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

Billing, Johan

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PO Box 117  
221 00 Lund  
+46 46-222 00 00

## SUPPLEMENTARY MATERIAL

### Synthesis of a C<sub>3</sub>-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<sub>2</sub>Cl<sub>2</sub> 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<sub>2</sub>Cl<sub>2</sub>:MeOH 1:1 was used for size-exclusion chromatography. Chemical shifts are reported relative to Me<sub>4</sub>Si 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<sup>t</sup>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<sub>3</sub> and 13.8 mL conc. H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> and evaporated. The crude oxidation product was dissolved in THF (75 mL) and H-Tyr-O<sup>t</sup>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<sub>f</sub> = 0.24) to give **2** (2.01 g, 53%) as a white amorphous solid. [α]<sub>D</sub><sup>22</sup> = +32 (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 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<sup>δ</sup>), 6.64 (d, *J*=8.0 Hz, 2H, Tyr-H<sup>ε</sup>), 5.51 (t, *J*=9.9 Hz, 1H, SAA-H<sup>3</sup>), 5.42 (t, *J*=9.3 Hz, 1H, SAA-H<sup>4</sup>), 4.70 (d, *J*=8.5 Hz, 1H, SAA-H<sup>1</sup>), 4.33 (d, *J*=9.4 Hz, 1H, SAA-H<sup>5</sup>), 4.27 (m, 3H, Tyr-H<sup>α</sup>+2×Fmoc-H), 4.02 (t, *J*=6.2 Hz, 1H, Fmoc), 3.76 (q, *J*=9.0 Hz, SAA-H<sup>2</sup>), 3.44 (s, 3H, OMe), 2.83 (d, *J*=6.4 Hz, Tyr-H<sup>β</sup>), 1.17 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>49</sub>H<sub>48</sub>N<sub>2</sub>O<sub>12</sub>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<sup>t</sup>Bu (3).** Compound **2** (250 mg, 0.292 mmol) was dissolved in THF (30 mL) and 1-octanethiol (510  $\mu$ L, 2.92 mmol) and TBAF $\cdot$ 3H<sub>2</sub>O (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<sub>2</sub>Cl<sub>2</sub>:MeOH 20:1, R<sub>f</sub> = 0.26) to give **3** (169 mg, 91%) as a white amorphous solid.  $[\alpha]_D^{22} = -27$  (*c* 0.5, DMSO); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz)  $\delta$  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 <sup>$\delta$</sup> ), 6.64 (d, *J*=8.3 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 5.38 (m, 2H, SAA-H<sup>3</sup>+SAA-H<sup>4</sup>), 4.46 (d, *J*=8.0 Hz, 1H, SAA-H<sup>1</sup>), 4.31 (d, *J*=9.3 Hz, 1H, SAA-H<sup>5</sup>), 4.24 (q, *J*=7.2 Hz, 1H, Tyr-H <sup>$\alpha$</sup> ), 3.48 (s, 3H, OMe), 2.84 (m, 3H, SAA-H<sup>2</sup>+Tyr-H <sup>$\beta$</sup> ), 1.74 (br s, 2H, NH<sub>2</sub>), 1.17 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>34</sub>H<sub>38</sub>N<sub>2</sub>O<sub>10</sub>Na (M+Na): 657.2424; found 657.2430.

**Fmoc-[SAA(di-OBz)-Tyr]<sub>2</sub>-O<sup>t</sup>Bu (4).** Compound **2** (445 mg, 0.519 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) and Et<sub>3</sub>SiH (205  $\mu$ L, 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  $\mu$ L, 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<sub>f</sub> = 0.13) followed by size-exclusion chromatography to give **4** (428 mg, 58%) as a white amorphous solid.  $[\alpha]_D^{22} = +9$  (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  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 $\times$ Fmoc-H), 7.57 (m, 6H, Bz-*p*+1 $\times$ Fmoc-H+1 $\times$ NH), 7.41 (m, 11H, Bz-*m*+3 $\times$ Fmoc-H), 7.14 (m, 2H, Fmoc), 6.98 (d, *J*=8.3 Hz, 2H, Tyr-H <sup>$\delta$</sup> ), 6.74 (d, *J*=8.4 Hz, 2H, Tyr-H <sup>$\delta$</sup> ), 6.64 (d, *J*=8.4 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 6.43 (d, *J*=8.3 Hz, 2H, Tyr-H <sup>$\epsilon$</sup> ), 5.46 (m, 3H, 2 $\times$ SAA-H<sup>3</sup>+1 $\times$ SAA-H<sup>4</sup>), 5.30 (t, *J*=9.6 Hz, 1H, SAA-H<sup>4</sup>), 4.66 (d, *J*=8.6 Hz, 1H, SAA-H<sup>1</sup>), 4.63 (d, *J*=8.5 Hz, 1H, SAA-H<sup>1</sup>), 4.35 (m, 2H, SAA-H<sup>5</sup>+Tyr-H <sup>$\alpha$</sup> ), 4.24 (m, 3H, SAA-H<sup>5</sup>+Tyr-H <sup>$\alpha$</sup> +Fmoc-H), 4.10 (m, 2H, SAA-H<sup>2</sup>+Fmoc-H), 4.01 (t, *J*=6.7 Hz, 1H, Fmoc-H), 3.69 (q, *J*=9.3 Hz, SAA-H<sup>2</sup>), 3.40 (s, 3H, OMe), 3.17 (s, 3H, OMe), 2.83 (d, *J*=6.6 Hz, Tyr-H <sup>$\beta$</sup> ), 2.54 (Tyr-H <sup>$\beta$</sup> , obscured by solvent signal), 2.31 (t, *J*=13.9 Hz, 1H, Tyr-H <sup>$\beta$</sup> ), 1.18 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>79</sub>H<sub>76</sub>N<sub>4</sub>O<sub>21</sub>Na (M+Na): 1439.4900; found 1439.4886.

**Fmoc-[SAA(di-OBz)-Tyr]<sub>3</sub>-O<sup>t</sup>Bu (5).** Compound **4** (200 mg, 0.141 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2.2 mL) and Et<sub>3</sub>SiH (56  $\mu$ L, 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  $\mu$ L, 0.169 mmol) were added. The mixture was stirred for 15 h and then evaporated. The product was purified with flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH 15:1, R<sub>f</sub> = 0.26) followed by size-exclusion chromatography to give **5** (190 mg, 68%) as a white amorphous solid.  $[\alpha]_D^{22} = +21$  (*c* 0.5, acetone); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  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 $\times$ Fmoc-H), 7.55 (m, 8H, Bz-*p*+1 $\times$ Fmoc-H+1 $\times$ NH), 7.40 (m, 15H, Bz-*m*+3 $\times$ Fmoc-H), 7.14 (m,

2H, Fmoc), 6.98 (d,  $J=8.4$  Hz, 2H, Tyr-H<sup>δ</sup>), 6.73 (d,  $J=8.4$  Hz, 4H, Tyr-H<sup>δ</sup>), 6.63 (d,  $J=8.4$  Hz, 2H, Tyr-H<sup>ε</sup>), 6.43 (d,  $J=8.3$  Hz, 4H, Tyr-H<sup>ε</sup>), 5.48 (m, 4H, 3×SAA-H<sup>3</sup>+1×SAA-H<sup>4</sup>), 5.32 (t,  $J=9.0$  Hz, 1H, SAA-H<sup>4</sup>), 5.29 (t,  $J=9.6$  Hz, 1H, SAA-H<sup>4</sup>), 4.66 (d,  $J=8.3$  Hz, 1H, SAA-H<sup>1</sup>), 4.62 (d,  $J=8.2$  Hz, 1H, SAA-H<sup>1</sup>), 4.58 (d,  $J=8.4$  Hz, 1H, SAA-H<sup>1</sup>), 4.30 (m, 7H, 3×SAA-H<sup>5</sup>+3×Tyr-H<sup>α</sup>+Fmoc-H), 4.10 (m, 2H, SAA-H<sup>2</sup>+Fmoc-H), 4.00 (m, 2H, SAA-H<sup>2</sup>+Fmoc-H), 3.68 (q,  $J=9.3$  Hz, SAA-H<sup>2</sup>), 3.40 (s, 3H, OMe), 3.18 (s, 3H, OMe), 3.15 (s, 3H, OMe), 2.83 (d,  $J=6.6$  Hz, Tyr-H<sup>β</sup>), 2.54 (Tyr-H<sup>β</sup>, obscured by solvent signal), 2.28 (t,  $J=11.1$  Hz, 1H, Tyr-H<sup>β</sup>), 1.18 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>109</sub>H<sub>104</sub>N<sub>6</sub>O<sub>30</sub>Na (M+Na): 1999.6695; found 1999.6700.

**H-[SAA(di-OBz)-Tyr]<sub>3</sub>-O<sup>t</sup>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<sub>2</sub>Cl<sub>2</sub>:MeOH 9:1 and filtered through silica. Evaporation of the filtrate gave **6** (146 mg, 98%) as a yellowish amorphous solid.  $[\alpha]_D^{22} = +10$  (*c* 0.5, DMSO); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 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<sup>δ</sup>), 6.84 (d,  $J=8.3$  Hz, 2H, Tyr-H<sup>δ</sup>), 6.82 (d,  $J=8.3$  Hz, 2H, Tyr-H<sup>δ</sup>), 6.71 (d,  $J=8.4$  Hz, 2H, Tyr-H<sup>ε</sup>), 6.53 (d,  $J=7.8$  Hz, 2H, Tyr-H<sup>ε</sup>), 6.51 (d,  $J=8.1$  Hz, 2H, Tyr-H<sup>ε</sup>), 5.72 (t,  $J=10.0$  Hz, 1H, SAA-H<sup>3</sup>), 5.65 (t,  $J=10.0$  Hz, 1H, SAA-H<sup>3</sup>), 5.49 (t,  $J=9.7$  Hz, 1H, SAA-H<sup>4</sup>), 5.45 (t,  $J=9.3$  Hz, 1H, SAA-H<sup>3</sup>), 5.37 (t,  $J=9.7$  Hz, 1H, SAA-H<sup>4</sup>), 5.36 (t,  $J=9.5$  Hz, 1H, SAA-H<sup>4</sup>), 4.72 (d,  $J=8.4$  Hz, 1H, SAA-H<sup>1</sup>), 4.66 (d,  $J=8.2$  Hz, 1H, SAA-H<sup>1</sup>), 4.43 (m, 4H, SAA-H<sup>1</sup>+3×Tyr-H<sup>α</sup>), 4.29 (d,  $J=10.0$  Hz, 1H, SAA-H<sup>5</sup>), 4.20 (d,  $J=9.7$  Hz, 1H, SAA-H<sup>5</sup>), 4.19 (d,  $J=9.9$  Hz, 1H, SAA-H<sup>5</sup>), 4.05 (dd,  $J=10.3$  Hz,  $J=8.6$  Hz, SAA-H<sup>2</sup>), 4.00 (dd,  $J=10.3$  Hz,  $J=8.4$  Hz, SAA-H<sup>2</sup>), 3.55 (s, 3H, OMe), 3.36 (s, 3H, OMe), 3.33 (s, 3H, OMe), 2.98 (m, 3H, 1×SAA-H<sup>2</sup>+2×Tyr-H<sup>β</sup>), 2.82 (m, 2H, Tyr-H<sup>β</sup>), 2.61 (m, 2H, Tyr-H<sup>β</sup>), 1.29 (s, 9H, O<sup>t</sup>Bu); HRMS (FAB) calcd. for C<sub>94</sub>H<sub>94</sub>N<sub>6</sub>O<sub>28</sub>Na (M+Na): 1777.6014; found 1777.6017.

**cyclo[SAA(di-OBz)-Tyr]<sub>3</sub> (7).** Compound **6** (156 mg, 88.7 μmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6.8 mL) and Et<sub>3</sub>SiH (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<sub>2</sub>Cl<sub>2</sub>:MeOH 15:1, R<sub>f</sub> = 0.20) followed by size-exclusion chromatography to give **7** (40.3 mg, 27%) as a white amorphous solid.  $[\alpha]_D^{22} = -62$  (*c* 0.5, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 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<sup>δ</sup>), 6.55 (d,  $J=8.5$  Hz, 6H, Tyr-H<sup>ε</sup>), 5.84 (t,  $J=7.8$  Hz, 3H, SAA-H<sup>4</sup>), 5.50 (t,  $J=8.0$  Hz, 3H, SAA-H<sup>3</sup>), 4.86 (obscured by solvent signal, SAA-H<sup>1</sup>), 4.41 (t,  $J=7.0$  Hz, 3H, SAA-H<sup>2</sup>), 4.25 (t,  $J=6.7$  Hz, 3H, Tyr-H<sup>α</sup>), 4.22 (d,  $J=7.7$  Hz, 3H, SAA-H<sup>5</sup>), 3.54 (s, 9H, OMe), 3.09 (dd,  $J=14.1$  Hz,  $J=6.2$  Hz, 3H, Tyr-H<sup>β</sup>), 2.92 (dd,  $J=13.9$  Hz,  $J=8.1$  Hz, 3H, Tyr-H<sup>β</sup>); HRMS (FAB) calcd. for C<sub>90</sub>H<sub>84</sub>N<sub>6</sub>O<sub>27</sub>Na (M+Na): 1703.5282; found 1703.5294.

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