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Cyclic hexapeptides with free carboxylate groups as new receptors for monosaccharides

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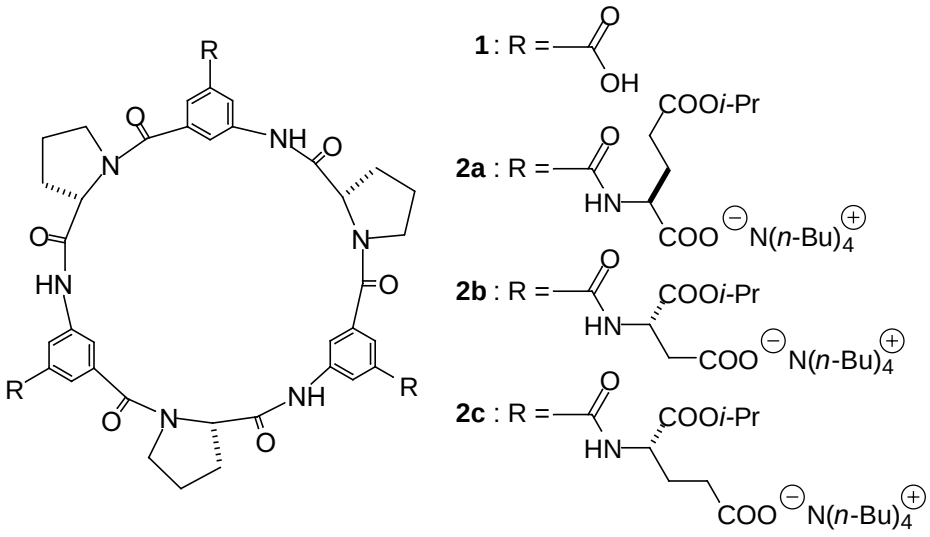
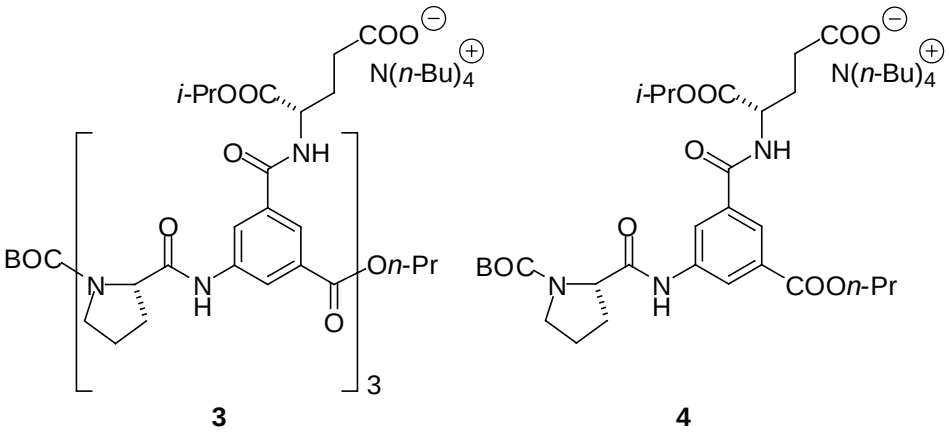
Structures of compounds **1** and **2a**-**c**:

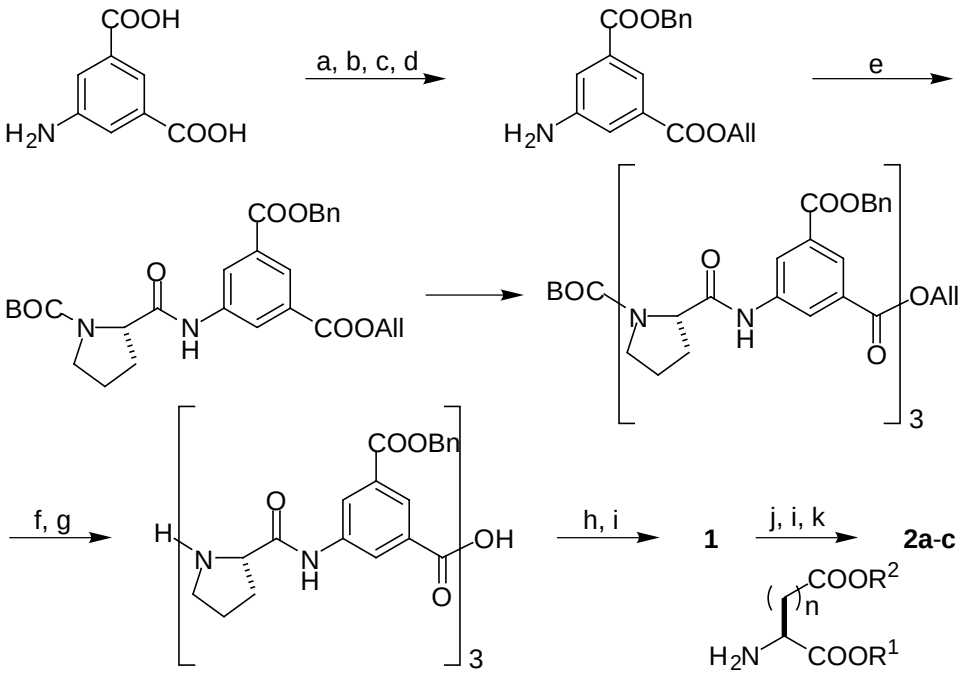


Structures of compounds **3** and **4**:



3 **4**

Synthesis of compounds **2a**-**2c**:



a. BOC_2O; b. $AllBr/NaHCO_3$; c. $BnBr/NaHCO_3$; d. TFA; e. BOC-L-proline/PyCloP/DIEA; f. $[Pd(P(4\text{-}Me_2NPh)Ph_2)_4]$/morpholine; g. HCl/1,4-dioxane; h. TBTU/DIEA; i. H_2/10% Pd/C; j. **2a**: n = 2, R^1 = Bn, R^2 = *i*-Pr; **2b**: n = 1, R^1 = *i*-Pr, R^2 = Bn; **2c**: n = 2, R^1 = *i*-Pr, R^2 = Bn, PyCloP/DIEA; k. $N(n\text{-}Bu)_4^+OH^-$.

General Methods. Analyses were carried out as follows: melting points, Büchi 510 apparatus;

optical rotation, Perkin Elmer MC digital polarimeter (d = 10 cm); NMR, Varian VXR 300, Bruker DRX 500 equipped with an automatic sampler; CI MS, Finnigan INCOS 50; FAB MS, Finnigan MAT 8200; ESI MS, Bruker Esquire 3000; elemental analysis, Pharmaceutical Institute of the Heinrich-Heine-University, Düsseldorf; chromatography, ICN silica gel 32-63 (ICN Biomedicals). The following abbreviations are used: BOC, *tert*-butoxy carbonyl, DIEA, *N*-ethyldiisopropylamine; TFA, trifluoroacetic acid; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; TBTU, O-(1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate; Pro, L-proline; AIA, 5-amino isophthalic acid; DMDPd, tetrakis[4-(*N,N*-dimethylamino)phenyldiphenylphosphino]-palladium(0); DCC, *N*,*N*′-dicyclohexylcarbodiimide; DCU *N*,*N*′-dicyclohexylurea.

Materials. All solvents were dried following standard procedures before use. DMF p.a. was purchased from Fluka and used without further purification. PyCloP (J. Coste, E. Frérot, P. Jouin, *J. Org. Chem.* **1994**, *59*, 2437-2446) and BOC-(L)-Glu(O*i*Pr)-OH (S. Kubik, *J. Am. Chem. Soc.* **1999**, *121*, 5846-5855) were prepared according to the literature procedures. TBTU was purchased from BACHEM. BOC-(L)-Asp(OBn)-OH, BOC-(L)-Proline, and BOC-(L)-Glu(OBn)-OH were purchased from NOVABIOCHEM.

Host-Guest Titrations. Stock solutions of the guest (0.1 µmol/100 µL) and the cyclopeptide (2 µmol/800 µl) in 4% $CD_3OD/CDCl_3$ were prepared. In total, 11 NMR tubes were set up by adding increasing amounts of the host solution (0 - 800 µl) to 100 µl of the guest solution. All samples were made up to a volume of 1 mL with 4% $CD_3OD/CDCl_3$ and the respective ^{1}H-NMR spectra were recorded. The chemical shift of prominent guest protons were plotted against the host concentration. From the resulting saturation curves, K_a and $\Delta\delta_{max}$ were calculated using the SIGMA Plot 3.0 (Jandel Scientific) software package.

Job Plots. Equimolar solutions (1 mM) of guest and host in 4% $CD_3OD/CDCl_3$ were prepared and mixed in various ratios. ^{1}H-NMR spectra of the resulting solutions were recorded, and the change in chemical shift of prominent protons was analyzed.

General Procedure for the Cleavage of *N-tert*-Butoxycarbonyl Groups. Method A. The carbamate was dissolved in CH_2Cl_2 (10 mL) After cooling with an ice bath, TFA (10 mL) was added dropwise. The mixture was stirred for 1.5 h at 0 - 5°C and then concentrated to dryness in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed twice with 10%

aqueous Na_2CO_3 and three times with water. The organic layer and was dried, HCl conc. (1.1 equiv.) was added, and the mixture was evaporated to dryness in vacuo. **Method B.** The carbamante was suspended in 1,4-dioxane (20 mL/mmol). After cooling with an ice bath, a 6 N solution of HCl in 1,4-dioxane (40 mL/mmol) was added dropwise. The reaction mixture was stirred for 2 h at 0°C and then evaporated in vacuo.

General Procedure for the Cleavage of Allyl Esters. Method C. The ester was dissolved in THF (20 mL/mmol) under inert conditions. DMDPd (10 mg) and morpholine (2 equiv.) were added, and the reaction mixture was stirred for 30 min at room temperature. Completion of the reaction was checked by TLC. Afterward, the solvent was evaporated in vacuo, the residue was redissolved in ethyl acetate, and the organic layer was extracted three times with 4 N hydrochloric acid and three times with water. After drying, the solvent was removed in vacuo.

General Procedure for the Cleavage of Benzyl Esters. Method D. The ester was dissolved in CH_2Cl_2/methanol 1:1 (300 mL/mmol). After the addition of 10% Pd/C (100 mg) the mixture was subjected to hydrogenation at atmospheric pressure for about 2 h. Completion of the reaction was checked by TLC. The catalyst was removed by filtering the solution first through a glass frit (P4) and then through a membrane filter. The filtrate was evaporated, and the product was dried at 50°C in vacuo.

General Procedure for the Syntheses of Tetra-*n*-butyl Ammonium Carboxylates. Method E. The carboxylic acid was dissolved in methanol (300 mL/mmol). Tetra-*n*-butyl ammonium hydroxide (1 equiv. per carboxylic acid group, 1.0 M solution in methanol) was added. The solution was evaporated to dryness, and the product was dried at 50°C in vacuo.

5-[(*tert*-Butyloxycarbonyl)amino]-isophthalic acid. 5-Aminoisophthalic acid (18.12 g, 0.10 mol) was dissolved in aqueous sodium hydroxide (1 N, 200 mL). The mixture was cooled with an ice bath, and a solution of di-*tert*-butyl dicarbonate (24.00 g, 0.11 mol) in 1,4-dioxane (200 mL) was added dropwise within 2 h. The reaction mixture was stirred at 0 - 5°C for 3 h and then overnight at room temperature. The solvent was subsequently evaporated to about half its original volume. After cooling with an ice bath, this solution was acidified to pH 5 with 20% aqueous $KHSO_4$. The precipitate was filtered off, washed with water, and dried over P_4O_{10} in vacuo. Yield 27.55 g (98%); mp. >250°C; ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS) δ 1.50 (s, 9H; *t*Bu), 8.09 (t, 1H, ^{4}J = 1.3

Hz; AIA-H(6)), 8.32 (s, 2H; AIA-H(2) + AIA-H(4)), 9.78 (s, 1H; NH), 13.19 (s, b, 2H; 2×COOH); $C_{13}H_{15}NO_6 \cdot 0.25\ H_2O$ (285.77): calcd C 54.64 H 5.47 N 4.90; found C 54.49 H 5.47 N 4.76; CI-MS (NH_3): m/z (relative intensity): 299 (100) [M + NH_4^+].

5-[(*tert*-Butyloxycarbonyl)amino]-isophthalic acid mono allyl ester. A mixture of 5-[(*tert*-butyloxycarbonyl)amino]-isophthalic acid (2.80 g, 10.0 mmol) and $NaHCO_3$ (0.84 g , 10.0 mmol) was suspended in DMF (50 mL) and stirred overnight. Allyl bromide (2.5 mL, 30.0 mmol) was then added, and stirring was continued for another 24 h. After the addition of aqueous Na_2CO_3 (2.12 g in 250 mL), the resulting mixture was extracted three times with diethyl ether. The organic layers were discarded. The aqueous layer was acidified with 10% aqueous $KHSO_4$ and extracted three times with ethyl acetate. The combined organic layers were dried and concentrated to dryness in vacuo. The remaining oil was heated to reflux in toluene (200 mL), and the hot suspension was passed through a layer of Celite. Upon cooling, pure product crystallized. It was filtered off, washed with a small amount of cold toluene, and dried in vacuo. Yield 1.10 g (34%); mp. 163°C (dec.); ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS) δ 1.50 (s, 9H; *t*Bu), 4.83 (ddd, 2H, ^3J = 5.1 Hz; $CH_2CH=CH_2$), 5.30 (ddt, 1H, ^3J = 10.8 Hz, ^2J = 1.6 Hz; $CH_2CH=CH_2$, *cis*), 5.42 (ddt, 1H, ^3J = 17.0 Hz, ^2J = 1.6 Hz; $CH_2CH=CH_2$, *trans*), 6.01 - 6.10 (m, 1H; $CH_2CH=CH_2$), 8.10 (s, 1H; AIA-H(6)), 8.36 (s, 2H; AIA-H(2) + AIA-H(4)), 9.83 (s, 1H; NH), 13.28 (s, b, 1H; COOH); $C_{16}H_{19}NO_6$ (321.33): calcd C 59.80 H 5.96 N 4.36; found C 60.00 H 5.77 N 4.33; CI-MS (NH_3): m/z (relative intensity): 339 (100) [M + NH_4^+].

5-[(*tert*-Butyloxycarbonyl)amino]-isophthalic acid mono allyl mono benzyl ester. 5-[(*tert*-Butyloxycarbonyl)amino]-isophthalic acid mono allyl ester (1.29 g, 4.0 mmol) and $NaHCO_3$ (1.01 g, 12.0 mmol) were suspended in DMF (50 mL). Benzyl bromide (1.5 mL, 12.0 mmol) was added, and the reaction mixture was stirred for 48 h at room temperature. After the addition of 120 mL ethyl acetate, the mixture was washed twice with 10 % aqueous $NaHCO_3$, and three times with water. The organic layer was dried and concentrated to dryness in vacuo. The product was suspended in a small amount of cold diethyl ether, filtered off, and dried in vacuo. Yield 1.33 g (81%); mp. 125 - 127°C; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS) δ 1.52 (s, 9H; *t*Bu), 4.83 (ddd, 2H, ^3J = 5.7 Hz; $CH_2CH=CH_2$), 5.29 (ddt, 1H, ^3J = 10.4 Hz, ^2J = 1.3 Hz; $CH_2CH=CH_2$, *cis*), 5.38 (s, 2H; Bn-CH_2), 5.40 (ddt, 1H, ^3J = 17.0 Hz, ^2J = 1.3 Hz; $CH_2CH=CH_2$, *trans*), 5.98 - 6.07 (m, 1H;

$CH_2C\underline{H}=CH_2$), 6.73 (s, b, 1H; N$\underline{H}$), 7.32 - 7.46 (m, 5H; Bn-$\underline{H}$), 8.24 + 8.30 (2 × s, b, 2H; AIA-$\underline{H}$(2) + AIA-$\underline{H}$(4)), 8.40 (t, 1H, ^4J = 1.6 Hz; AIA-$\underline{H}$(6)); $C_{23}H_{25}NO_6$ (411.45): calcd C 67.14 H 6.12 N 3.40; found C 66.89 H 6.26 N 3.18; CI-MS (NH_3): m/z (relative intensity): 429 (100) [M + NH_4^+].

Dipeptide BOC-(L)-Pro-AIA(OBn)-OAll. Prior to coupling, 5-[(*tert*-butyloxycarbonyl)amino]-isophthalic acid mono allyl mono benzyl ester, was deprotected at the amino group according to **Method A** only that the free amine was not converted to the hydrochloride. The resulting free amine (3.81 g, 12.2 mmol), BOC-L-proline (3.93 g, 18.4 mmol), and PyCloP (7.67 g, 18.4 mmol) were dissolved in CH_2Cl_2 (260 mL). DIEA (6.35 mL, 36.7 mmol) was added, and the solution was stirred overnight. The solvent was removed in vacuo, and the dipeptide was isolated from the residue by chromatographic workup (hexane/ethyl acetate 1:1). The product crystallized on drying in vacuo. Yield 6.15 g (99%); mp. 118 - 119°C; $[\alpha]_D^{25}$= -82.0° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.34 (s, 9H; *t*Bu), 1.75 - 1.90 (m, 1H; Pro-$\underline{H}$(β)), 1.90 - 2.00 (m, 2H; Pro-$\underline{H}$(γ)), 2.13 - 2.31 (m, 1H; Pro-$\underline{H}$(β)), 3.33 - 3.51 (m, 2H; Pro-$\underline{H}$(δ)), 4.24 (dd, 1H; $^3J_{ae}$ = 4.1 Hz, $^3J_{aa}$ = 8.3 Hz, Pro-$\underline{H}$(α)), 4.83 (ddd, 2H, ^3J = 5.6 Hz; $C\underline{H}_2CH=CH_2$), 5.28 (ddt, 1H, ^3J = 10.5 Hz, ^2J = 1.5 Hz; $CH_2CH=C\underline{H}_2$, *cis*), 5.39 (s, 2H; Bn-$C\underline{H}_2$), 5.40 (ddt, 1H, ^3J = 17.2 Hz, ^2J = 1.5 Hz; $CH_2CH=C\underline{H}_2$, *trans*), 6.00 - 6.12 (m, 1H; $CH_2C\underline{H}=CH_2$), 7.31 - 7.49 (m, 5H; Bn-$\underline{H}$), 8.22 (t, 1H, ^4J = 1.6 Hz; AIA-$\underline{H}$(6)), 8.49 + 8.53 (2 × t, 2 × 1H, ^4J = 1.6 Hz; AIA-$\underline{H}$(2) + AIA-$\underline{H}$(4)), 10.12 (s, 1H, N$\underline{H}$); $C_{28}H_{32}N_2O_7$ (508.57): calcd C 66.13 H 6.34 N 5.51; found C 66.18 H 6.33 N 5.51; CI-MS (NH_3): m/z (relative intensity): 526 (100) [M + NH_4^+], 509 (4) [M + H^+].

Tetrapeptide BOC-[(L)-Pro-AIA(OBn)]$_2$-OAll. Prior to coupling, equimolar amounts of BOC-(L)-Pro-AIA(OBn)-OAll were deprotected at the amino group as described in **Method A** and at the allyl ester carboxyl group as described in **Method C**. Both products were dissolved in CH_2Cl_2 (25 mL/μmol). PyCloP (1.5 equiv) and DIEA (4.0 equiv) were added, and the solution was stirred overnight. The solvent was then evaporated in vacuo, and the residue was purified chromatographically (ethyl acetate). The product crystallized upon drying in vacuo.

Hexapeptide BOC-[(L)-Pro-AIA(OBn)]$_3$-OAll. Prior to coupling, the dipeptide BOC-(L)-Pro-AIA(OBn)-OAll was deprotected at the allyl ester carboxyl group according to **Method C.** The tetrapeptide BOC-[(L)-Pro-AIA(OBn)]$_2$-OAll was deprotected at the amino group according to **Method A.** Both products as well as PyCloP (1.5 equiv.) were dissolved in CH_2Cl_2 (25 mL/μmol).

DIEA (4 equiv) was added, and the solution was stirred overnight. The solvent was then evaporated in vacuo, and the residue was purified chromatographically (ethyl acetate/isopropanol 10:1). After evaporation of the solvent the product was dried in vacuo.

Cyclopeptide *cyclo*-[(L)-Pro-AIA(OBn)]$_3$. The linear hexapeptide BOC-[(L)-Pro-AIA(OBn)]$_3$-OAll (3.40 g, 2.81 mmol) was deprotected at the allyl ester carboxyl group according to **Method C**. The BOC group was cleaved as described in **Method B**. The product was triturated with diethyl ether to afford a white solid. The resulting deprotected hexapeptide (3.10 g, 2.77 mmol) and DIEA (1.54 mL, 8.9 mmol) were dissolved in DMF (300 mL), and the solution was heated to 90°C. A solution of TBTU (0.99 g, 3.1 mmol) in DMF (40 mL) was added dropwise within 2 h. After stirring for 3 h at the same temperature, the solvent was evaporated in vacuo. The product was isolated from the residue by two successive chromatographic steps (1. methanol/isopropanol 10:1; 2. chloroform/acetone 2:1). Finally, the product was recrystallized from methanol. Yield 1.27 g (43%); mp. >250°C (softening from 164°C); $[\alpha]_D^{25}$= 7.8° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D6]DMSO , 25°C, TMS) δ 1.81 - 2.09 (m, 9H; 3 × Pro-H(β) + 6 × Pro-H(γ)), 2.25 - 2.38 (m, 3H; 3 × Pro-H(β)), 3.22 - 3.51 (m, 6H; Pro-H(δ)), 4.65 (dd, 3H; $^3J_{ae}$ = 4.1 Hz, $^3J_{aa}$ = 8.4 Hz, Pro-H(α)), 5.37 (s, 6H; Bn-CH_2), 7.35 - 7.54 (m, 15H; Bn-H), 7.67 (t, 3H, ^{4}J = 1.7 Hz; AIA-H(6)), 7.91 (t, 3H, ^{4}J = 1.7 Hz; AIA-H(4)), 8.66 (t, 3H, ^{4}J = 1.7 Hz; AIA-H(2)), 10.61 (s, 3H, NH); $C_{60}H_{54}N_6O_{21}\cdot 2\ H_2O$ (508.57): calcd C 66.29 H 5.38 N 7.73; found C 66.25 H 5.17 N 7.52; FAB-MS: m/z (relative intensity): 1051 (7) [M + H$^+$].

***cyclo*-[(L)-Pro-AIA(OH)]$_3$ (1).** *cyclo*-[(L)-Pro-AIA(OBn)]$_3$ (0.46 g, 0.44 mmol) was dissolved in CH_2Cl_2 (80 mL). After the addition of methanol (200 mL) and 10% Pd/C (100 mg) the mixture was subjected to hydrogenation at atmospheric pressure for about 2 h. Completion of the reaction was checked by TLC. The catalyst was then filtered off by passage through a layer of Celite and washed with CH_2Cl_2/methanol (1:2.5). The combined filtrate and washings were evaporated to dryness in vacuo. The product was used for the next step without further purification. Yield: 0.34 g (99%).

BOC-(L)-Glu(O*i*Pr)-OBn. BOC-(L)-Glu(O*i*Pr)-OH (5.79 g, 20.0 mmol) and $NaHCO_3$ (5.04 g, 60.0 mmol) were suspended in DMF (90 mL). Benzyl bromide (7.5 mL, 60.0 mmol) was added and the reaction mixture was stirred for 48 h at room temperature. After the addition of ethyl acetate (220 mL), the mixture was washed twice with 10 % aqueous $NaHCO_3$, and three times with water.

The organic layer was dried and concentrated to dryness in vacuo. The product was dissolved in a small amount of diethyl ether (50 mL). Hexane (25 mL) was added, and the solution was cooled to 2°C. The precipitate was filtered off, washed with hexane, and dried in vacuo. Yield 6.22 g (82%); mp. 74°C; $[\alpha]_D^{25}$ = -2.0° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.24 (d, 6H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>$_3$), 1.36 (s, 9H; *t*Bu), 1.78 - 2.05 (m, 2H; Glu-<u>H</u>(β)), 2.27 - 2.35 (m, 2H; Glu-<u>H</u>(γ)), 4.13 - 4.03 (m, 1H; Glu-<u>H</u>(α)), 4.89 (sept, 1H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>), 5.10 + 5.15 (2 × d, 2 × 1H, ^{2}J = 12.6 Hz; Bn-C<u>H</u>$_2$), 6.82 (s, b, 1H; N<u>H</u>), 7.26 - 7.37 (m, 5H; Bn-<u>H</u>); $C_{20}H_{29}NO_6$ (379.45): calcd C 63.31 7.70 N 3.69; found C 63.45 H 7.96 N 3.69.

BOC-(L)-Glu(OBn)-O*i*Pr. BOC-(L)-Glu(OBn)-OH (5.07 g, 15.0 mmol), isopropanol (1.13 g, 18.6 mmol), and DMAP (0.16 g, 1.3 mmol) were dissolved in THF (100 mL). After cooling with an ice bath, a solution of DCC (3.87 g, 18.6 mmol) in THF (30 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0°C, and then for 24 h at room temperature. The precipitated DCU was filtered off, and the solvent was evaporated in vacuo. The product was isolated from the residue by column chromatography (ethyl acetate/hexane 1:1). It crystallized on drying in vacuo. Yield 4.60 g (81%); mp. 65°C; $[\alpha]_D^{25}$ = 12.3° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.18 + 1.19 (2 × d, 2 × 3H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>$_3$), 1.38 (s, 9H; *t*Bu), 1.80 - 2.04 (m, 2H; Glu-<u>H</u>(β)), 2.40 - 2.47 (m, 2H; Glu-<u>H</u>(γ)), 3.92 - 4.00 (m, 1H; Glu-<u>H</u>(α)), 4.91 (sept, 1H, ^{3}J = 6.3Hz; *i*Pr-C<u>H</u>), 5.10 (s, 2H; Bn-C<u>H</u>$_2$), 6.70 (d, 1H, ^{3}J = 7.0 Hz; N<u>H</u>), 7.25 - 7.36 (m, 5H; Bn-<u>H</u>); $C_{20}H_{29}NO_6$ (379.45): calcd C 63.31 7.70 N 3.69; found C 63.50 H 7.89 N 3.82; CI-MS (NH_3): m/z (relative intensity): 397 (100) [M + NH_4^+], 380 (48) [M + H^+].

BOC-(L)-Asp(OBn)-O*i*Pr. BOC-(L)-Asp(OBn)-OH, isopropanol (0.54 g, 8.9 mmol), and DMAP were dissolved in THF (25 mL). After cooling with an ice bath, a solution of DCC (1.85 g, 8.88 mmol) in THF (15 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0°C, and then for 24 h at room temperature. The precipitated DCU was filtered off, and the solvent was evaporated in vacuo. The product was isolated from the residue by column chromatography (ethyl acetate/hexane 1:1). It crystallized on drying in vacuo. Yield 1.98 (73%); mp. 60°C; $[\alpha]_D^{25}$ = 21.7° (*c* = 1, $CHCl_3$); ^{1}H NMR (200 MHz, [D_6]DMSO, 25°C, TMS) δ 1.15 + 1.16 (2 × d, 2 × 3H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>$_3$), 1.40 (s, 9H; *t*Bu), 2.78 (m, 2H, ^{2}J = 16.2 Hz; Asp-<u>H</u>(β)), 4.25 - 4.41 (m, 1H; Asp-<u>H</u>(α)), 4.90 (sept, 1H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>), 5.13 (s, 2H; Bn-C<u>H</u>$_2$), 7.28 - 7.46 (m, 6H; Bn-<u>H</u> + N<u>H</u>);

$C_{19}H_{27}NO_6$ (365.43): calcd C 62.45 7.45 N 3.83; found C 62.69 H 7.73 N 4.10; CI-MS (NH_3): m/z (relative intensity): 383 (100) [M + NH_4^+], 366 (52) [M + H^+].

General Procedure for the Synthesis of the Benzyl Protected Receptors. Prior to coupling, BOC-(L)-Glu(O*i*Pr)-OBn (for **2a**), BOC-(L)-Asp(OBn)-O*i*Pr (for **2b**), or BOC-(L)-Glu(OBn)-O*i*Pr (for **2c**) was deprotected at the amino group according to **Method A**. *cyclo*-[(L)-Pro-AIA(OH)]$_3$ **(1)** (0.34 g, 0.44 mmol), the corresponding amine (4.5 equiv), PyCloP (4.5 equiv, 0.84 g, 1.98 mmol), and DIEA (13.5 equiv, 1.1 mL, 5.94 mmol) were suspended in CH_2Cl_2 (60 mL). The reaction mixture was stirred at room temperature. After about 6 h, all of the starting material was completely dissolved and the suspension became clear. After 48 h, the solvent was removed, and the product was isolated from the residue by chromatographic workup (methanol/ethyl acetate 10:1).

***cyclo*-{(L)-Pro-AIA[(L)-Glu(O*i*Pr)-OBn]}$_3$.** Yield 0.58 g (84%); mp. 168°C (softening from 160°C); $[\alpha]_D^{25}$ = -19.7° (*c* = 1, $CHCl_3$); ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS) δ 1.16 + 1.17 (2 × d, 2 × 9H, ^{3}J = 6.5 Hz; *i*Pr-C$\underline{H}_3$), 1.83 - 2.19 (m, 18H; Pro-$\underline{H}$(β) + Pro-$\underline{H}$(γ) + Glu-$\underline{H}$(β)), 2.39 - 2.46 (m, 6H; Glu-$\underline{H}$(γ)), 3.37 - 3.53 (m, 6H; Pro-$\underline{H}$(δ)), 4.50 - 4.57 (m, 3H; Glu-$\underline{H}$(α)), 4.70 (dd, 3H, $^3J_{ae}$ = 3.9 Hz, $^3J_{aa}$ = 8.0 Hz, Pro-$\underline{H}$(α)), 4.88 (sept, 3H, ^{3}J = 6.2 Hz; *i*Pr-C$\underline{H}$), 5.17 (s, 6H; Bn-C$\underline{H}_2$), 7.30 - 7.38 (m, 15H; Bn-$\underline{H}$), 7.66 (t, 3H, ^{4}J = 1.4 Hz; AIA-$\underline{H}$(6)), 7.75 (t, 3H, ^{4}J = 1.4 Hz; AIA-$\underline{H}$(4)), 8.57 (t, 3H, ^{4}J = 1.4 Hz; AIA-$\underline{H}$(2)), 8.94 (d, 3H, ^{3}J = 7.4 Hz; Glu-N$\underline{H}$), 10.53 (s, 3H; AIA-N$\underline{H}$); $C_{84}H_{93}N_9O_{21}\cdot 1\ H_2O$ (1582.73): calcd C 63.75 H 6.05 N 7.96; found C 63.90 H 5.86 N 7.68; FAB-MS: m/z (relative intensity): 1567 (100) [M + Na^+].

***cyclo*-{(L)-Pro-AIA[(L)-Asp(OBn)-O*i*Pr]}$_3$.** Yield: 0.55 g (82 %); mp. 161°C (softening from 150°C); $[\alpha]_D^{25}$ = -27.3° (*c* = 1, $CHCl_3$); ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS)) δ 1.16 + 1.17 (2 × d, 2 × 9H, ^{3}J = 6.3 Hz; *i*Pr-C$\underline{H}_3$), 1.81 - 2.08 (m, 9H; Pro-$\underline{H}$(β) + Pro-$\underline{H}$(γ)), 2.27 - 2.35 (m, 6H; Pro-$\underline{H}$(β)), 2.95 (m, 6H, ^{2}J = 16.4 Hz; Asp-$\underline{H}$(β)), 3.37 - 3.53 (m, 6H; Pro-$\underline{H}$(δ)), 4.70 (dd, 3H, $^3J_{ae}$ = 4.4 Hz, $^3J_{aa}$ = 8.8 Hz, Pro-$\underline{H}$(α)), 4.79 - 4.85 (m, 3H; Asp-$\underline{H}$(α)), 4.92 (sept, 3H, ^{3}J = 6.3 Hz; *i*Pr-C$\underline{H}$), 5.13 (s, 6H; Bn-C$\underline{H}_2$), 7.28 - 7.40 (m, 15H; Bn-$\underline{H}$), 7.58 (s, 3H; AIA-$\underline{H}$(6)), 7.73 (s, 3H; AIA-$\underline{H}$(4)), 8.58 (s, 3H; AIA-$\underline{H}$(2)), 9.05 (d, 3H, ^{3}J = 7.6 Hz; Asp-N$\underline{H}$), 10.55 (s, b, 3H; AIA-N$\underline{H}$); $C_{81}H_{87}N_9O_{21}\cdot 1.5\ H_2O$ (1549.65): calcd C 62.45 H 5.85 N 8.13; found C 62.84 H 6.05 N 8.14; FAB MS m/z (relative intensity): 1522 (73) [M + H^+], 1544 (100) [M + Na^+].

***cyclo*-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr)}$_3$.** Yield 0.62 g (91%); mp. 162°C (softening from

131°C); $[\alpha]_D^{25}$ = -16.6° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.20 + 1.21 (2 × d, 2 × 9H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>$_3$), 1.85 - 2.40 (m, 18H; Pro-<u>H</u>(β) + Pro-<u>H</u>(γ) + Glu-<u>H</u>(β)), 2.41 - 2.57 (m, 6H; Glu-<u>H</u>(γ)), 3.38 - 3.60 (m, 6H; Pro-<u>H</u>(δ)), 4.43 - 4.50 (m, 3H; Glu-<u>H</u>(α)), 4.73 (dd, 3H, $^3J_{ae}$ = 4.0 Hz, $^3J_{aa}$ = 8.6 Hz, Pro-<u>H</u>(α)), 4.94 (sept, 3H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>), 5.10 (s, 6H; Bn-C<u>H</u>$_2$), 7.27 - 7.40 (m, 15H; Bn-<u>H</u>), 7.61 (t, 3H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(6)), 7.78 (t, 3H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(4)), 8.47 (s, b, 3H; AIA-<u>H</u>(2)), 8.51 (d, 3H, ^{3}J = 7.7 Hz; Glu-N<u>H</u>), 10.16 (s, b, 3H; AIA-N<u>H</u>); $C_{84}H_{93}N_9O_{21}\cdot 3\ H_2O$ (1618,76): calcd C 62.33 H 6.16 N 7.79; found C 62.42 H 6.24 N 7.61; FAB-MS: m/z (relative intensity): 1564 (100) [M + H^+].

Receptors 2a-c. The benzyl protected receptors *cyclo*-{(L)-Pro-AIA[(L)-Glu(O*i*Pr)-OBn]}$_3$, *cyclo*-{(L)-Pro-AIA[(L)-Asp(OBn)-O*i*Pr]}$_3$, and *cyclo*-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}$_3$ (300 µmol) were hydrogenated according to **Method D** and then converted to the tetra-*n*-butyl ammonium salts as described in **Method E**.

***cyclo*-{(L)-Pro-AIA[(L)-Glu(O*i*Pr)O$^-$]}$_3$ (NBu_4^+)$_3$ (2a).** Yield: 0.59 g (100%); mp. 196°C (dec., softening from 183°C); $[\alpha]_D^{25}$ = -26.6° (*c* = 1, MeOH); ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS) δ 0.93 (t, 36H, ^{3}J = 7.4 Hz; *n*Bu-C<u>H</u>$_3$), 1.14 (d, 18H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>$_3$), 1.30 - 1.41 (m, 24H; *n*Bu-γC<u>H</u>$_2$), 1.57 - 1.67 (m, 24H; *n*Bu-βC<u>H</u>$_2$), 1.87 - 2.27 (m, 18H; Pro-<u>H</u>(β) + Pro-<u>H</u>(γ) + Glu-<u>H</u>(β)), 2.29 - 2.41 (m, 6H; Glu-<u>H</u>(γ)), 3.11 - 3.20 (m, 24H; *n*Bu-αC<u>H</u>$_2$), 3.42 - 3.57 (m, 6H; Pro-<u>H</u>(δ)), 3.87 - 3.96 (m, 3H; Glu-<u>H</u>(α)), 4.70 (dd, 3H, $^3J_{ae}$ = 3.6 Hz, $^3J_{aa}$ = 7.9 Hz, Pro-<u>H</u>(α)), 4.88 (sept, 3H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>), 7.48 (s, 3H; AIA-<u>H</u>(6)), 7.68 (s, 3H; AIA-<u>H</u>(4)), 7.98 (s, b, 3H; Glu-N<u>H</u>), 8.54 (s, 3H; AIA-<u>H</u>(2)), 10.54 (s, b, 3H; AIA-N<u>H</u>); $C_{111}H_{180}N_{21}O_{21}\cdot 2\ H_2O$ (2072.76): calcd C 64.88 H 9.03 N 8.18; found C 64.88 H 9.12 N 8.18; ESI MS m/z (relative intensity): 1292 (100) [M - 3 NBu_4^+ + 2 H^+], 1534 (46) [M - 2 NBu_4^+ + H^+].

***cyclo*-{(L)-Pro-AIA[(L)-Asp(O$^-$)-O*i*Pr]}$_3$ (NBu_4^+)$_3$ (2b).** Yield: 0.58 g (100%); mp. 159°C (dec.); $[\alpha]_D^{25}$ = -28.7° (*c* = 1, $CHCl_3$); ^{1}H NMR (500 MHz, [D_6]DMSO, 25°C, TMS) δ 0.93 (t, 36H, ^{3}J = 7.3 Hz; *n*Bu-C<u>H</u>$_3$), 1.15 + 1.19 (2 × d, 2 × 18H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>$_3$), 1.26 - 1.36 (m, 24H; *n*Bu-γC<u>H</u>$_2$), 1.50 - 1.62 (m, 24H; *n*Bu-βC<u>H</u>$_2$), 1.83 - 2.08 (m, 9H; Pro-<u>H</u>(β) + Pro-<u>H</u>(γ)), 2.21 - 2.43 (m, 9H; Pro-<u>H</u>(β) + Asp-<u>H</u>(β)), 3.09 - 3.21 (m, 24H; *n*Bu-αC<u>H</u>$_2$), 3.37 - 3.52 (m, 6H; Pro-<u>H</u>(δ)), 4.40 - 4.46 (m, 3H; Asp-<u>H</u>(α)), 4.70 (dd, 3H, $^3J_{ae}$ = 3.9 Hz, $^3J_{aa}$ = 8.5 Hz, Pro-<u>H</u>(α)), 4.87 (sept, 3H, ^{3}J = 6.3 Hz; *i*Pr-C<u>H</u>), 7.48 (s, 3H; AIA-<u>H</u>(6)), 7.70 (s, 3H; AIA-<u>H</u>(4)), 8.57 (s, 3H; AIA-<u>H</u>(2)), 10.14 (s, 3H;

Asp-NH), 10.59 (s, 3H; AIA-NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25°C, TMS) δ 13.5 (*n*Bu-CH$_3$), 19.2 (*n*Bu-γCH$_2$), 21.5 + 21.6 (*i*Pr-CH$_3$), 23.0 (*n*Bu-βCH$_2$), 24.6 (Pro-C(γ)), 29.5 (Pro-C(β)), 38.2 (Asp-C(β)), 49.5 (Pro-C(δ)), 50.1 (Asp-C(α)), 57.5 (*n*Bu-αCH$_2$), 60.0 (Pro-C(α)), 67.3 (*i*Pr-CH), 118.4 + 118.7 (AIA-C(4) + AIA-C(6)), 120.5 (AIA-C(2)), 134.9 (AIA-C(1)), 138.3 (AIA-C(5)), 139.4 (AIA-C(3)), 164.8 (AIA-C(1)CO), 168.2 + 168.3 (Pro-CO + AIA-C(5)CO), 170.6 (Asp-C(α)CO), 171.6 (Asp-C(β)CO); $C_{108}H_{174}N_{21}O_{21}\cdot 4\ H_2O$ (2048.70): calcd C 63.32 H 8.95 N 8.20; found C 63.51 H 8.83 N 8.21; ESI MS m/z (relative intensity): 1251 (14) [M - 3 NBu_4^+ + 2 H^+], 1733 (100) [M - NBu_4^+].

***cyclo*-{(L)-Pro-AIA[(L)-Glu(O$^-$)-O*i*Pr]}$_3$ (NBu_4^+)$_3$ (2c).** Yield: 0.59 g (100%); mp. 187-188°C (dec., softening from 174°C); $[\alpha]_D^{25}$ = -50.4° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 60°C, TMS) δ 0.93 (t, 36H, ^{3}J = 7.2 Hz; *n*Bu-CH$_3$), 1.16 + 1.21 (2 × d, 2 × 18H, ^{3}J = 6.2 Hz; *i*Pr-CH$_3$), 1.24 - 1.38 (m, 24H; *n*Bu-γCH$_2$), 1.51 - 1.63 (m, 24H; *n*Bu-βCH$_2$), 1.84 - 2.40 (m, 24H; Pro-H(β) + Pro-H(γ) + Glu-H(β) + Glu-H(γ)), 3.11 - 3.19 (m, 24H; *n*Bu-αCH$_2$), 3.47 - 3.60 (m, 6H; Pro-H(δ)), 4.10 - 4.16 (m, 3H; Glu-H(α)), 4.73 (dd, 3H, $^3J_{ae}$ = 3.4 Hz, $^3J_{aa}$ = 7.4 Hz, Pro-H(α)), 4.92 (sept, 3H, ^{3}J = 6.4 Hz; *i*Pr-CH), 7.73 (s, 3H; AIA-H(6)), 7.81 (s, 3H; AIA-H(4)), 8.49 (s, 3H; AIA-H(2)), 10.50 (s, 3H; AIA-NH), 11.85 (s, 3H; Glu-NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25°C, TMS) δ 13.4 (*n*Bu-CH$_3$), 19.1 (*n*Bu-γCH$_2$), 21.4 + 21.5 (*i*Pr-CH$_3$), 22.9 (*n*Bu-βCH$_2$), 24.4 (Pro-C(γ)), 26.4 + 29.5 (Pro-C(β) + Glu-H(β)), 35.0 (Glu-C(γ)), 49.2 (Pro-C(δ)), 54.8 (Glu-C(α)), 57.4 (*n*Bu-αCH$_2$), 59.7 (Pro-C(α)), 67.1 (*i*Pr-CH), 118.8 + 118.9 (AIA-C(4) + AIA-C(6)), 120.6 (AIA-C(2)), 134.5 (AIA-C(1)), 138.2 (AIA-C(5)), 139.1 (AIA-C(3)), 164.6 (AIA-C(1)CO), 168.1 (Pro-CO), 170.4 (AIA-C(5)CO), 171.6 (Glu-C(α)CO), 175.1 (Glu-C(β)CO); $C_{111}H_{180}N_{21}O_{21}\cdot 3\ H_2O$ (2072.76): calcd C 64.34 H 9.05 N 8.02; found C 64.32 H 9.04 N 8.11; ESI MS m/z (relative intensity): 1292 (100) [M - 3 NBu_4^+ + 2 H^+], 1776 (46) [M - NBu_4^+].

BOC-AIA[(L)-Glu(OBn)-O*i*Pr]-OAll. Prior to coupling, BOC-(L)-Glu(OBn)-O*i*Pr was deprotected at the amino group according to **Method A.** (L)-Glu(OBn)-O*i*Pr · HCl (1.40 g, 4.43 mmol), 5-[(*tert*-butyloxycarbonyl)amino]-isophthalic acid mono allyl ester (2.14 g, 6.65 mmol) and PyCloP (2.80 g, 6.65 mmol) were dissolved in CH_2Cl_2 (90 mL). DIEA (3.10 mL, 17.73 mmol) was added and then the solution was stirred overnight. The solvent was removed in vacuo and the dipeptide was isolated from the residue by chromatographic workup ($CHCl_3$/acetone, 10:1). The

product was dried in vacuo. Yield 2.43 g (94%); mp. 66 - 67°C; $[\alpha]_D^{25}$= 8.1° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.12 + 1.24 (2 × d, 2 × 3H, ^{3}J = 5.1 Hz; *i*Pr-C<u>H</u>$_3$), 1.52 (s, 9H; *t*Bu), 2.02 - 2.28 (m, 2H; Glu-<u>H</u>(β)), 2.52 - 2.57 (m, 2H; Glu-<u>H</u>(γ)), 4.44 - 4.54 (m, 1H; Glu-<u>H</u>(α)), 4.85 (ddd, 2H, ^{3}J = 5.3 Hz; C<u>H</u>$_2$CH=CH$_2$), 4.97 (sept, 1H, ^{3}J = 6.2Hz; *i*Pr-C<u>H</u>), 5.12 (s, 2H; Bn-C<u>H</u>$_2$), 5.30 (ddt, 1H, ^{3}J = 10.6 Hz, ^{2}J = 1.7 Hz; CH$_2$CH=C<u>H</u>$_2$, *cis*), 5.43 (ddt, 1H, ^{3}J = 17.2 Hz, ^{2}J = 1.7 Hz; CH$_2$CH=C<u>H</u>$_2$, *trans*), 6.00 - 6.14 (m, 1H; CH$_2$C<u>H</u>=CH$_2$), 7.28 - 7.38 (m, 5H; Bn-<u>H</u>), 8.04 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(6)), 8.16 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(4)), 8.27 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(2)), 8.58 (d, b, 1H, ^{3}J = 7.3 Hz; Glu-N<u>H</u>), 9.40 (s, 1H; AIA-N<u>H</u>); $C_{31}H_{38}N_2O_9$ (582.66): calcd C 63.90 H 6.57 N 4.81; found C 63.67 H 6.54 N 4.71.

Dipeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}-OAll. Prior to coupling, BOC-AIA[(L)-Glu(OBn)-O*i*Pr]-OAll was deprotected at the amino group according to **Method A** only that the free amine was not converted to the hydrochloride. The free amine (1.90 g, 4.0 mmol), BOC-(L)-Proline (1.28 g, 6.0 mmol), and PyCloP (2.53 g, 6.0 mmol) were dissolved in CH_2Cl_2 (80 mL). DIEA (2.78 mL, 16.0 mmol) was added and then the solution was stirred overnight. The solvent was removed in vacuo and the product was isolated from the residue by chromatographic workup ($CHCl_3$/acetone, 5:1). It crystallized on drying in vacuo. Yield 2.46 g (91%); mp. 69°C (softening from 52°C); $[\alpha]_D^{25}$= -51.5° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 1.19 + 1.21 (2 × d, 2 × 3H, ^{3}J = 4.8 Hz; *i*Pr-C<u>H</u>$_3$), 1.34 (s, 9H; *t*Bu), 1.76 - 2.28 (m, 6H; Pro-<u>H</u>(β) + Pro-<u>H</u>(γ) + Glu-<u>H</u>(β)), 2.49 - 2.52 (m, 2H; Glu-<u>H</u>(γ)), 3.33 - 3.51 (m, 2H; Pro-<u>H</u>(δ)), 4.26 (dd, 1H; $^3J_{ae}$ = 4.2 Hz, $^3J_{aa}$ = 8.8 Hz, Pro-<u>H</u>(α)), 4.42 - 4.52 (m, 1H; Glu-<u>H</u>(α)), 4.84 (ddd, 2H, ^{3}J = 5.5 Hz; C<u>H</u>$_2$CH=CH$_2$), 4.94 (sept, 1H, ^{3}J = 6.2 Hz; *i*Pr-C<u>H</u>), 5.10 (s, 2H; Bn-C<u>H</u>$_2$), 5.28 (ddt, 1H, ^{3}J = 10.1 Hz, ^{2}J = 1.5 Hz; CH$_2$CH=C<u>H</u>$_2$, *cis*), 5.40 (ddt, 1H, ^{3}J = 17.2 Hz, ^{2}J = 1.5 Hz; CH$_2$CH=C<u>H</u>$_2$, *trans*), 5.98 - 6.12 (m, 1H; CH$_2$C<u>H</u>=CH$_2$), 7.23 - 7.35 (m, 5H; Bn-<u>H</u>), 8.11 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(6)), 8.26 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(4)), 8.42 (t, 1H, ^{4}J = 1.5 Hz; AIA-<u>H</u>(2)), 8.61 (d, 1H, ^{3}J = 7.7 Hz; Glu-N<u>H</u>), 10.00 (s, 1H; AIA-N<u>H</u>); $C_{36}H_{45}N_3O_{10}$ (679.77): calcd C 63.61 H 6.67 N 6.18; found C 63.44 H 6.88 N 6.16; FAB-MS: m/z (relative intensity): 702 (22) [M + Na^+].

Tetrapeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}$_2$-OAll. Prior to coupling, equimolar amounts of the dipeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}-OAll were deprotected at the amino group as described in **Method A** and at the allyl ester carboxyl group as described in

Method C. Both products were dissolved in CH_2Cl_2. PyCloP (1.5 equiv.) and DIEA (4.0 equiv.) were added, and the solution was stirred overnight. The solvent was then evaporated in vacuo, and the residue was purified chromatographically (acetone/$CHCl_3$ 1:1). The product crystallized upon drying in vacuo.

Hexapeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}$_3$-OAll. Prior to coupling, the dipeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}-OAll (0.74 g, 1.10 mmol) was deprotected at the allyl ester carboxyl group according to **Method C.** The tetrapeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}$_2$-OAll (1.14 g, 0.95 mmol) was deprotected at the amino group according to **Method B.** Both products as well as PyCloP (0.50 g, 1.19 mmol) were dissolved in CH_2Cl_2 (25 mL). DIEA (0.58 mL, 3.3 mmol) was added, and the solution was stirred overnight. After removing the solvent in vacuo, the product was isolated chromatographically in two steps (1. $CHCl_3$/acetone/MeOH 20:10:1, 2. CH_2Cl_2/MeOH 10:1). After evaporation of the solvent the product was dried in vacuo. Yield 1.05 g (65%); mp. 122°C; $[\alpha]_D^{25}$= -54.7° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 100°C, TMS) δ 1.20 - 1.24 (m, 18H; 3 × *i*Pr-C<u>H</u>$_3$), 1.36 (s, 9H; *t*Bu), 1.78 - 2.42 (m, 18H; Pro(1 + 3 + 5)-<u>H</u>(β) + Pro(1 + 3 + 5)-<u>H</u>(γ) + Glu(2' + 4' + 6')-<u>H</u>(β)), 2.50 - 2.58 (m, 6H; Glu(2' + 4' + 6')-<u>H</u>(γ)), 3.34 - 3.72 (m, 6H; Pro(1 + 3 + 5)-<u>H</u>(δ)), 4.27 (dd, 1H; $^3J_{ae}$ = 4.0 Hz, $^3J_{aa}$ = 8.2 Hz, Pro(1)-<u>H</u>(α)), 4.44 - 4.55 (m, 3H; Glu(2' + 4' + 6')-<u>H</u>(α)), 4.44 - 4.55 (m, 3H; Pro(3 + 5)-<u>H</u>(α)), 4.86 (ddd, 2H, ^3J = 5.5 Hz; C<u>H</u>$_2$CH=CH$_2$), 4.91 - 5.02 (m, 3H; 3 × *i*Pr-C<u>H</u>), 5.11+5.12 (2 × s, 6H; 3 × Bn-C<u>H</u>$_2$), 5.30 (ddt, 1H, ^3J = 10.4 Hz, ^2J = 1.5 Hz; CH$_2$CH=C<u>H</u>$_2$, *cis*), 5.43 (ddt, 1H, ^3J = 17.2 Hz, ^2J = 1.5 Hz; CH$_2$CH=C<u>H</u>$_2$, *trans*), 6.00 - 6.15 (m, 1H; CH$_2$C<u>H</u>=CH$_2$), 7.26 - 7.37 (m, 15H; 3 × Bn-<u>H</u>), 7.72 + 7.99 (2 × s, b, 2 × 2H; AIA(2 + 4)-<u>H</u>(2) + AIA(2 + 4)-<u>H</u>(4)), 8.11 - 8.17 (m, 3H; AIA(2 + 4 + 6)-<u>H</u>(6)), 8.30 (s, b, 1H; AIA(6)-<u>H</u>(4)), 8.47 (s, b, 1H; AIA(6)-<u>H</u>(2)), 8.49 - 8.58 (m, 2H; Glu(2' + 4')-N<u>H</u>), 8.65 (d, b, 1H, ^3J = 7.1 Hz, Glu(6')-N<u>H</u>), 9.92 + 10.11 + 10.21 (3 × s, b, 3H, AIA(2 + 4 + 6)-N<u>H</u>); $C_{92}H_{107}N_9O_{24}$ (1722.92): calcd C 64.14 H 6.26 N 7.32; found C 63.87 H 6.46 N 7.29; FAB-MS: m/z (relative intensity): 1745 (100) [M + Na$^+$].

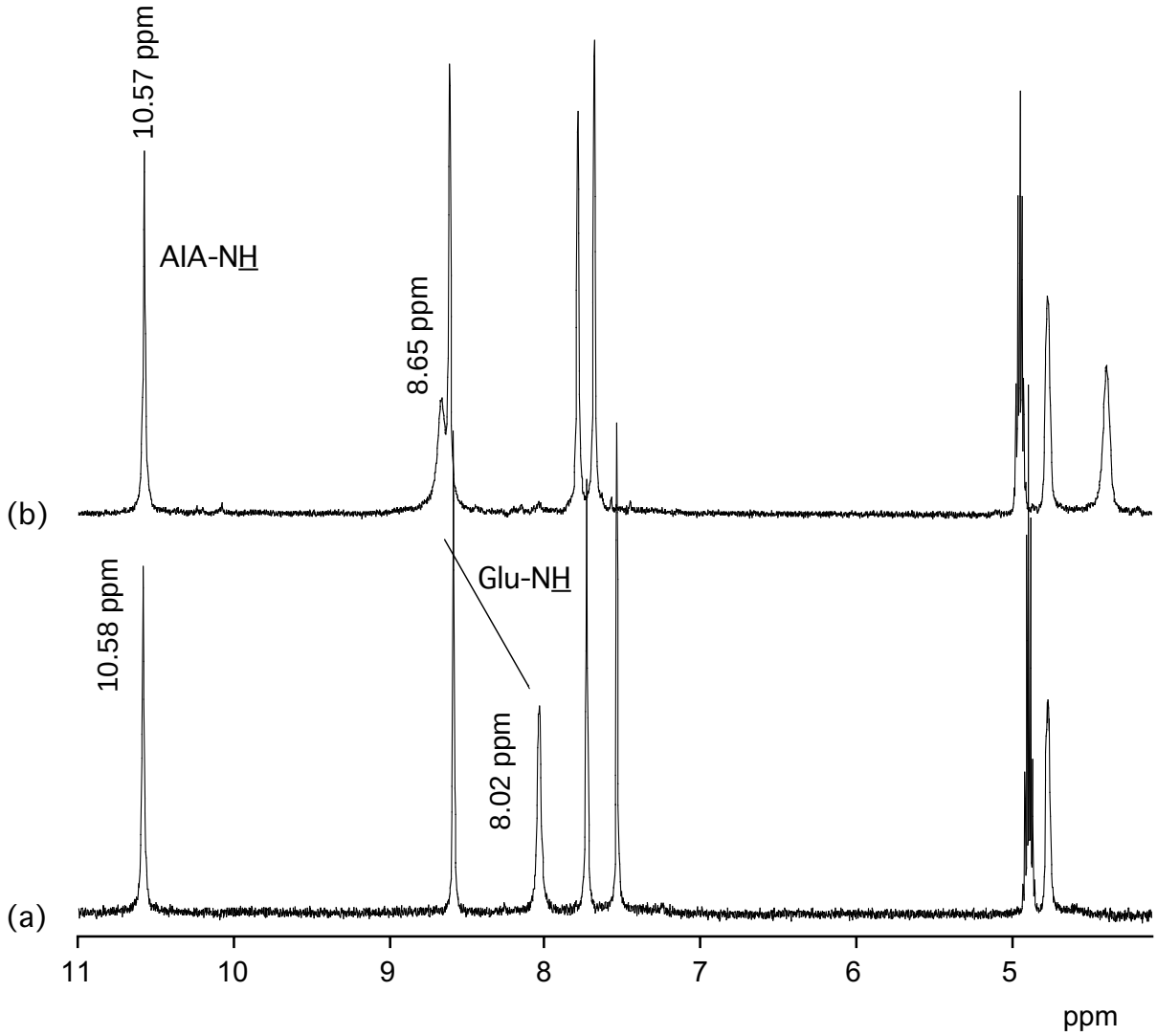
Linear Hexapeptide BOC-{(L)-Pro-AIA[(L)-Glu(O$^-$)-O*i*Pr]}$_3$-O*n*Pr (NBu$_4^+$)$_3$ (3). The hexapeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}$_3$-OAll (0.4 g, 0.23 mmol) was hydrogenated according to **Method D**, using 1,4-dioxane/water 9:1 instead of methanol as solvent. The product (237.1 mg, 0.16 mmol) was converted to the tetra-*n*-butyl ammonium salt according to **Method E**.

Yield 365 mg (100%); mp. 145°C (dec., softening from 95°C); $[\alpha]_D^{25}$= -58.4° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 0.80 - 1.02 (m, 39H; *n*Bu-C$\underline{H}_3$ + *n*Pr-C$\underline{H}_3$), 1.10 - 1.24 (m, 16H; *i*Pr-C$\underline{H}_3$), 1.24 - 1.42 (m, 33H; *n*Bu-γC$\underline{H}_2$ + *t*Bu), 1.54 - 1.67 (m, 24H; *n*Bu-βC$\underline{H}_2$), 1.68 - 2.28 (m, 26H; Pro(1 + 3 + 5)-$\underline{H}$(β) + Pro(1 + 3 + 5)-$\underline{H}$(γ) + Glu(2' + 4' + 6')-$\underline{H}$(β) + Glu(2' + 4' + 6')-$\underline{H}$(γ) + *n*Pr-βC$\underline{H}_2$), 3.13 - 3.23 (m, 24H; *n*Bu-αC$\underline{H}_2$), 3.30 - 4.35 (m, 14H; Glu(2' + 4' + 6')-$\underline{H}$(α) + Pro(1 + 3 + 5)-$\underline{H}$(α) + Pro(1 + 3 + 5)-$\underline{H}$(δ) + *n*Pr-αC$\underline{H}_2$), 4.84 - 4.99 (m, 3H; *i*Pr-C$\underline{H}$), 7.40 - 8.75 (m, b, 9H; AIA(2 + 4 + 6)-$\underline{H}$(2) + AIA(2 + 4 + 6)-$\underline{H}$(4) + AIA(2 + 4 + 6)-$\underline{H}$(6)), 9.90 - 12.00 (m, b, 6H; AIA-N$\underline{H}$ + Glu-N$\underline{H}$); ^{13}C NMR (75 MHz, [D_6]DMSO, 100°C, TMS) δ 9.5 (*n*Pr-C$\underline{H}_3$), 12.6 (*n*Bu-C$\underline{H}_3$), 18.6 (*n*Bu-γC$\underline{H}_2$), 20.8 - 21.2 (m, *i*Pr-C$\underline{H}$), 22.7 (*n*Bu-βC$\underline{H}_2$), 27.6 (*t*Bu-C$\underline{H}_3$) (in spite of prolonged measurements, the other signals in the ^{13}C-NMR spectrum are too small to allow an accurate assignment); ESI MS m/z (relative intensity): 1935 (100) [M - NBu_4^+].

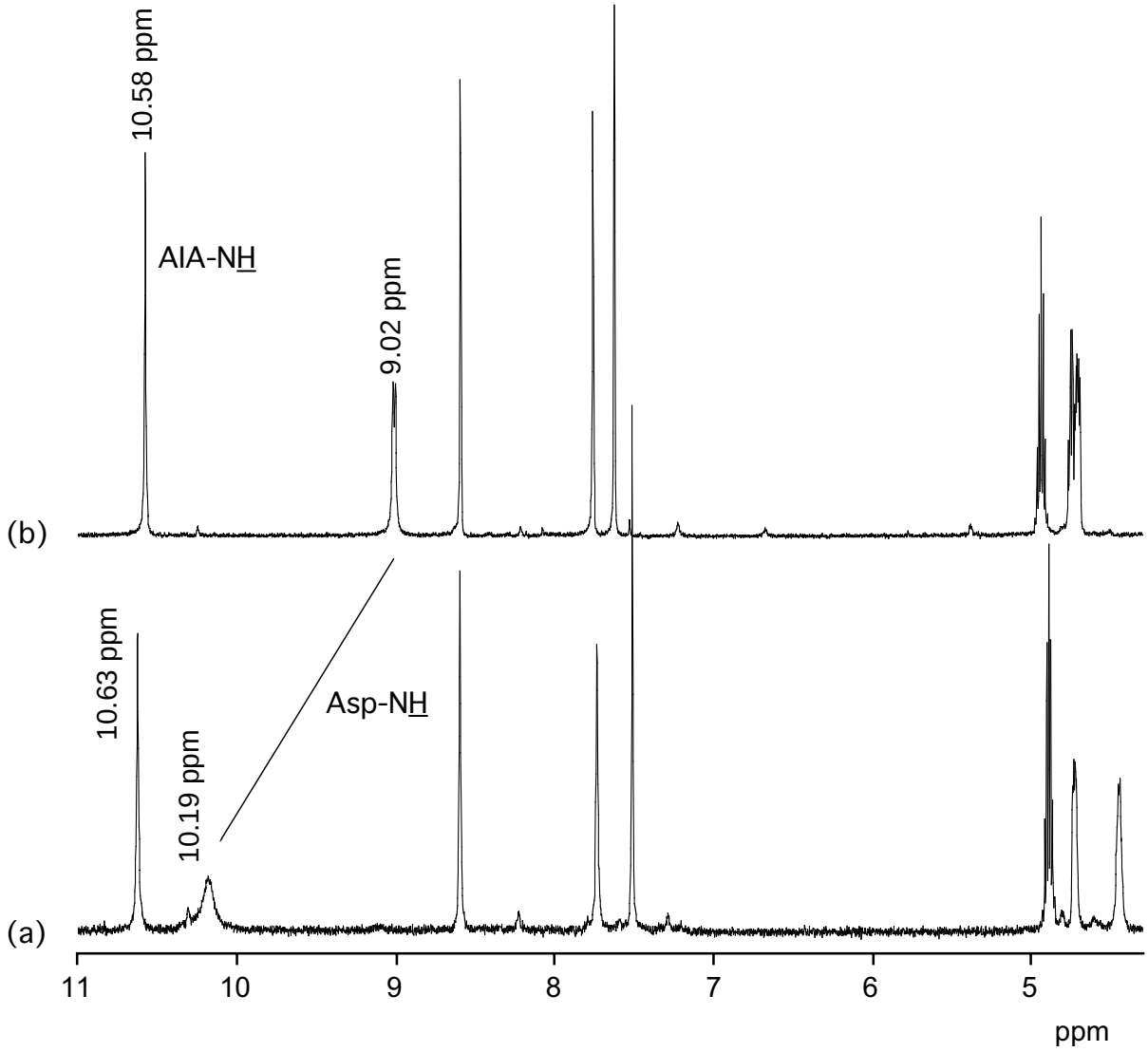
Linear Dipeptide BOC-{(L)-Pro-AIA[(L)-Glu(O$^-$)-O*i*Pr]}-O*n*Pr NBu_4^+ (4). The dipeptide BOC-{(L)-Pro-AIA[(L)-Glu(OBn)-O*i*Pr]}-OAll (0.86 g, 1.26 mmol) was hydrogenated according to **Method D**, using 1,4-dioxane/water 9:1 instead of methanol as solvent. The product (295.8 mg, 0.50 mmol) was converted to the tetra-*n*-butyl ammonium salt according to **Method E**. Yield 420 mg (100%); mp. 96°C (softening from 79°C); $[\alpha]_D^{25}$= -34.3° (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, [D_6]DMSO, 100°C, TMS) δ 0.94 (t, 12H, ^{3}J = 7.3 Hz; *n*Bu-C$\underline{H}_3$), 0.99 (t, 3H, ^{3}J = 7.3 Hz; *n*Pr-C$\underline{H}_3$), 1.16 + 1.21 (2 × d, 2 × 3H, ^{3}J = 6.2 Hz; *i*Pr-C$\underline{H}_3$), 1.26 - 1.42 (m, 8H; *n*Bu-γC$\underline{H}_2$), 1.34 (s, 9H; *t*Bu), 1.54 - 1.68 (m, 8H; *n*Bu-βC$\underline{H}_2$), 1.68 - 2.28 (m, 10H; Pro-$\underline{H}$(β) + Pro-$\underline{H}$(γ) + Glu-$\underline{H}$(β) + Glu-$\underline{H}$(γ) + *n*Pr-βC$\underline{H}_2$), 3.13 - 3.22 (m, 8H; *n*Bu-αC$\underline{H}_2$), 3.32 - 3.50 (m, 2H; Pro-$\underline{H}$(δ)), 4.12 - 4.21 (m, 1H; Glu-$\underline{H}$(α)), 4.22 - 4.28 (m, 2H; *n*Pr-αC$\underline{H}_2$), 4.30 (dd, 1H; $^3J_{ae}$ = 3.3 Hz, $^3J_{aa}$ = 8.2 Hz, Pro-$\underline{H}$(α)), 4.91 (sept, 1H, ^{3}J = 6.2 Hz; *i*Pr-C$\underline{H}$), 8.18 (s, b, 1H; AIA-$\underline{H}$(6)), 8.34 - 8.38 (m, 1H; AIA-$\underline{H}$(4)), 8.52 - 8.55 (m, 1H; AIA-$\underline{H}$(2)), 10.41 (s, b, 1H; AIA-N$\underline{H}$), 11.49 (s, b, 1H; Glu-N$\underline{H}$); ^{13}C NMR (75 MHz, [D_6]DMSO, 100°C, TMS) δ 10.2 (*n*Pr-C$\underline{H}_3$), 13.2 (*n*Bu-C$\underline{H}_3$), 19.2 (*n*Bu-γC$\underline{H}_2$), 21.5 + 21.6 (*i*Pr-C$\underline{H}$), 10.2 (*n*Pr-βC$\underline{H}_2$), 23.4 (*n*Bu-βC$\underline{H}_2$), 23.5 (Pro-C$\underline{(γ)}$), 27.0 (Glu-C$\underline{(γ)}$), 28.2 (*t*Bu-C$\underline{H}_3$), 30.6 (Pro-C$\underline{(β)}$), 35.2 (Glu-C$\underline{(β)}$), 46.7 (Pro-C$\underline{(δ)}$), 55.0 (Glu-C$\underline{(α)}$), 58.4 (*n*Bu-αC$\underline{H}_2$), 60.5 (Pro-C$\underline{(α)}$), 66.3 (*n*Pr-αC$\underline{H}_2$), 67.2 (*i*Pr-C$\underline{H}$), 78.7 (*t*Bu-C$\underline{(CH_3)_3}$), 122.3 (AIA-C$\underline{(2)}$), 123.0 (AIA-C$\underline{(6)}$), 123.2 (AIA-C$\underline{(4)}$), 130.8 (AIA-C$\underline{(1)}$), 136.0 (AIA-C$\underline{(5)}$), 139.5 (AIA-C$\underline{(3)}$), 153.6 (BOC-C$\underline{O}$), 165.4 (AIA-C(1)C$\underline{O}$), 165.6 (Pro-C$\underline{O}$), 171.7 (AIA-C(5)CO), 171.8 (Glu-C(α)C$\underline{O}$),

174.8 (Glu-C(β)CO); $C_{45}H_{76}N_4O_{10}\cdot 0.5\ H_2O$ (842.13) calcd C 64.18 H 9.22 N 6.65; found C 63.97 H 9.12 N 6.63; ESI MS m/z (relative intensity): 590 (100) [M - NBu_4^+].

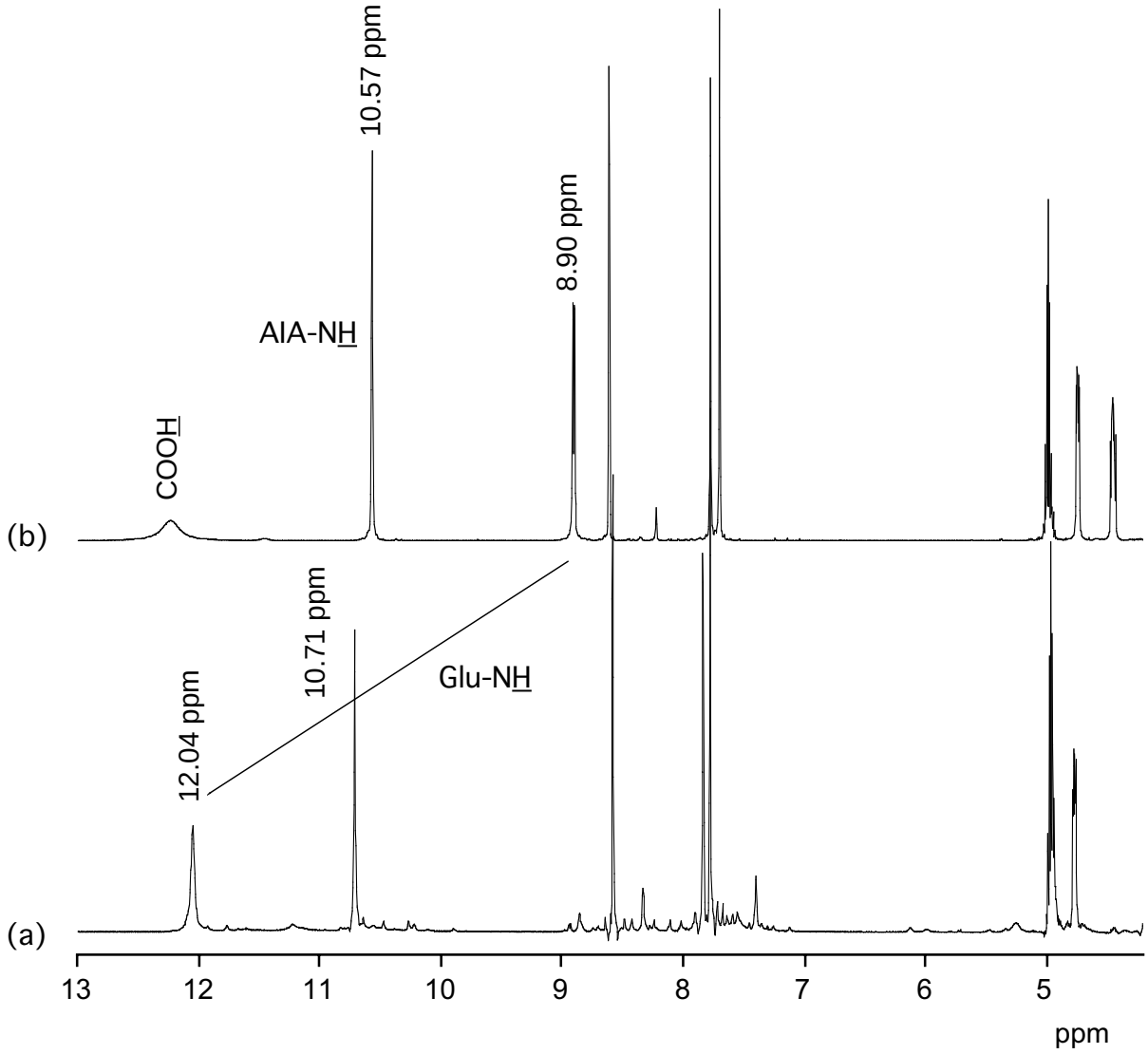
^{1}H-NMR spectra: **2a** (1 mg/mL) in [D_6]DMSO (a), protonated precursor of **2a** (1 mg/mL) in [D_6]DMSO (b)



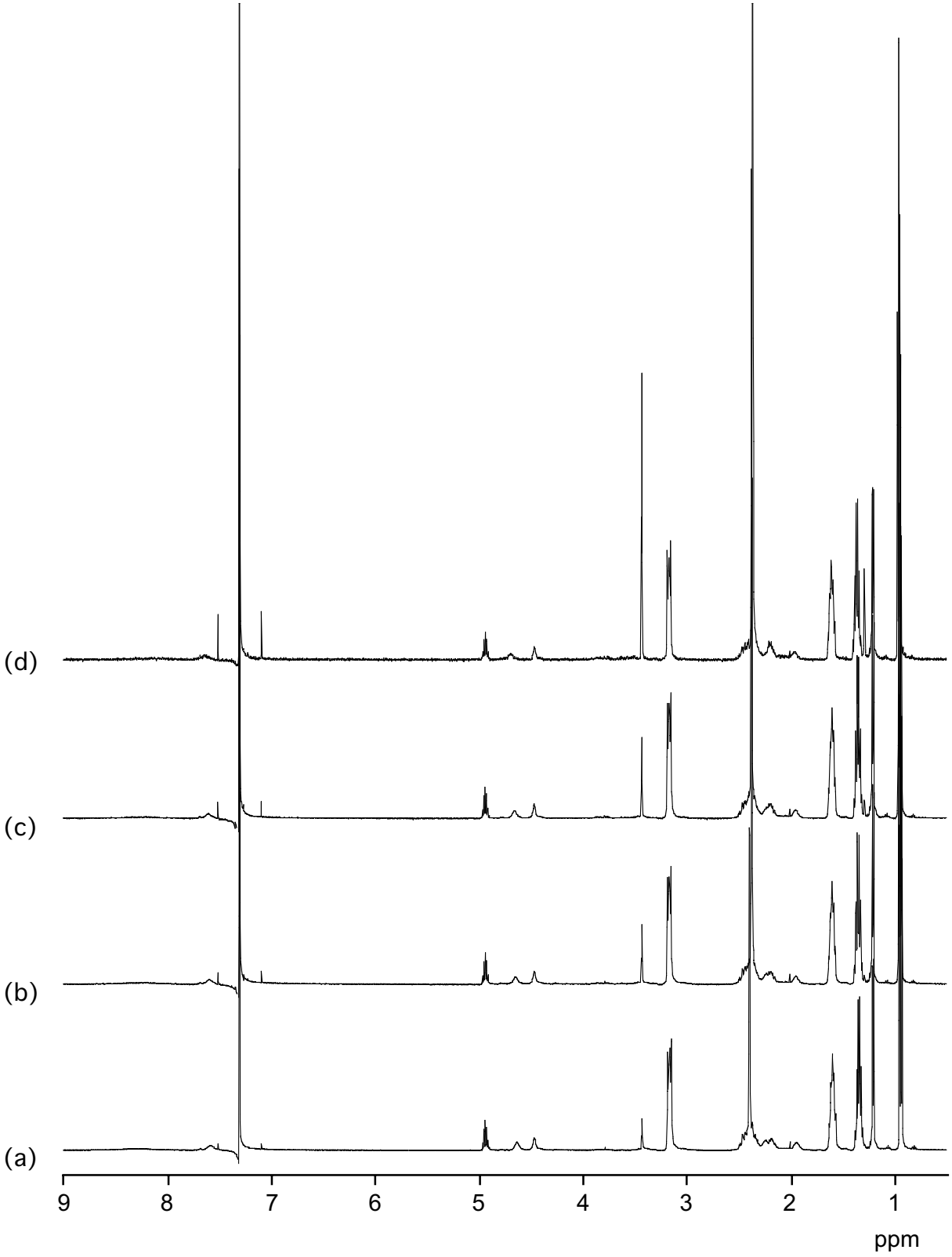
[1]H-NMR spectra: **2b** (1 mg/mL) in [D_6]DMSO (a), protonated precursor of **2b** (1 mg/mL) in [D_6]DMSO (b)



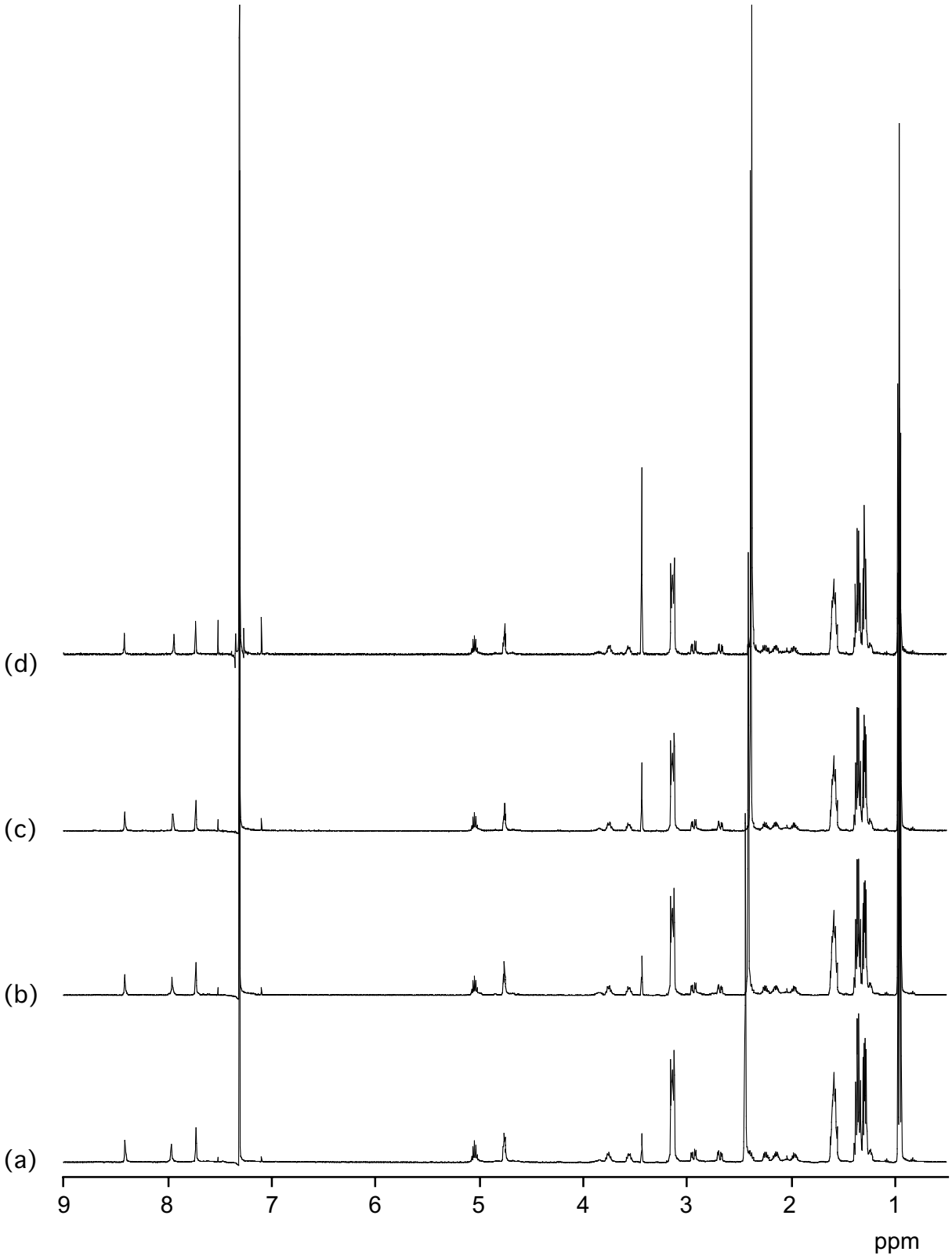
^{1}H-NMR spectra: **2c** (1 mg/mL) in [D_6]DMSO (a), protonated precursor of **2c** (1 mg/mL) in [D_6]DMSO (b)



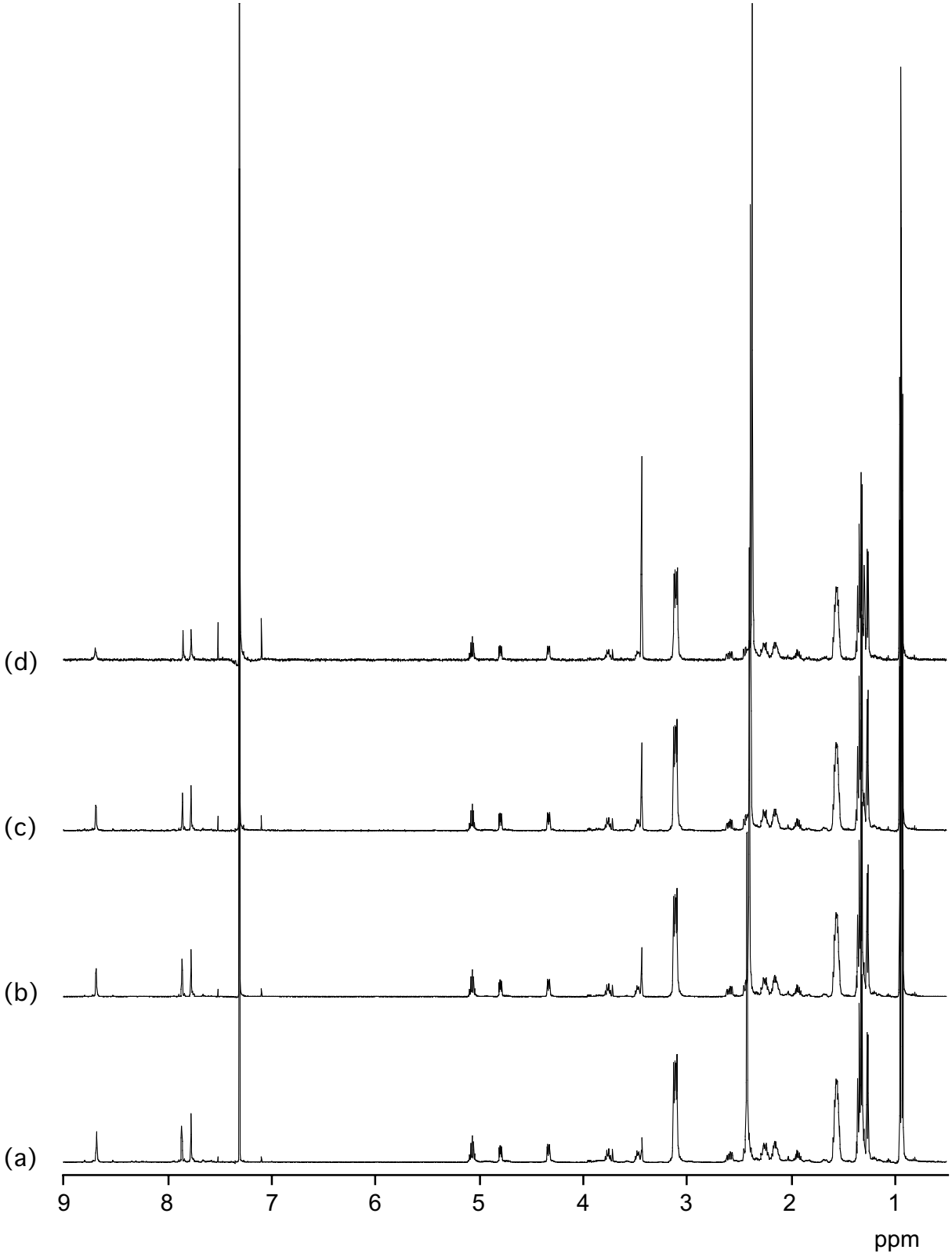
NMR dilution titration: **2a** in 4% $CD_3OD/CDCl_3$ (a) 2mM, (b) 1.4 mM, (c) 0.8 mM, (d) 0.2 mM



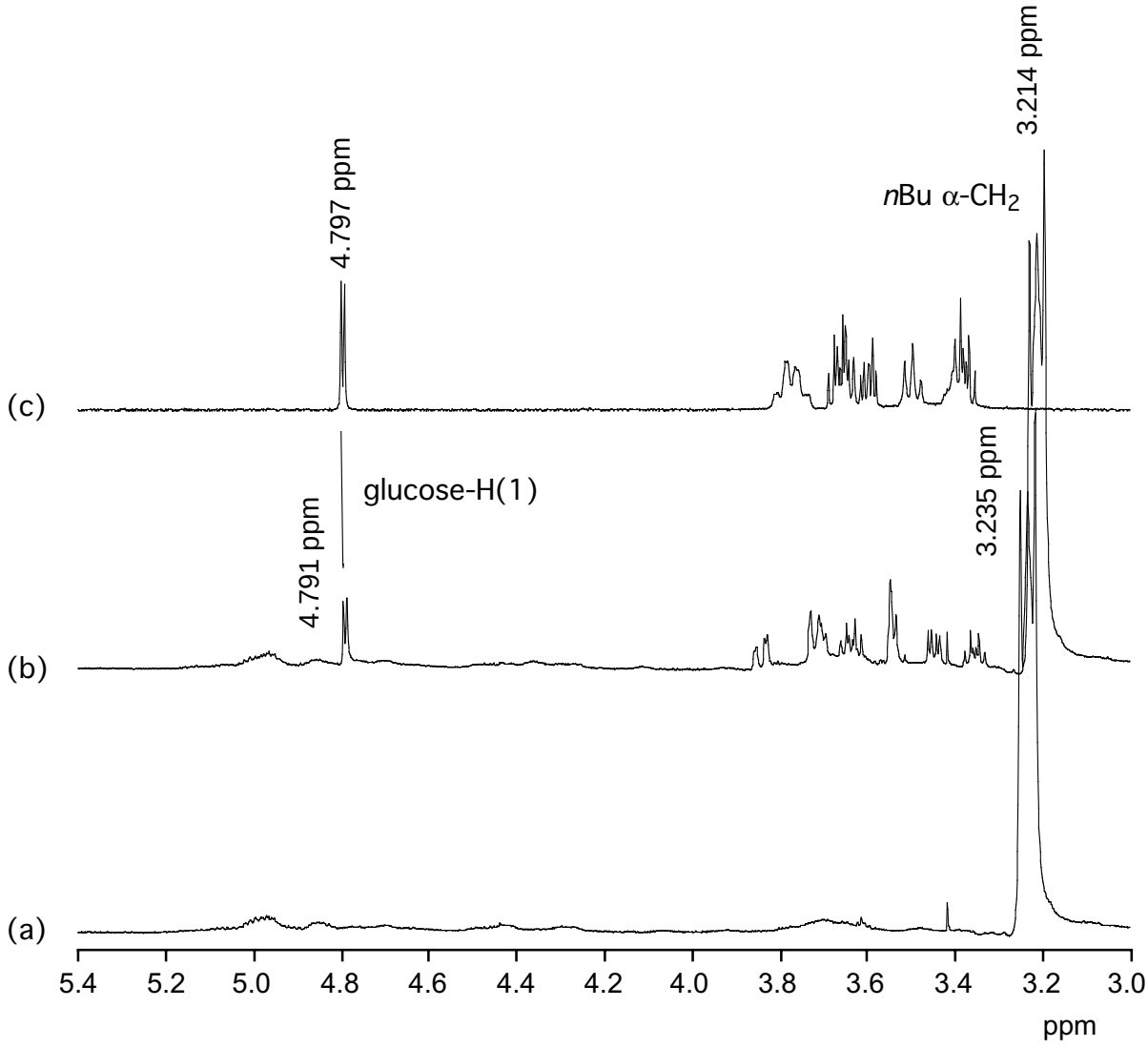
NMR dilution titration: **2b** in 4% $CD_3OD/CDCl_3$ (a) 2mM, (b) 1.4 mM, (c) 0.8 mM, (d) 0.2 mM



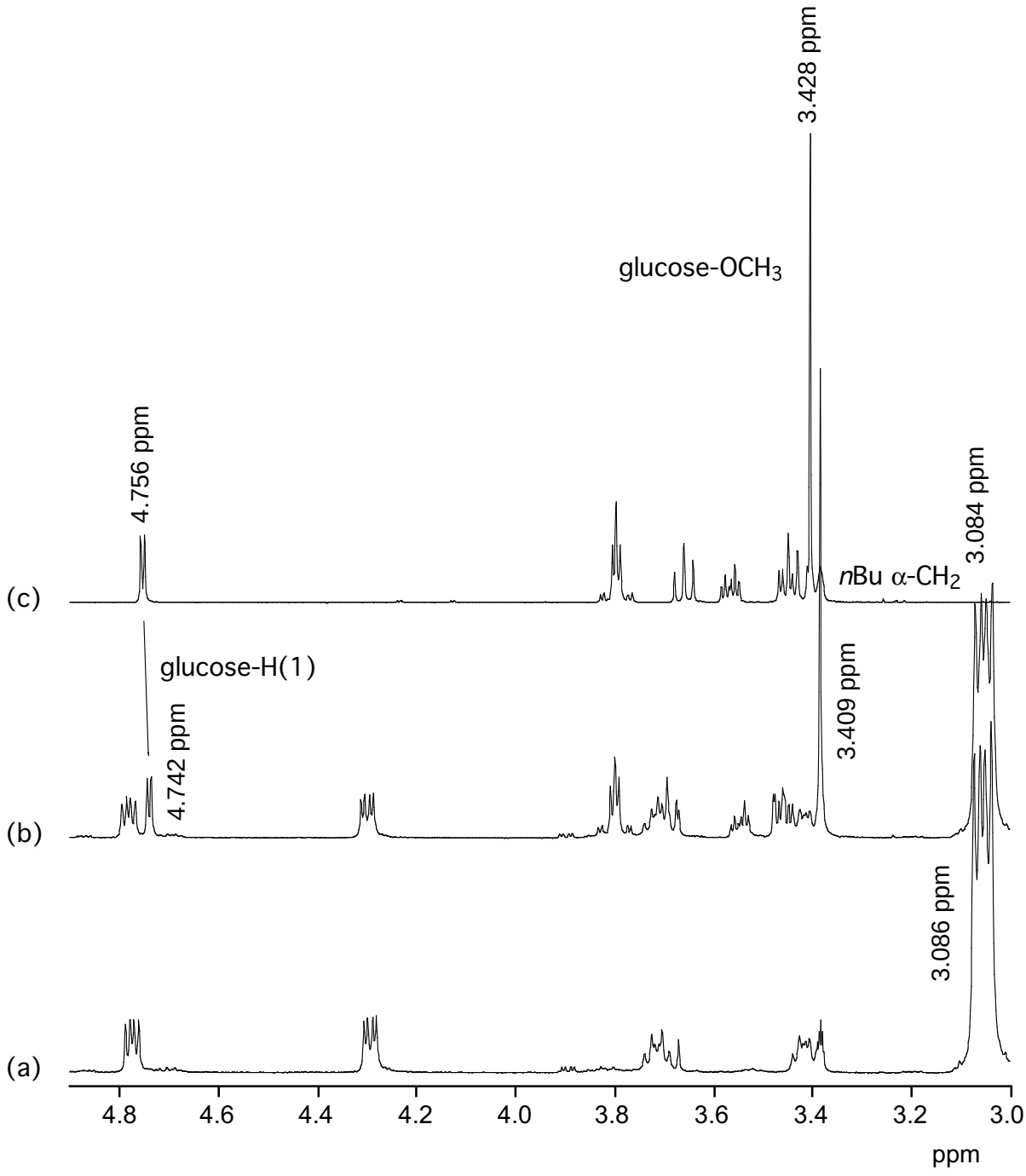
NMR dilution titration: **2a** in 4% $CD_3OD/CDCl_3$ (a) 2mM, (b) 1.4 mM, (c) 0.8 mM, (d) 0.2 mM



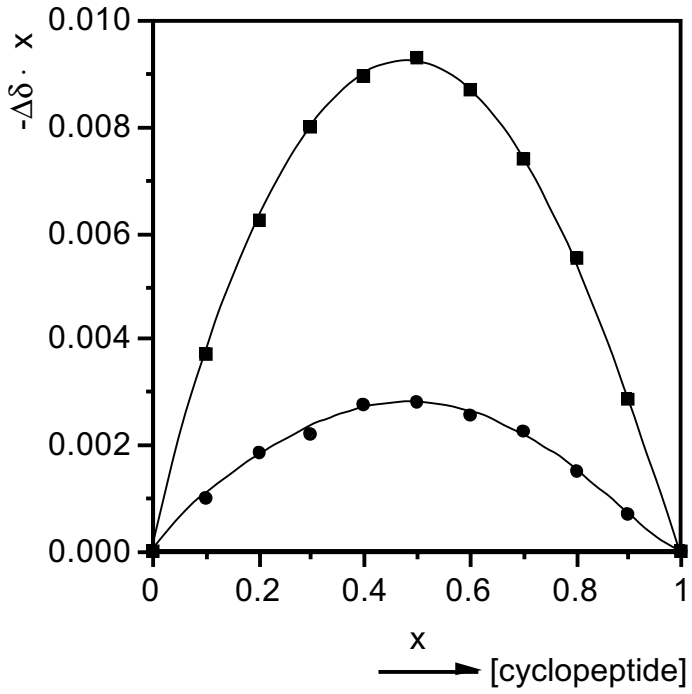
^{1}H-NMR spectra: **2c** (1 mM) in $CDCl_3$ (a), α-D-octylglucopyranoside (1 mM) in $CDCl_3$ (c), 1:1 mixture of **2c** and α-D-octylglucopyranoside in $CDCl_3$ (b)



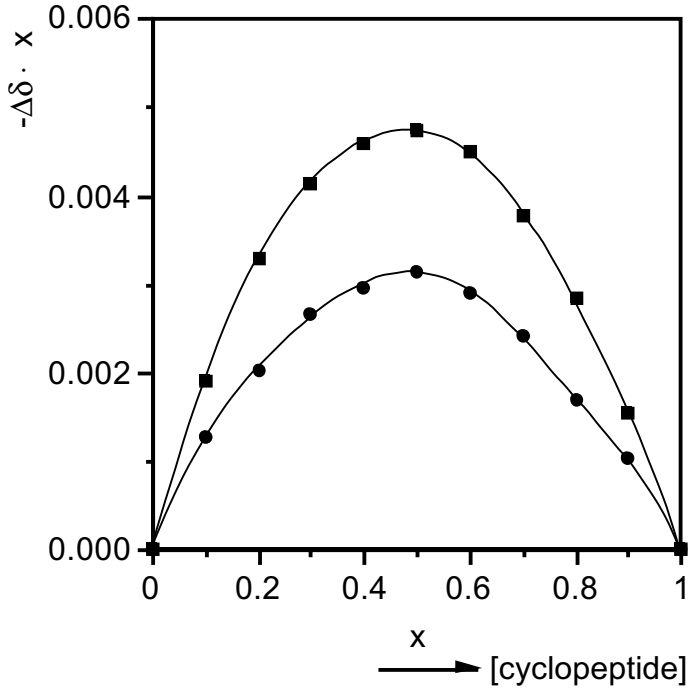
^{1}H-NMR spectra: **2c** (1 mM) in 4% $CD_3OD/CDCl_3$ (a), α-D-methylglucopyranoside (1 mM) in 4% $CD_3OD/CDCl_3$ (c), 1:1 mixture of **2c** and α-D-methylglucopyranoside in 4% $CD_3OD/CDCl_3$ (b)



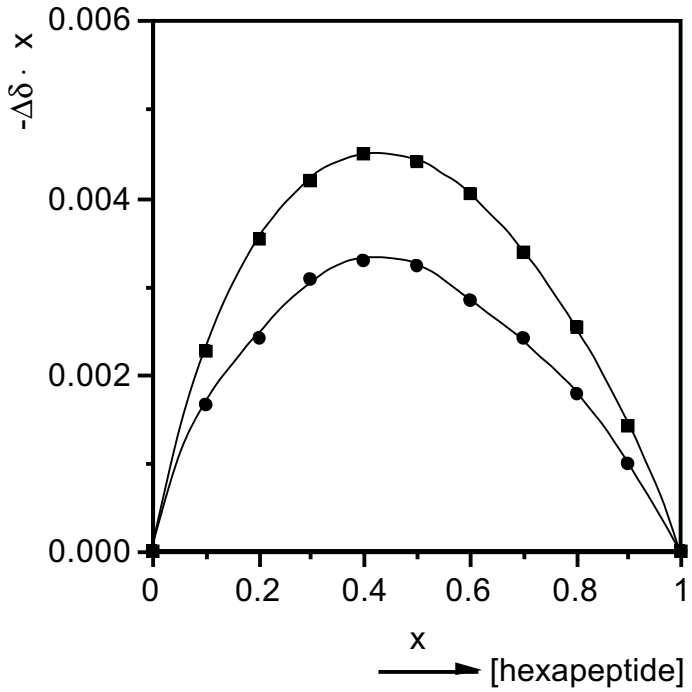
Job-Plot: **2b** + β-D-methylglucopyranoside in 4% $CD_3OD/CDCl_3$ ($\Delta\delta$ = variation of the shift of glucose-H(1) and glucose-(OCH_3))



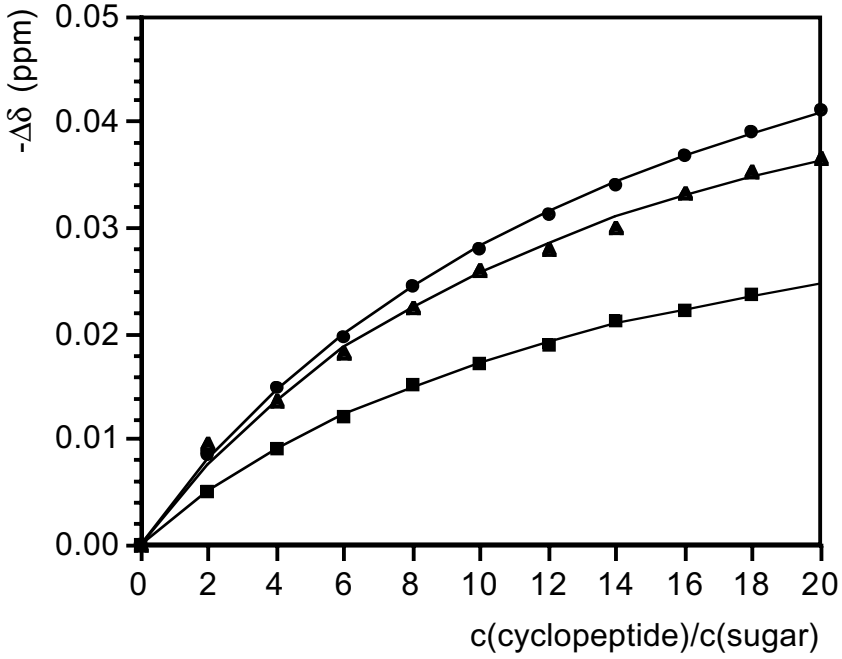
Job-Plot: **2c** + α-D-methylglucopyranoside in 4% $CD_3OD/CDCl_3$ ($\Delta\delta$ = variation of the shift of glucose-H(1) and glucose-(OCH_3))



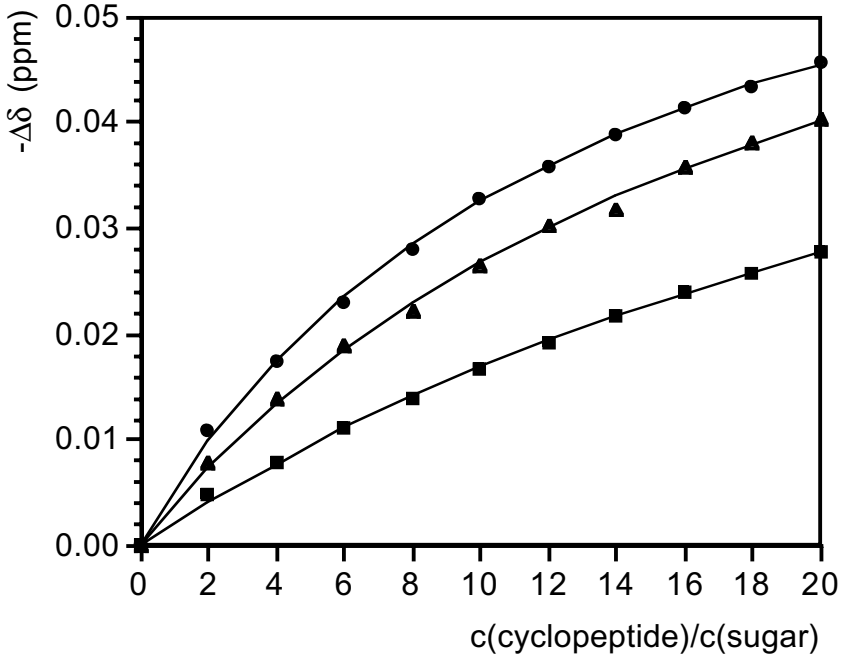
Job-Plot: **3** + α-D-methylglucopyranoside in 4% $CD_3OD/CDCl_3$ (Δδ = variation of the shift of glucose-H(1) and glucose-(OCH_3))



NMR-Titration: Experimental points and calculated curves of **2b** + α-D-methylglucopyranoside (circles), α-D-methylmannopyranoside (squares), and α-D-methylgalactopyranoside (triangles) in 4% $CD_3OD/CDCl_3$ (Δδ = variation of the shift of glycoside-(OCH_3))



NMR-Titration: Experimental points and calculated curves of **2b** + β-D-methylglucopyranoside (circles), β-D-methylribofuranoside (squares), and β-D-methylgalactopyranoside (triangles) in 4% $CD_3OD/CDCl_3$ ($\Delta\delta$ = variation of the shift of glycoside-(OCH_3))



NMR-Titration: Experimental points and calculated curves of β-D-methylglucopyranoside + **2a** (circles), **2b** (squares), and **2c** (triangles) in 4% $CD_3OD/CDCl_3$ ($\Delta\delta$ = variation of the shift of glycoside-(OCH_3))