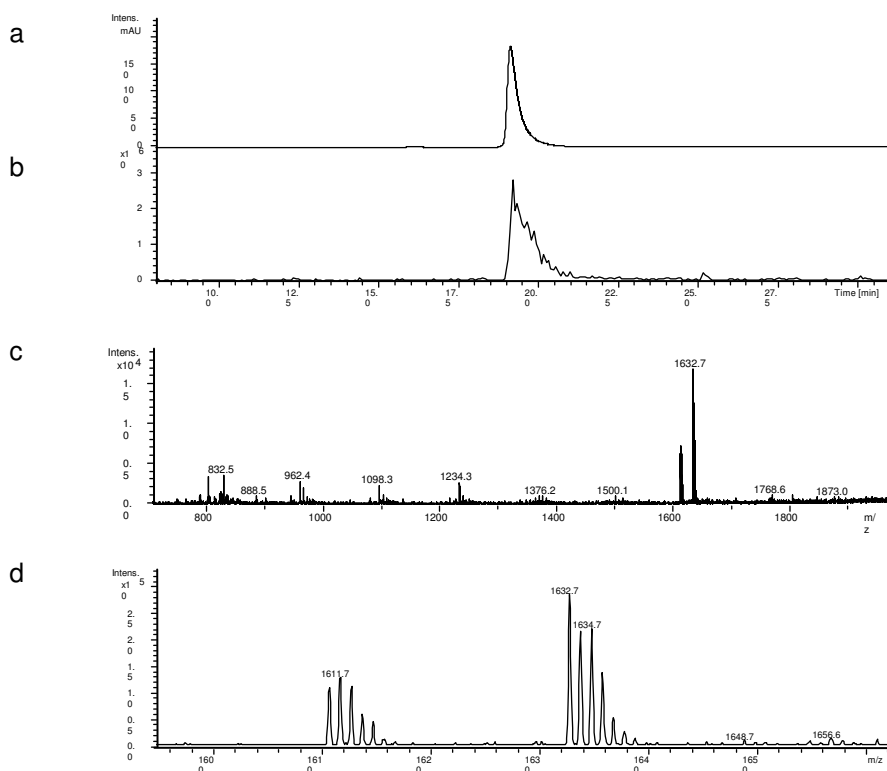


Figure S4: LC-MS analysis of receptor **3c**. (a) UV-chromatogram monitored at 252-256 nm. (b) Base-peak mass chromatogram. (c) Mass spectrum of the main peak in the chromatogram (positive ion mode). (d) Expansion of the mass spectrum showing the singly charged receptor ion ($M+H^+$: obsd $m/z = 1610.7$; calcd 1611.33 and $M+Na^+$: obsd $m/z = 1632.7$; calcd 1633.31).

The 1H -NMR spectrum of the free receptor **3c** (Figure S5b) was difficult to interpret due to the broadness of the signals presumably caused by the existence of multiple conformations. The NMR data presented below corresponds to the sulfate complex of receptor **3c** (Figure S5a).

1H -NMR (500 MHz, 8:2 CD_3OD/D_2O) δ 1.74 (m, 6H, ProH(γ) + ProH(β)), 1.97 (m, 4H, ProH(β) + TproH(β)), 2.33 (m, 6H, TproH(β)), 2.49 (m, 4H, 1,2-EDT(CH_2)), 2.79 (m, 4H, 1,2-EDT(CH_2)), 3.14 (m, 4H, TproH(γ)), 3.50-3.88 (m, 8H, TproH(δ) + ProH(δ)), 3.96 (dd, $^2J(H,H) = 12.3$ Hz, $^3J(H,H) = 7.1$ Hz, 2H, TproH(δ)), 4.04 (dd, $^2J(H,H) = 12.6$ Hz, $^3J(H,H) = 7.2$ Hz, 2H, TproH(δ)), 6.70 (dd, 2H, ProH(α)), 6.93 (dd, 2H, TproH(α)), 7.04 (dd, 2H, TproH(α)), 7.62-7.56 (m, 6H, APAH(3)), 7.67 (m,

4H, APAH(4) + APAH(5)), 7.78 (m, 4H, APAH(4) + APAH(5)), 7.84 (m, 4H, APAH(4) + APAH(5)). The NHs were not observed because they exchanged with the deuterium of the solvent.

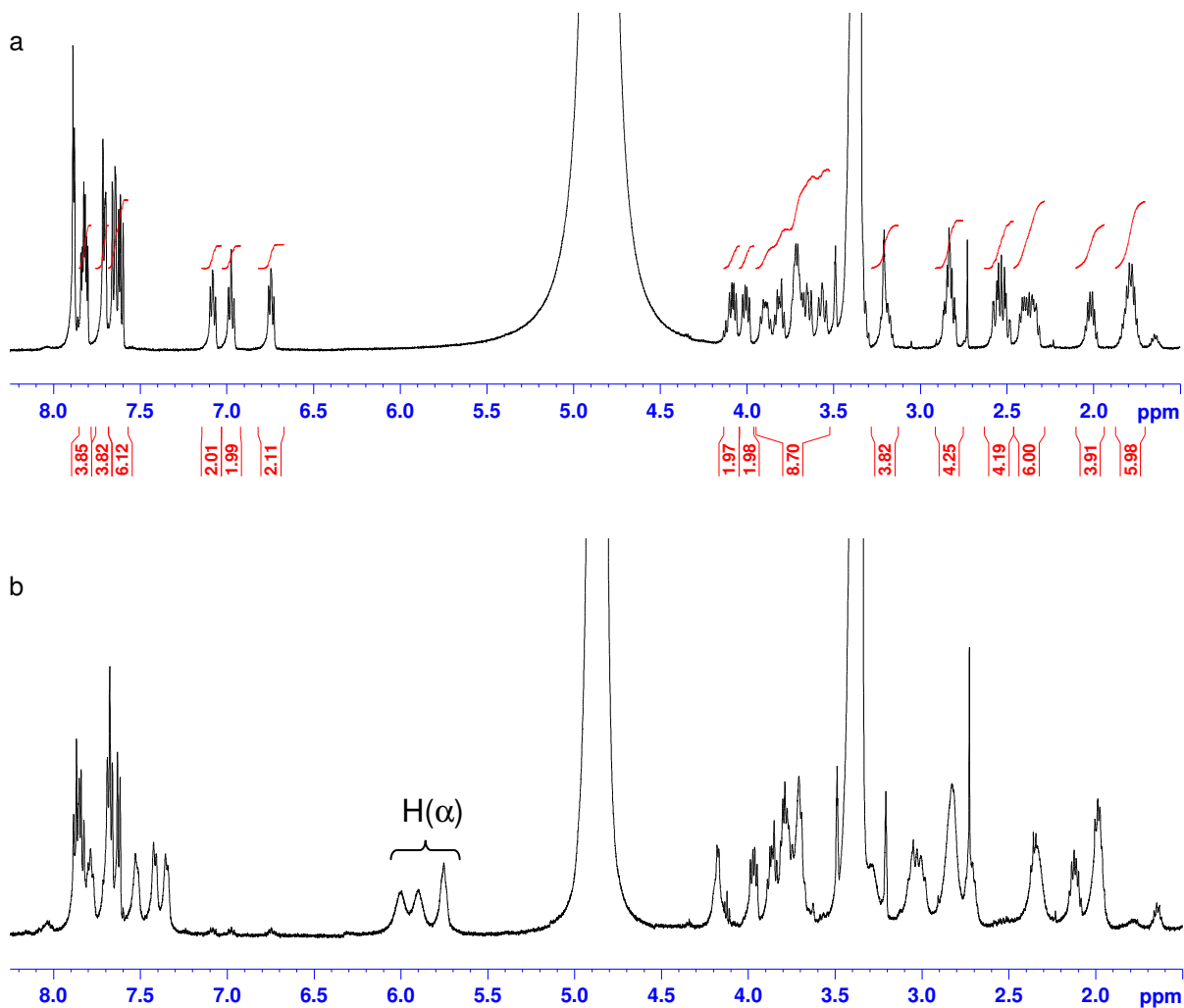
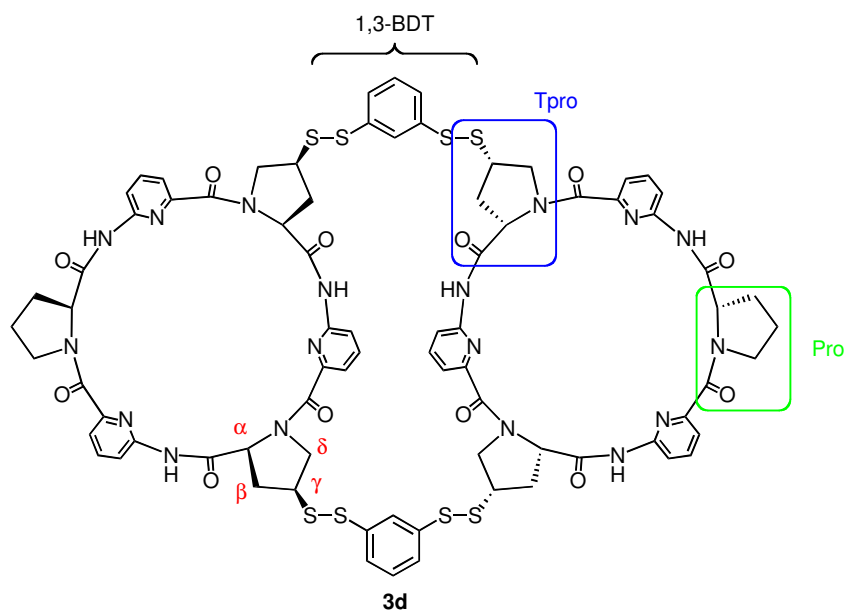


Figure S5: $^1\text{H-NMR}$ spectra of receptor **3c** in 8:2 $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ (a) as the sulfate complex, and (b) in the absence of sulfate. Note the pronounced downfield shifts of the three $\text{H}(\alpha)$ signals caused by the addition of sodium sulfate that are indicative of complex formation.

3.2 Synthesis of receptor **3d**



A solution containing cyclopeptide **3a** (21.33 mg, 0.66 mM, 1 equiv.), 1,3-benzenedithiol **d** (3.27 mL of a 8 mM solution in CH₃CN, 1.35 mM, 2.04 equiv.), Na₂SO₄ (200 μ L of a 0.5 M solution in water, 5 mM, 7.6 equiv.), KOH (170 μ L of a 0.5 M solution in water), water (6.30 mL) and CH₃CN (10.06 mL) was prepared and stirred for 22 days. The solution was acidified with formic acid (400 μ L). BaCl₂ (500 μ L of a solution of 0.25 M in water) was added, causing the precipitation of BaSO₄. The suspension was stirred for 20 min and the precipitate allowed to settle for 30 min. The supernatant was filtered (regular cellulose, 0.45 μ m, 17 mm internal diameter). The solid was washed with a mixture of 2:1 CH₃CN/water and the resulting supernatants filtered. The combination of all the supernatants was reduced under vacuum until a cloudy solution was obtained (~3 mL). DMSO was added until a clear solution was obtained. The resulting solution was immediately purified by semi-preparative HPLC (column: Zorbax Eclipse XDB-C8, 9.4 \times 250 mm, 5 μ m, solvents: water + 0.1% TFA and CH₃CN + 0.1% TFA, elution: 40 to 60% of CH₃CN in 15 min at 4 mL/min, retention time: 14.6-15.4 min). Fractions were concentrated and dried using a Genevac Evaporator (mode: HPLC fraction dry pure at 25 $^{\circ}$ C) to give a white solid [11.67 mg, 52% yield].

The UV trace, the base peak chromatogram and the ESI-MS spectra corresponding to the LC-MS analysis of receptor **3d** are shown in Figure S6.

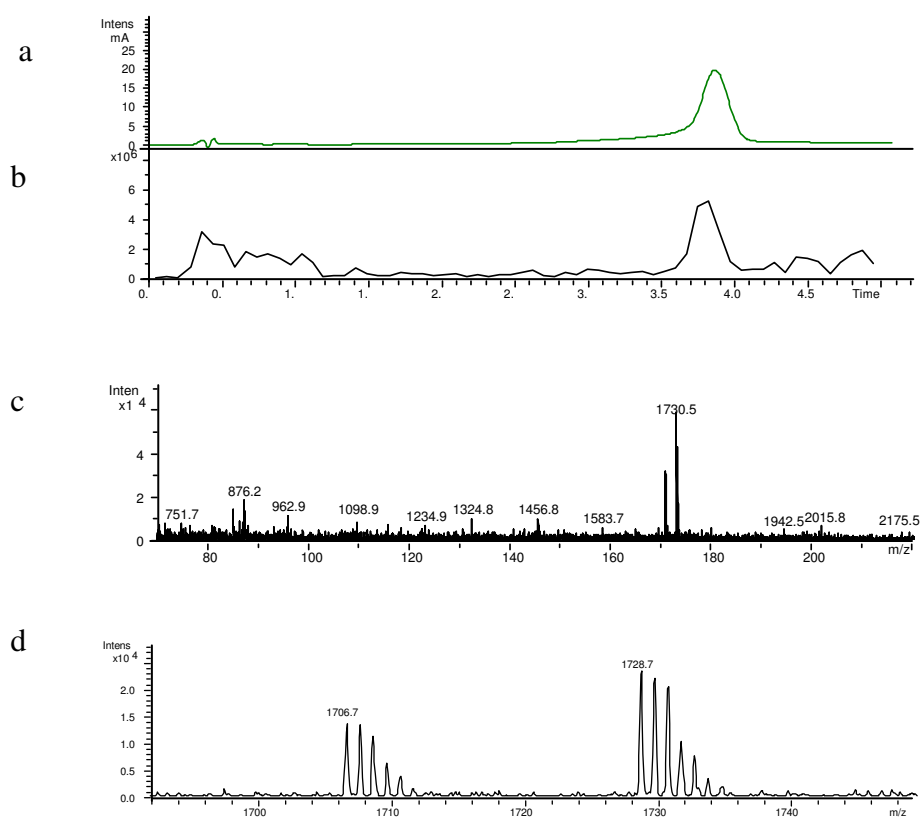


Figure S6: LC-MS analysis of receptor **3d**. (a) UV-chromatogram monitored at 252-256 nm. (b) Base-peak mass chromatogram. (c) Mass spectrum of the main peak in the chromatogram (positive ion mode). (d) Expansion of the mass spectrum showing the singly-charged receptor ion ($M+H^+$: obsd $m/z = 1706.7$; calcd 1707.3 and $M+Na^+$: obsd $m/z = 1728.7$; calcd 1729.31).

The 1H -NMR spectrum of the free receptor **3d** (Figure S7b) was difficult to interpret due to the broadness of the signals presumably caused by the existence of multiple conformations. The NMR data presented below corresponds to the sulfate complex of receptor **3d** (Figure S7a). The inaccuracies observed in the integration are due to the poor phasing produced by the large solvent peaks.

1H -NMR (500 MHz, 8:2 CD_3OD/D_2O) δ 1.72-2.04 (m, 16H, TproH(β) + ProH(β) + ProH(γ)), 3.12 (m, 4H, TproH(γ)), 3.71-3.91 (m, 8H, ProH(δ) + TproH(δ)), 4.07 (m, 4H, TproH(δ)), 6.60 (m, 6H,

TproH(α) + ProH(α), 6.94 (m, 2H, 1,3-BDTH(5)), 7.13 (m, 4H, 1,3-BDTH(4,6)), 7.55-7.49 (m, 2H, 1,3-BDTH(2)), 7.66-7.58 (m, 12H, APAH(3) + APAH(4) + APAH(5)), 7.78 (t, 2H, $^3J(\text{H,H}) = 8.0$ Hz, APAH(4)), 7.83 (2d, 4H, $^3J(\text{H,H}) = 8.0$ Hz, APAH(5)). The NHs were not observed because they exchanged with the deuterium of the solvent.

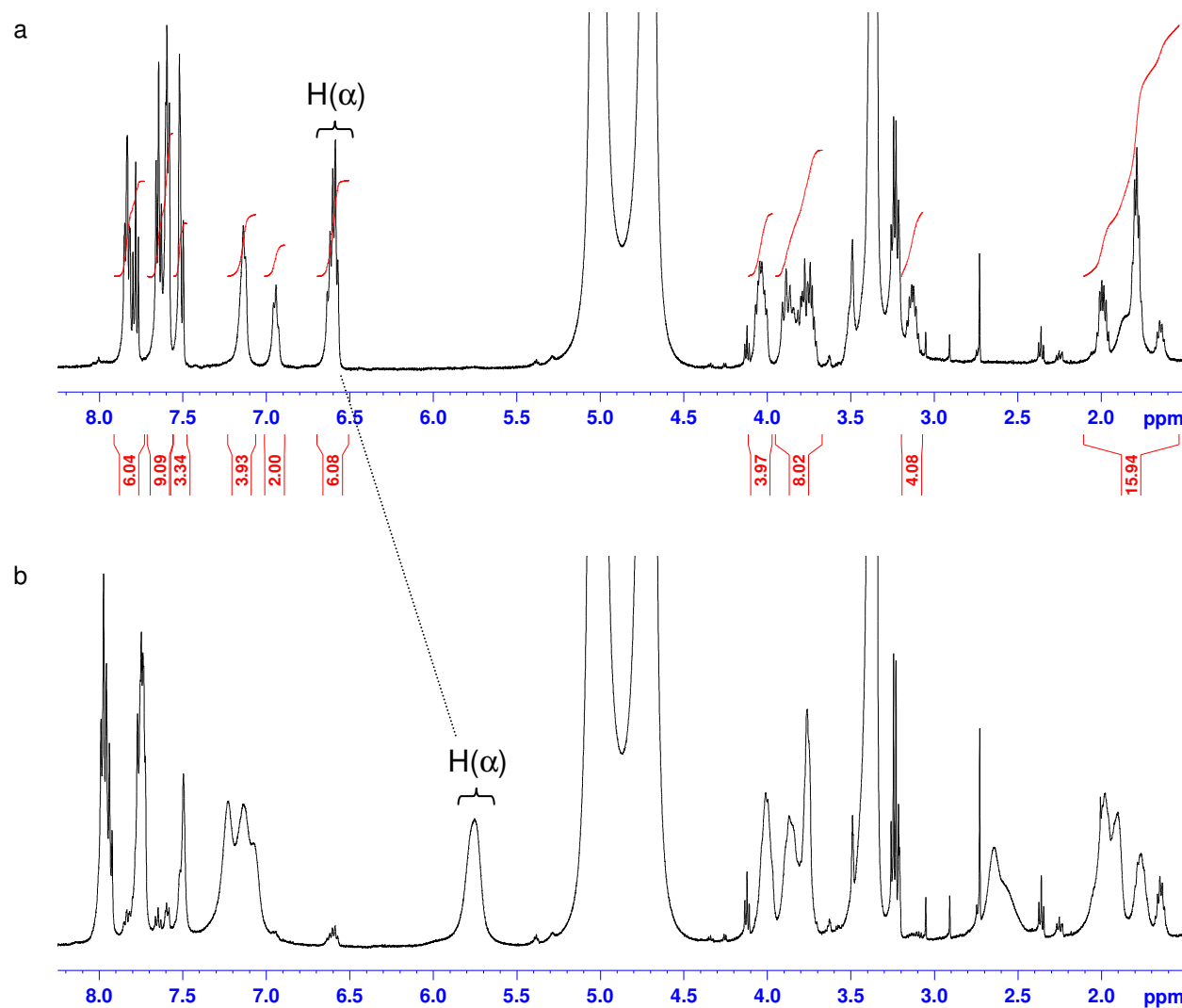


Figure S7: $^1\text{H-NMR}$ spectra in 8:2 $\text{CD}_3\text{OD}/\text{D}_2\text{O}$ of receptor **3d** (a) as the sulfate complex and (b) in the absence of sulfate. Note the pronounced downfield shift of the $\text{H}(\alpha)$ signal caused by the addition of sodium sulfate that is indicative of complex formation.

4. ITC titrations

Equilibrium constants, enthalpies and entropies of binding were determined using isothermal titration calorimetry. The titrations were carried out in mixtures of water and acetonitrile using a MCS-ITC system from Microcal LLC.

The standard ITC experiment involved the titration of a solution of the anion into a solution of the receptor at 298K. For the competition experiments, the solution of the strong binder was titrated into a solution of the receptor saturated with a weaker binder.

In both cases, the anion solution was added in 30 injections of 10 μL , separated by an interval of 180 sec between injections, with the exception of the first addition which was 3 μL . The peak produced by this injection was discarded during data processing. Binding constants and enthalpies of binding were obtained by curve fitting of the titration data using the one-site binding (iodide and bromide) and competitive binding (sulfate and selenate) models available in the Origin 7.0 software.

The different anions and receptors were weighed using an analytical precision balance and dissolved in a known volume with freshly degassed solvent or mixture of solvents and loaded into the system for immediate analysis. Solutions involved in the same titration experiment were made up from the same batch of solvent.

The exact concentrations used and the ITC data is shown in Figures S8 and S9 below.

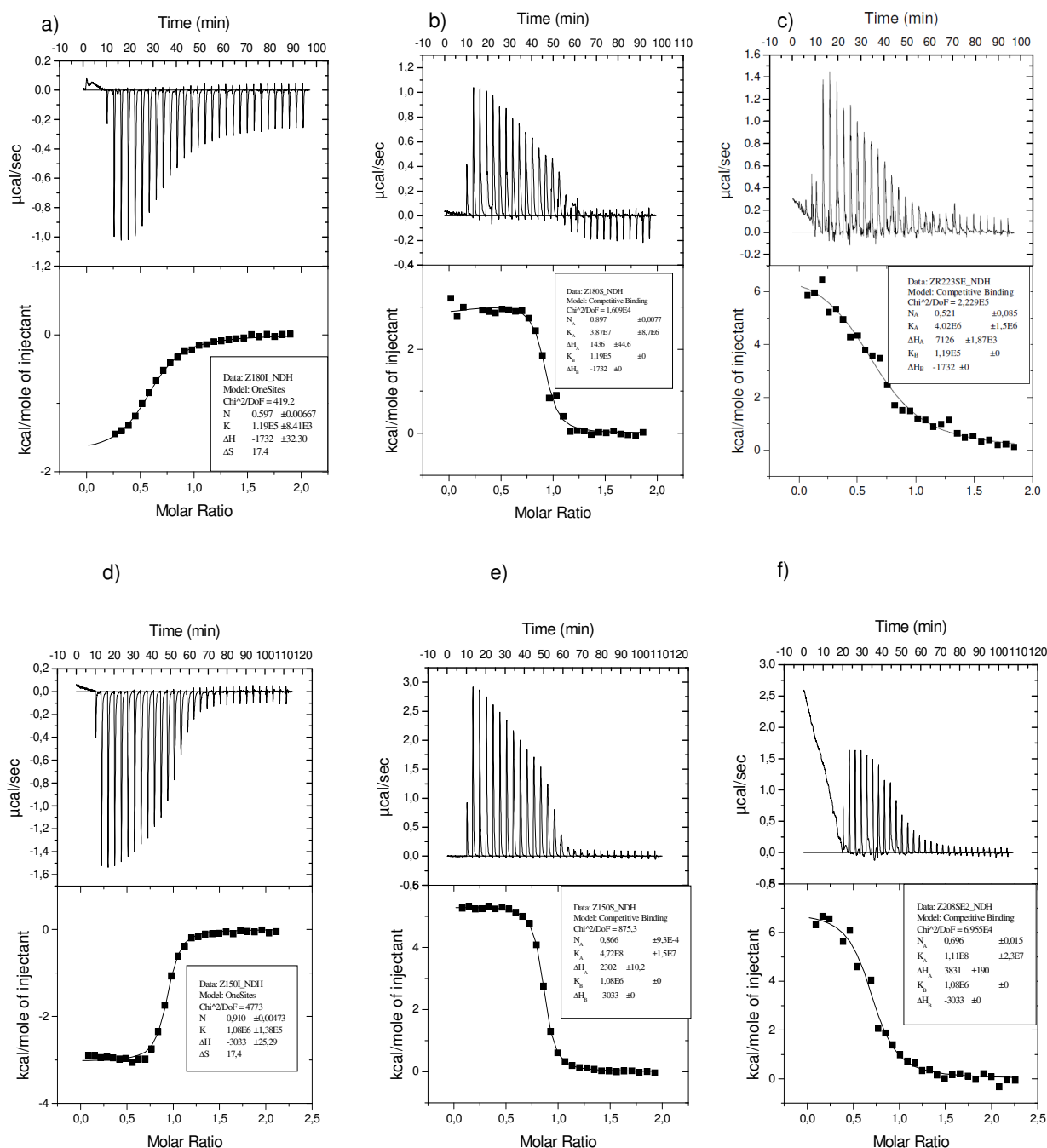


Figure S8: ITC traces and binding isotherms for the titrations of (a) receptor **3d** (0.15 mM) with KI (1.6 mM) (b) receptor **3d** (0.15 mM) and KI (0.22 mM) with Na_2SO_4 (1.2 mM), (c) receptor **3d** (0.093 mM) and KI (0.28 mM) with Na_2SeO_4 (0.73 mM), (d) receptor **3c** (0.18 mM) with KI (1.6 mM) and (e) receptor **3c** (0.19 mM) and KI (0.29 mM) with Na_2SO_4 (1.6 mM) and (f) receptor **3c** (0.068 mM) and KI (0.20 mM) with Na_2SeO_4 (0.66 mM) in 41 mol% acetonitrile in water at 298 K.

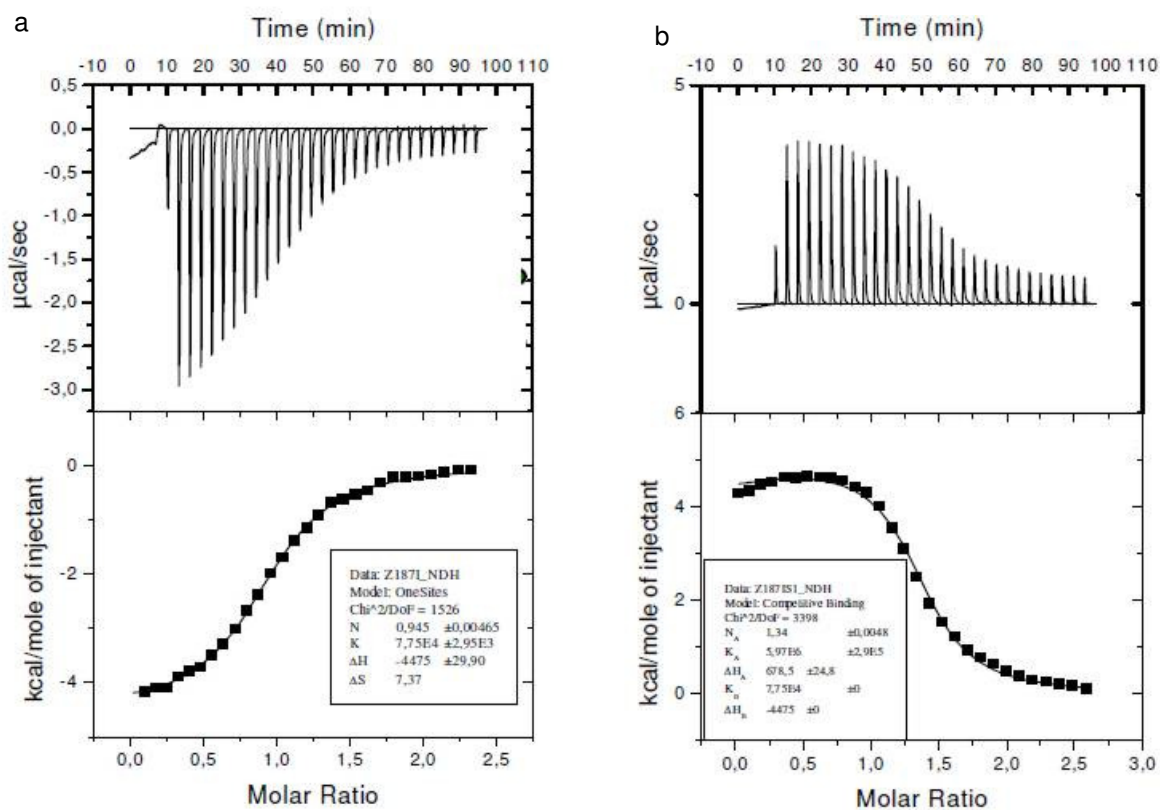


Figure S9: ITC traces and binding isotherms for the titrations of (a) receptor **2c** (0.20 mM) with KI (2.0 mM) (b) receptor **3c** (0.16 mM) and KI (0.38 mM) with Na_2SO_4 (1.8 mM) in 41 mol% acetonitrile in water at 298 K.

5. References

- S1. Kubik, S.; Goddard, R.; Kirchner, R.; Nolting, D.; Seidel J. *Angew. Chem.* **2001**, *113*, 2722-2725; *Angew. Chem. Int. Ed.* **2001**, *40*, 2648-2651 (Supporting Information).
- S2. Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437-2446.
- S3. Fiehn, T.; Goddard, R.; Seidel, R. W.; Kubik, S. *Chem. Eur. J.* **2010**, *16*, 7241-7255.
- S4. Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804-7805.