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Dynamic Combinatorial Optimization of a Neutral Receptor That Binds Inorganic Anions in Aqueous Solution

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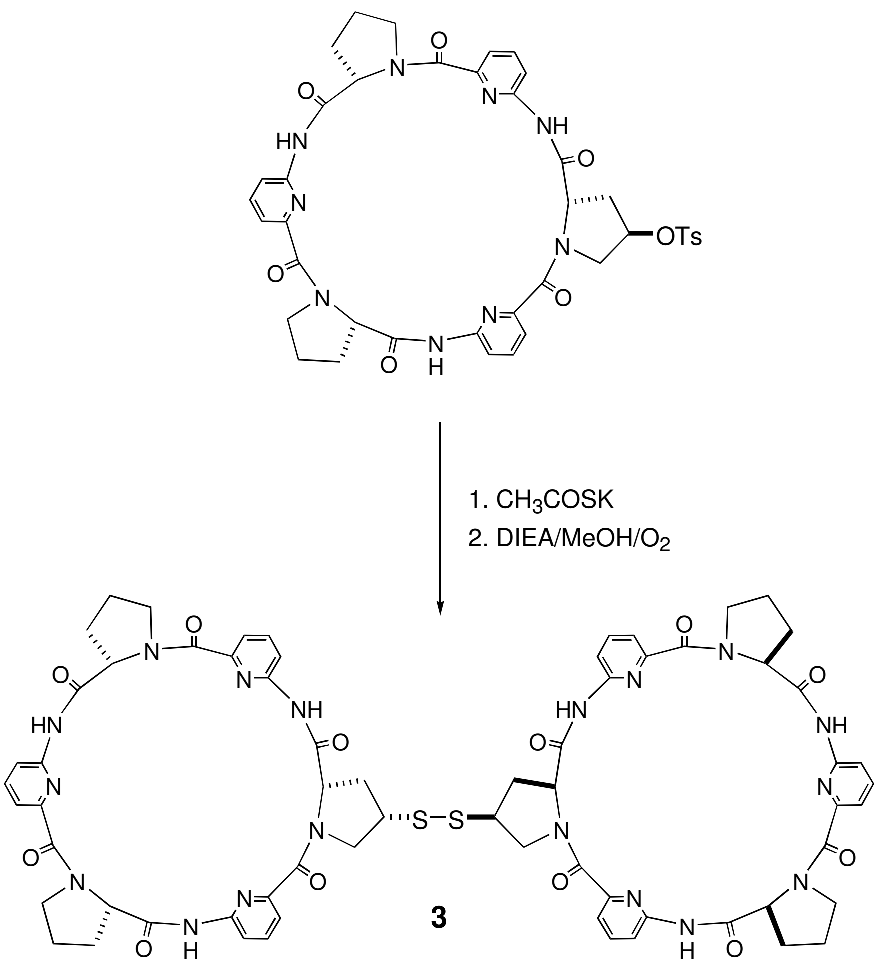
Synthesis of compound **3**:



General Methods. Analyses were carried out as follows: melting points, Büchi 510 apparatus; optical rotation, Perkin Elmer 241 MC digital polarimeter (d = 10 cm); NMR, Bruker DRX 500 equipped with an automatic sampler; FAB MS, Finnigan MAT 8200; ESI MS, Bruker Esquire 3000; elemental analysis, Pharmaceutical Institute of the Heinrich-Heine-University, Düsseldorf; RP chromatography, MERCK LiChroprep RP-8 (40-63 μm) prepacked column size B (310-25), and MERCK LiChroprep RP-18 (40-63 μm) prepacked column size A (240-10). The following abbreviations are used: DIEA, *N*-ethyldiisopropylamine; TFA, trifluoroacetic acid; Hyp, L-hydroxyproline; Pro, L-proline; APA, 6-aminopicolinic acid; BDT, 1,3-benzenedithiol.

Bis(cyclopeptide) disulfide (3). The previously described cyclohexapeptide tosylate[1] (660 mg, 0.8 mmol) and potassium thioacetate (330 mg, 8.0 mmol) were stirred at 80°C in DMF (20 mL) for 3 h. Afterward, the solvent was evaporated *in vacuo*, and the product was isolated from the residue by chromatographic workup using a silica column and acetone as eluent. Ca. 1 L of acetone is required for complete elution of the product. The fractions containing pure product were combined, the solvent was removed *in vacuo*, and the residue was triturated with water. The solid formed was filtered off, washed with water and diethyl ether, and dried. It was used for the next step without further purification. Yield: 0.52 g (90%). For this, the acetylated cyclopeptide thiol (520 mg, 0.72 mmol) was dissolved in dry methanol (50 mL). After the addition of DIEA (0.5 mL), the resulting reaction mixture was stirred for 2 d at room temperature while passing a light stream of air through. Then, the solvent was removed *in vacuo*, and the remaining crude product was 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/H_2O, 1:10. The eluent composition was gradually changed to 1,4-dioxane/H_2O, 1:2, with which pure product eluted. Trituration of the residue remaining after evaporation of the solvent with diethyl ether afforded an off white solid, which was dried *in vacuo*. Yield 0.36 g (73%); mp. > 250°C; $[\alpha]_D^{25}$ = -568.4 (c = 2, DMF); ^{1}H-NMR (500 MHz, [d$_6$]DMSO, 25°C, TMS) δ 1.85 (m, 8H; ProC(γ)H_2), 2.04 (m, 4H; ProC(β)H), 2.15 (m, 2H; HypC(β)H), 2.57 (m, 4H; ProC(β)CH), 2.98 (m, 2H; HypC(β)H), 3.54 (m, 2H; HypC(δ)H), 3.60 (m, 4H; ProC(δ)H), 3.71 (m,

[1] Kubik, S.; Kirchner, R.; Nolting, D.; Seidel, J. *J. Am. Chem. Soc.* **2002**, *124*, 12752-12760.

6H; ProC(δ)H + HypC(γ)H), 4.05 (m, 2H; HypC(δ)H), 5.56 (m, 2H; HypC(α)H), 5.63 (m, 4H; ProC(α)H), 7.22 (d, ^{3}J = 8.2 Hz, 4H; APAH(3)), 7.26 (d, ^{3}J = 8.2 Hz, 2H; APAH(3)), 7.42 (m, 4H; APAH(5)), 7.47 (d, ^{3}J = 7.6 Hz, 2H; APAH(5)), 7.74 (m, 6H; APAH(4)), 9.59 (s, 4H; APANH), 9.72 (s, 2H; APANH); ^{13}C-NMR (125 MHz, [d_6]DMSO, 25°C, TMS) δ 22.2 + 22.3 (ProC(γ)), 32.4 (ProC(β)), 37.4 (HypC(β)), 44.4 (HypC(γ)), 48.0 (ProC(δ)), 53.2 (HypC(δ)), 61.2 + 61.3 + 61.4 (ProC(α) + HypC(α)), 115.4 + 115.7 + 115.9 (APAC(3)), 119.6 + 119.7 (APAC(5)), 138.8 + 138.9 + 139.0 (APAC(4)), 148.4 (APAC(2)), 151.3 + 151.8 + 151.9 (APAC(6)), 165.7 + 165.8 + 165.9 (APACO), 170.8 + 171.0 (HypCO/ProCO); $C_{66}H_{64}N_{18}O_{12}S_2 \cdot 7H_2O$ (1491.6): calcd C 53.15, H 5.27, N 16.90; found C 53.18, H 4.98, N 16.64; FAB-MS: *m/z* (relative intensity): 1365 (30) [$M+H^+$], 1387 (20) [$M+Na^+$].

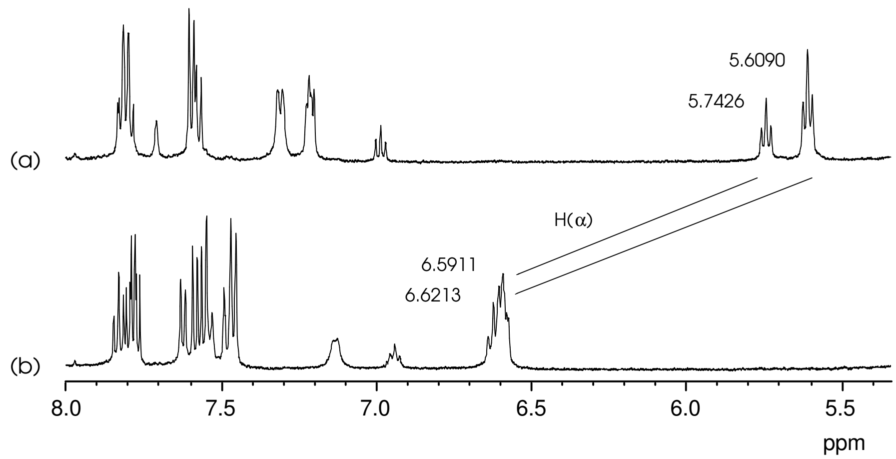
General procedures for generating and analyzing DCLs. A stock solution was prepared containing the required dithiols at an overall concentration of 4.0 mM (for the biased libraries) or 8.0 mM (for the library shown in Fig. 1) in 10 mL of water and the pH was adjusted to 8.0 using KOH. To 0.5 mL aliquots of this mixture was added a solution of disulfide **3** (2.74 mg, 2.0 mmol) in 1 mL of acetonitrile. Aliquots of 0.5 mL of the resulting solution were transferred into 2 mL HPLC vials containing the guest (5 mmol), as desired. The vials were capped and stirred for 7 days. The resulting DCLs were analyzed using ESI-MS (injection of 10 µl into a 4 µl/min stream of 1:1 acetonitrile/water entering a Micromass Quattro LC mass spectrometer) and HPLC (Waters Symmetry C18 column, acetonitrile/water gradient using 0.1% TFA).

Synthesis of receptors 3b and 3c. Cyclopeptide disulfide **3** (75 mg, 0.055 mmol) was dissolved in a 10 mM solution of Na_2SO_4 in 2:1 (v/v) acetonitrile/water (75 mL). After the addition of dithiol linker **b** or **c** (0.055 mmol), the pH of the reaction mixture was adjusted to 8.5 with 1M aqueous NaOH. The resulting solution was stirred for 4 d at room temperature in an open flask. Then, 1M HCl was added until a pH of 3 was reached, and the solvent was evaporated *in vacuo*. During this and every other evaporation step, care was taken that the solution did not exceed a temperature of 30°C. The residue was suspended in CH_2Cl_2/MeOH, 10:1 and subjected to a silica column. The methanol content of the eluent was increased gradually until the product eluted. Product fractions were evaporated to dryness, and a second chromatographic purification step was carried out. For this, the product was dissolved in a small amount of DMSO and applied to a RP-18 column conditioned with 1,4-dioxane/H_2O, 1:10. The eluent composition was gradually changed until pure product eluted. Trituration of the residue remaining after evaporation of the solvent with diethyl ether afforded a white solid, which was dried *in vacuo*.

3b: Eluent gradient for the silica column: CH_2Cl_2/MeOH, 10:1 - 7.5:1 - 5:1 - 2.5:1. Eluent gradient for the RP-18 column: 1,4-dioxane/H_2O, 1:10 - 1:6 - 1:3 - 1:2. A mixture of the product and a side product was eluted from the RP-18 column with 1,4-dioxane/H_2O 1:3. Therefore, elution was continued with this solvent until no side product could be detected in the collected fractions any more. Only then was the last solvent mixture used. $C_{69}H_{70}N_{18}O_{13}S_4\cdot 7.5H_2O$ (1622.8): calcd C 51.07, H 5.28, N 15.54; found C 51.41, H 5.14, N 15.16; ESI-MS: *m/z* (relative intensity): 1489.2 (10) [M+H$^+$; calcd 1487.4]; 1510.0 (100) [M+Na$^+$; calcd 1509.4].

3c: Eluent gradient for the silica column: CH_2Cl_2/MeOH, 10:1 - 7.5:1 - 5:1 - 2.5:1. Eluent gradient for the RP-18 column: 1,4-dioxane/H_2O, 1:10 - 1:6 - 1:3 - 1:1.5. ^{1}H-NMR (500 MHz, D_2O/CD_3OD 1:2, 25°C, TMS) δ 1.95 (m, 8H; ProC(γ)H_2), 2.06 (m, 4H; ProC(β)H), 2.22 (m, 2H; HypC(β)H), 2.65 (m, 4H; ProC(β)H), 2.81 (m, 2H; HypC(β)H), 3.66 - 3.85 (m, b, 12H; ProC(δ)H + HypC(δ)H), 4.03 (q, 2H; HypC(γ)H), 5.61 (t, ^3J = 7.6 Hz, 4H; ProC(α)H), 5.74 (t, ^3J = 7.6 Hz, 2H; HypC(α)H), 6.99 (t, ^3J = 7.6 Hz, 1H; BDTH(5)), 7.21 (m, 4H; APAH(3)), 7.31 (m, 4H; APAH(5)), 7.59 (m, 6H; APAH(3) + APAH(5) + BDTH(3) + BDTH(5)), 7.71 (s, 1H; BDTH(2)), 7.81 (m, 6H; APAH(4)); $C_{72}H_{68}N_{18}O_{12}S_4\cdot 6.5H_2O$ (1622.8): calcd C 53.29, H 5.03, N 15.54; found C 53.56, H 4.70, N 15.23; ESI-MS: *m/z* (relative intensity): 1505.5 (5) [M+H$^+$; calcd 1505.4]; 1527.9 (100) [M+Na$^+$; calcd 1527.4].

[1]H-NMR spectra: **3c** (0.5 mM) in D_2O/CD_3OD 1:2 (a), **3c** (0.5 mM) + 2 equiv of Na_2SO_4 in D_2O/CD_3OD 1:2 (b).



Isothermal microcalorimetric titration: **3c** (0.052 mM) with K_2SO_4 (0.50 mM) recorded in 2:1 (v/v) acetonitrile/water at 298 K. The upper graph shows the measured heat pulses. The molar heats per pulse are depicted along with the curve fit (solid line) in the lower diagram.