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Macrocyclic Carbohydrate/Amino Acid Hybrid Molecules - Synthesis and Evaluation as Artificial Receptors

Billing, Johan

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SUPPLEMENTARY MATERIAL

Synthesis of a C_3-symmetric macrocycle with alternating sugar amino acid and tyrosine residues

Johan F. Billing and Ulf J. Nilsson*

Organic and Bioorganic Chemistry, Lund University, PO Box 124, SE-221 00 Lund, Sweden

General methods. THF and CH_2Cl_2 were dried over 4 Å molecular sieves before use and MeOH was dried over 3 Å molecular sieves. Matrex 35-70 mm 60 Å silica (Millipore) was used for flash chromatography and Sephadex LH-20 in CH_2Cl_2:MeOH 1:1 was used for size-exclusion chromatography. Chemical shifts are reported relative to Me_4Si and were calculated using the residual solvent peak as a reference. NMR spectra were assigned with the help of correlation spectroscopy (COSY).

Fmoc-SAA(di-OBz)-Tyr-O^{t}Bu (2). Methyl 3,4-di-*O*-benzoyl-2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside **1** (2.74 g, 4.40 mmol) was dissolved in acetone (600 mL) and the mixture was cooled to 0 °C. Jones's reagent (4 M, 44 mL, prepared by dissolving 24.0 g CrO_3 and 13.8 mL conc. H_2SO_4 in 46.2 mL water) was added. The solution was stirred at room temperature for 2 h and then quenched by the addition of MeOH (120 mL). The mixture was carefully evaporated (caution: bumping) and the residue was dissolved in water (200 mL) and EtOAc (200 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 × 200 mL). The organic phases were combined and washed with water (3 × 200 ml), dried over Na_2SO_4 and evaporated. The crude oxidation product was dissolved in THF (75 mL) and H-Tyr-O^{t}Bu (1.04 g, 4.40 mmol), HOBt (0.594 g, 4.40 mmol), EDC•HCl (0.885 g, 4.62 mmol) and *N* -methylmorpholine (0.97 mL, 8.80 mmol) were added. After 16 h, the mixture was concentrated, dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (Toluene:EtOAc 5:2, R_f = 0.24) to give **2** (2.01 g, 53%) as a white amorphous solid. $[\alpha]^{22}_D$ = +32 (*c* 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.22 (s, 1H, Tyr-OH), 8.43 (d, *J*=7.5 Hz, 1H, NH), 7.80 (m, 6H, Bz-*o*+2×Fmoc-H), 7.57 (m, 4H, Bz-*p*+1×Fmoc-H+ 1×NH), 7.38 (m, 7H, Bz-*m*+3×Fmoc-H), 7.17 (m, 2H, Fmoc), 6.98 (d, *J*=8.1 Hz, 2H, Tyr-H$^{\delta}$), 6.64 (d, *J*=8.0 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.51 (t, *J*=9.9 Hz, 1H, SAA-H^3), 5.42 (t, *J*=9.3 Hz, 1H, SAA-H^4), 4.70 (d, *J*=8.5 Hz, 1H, SAA-H^1), 4.33 (d, *J*=9.4 Hz, 1H, SAA-H^5), 4.27 (m, 3H, Tyr-H$^{\alpha}$+2×Fmoc-H), 4.02 (t, *J*=6.2 Hz, 1H, Fmoc), 3.76 (q, *J*=9.0 Hz, SAA-H^2), 3.44 (s, 3H, OMe), 2.83 (d, *J*=6.4 Hz, Tyr-H$^{\beta}$), 1.17 (s, 9H, O^{t}Bu); HRMS (FAB) calcd. for $C_{49}H_{48}N_2O_{12}Na$ (M+Na): 879.3105; found 879.3111.

* Corresponding author. Tel.: +46-46-2228218; fax: +46-46-2228209; e-mail: ulf.nilsson@bioorganic.lth.se.

H-SAA(di-OBz)-Tyr-O^{t}Bu (3). Compound **2** (250 mg, 0.292 mmol) was dissolved in THF (30 mL) and 1-octanethiol (510 μL, 2.92 mmol) and TBAF•$3H_2O$ (184 mg, 0.583 mmol) were added. The mixture was sonicated for 5 minutes and then evaporated. The residue was purified with flash chromatography (CH_2Cl_2:MeOH 20:1, R_f = 0.26) to give **3** (169 mg, 91%) as a white amorphous solid. $[\alpha]^{22}_D$ = -27 (*c* 0.5, DMSO); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.24 (s, 1H, Tyr-OH), 8.32 (d, *J*=7.6 Hz, 1H, NH), 7.85 (d, *J*=7.6 Hz, 2H, Bz-*o*), 7.75 (d, *J*=7.8 Hz, 2H, Bz-*o*), 7.57 (q, *J*=7.6 Hz, 2H, Bz-*p*), 7.46 (t, *J*=7.6 Hz, 2H, Bz-*m*), 7.42 (t, *J*=7.6 Hz, 2H, Bz-*m*), 6.98 (d, *J*=8.3 Hz, 2H, Tyr-H$^\delta$), 6.64 (d, *J*=8.3 Hz, 2H, Tyr-H$^\varepsilon$), 5.38 (m, 2H, SAA-H^3+SAA-H^4), 4.46 (d, *J*=8.0 Hz, 1H, SAA-H^1), 4.31 (d, *J*=9.3 Hz, 1H, SAA-H^5), 4.24 (q, *J*=7.2 Hz, 1H, Tyr-H$^\alpha$), 3.48 (s, 3H, OMe), 2.84 (m, 3H, SAA-H^2+Tyr-H$^\beta$), 1.74 (br s, 2H, NH_2), 1.17 (s, 9H, O^{t}Bu); HRMS (FAB) calcd. for $C_{34}H_{38}N_2O_{10}Na$ (M+Na): 657.2424; found 657.2430.

Fmoc-[SAA(di-OBz)-Tyr]$_2$-O^{t}Bu (4). Compound **2** (445 mg, 0.519 mmol) was dissolved in CH_2Cl_2 (8 mL) and Et_3SiH (205 μL, 1.30 mmol) and TFA (4 mL, 51.9 mmol) were added. The mixture was stirred for 4 h and then coevaporated with toluene. The residue was dissolved together with compound **3** (330 mg, 0.519 mmol) in THF (25 mL) and HOBt (70.2 mg, 0.519 mmol) and DIC (98 μL, 0.623 mmol) were added. The mixture was stirred for 16 h and then evaporated. The product was purified with flash chromatography (Toluene:EtOAc 1:1, R_f = 0.13) followed by size-exclusion chromatography to give **4** (428 mg, 58%) as a white amorphous solid. $[\alpha]^{22}_D$ = +9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.22 (s, 1H, Tyr-OH), 9.08 (s, 1H, Tyr-OH), 8.41 (d, *J*=9.1 Hz, 1H, NH), 8.36 (d, *J*=7.5 Hz, 1H, NH), 8.18 (d, *J*=8.4 Hz, 1H, NH), 7.76 (m, 10H, Bz-*o*+2×Fmoc-H), 7.57 (m, 6H, Bz-*p*+1×Fmoc-H+1×NH), 7.41 (m, 11H, Bz-*m*+3×Fmoc-H), 7.14 (m, 2H, Fmoc), 6.98 (d, *J*=8.3 Hz, 2H, Tyr-H$^\delta$), 6.74 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.64 (d, *J*=8.4 Hz, 2H, Tyr-H$^\varepsilon$), 6.43 (d, *J*=8.3 Hz, 2H, Tyr-H$^\varepsilon$), 5.46 (m, 3H, 2×SAA-H^3+1×SAA-H^4), 5.30 (t, *J*=9.6 Hz, 1H, SAA-H^4), 4.66 (d, *J*=8.6 Hz, 1H, SAA-H^1), 4.63 (d, *J*=8.5 Hz, 1H, SAA-H^1), 4.35 (m, 2H, SAA-H^5+Tyr-H$^\alpha$), 4.24 (m, 3H, SAA-H^5+Tyr-H$^\alpha$+ Fmoc-H), 4.10 (m, 2H, SAA-H^2+Fmoc-H), 4.01 (t, *J*=6.7 Hz, 1H, Fmoc-H), 3.69 (q, *J*=9.3 Hz, SAA-H^2), 3.40 (s, 3H, OMe), 3.17 (s, 3H, OMe), 2.83 (d, *J*=6.6 Hz, Tyr-H$^\beta$), 2.54 (Tyr-H$^\beta$, obscured by solvent signal), 2.31 (t, *J*=13.9 Hz, 1H, Tyr-H$^\beta$), 1.18 (s, 9H, O^{t}Bu); HRMS (FAB) calcd. for $C_{79}H_{76}N_4O_{21}Na$ (M+Na): 1439.4900; found 1439.4886.

Fmoc-[SAA(di-OBz)-Tyr]$_3$-O^{t}Bu (5). Compound **4** (200 mg, 0.141 mmol) was dissolved in CH_2Cl_2 (2.2 mL) and Et_3SiH (56 μL, 0.352 mmol) and TFA (1.1 mL, 14.1 mmol) were added. The mixture was stirred for 4 h and then coevaporated with toluene. The residue was dissolved together with compound **3** (89.5 mg, 0.141 mmol) in THF (7 mL) and HOBt (19.0 mg, 0.141 mmol) and DIC (26 μL, 0.169 mmol) were added. The mixture was stirred for 15 h and then evaporated. The product was purified with flash chromatography (CH_2Cl_2:MeOH 15:1, R_f = 0.26) followed by size-exclusion chromatography to give **5** (190 mg, 68%) as a white amorphous solid. $[\alpha]^{22}_D$ = +21 (*c* 0.5, acetone); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.23 (s, 1H, Tyr-OH), 9.10 (s, 1H, Tyr-OH), 9.09 (s, 1H, Tyr-OH), 8.39 (m, 3H, NH), 8.16 (d, *J*=8.7 Hz, 1H, NH), 8.11 (d, *J*=7.9 Hz, 1H, NH), 7.77 (m, 14H, Bz-*o*+2×Fmoc-H), 7.55 (m, 8H, Bz-*p*+1×Fmoc-H+1×NH), 7.40 (m, 15H, Bz-*m*+3×Fmoc-H), 7.14 (m,

2H, Fmoc), 6.98 (d, *J*=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.73 (d, *J*=8.4 Hz, 4H, Tyr-H$^{\delta}$), 6.63 (d, *J*=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.43 (d, *J*=8.3 Hz, 4H, Tyr-H$^{\varepsilon}$), 5.48 (m, 4H, 3×SAA-H^{3}+ 1×SAA-H^{4}), 5.32 (t, *J*=9.0 Hz, 1H, SAA-H^{4}), 5.29 (t, *J*=9.6 Hz, 1H, SAA-H^{4}), 4.66 (d, *J*=8.3 Hz, 1H, SAA-H^{1}), 4.62 (d, *J*=8.2 Hz, 1H, SAA-H^{1}), 4.58 (d, *J*=8.4 Hz, 1H, SAA-H^{1}), 4.30 (m, 7H, 3× S A A - H^{5}+3×Tyr-H$^{\alpha}$+Fmoc-H), 4.10 (m, 2H, SAA-H^{2}+Fmoc-H), 4.00 (m, 2H, SAA-H^{2}+Fmoc-H), 3.68 (q, *J*=9.3 Hz, SAA-H^{2}), 3.40 (s, 3H, OMe), 3.18 (s, 3H, OMe), 3.15 (s, 3H, OMe), 2.83 (d, *J*=6.6 Hz, Tyr-H$^{\beta}$), 2.54 (Tyr-H$^{\beta}$, obscured by solvent signal), 2.28 (t, *J*=11.1 Hz, 1H, Tyr-H$^{\beta}$), 1.18 (s, 9H, O^{t}Bu); HRMS (FAB) calcd. for $C_{109}H_{104}N_6O_{30}Na$ (M+Na): 1999.6695; found 1999.6700.

H-[SAA(di-OBz)-Tyr]$_3$-O^{t}Bu (6). Compound **5** (168 mg, 84.7 μmol) was dissolved in THF (3 mL) and *N*-(2-mercaptoethyl)aminomethyl polystyrene (2.1 mmol/g, 400 mg) and DBU (25 μL, 169 μmol) were added. After stirring the mixture for 6 h, the solid phase was filtered off and washed with THF (2×5 mL) and MeOH (3×5 mL). The filtrate and washings were combined and evaporated. The residue was dissolved in CH_2Cl_2:MeOH 9:1 and filtered through silica. Evaporation of the filtrate gave **6** (146 mg, 98%) as a yellowish amorphous solid. $[\alpha]^{22}_{D}$ = +10 (*c* 0.5, DMSO); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.86 (m, 12H, Bz-*o*), 7.51 (m, 6H, Bz-*p*), 7.35 (m, 12H, Bz-*m*), 7.04 (d, *J*=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.84 (d, *J*=8.3 Hz, 2H, Tyr-H$^{\delta}$), 6.82 (d, *J*=8.3 Hz, 2H, Tyr-H$^{\delta}$), 6.71 (d, *J*=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.53 (d, *J*=7.8 Hz, 2 H, Tyr-H$^{\varepsilon}$), 6.51 (d, *J*=8.1 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.72 (t, *J*=10.0 Hz, 1H, SAA-H^{3}), 5.65 (t, *J*=10.0 Hz, 1H, SAA-H^{3}), 5.49 (t, *J*=9.7 Hz, 1H, SAA-H^{4}), 5.45 (t, *J*=9.3 Hz, 1H, SAA-H^{3}), 5.37 (t, *J*=9.7 Hz, 1H, SAA-H^{4}), 5.36 (t, *J*=9.5 Hz, 1H, SAA-H^{4}), 4.72 (d, *J*=8.4 Hz, 1H, SAA-H^{1}), 4.66 (d, *J*=8.2 Hz, 1H, SAA-H^{1}), 4.43 (m, 4H, SAA-H^{1}+3×Tyr-H$^{\alpha}$), 4.29 (d, *J*=10.0 Hz, 1H, SAA-H^{5}), 4.20 (d, *J*=9.7 Hz, 1H, SAA-H^{5}), 4.19 (d, *J*=9.9 Hz, 1H, SAA-H^{5}), 4.05 (dd, *J*=10.3 Hz, *J*=8.6 Hz, SAA-H^{2}), 4.00 (dd, *J*=10.3 Hz, *J*=8.4 Hz, SAA-H^{2}), 3.55 (s, 3H, OMe), 3.36 (s, 3H, OMe), 3.33 (s, 3H, OMe), 2.98 (m, 3H, 1×SAA-H^{2}+2×Tyr-H$^{\beta}$), 2.82 (m, 2H, Tyr-H$^{\beta}$), 2.61 (m, 2H, Tyr-H$^{\beta}$), 1.29 (s, 9H, O^{t}Bu); HRMS (FAB) calcd. for $C_{94}H_{94}N_6O_{28}Na$ (M+Na): 1777.6014; found 1777.6017.

cyclo[SAA(di-OBz)-Tyr]$_3$ (7). Compound **6** (156 mg, 88.7 μmol) was dissolved in CH_2Cl_2 (6.8 mL) and Et_3SiH (35 μL, 222 μmol) and TFA (3.4 mL, 44.3 mmol) were added. The mixture was stirred for 4 h and then coevaporated with toluene. The residue was dissolved in THF (89 mL) and DIPEA (45 μL, 266 μmol) and HAPyU (46.0 mg, 106 μmol) were added. After stirring for 5 h, the reaction was quenched by addition of MeOH (5 mL). The reaction mixture was evaporated and the product was purified with flash chromatography (CH_2Cl_2:MeOH 15:1, R_f = 0.20) followed by size-exclusion chromatography to give **7** (40.3 mg, 27%) as a white amorphous solid. $[\alpha]^{22}_{D}$ = -62 (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.95 (d, *J*=8.1 Hz, 12H, Bz-*o*), 7.54 (m, 6H, Bz-*p*), 7.43 (t, *J*=8.0 Hz, 6H, Bz-*m*), 7.40 (t, *J*=7.7 Hz, 6H, Bz-*m*), 6.91 (d, *J*=8.5 Hz, 6H, Tyr-H$^{\delta}$), 6.55 (d, *J*=8.5 Hz, 6H, Tyr-H$^{\varepsilon}$), 5.84 (t, *J*=7.8 Hz, 3H, SAA-H^{4}), 5.50 (t, *J*=8.0 Hz, 3H, SAA-H^{3}), 4.86 (obscured by solvent signal, SAA-H^{1}), 4.41 (t, *J*=7.0 Hz, 3H, SAA-H^{2}), 4.25 (t, *J*=6.7 Hz, 3H, Tyr-H$^{\alpha}$), 4.22 (d, *J*=7.7 Hz, 3H, SAA-H^{5}), 3.54 (s, 9H, OMe), 3.09 (dd, *J*=14.1 Hz, *J*=6.2 Hz, 3H, Tyr-H$^{\beta}$), 2.92 (dd, *J*=13.9 Hz, *J*=8.1 Hz, 3H, Tyr-H$^{\beta}$); HRMS (FAB) calcd. for $C_{90}H_{84}N_6O_{27}Na$ (M+Na): 1703.5282; found 1703.5294.

cyclo(SAA-Tyr)$_3$ (8). Compound **7** (11.5 mg, 6.84 μmol) was dissolved in MeOH (2 mL) and NaOMe/MeOH (0.1 M, 40 μL) was added. The mixture was stirred for two days followed by addition of more NaOMe/MeOH (0.1 M, 40 μL). The mixture was stirred for three more days, neutralized with AcOH and evaporated. The residue was dissolved in H_2O:DMSO 1:1 and purified using preparative HPLC (C_{18} column, 10→20% B in A over 40 min, A: H_2O + 0.1% TFA, B: CH_3CN + 0.1% TFA, t_R=18 min) to afford **8** (1.15 mg, 16%) as a fluffy white powder after lyophilization. $[\alpha]^{22}_D$ = -76 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.05 (d, *J*=8.5 Hz, 6H, Tyr-H$^{\delta}$), 6.68 (d, *J*=8.5 Hz, 6H, Tyr-H$^{\varepsilon}$), 4.64 (d, *J*=6.1 Hz, 3H, SAA-H^1), 4.36 (t, *J*=6.9 Hz, 3H, Tyr-H$^{\alpha}$), 3.97 (t, *J*=7.8 Hz, 3H, SAA-H^4), 3.83 (d, *J*=7.4 Hz, 3H, SAA-H^5), 3.81 (t, *J*=7.8 Hz, 3H, SAA-H^2), 3.54 (t, *J*=7.8 Hz, 3H, SAA-H^3), 3.36 (s, 9H, OMe), 3.21 (dd, *J*=14.2 Hz, *J*=5.5 Hz, 3H, Tyr-H$^{\beta}$), 3.05 (dd, *J*=13.7 Hz, *J*=8.1 Hz, 3H, Tyr-H$^{\beta}$); HRMS (FAB) calcd. for $C_{48}H_{60}N_6O_{21}Na$ (M+Na): 1079.3709; found 1079.3712.