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

Billing, Johan

2005

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Tetrahedron

Tetrahedron 61 (2005) 863–874

Cyclic peptides containing a δ-sugar amino acid—synthesis and evaluation as artificial receptors

Johan F. Billing and Ulf J. Nilsson*

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

Received 25 August 2004; revised 21 October 2004; accepted 12 November 2004

Available online 10 December 2004

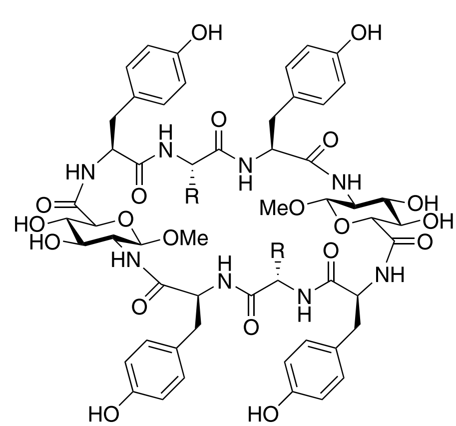
Abstract—An Fmoc-protected δ-sugar amino acid, prepared by oxidation of a glucosamine derivative, was coupled to three different tripeptide *tert*-butyl esters (H-Tyr-Tyr-Tyr-O^{t}Bu, H-Tyr-Glu(OBzl)-Tyr-O^{t}Bu and H-Tyr-Arg(Mtr)-Tyr-O^{t}Bu) and the resulting sugar amino acid/amino acid hybrids were transformed into dimers that were subsequently cyclized to give three C_2-symmetric macrocycles. The macrocycles were deprotected and their binding properties towards *p*-nitrophenyl glycosides, nucleotides, and purines were examined. Of the ligands screened, only some of the purines showed weak, but significant, binding.

1. Introduction

Interactions of small ligands, such as carbohydrates, metabolites or hormones, with binding sites in proteins are vital to life processes and the synthesis of artificial receptors that mimic such interactions has been an ongoing goal in many research groups for a long time.[1] A basic design for biomimetic artificial receptors involves amphiphilic molecules, often macrocycles, with both polar and non-polar regions, thus enabling interactions with both polar and non-polar regions of a ligand.

Sugar amino acids[2–5] are carbohydrates that contain at least one amino and one carboxylic acid functionality, which allows for the use of peptide coupling chemistry in order to combine them with amino acids or other building blocks. Sugar amino acids have been used to prepare cyclic homo-oligomers[6–8] and cyclic sugar amino acid/amino acid hybrids,[9–15] that have been used in various studies. It has been proposed that such molecules could be interesting artificial receptors, and in one case it has been shown that a cyclodextrin-like cyclic hexamer could bind to benzoic acid and *p*-nitrophenol in water, although no binding constants were given.[6] We decided to explore the use of sugar amino acids as polar structural elements combined with non-polar aromatic amino acids for the construction of amphiphilic molecules as biomimetic receptors.

Herein, we report the synthesis of polyamphiphilic water-soluble macrocyclic sugar amino acid/amino acid hybrid molecules **1a–c** (see Fig. 1) and an investigation of their binding properties against biomolecules. We chose to use the δ-sugar amino acid obtained by oxidation of a partially protected methyl β-glycoside of glucosamine together with the aromatic amino acid tyrosine as building blocks for our macrocycles. The δ-sugar amino acid was chosen because of its extended geometry, which prevents turn formation and presumably thus gives rise to more accessible cores of the macrocycles. In addition, we introduced amino acids with charged side chains to enhance solubility and potentially



1a: R = tyrosine side chain
1b: R = CH_2CH_2COOH
1c: R = $CH_2CH_2CH_2NHC(NH)NH_2$

Figure 1. Synthesized macrocycles.

Keywords: Sugar amino acids; Macrocycles; Cyclic peptides; Artificial receptors; Molecular recognition.

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

0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.11.024

also binding. Two monosaccharides and six amino acids were used in each macrocyclic ring in order to obtain macrocycles large enough to form a central pocket where ligands might bind.

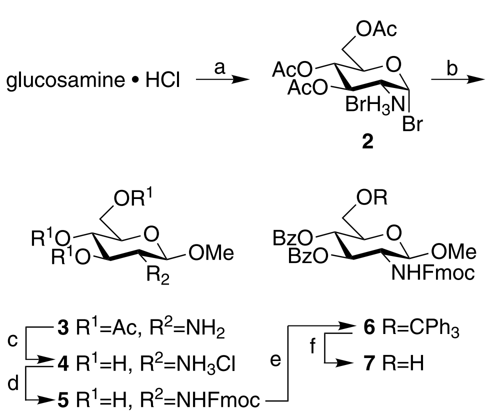
2. Results and discussion

2.1. Synthesis

The synthetic strategy towards the macrocycles involved two building blocks for each macrocycle, a C-protected tripeptide and an amino sugar precursor, which upon oxidation gives the N-protected sugar amino acid (SAA). The tripeptide and the sugar amino acid were coupled together to give a linear sugar amino acid/amino acid hybrid, which was then transformed into a dimer that was subsequently cyclized to give the desired macrocycle. To achieve this, it was necessary to use orthogonal protecting groups for N- and C-protection. The use of the base-labile Fmoc group and acid-labile *tert*-butyl esters met this requirement.

The starting material glucosamine hydrochloride was transformed into the known tri-*O*-acetylated methyl pyranoside **3** in a two-step procedure using a combination of previously described methods[16,17] (Scheme 1). Attempts to deacetylate **3** using base-catalyzed transesterfication with Me_2NEt in MeOH gave ca. 12% of an *N*-acetyl side-product, while acidic transesterfication cleanly produced known hydrochloride **4**.[18] The amine was selectively protected using *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu) to give **5**. The primary hydroxyl group was protected as the triphenylmethyl ether and the secondary hydroxyl groups as benzoates to give **6**. Cleavage of the triphenylmethyl ether using hydrogen bromide in acetic acid completed the synthesis of sugar amino acid precursor **7**. A similar sequence leading to the α anomer of the same sugar amino acid has been disclosed.[19]

Three different tripeptide *tert*-butyl esters **10a–c** were prepared in solution in good yields (Scheme 2) to serve as the required C-protected tripeptides. Peptide couplings were made using *N*-(3-dimethylaminopropyl)-*N*′-ethyl carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine in THF, *tert*-butyl esters were cleaved with 33% TFA in CH_2Cl_2 using Et_3SiH as a scavenger,[20] and piperidine was used to cleave the Fmoc group. The 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group has previously been reported to be stable at 25% TFA in CH_2Cl_2,[15] and this was also the case at 33% TFA in CH_2Cl_2.



Scheme 1. Synthesis of the δ-sugar amino acid precursor **7**: (a) AcBr (neat), 3 days, 82%; (b) MeOH, pyridine, 1 h, 76%; (c) HCl/MeOH, 24 h, 95%; (d) Fmoc-OSu, $NaHCO_3$, 15 h, 76%; (e) chlorotriphenylmethane, pyridine, 85 °C, 2 h, then BzCl, pyridine, r.t., 4 h, 63%; (f) HBr/AcOH, 3 min, 81%.

Sugar amino acid precursor **7** was oxidized with Jones's reagent and the crude sugar amino acid was directly coupled to a C-protected tripeptide **10a–c** (Scheme 3) to give sugar amino acid/amino acid hybrids **14a–c**. The hybrids **14a–c** were either deprotected at the N-terminal using DBU in the presence of a solid-phase thiol as a scavenger for the liberated dibenzofulvene[21] to give **15a–c** or at the C-terminal using TFA/Et_3SiH/CH_2Cl_2[20] to give **16a–c**. The N-deprotected hybrids **15a–c** and the C-deprotected hybrids **16a–c** were coupled together using *N*,*N*′-diisopropylcarbodiimide (DIC) and HOBt to give linear dimers **17a–c**. The EDC·HCl/HOBt/*N*-methylmorpholine coupling protocol that had been used earlier in the synthetic scheme gave excessive epimerization in this coupling (ca. 20% epimer of **17a** formed according to NMR) and could not be used here. The DIC/HOBt protocol without base has been reported to give good results in difficult couplings[22] and gave good results with **17a–c** (no epimerization according to ^{1}H NMR spectrum). The linear dimers were N-deprotected as above to give **18a–c**.

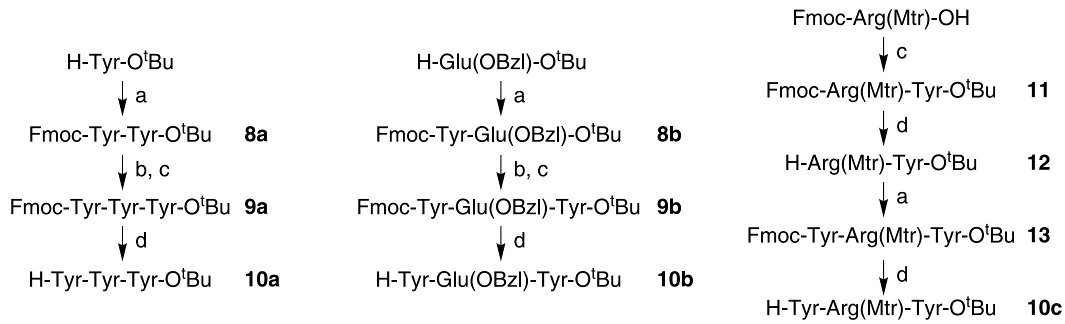
In order to evaluate cyclization conditions, a portion of **18a** was C-deprotected and initial cyclization attempts were made using the following conditions:

(a) EDC·HCl/HOBt and *N*-methylmorpholine in THF.
(b) DIC/HOBt both with and without *N*,*N*-diisopropylethylamine (DIPEA) in both THF and DMF.
(c) Diphenylphosphoryl azide (DPPA) both with $NaHCO_3$ in DMF and with DIPEA in THF.
(d) 1-[bis-(Dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide (TBTU)†, HOBt, and DIPEA in THF.
(e) 1-[bis-(Dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU) with both DIPEA and 2,4,6-collidine in THF.
(f) 1-(1-Pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridinylmethylene)pyrrolidinium hexafluorophosphate 3-oxide (HAPyU)‡ with both DIPEA and 2,4,6-collidine in THF.

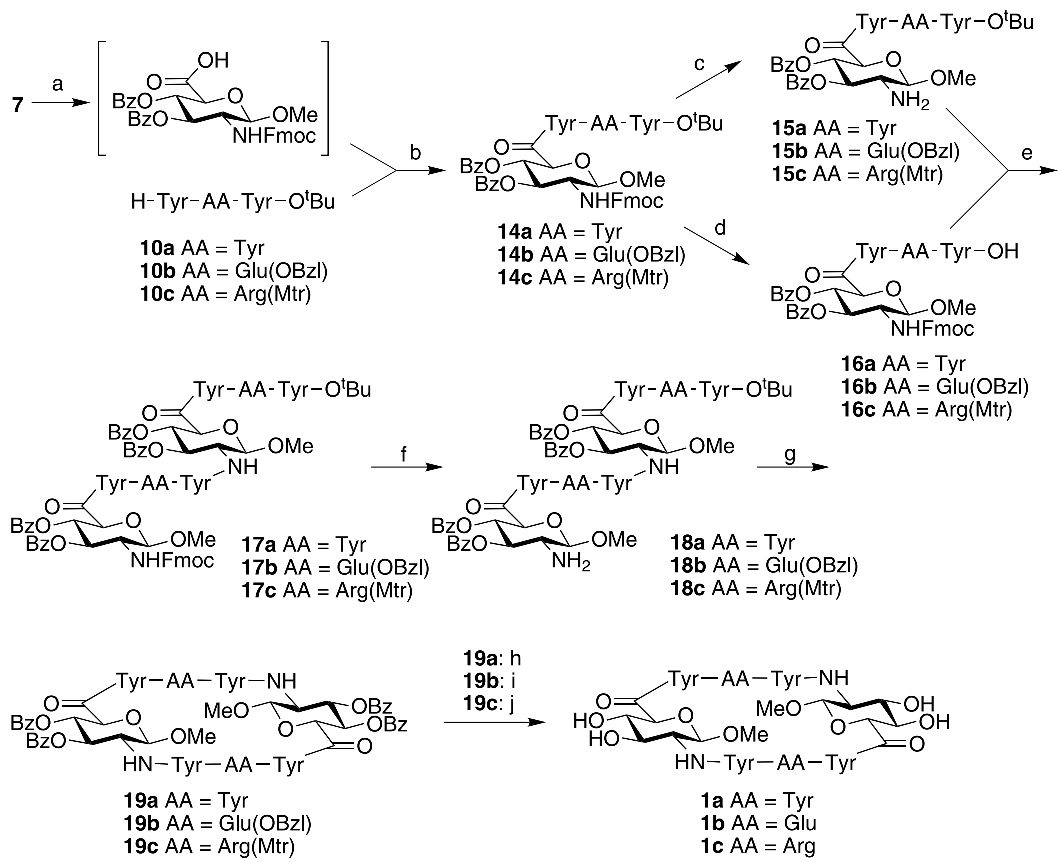
All attempts were carried out at 1 mM concentration of the linear starting material. Only TBTU, HATU, and HAPyU

† HATU and HBTU were long believed to be uronium salts, but have been shown to be guanidinium salts when prepared using the conventional methods.[23] This is likely to be true for TBTU and HAPyU as well. We have chosen to name all these reagents as guanidinium salts.

‡ HAPyU was prepared from 1-hydroxy-7-azabenzotriazole potassium salt (KOAt)[23] and commercially available chloro-*N*,*N*,*N*′,*N*′-bis(tetramethylene)-formamidinium hexafluorophosphate using Knorr's method for the preparation of similar coupling reagents.[24,25]



Scheme 2. Synthesis of tripeptide *tert*-butyl esters **10a–c**: (a) Fmoc-Tyr-OH, EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 83–88%; (b) TFA, Et_3SiH, CH_2Cl_2, 4 h; (c) H-Tyr-O^{t}Bu, EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 83–94%; (d) piperidine, CH_2Cl_2, 30 min, 83–90%.



Scheme 3. Synthesis of macrocycles **1a–c**: (a) CrO_3, H_2SO_4(aq), acetone, 1.5 h; (b) EDC·HCl, HOBt, *N*-methylmorpholine, THF, 16 h, 45–64% (two steps); (c) DBU, *N*-(2-mercaptoethyl) aminomethyl polystyrene, 6 h, 82–99%; (d) TFA, Et_3SiH, CH_2Cl_2, 3–4 h; (e) DIC, HOBt, THF, 16 h, 48–72%; (f) DBU, *N*-(2-mercaptoethyl)aminomethyl polystyrene, 6 h, 88–99%; (g) (i) TFA, Et_3SiH, CH_2Cl_2, 3–4 h; (ii) HAPyU, DIPEA, THF, 2 h, 39–53%; (h) NaOMe/MeOH, 24 h, 61%; (i) (i) HCOOH, Pd black, MeOH, 15 min; (ii) NaOMe/MeOH, 24 h, 81%; (j) (i) TFA, PhSMe, 24 h; (ii) NaOMe/MeOH, 24 h, 59%.

gave the macrocycle **19a** as a major product according to MALDI-TOF analysis of the reaction mixtures.

The HATU and HAPyU reagents have been shown to give less epimerization than TBTU in the cyclization of pentapeptides[26] and these reagents were thus investigated further. Furthermore, it has been shown that DIPEA gives less epimerization than 2,4,6-collidine for the cyclization of pentapeptides by HAPyU,[26] while for segment condensations the opposite is true.[27] Hence, both DIPEA and 2,4,6-collidine were evaluated as bases. In the cyclization of **18a** to **19a**, DIPEA gave a higher yield and less epimerization. Compounds **18b–c** could also be cyclized to **19b–c** using this method (see Table 1). In the cyclization of **18b** to **19b**, HAPyU gave a better yield than HATU.

The ^{1}H NMR spectra of the protected macrocycles **19a–c** only gave poorly resolved spectra with broad peaks at room temperature, presumably due to slow conformational exchange. Resolved spectra of **19a** and **19b** could be obtained at 150 and 120 °C, respectively, but compound **19c** only gave poorly resolved spectra even at these temperatures.

Macrocycle **19a** was deprotected using 10 mM NaOMe in

Table 1. Cyclization conditions, yields and epimerizations

Starting material	Reagents[a]	Reaction time (h)[b]	Isolated yield (%)[c]	Epimerization
18a	HAPyU/2,4,6-collidine	3	35	5% isolated yield
18a	HAPyU/DIPEA	1	37	Not observed
18b	HAPyU/DIPEA	3	53	Traces on TLC
18b	HATU/DIPEA	2.5	32	Traces on TLC
18c	HAPyU/DIPEA	4	34	Traces on TLC

[a] At room temperature in THF with 1 mM concentration of linear starting material.
[b] Reactions were monitored with MALDI-TOF until the deprotected starting material was consumed.
[c] For both C-deprotection and cyclization.

MeOH to afford **1a** in 61% yield. The deprotection of macrocycle **19b** started with the cleavage of the benzyl esters using catalytic transfer hydrogenation with palladium black and formic acid as the hydrogen source,[28] followed by treatment of the crude product with NaOMe/MeOH to give **1b** in 81% yield. In the case of the macrocycle **19c**, the Mtr groups were first cleaved using neat TFA with thioanisole as a scavenger[29] and the crude product was then treated with NaOMe/MeOH to afford **1c** in 59% yield after HPLC purification.

2.2. Conformational analysis

The deprotected macrocycles **1a–c** all gave well resolved NMR spectra at room temperature. Macrocycle **1a** in MeOH-d_4 and macrocycles **1b–c** in D_2O all gave the expected ^{1}H NMR spectra for symmetrical compounds. In addition to the major peaks, macrocycle **1c** in also gave smaller peaks at 2.81 and 2.56 ppm, as well as some overlapping smaller peaks at 3.74 ppm and in the aromatic region. Heating of **1c** in DMSO-d_6 brought the ^{1}H NMR signals to coalescence, which shows that the multiple peaks of **1c** at ambient temperature were due to slow conformational exchange (Fig. 2).

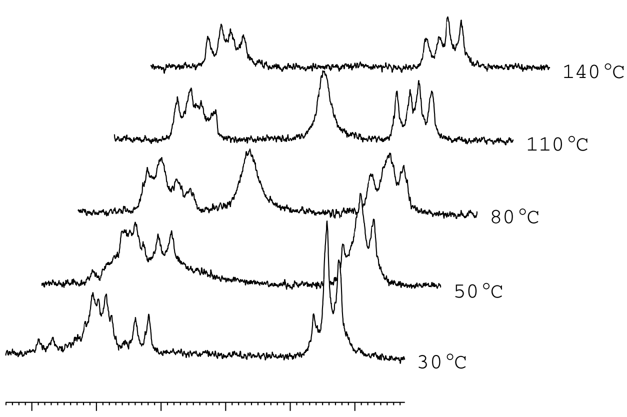


Figure 2. The aromatic region of the ^{1}H NMR spectrum of **1c** at different temperatures (400 MHz, DMSO-d_6).

Monte–Carlo conformational searches were performed on **1a–c** using MacroModel 8.5 (MMFFs force field with water as solvent, 20,000 steps, all backbone torsions were selected for random variation) to give for each macrocycle 110–240 conformers within 5 kcal/mol of the global minimum. When these conformers were studied, a coherent picture emerged. The dominant low-energy conformers for macrocycles **1a–c** were twisted, oblong structures with extended sugar amino acids and turns formed by the tripeptides (Fig. 3). In the conformers with lowest energy, the axial hydrogen atoms in the two sugar amino acids were facing each other, but conformers where one of the sugar amino acids had rotated to place the hydroxyl groups in position to form hydrogen bonds to the tripeptide, or to the other sugar amino acid, were also found. Hydrogen bonds were occasionally found within the tripeptides, but no pattern could be discerned. The $^3J_{HH}$ coupling constants for all three macrocycles indicate that the sugar amino acids are in the $^4C_1$ conformation. This was the case for the calculated conformers of **1a–b**, but many conformers of macrocycle **1c** deviated from the expected $^4C_1$ conformation of the pyranose rings. As there is no support for this in the coupling constants, we conclude that it is an artefact in the calculations possibly induced by the strong hydrogen bond formed between the arginine and tyrosine side chains.

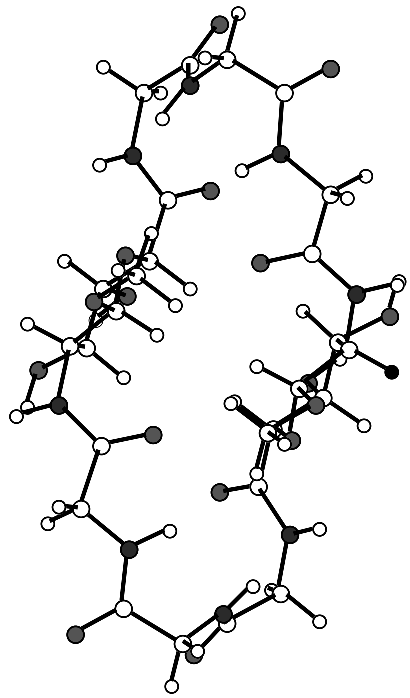


Figure 3. Calculated global energy minimum of macrocycle **1a** (side chains omitted for clarity).

2.3. Molecular recognition properties

Compound **1a** was not soluble in water and its binding properties were not examined. Macrocycles **1b–c** were screened using NMR titrations against a number of putative ligands: *p*-nitrophenyl glycosides, nucleotides, aromatic amino acids, aromatic amines and purines. Of all the ligands screened, only **1b** and caffeine and **1c** and the purine nucleotides 2′-deoxyadenosine 5′-monophosphate (dAMP) and 2′-deoxyguanosine 5′-monophosphate (dGMP) showed weak, but significant, interactions ($K_a \approx 10\ M^{-1}$). For comparison, reference peptide Ac-Tyr-Arg-Tyr-OMe[§] was also titrated with dAMP and dGMP and was found to bind more weakly ($K_a \approx 5\ M^{-1}$). The binding is most likely due to hydrophobic interaction between the purine and tyrosine rings, and the small increase in affinity for **1c** is thus due to its dimeric cyclic structure and/or the presence of the sugar amino acid moieties. However, the weak affinities preclude conclusions regarding detailed structure–affinity relationships.

3. Conclusions

We have described the synthesis of three δ-sugar amino acid/tripeptide dimeric macrocycles and evaluated their binding properties. Macrocycles **1b–c** were found to bind some purine derivatives with weak, but significant, binding constants. Apparently, the structures have to be modified in order to present binding sites pre-organized for higher affinity binding of biomolecules. However, although the binding is weak, this shows that sugar amino acid containing peptides can act as artificial receptors and serves as a starting point for further research.

4. Experimental

4.1. 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 before use. Other solvents were not dried unless specified. Matrex 35–70 μm 60 Å silica (Millipore) was used for flash chromatography. Sephadex LH-20 in CH_2Cl_2/MeOH 1:1 was used for size-exclusion chromatography. Sep-Pak Plus C_{18} cartridges (Waters) were used for solid-phase extraction. 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). All compounds were estimated to be >95% pure by ^1H NMR spectroscopy.

4.2. Preparation of sugar amino acid precursor 7

4.2.1. Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (2).[16,17] Acetyl bromide (70 mL, 0.94 mol) was added to D-glucosamine hydrochloride (21.9 g, 101.6 mmol, dried 24 h in vacuo at 70 °C over P_2O_5) and the mixture was stirred for 3 days at room temperature. Residual acetyl bromide was removed in vacuo (water aspirator) and the crude product was dissolved in hot chloroform (distilled from P_2O_5) and filtered while still hot. Crystals began to form as the solution cooled and diethyl ether was added to the stirred solution until the product precipitated from the mixture to afford **2** (37.6 g, 82%) as small white needles. Mp 143–148 °C (dec.), lit.[16] 149–150 °C (dec.); $[\alpha]_D^{22} = +130$ (*c* 1.0, acetone), lit.[5] $[\alpha]_D = +148.4$ (*c* 5.01, acetone); ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (br s, 3H, NH_3), 7.10 (d, *J* = 3.6 Hz, 1H, H^1), 5.50 (t, *J* = 9.8 Hz, 1H, H^3), 5.25 (t, *J* = 9.7 Hz, 1H, H^4), 4.33 (m, 2H, $H^5 + H^6$), 4.17 (d, *J* = 10.9 Hz, H^6), 3.93 (dd, *J* = 10.3, 3.7 Hz, 1H, H^5), 2.26, 2.13, 2.09 (3s, 3H each, OAc)[¶]; HRMS (FAB) calcd for $C_{12}H_{18}BrNO_7Na$ (M − HBr + Na): 390.0164; found 390.0171.

4.2.2. Methyl tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (3). Hydrobromide **2** (37.6 g, 84 mmol) was dissolved in MeOH (800 mL) and pyridine (8 mL, distilled from CaH_2) was added. After 1 h, toluene (150 mL) was added and the mixture was concentrated. The residue was dissolved in chloroform (750 mL), washed with Na_2CO_3-(aq) (5%, 2 × 100 mL) and water (100 mL), dried over Na_2SO_4, and concentrated. The crude product was recrystallized in chloroform/heptane to give **3** (20.3 g in three crops, 76%) as small white needles. Mp 146–148 °C, lit.[17] 151–152 °C; $[\alpha]_D^{22} = +14$ (*c* 1.0, MeOH), lit.[17] $[\alpha]_D^{27} = +15$ (*c* 1, MeOH); ^1H NMR spectrum is in agreement with published data[17]; HRMS (FAB) calcd for $C_{13}H_{21}NO_8Na$ (M + Na): 342.1165, found 342.1158.

4.2.3. Methyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (4). Acetyl chloride (67 mL, 0.95 mol) was slowly added to MeOH (320 mL) at 0 °C. Compound **3** (11.2 g, 35.1 mmol) was added and the solution was stirred for 24 h at room temperature. The solution was concentrated and the crude product was recrystallized in MeOH/EtOAc to give **4** (7.64 g in two crops, 95%) as small white needles. Mp 192–193 °C, lit.[18] 190 °C; $[\alpha]_D^{22} = -25$ (*c* 1.0, water), lit.[18] $[\alpha]_D^{22} = -23.4$ (*c* 1, water); ^1H NMR (300 MHz, D_2O) δ 4.51 (d, *J* = 8.6 Hz, 1H, H^1), 3.79 (dd, *J* = 12.4, 2.0 Hz, 1H, H^6), 3.61 (dd, *J* = 12.4, 5.2 Hz, 1H, H^6), 3.54 (dd, *J* = 10.5, 8.3 Hz, 1H, H^3), 3.45 (s, 3H, OMe), 3.34 (m, 2H, $H^4 + H^5$), 2.88 (dd, *J* = 10.6, 8.5 Hz, 1H, H^2); HRMS (FAB) calcd for $C_7H_{15}NO_5Na$ (M − HCl + Na): 216.0848; found 216.0855.

4.2.4. Methyl 2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (5). Compound **4** (2.78 g, 12.1 mmol) was dissolved in water (22 mL) and $NaHCO_3$ (1.02 g, 12.1 mmol) was added. After the evolution of gas had ceased, additional $NaHCO_3$ (1.02 g, 12.1 mmol), acetone (22 mL), and *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide (4.08 g, 12.1 mmol) were added. The reaction mixture was stirred overnight and solidified during the reaction. The product was suspended in water (100 mL) and chloroform (100 mL), filtered off, and washed with water and chloroform. The crude product was recrystallized in methanol to give **5** (3.81 g in three crops, 76%) as tiny white needles. Mp 163–164 °C; $[\alpha]_D^{22} = -20$ (*c* 0.5, MeOH); ^1H NMR (400 MHz, MeOH-d_4) δ 7.79 (d, *J* = 7.5 Hz, 2H, Fmoc), 7.68 (d, *J* = 6.9 Hz, 2H, Fmoc), 7.38 (t, *J* = 7.2 Hz, 2H, Fmoc), 7.30 (t, *J* = 7.5 Hz, 2H, Fmoc), 4.28 (m, 4H, 3 × Fmoc-H + H^1), 3.88 (dd, *J* = 11.9, 2.1 Hz, 1H, H^6), 3.68 (dd, *J* = 11.8, 5.8 Hz, 1H, H^6), 3.46 (s, 3 H, OMe), ∼3.34

§ Ac-Tyr-Arg(Mtr)-Tyr-OMe was prepared from Ac-Tyr-OH, H-Arg(Mtr)-OtBu and H-Tyr-OMe analogously to the synthesis of **9a–b**. The Mtr group was cleaved as in the synthesis of **1c**.

¶ In agreement with previously published data,[30] but a COSY experiment shows that the signals had been incorrectly assigned.

($H^2+H^3+H^4+H^5$, partially obscured by solvent signal); HRMS (FAB) calcd for $C_{22}H_{25}NO_7Na$ (M+Na): 438.1529; found 438.1530.

4.2.5. Methyl 3,4-di-*O*-benzoyl-2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-6-*O*-triphenylmethyl-β-D-glucopyranoside (6). Compound **5** (4.00 g, 9.63 mmol) was dissolved in pyridine (200 mL, distilled from CaH_2). Chlorotriphenylmethane (8.05 g, 28.9 mmol) was added and the mixture was stirred at 85 °C for 2 h. Benzoyl chloride (5.6 mL, 48.2 mmol) was added at 0 °C and the mixture was stirred for 3.5 h in room temperature. The mixture was poured over ice and the product was extracted with EtOAc (3×300 mL). The extract was washed with H_2SO_4(aq) (0.5 M, 2×200 mL), $NaHCO_3$(aq) (sat., 2×200 mL) and water (100 mL), dried over Na_2SO_4 and evaporated. The product was purified with flash chromatography (toluene/EtOAc 10:1, $R_f=0.20$) and lyophilized from benzene to give **6** (5.27 g, 63%) as a fluffy white powder. $[\alpha]_D^{22}=-20$ (*c* 0.4, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (d, $J=7.5$ Hz, 2H, Ar), 7.77 (d, $J=7.5$ Hz, 2H, Ar), 7.63 (m, 4H, NH+3×Ar), 7.53 (m, 2H, Ar), 7.37 (m, 14H, Ar), 7.17 (m, 10H, Ar), 5.49 (m, 2H, H^3+H^4), 4.68 (d, $J=8.4$ Hz, 1H, H^1), 4.22 (m, 2H, Fmoc), 4.04 (t, $J=6.5$ Hz, 1H, Fmoc), 3.93 (br d, $J=7.8$ Hz, 1H, H^5), 3.82 (q, $J=9.2$ Hz, 1H, H^2), 3.50 (s, 3H, OMe), ~3.28 (H^6, obscured by HDO signal), 2.95 (dd, $J=10.0$, 3.4 Hz, 1H, H6); HRMS (FAB) calcd for $C_{55}H_{47}NO_9Na$ (M+Na): 888.3149; found 888.3161.

4.2.6. Methyl 3,4-di-*O*-benzoyl-2-(9-fluorenylmethoxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (7). Compound **6** (3.66 g, 4.23 mmol) was dissolved in glacial acetic acid (150 mL) and HBr in AcOH (4.1 M, 2.1 mL, 8.5 mmol) was added. The mixture was stirred for 3 min and then poured over ice. The product was extracted with chloroform (4×100 mL) and the extract was dried over $MgSO_4$ and evaporated. The product was purified with flash chromatography (toluene/EtOAc 2:1, $R_f=0.14$) and lyophilized from benzene to give **7** (2.14 g, 81%) as a fluffy white powder. $[\alpha]_D^{22}=-41$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.83 (m, 4H, Bz-*o*+2×Fmoc-H), 7.76 (d, $J=7.7$ Hz, 2H, Bz-*o*), 7.65–7.30 (m, 11H, Bz-*m*+Bz-*p*+NH+4×Fmoc-H), 7.14 (q, $J=7.6$ Hz, 2H, Fmoc), 5.50 (t, $J=9.9$ Hz, 1H, H^3), 5.25 (t, $J=9.8$ Hz, 1H, H^4), 4.89 (t, $J=5.7$ Hz, 1H, OH), 4.64 (d, $J=8.4$ Hz, 1H, H^1), 4.18 (m, 2H, Fmoc), 4.01 (t, $J=6.7$ Hz, 1H, Fmoc), 3.72 (m, 2H, H^2+H^5), 3.54 (m, 2H, 2×H^6), 3.44 (s, 3H, OMe); HRMS (FAB) calcd for $C_{36}H_{33}NO_9Na$ (M+Na): 646.2053; found 646.2059.

4.3. Preparation of tripeptide *tert*-butyl esters 11a–c

4.3.1. Fmoc-Tyr-Tyr-O^{t}Bu (8a). Fmoc-Tyr-OH (700 mg, 1.74 mmol) was dissolved in THF (17 mL) and H-Tyr-O^{t}Bu (412 mg, 1.74 mmol), HOBt (234 mg, 1.74 mmol), EDC·HCl (349 mg, 1.82 mmol) and *N*-methylmorpholine (0.380 mL, 3.47 mmol) were added. The mixture was stirred overnight and then evaporated. The residue was dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (toluene/EtOAc 2:1, $R_f=0.20$) to give **8a** (948 mg, 88%) as a white amorphous solid. $[\alpha]_D^{22}=-16$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.56 (d, $J=7.7$ Hz, 2H, Fmoc), 7.37 (t, $J=7.5$ Hz, 2H, Fmoc), 7.28 (m, 2H, Fmoc), 7.03 (d, $J=8.5$ Hz, 2H, Tyr-H$^\delta$), 7.00 (d, $J=8.4$ Hz, 2H, Tyr-H$^\delta$), 6.68 (d, $J=8.5$ Hz, 2H, Tyr-H$^\varepsilon$), 6.67 (d, $J=8.5$ Hz, 2H, Tyr-H$^\varepsilon$), 4.45 (t, $J=7.0$ Hz, 1H, Tyr-H$^\alpha$), 4.30 (m, 2H, 1×Tyr-H$^\alpha$+1×Fmoc-H), 4.16 (m, 2H, Fmoc), 3.05–2.85 (m, 3H, 3×Tyr-H$^\beta$), 2.71 (dd, $J=13.9$, 9.3 Hz, 1H, Tyr-H$^\beta$), 1.37 (s, 9H, O^{t}Bu). HRMS (FAB) calcd for $C_{37}H_{39}N_2O_7$ (M+H): 623.2757; found 623.2748.

4.3.2. Fmoc-Tyr-Tyr-Tyr-O^{t}Bu (9a). Compound **8a** (951 mg, 1.53 mmol) was dissolved in CH_2Cl_2 (12 mL) and Et_3SiH (0.61 mL, 3.8 mmol) and TFA (6 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude free acid was dissolved in THF (14 mL) and H-Tyr-O^{t}Bu (363 mg, 1.53 mmol), HOBt (206 mg, 1.53 mmol), EDC·HCl (308 mg, 1.60 mmol) and *N*-methylmorpholine (0.340 mL, 3.06 mmol) were added. The mixture was stirred overnight and then evaporated. The residue was dissolved in MeOH and impregnated on silica. The product was purified with flash chromatography (toluene/MeOH 5:1, $R_f=0.36$) to give **9a** (1.13 g, 94%) as a white amorphous solid. $[\alpha]_D^{22}=-22$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.55 (d, $J=7.5$ Hz, 2H, Fmoc), 7.37 (t, $J=7.5$ Hz, 2H, Fmoc), 7.26 (m, 2H, Fmoc), 7.00 (d, $J=8.5$ Hz, 6H, Tyr-H$^\delta$), 6.66 (m, 6H, Tyr-H$^\varepsilon$), 4.55 (t, $J=6.4$ Hz, 1H, Tyr-H$^\alpha$), 4.42 (t, $J=6.9$ Hz, 1H, Tyr-H$^\alpha$), 4.20 (m, 4H, 1×Tyr-H$^\alpha$+Fmoc), 3.05–2.75 (m, 5H, 5×Tyr-H$^\beta$), 2.66 (dd, $J=14.5$, 9.5 Hz, 1H, Tyr-H$^\beta$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{46}H_{48}N_3O_9$ (M+H): 786.3391; found 786.3398.

4.3.3. H-Tyr-Tyr-Tyr-O^{t}Bu (10a). Compound **9a** (2.27 g, 2.89 mmol) was suspended in CH_2Cl_2 (80 mL) and piperidine (14.3 mL) was added. After stirring for 30 min, toluene (50 mL) was added and the mixture was evaporated. The residue was dissolved in CH_2Cl_2 and purified with flash chromatography (toluene/MeOH 3:1, $R_f=0.13$) to give **10a** (1.42 g, 87%) as a white foam. $[\alpha]_D^{24}=-14$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 6.97 (m, 6H, Tyr-H$^\delta$), 6.67 (m, 6H, Tyr-H$^\varepsilon$), 4.56 (dd, $J=7.9$, 6.0 Hz, 1H, Tyr-H$^\alpha$), 4.44 (t, $J=7.1$ Hz, 1H, Tyr-H$^\alpha$), 3.44 (dd, $J=8.0$, 5.0 Hz, 1H, Tyr-H$^\alpha$), 3.00–2.70 (m, 5H, 5×Tyr-H$^\beta$), 2.52 (dd, $J=13.8$, 8.0 Hz, 1H, Tyr-H$^\beta$), 1.38 (s, 9H, O^{t}Bu); HRMS calcd for $C_{31}H_{38}N_3O_7$ (M+H): 564.2701; found 564.2710.

4.3.4. Fmoc-Tyr-Glu(OBzl)-O^{t}Bu (8b). The title compound was prepared from Fmoc-Tyr-OH (1.26 g, 3.12 mmol) and H-Glu(OBzl)-O^{t}Bu·HCl (1.03 g, 3.12 mmol) using the method described in the synthesis of **8a**, but using an additional equivalent of base to neutralize the hydrochloride salt. The product was purified with flash chromatography (toluene/EtOAc 3:1, $R_f=0.19$) to give **8b** (1.82 g, 86%) as a white foam. $[\alpha]_D^{22}=-16$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d_4, 300 MHz) δ 7.77 (d, $J=7.5$ Hz, 2H, Fmoc), 7.55 (d, $J=6.6$ Hz, 2H, Fmoc), 7.37 (d, $J=7.4$ Hz, 2H, Fmoc), 7.27 (m, 7H, Fmoc+Bzl), 7.06 (d, $J=8.4$ Hz, 2H, Tyr-H$^\delta$), 6.68 (d, $J=8.3$ Hz, 2H, Tyr-H$^\varepsilon$), 5.06 (m, 2H, Bzl), 4.30 (m, 3H, Tyr-H$^\alpha$+Glu-H$^\alpha$+1×Fmoc-H), 4.16 (m, 2H, Fmoc), 3.02 (dd, $J=13.9$ Hz, $J=5.2$ Hz, 1H, Tyr-H$^\beta$), 2.77 (dd, $J=14.0$, 9.4 Hz, 1H, Tyr-H$^\beta$), 2.43 (t,

$J=7.4$ Hz, 2H, Glu-H$^{\gamma}$), 2.15 (m, 1H, Glu-H$^{\beta}$), 1.90 (m, 1H, Glu-H$^{\beta}$), 1.43 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{40}H_{43}N_2O_8$ (M+H): 679.3019; found 679.3014.

4.3.5. Fmoc-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (9b). The title compound was prepared from **8b** (1.73 g, 2.55 mmol) and H-Tyr-O^{t}Bu (605 mg, 2.55 mmol) using the method described in the synthesis of **9a**. The product was purified with flash chromatography (toluene/EtOAc 3:2, $R_f=0.11$) to give **9b** (1.77 g, 83%) as a white foam. $[\alpha]_D^{22}=-15$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.77 (d, $J=$ 7.5 Hz, 2H, Fmoc), 7.55 (dd, $J=7.3$, 3.0 Hz, 2H, Fmoc), 7.37 (t, $J=7.4$ Hz, 2H, Fmoc), 7.28 (m, 7H, Fmoc+Bzl), 7.04 (d, $J=8.2$ Hz, 2H, Tyr-H$^{\delta}$), 7.02 (d, $J=8.2$ Hz, 2H, Tyr-H$^{\delta}$), 6.69 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, $J=$ 8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.05 (m, 2H, Bzl), 4.41 (m, 2H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$), 4.28 (m, 2H, 1×Tyr-H$^{\alpha}$+1×Fmoc-H), 4.25 (m, 2H, Fmoc), 2.92 (m, 3H, 3×Tyr-H$^{\beta}$), 2.75 (dd, $J=13.8$, 9.5 Hz, 1H, Tyr-H$^{\beta}$), 2.40 (t, $J=7.7$ Hz, 2H, Tyr-H$^{\gamma}$), 2.07 (m, 1H, Glu-H$^{\beta}$), 1.89 (m, 1H, Glu-H$^{\beta}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{49}H_{52}N_3O_{10}$ (M+H): 842.3653; found 842.3646.

4.3.6. H-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (10b). The Fmoc group of **9b** (1.74 g, 2.06 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 40:1, $R_f=0.21$) to give **10b** (1.05 g, 88%) as a white foam. $[\alpha]_D^{22}=-19$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.32 (m, 5H, Bzl), 7.03 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\delta}$), 6.99 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\delta}$), 6.68 (d, $J=8.5$ Hz, 4H, Tyr-H$^{\varepsilon}$), 5.11 (s, 2H, Bzl), 4.40 (m, 2H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$), 3.50 (dd, $J=7.5$, 5.3 Hz, 1H, Tyr-H$^{\alpha}$), 3.00–2.80 (m, 3H, Tyr-H$^{\beta}$), 2.68 (dd, $J=13.6$, 7.6 Hz, 1H, Tyr-H$^{\beta}$), 2.36 (t, $J=7.7$ Hz, 2H, Glu-H$^{\gamma}$), 2.05 (m, 1H, Glu-H$^{\beta}$), 1.87 (m, 1H, Glu-H$^{\beta}$), 1.36 (s, 9H, O^{t}Bu). HRMS (FAB) calcd for $C_{34}H_{42}N_3O_8$ (M+H): 620.2972; found 620.2969.

4.3.7. Fmoc-Arg(Mtr)-Tyr-O^{t}Bu (11). The title compound was prepared from Fmoc-Arg(Mtr)-OH (1.82 g, 2.99 mmol) and H-Tyr-O^{t}Bu (709 mg, 2.99 mmol) using the method described in the synthesis of **8a**. The product was purified with flash chromatography (toluene/EtOAc 1:3, $R_f=0.17$) to give **11** (2.06 g, 83%) as a white foam. $[\alpha]_D^{22}=-6$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.78 (d, $J=$ 7.5 Hz, 2H, Fmoc), 7.64 (t, $J=5.6$ Hz, 2H, Fmoc), 7.37 (t, $J=7.4$ Hz, 2H, Fmoc), 7.28 (t, $J=7.4$ Hz, 2H, Fmoc), 7.00 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\delta}$), 6.67 (d, $J=8.3$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.62 (s, 1H, Mtr-ArH), 4.45 (t, $J=7.0$ Hz, 1H, Tyr-H$^{\alpha}$), 4.34 (m, 2H, Fmoc), 4.19 (t, $J=6.5$ Hz, 1H, Fmoc), 4.05 (t, $J=6.9$ Hz, 1H, Arg-H$^{\alpha}$), 3.78 (s, 3H, Mtr-OMe), 3.14 (br s, 2H, Arg-H$^{\delta}$), 2.01 (m, 2H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.09 (s, 3H, Mtr-Me), 1.68 (m, 1H, Arg-H$^{\beta}$), 1.54 (m, 1H, Arg-H$^{\beta}$), 1.47 (m, 2H, Arg-H$^{\gamma}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{44}H_{54}N_5O_9S$ (M+H): 828.3642; found 828.3654.

4.3.8. H-Arg(Mtr)-Tyr-O^{t}Bu (12). The Fmoc group of **11** (2.01 g, 2.42 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 10:1, $R_f=0.31$) to give **12** (1.33 g, 90%) as a white foam. $[\alpha]_D^{22}=+7$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.01 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\delta}$), 6.69 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.65 (s, 1H, Mtr-ArH), 4.48 (dd, $J=8.1$, 6.5 Hz, 1H, Tyr-H$^{\alpha}$), 3.82 (s, 3H, Mtr-OMe), ~3.28 (Arg-H$^{\alpha}$, partially obscured by solvent signal), 3.12 (br s, 2H, Arg-H$^{\delta}$), 2.98 (dd, $J=13.9$, 6.4 Hz, 1H, Tyr-H$^{\beta}$), 2.86 (dd, $J=13.9$, 8.2 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.60 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.58 (m, 1H, Arg-H$^{\beta}$), 1.45 (m, 3H, 1×Arg-H$^{\beta}$+2×Arg-H$^{\gamma}$), 1.38 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{29}H_{44}N_5O_7S$ (M+H): 606.2961; found 606.2958.

4.3.9. Fmoc-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (13). The title compound was prepared from Fmoc-Tyr-OH (854 mg, 2.12 mmol) and **12** (1.28 g, 2.12 mmol) using the method described in the synthesis of **8a**. The product was purified with flash chromatography (toluene/EtOAc 1:4, $R_f=0.16$) to give **13** (1.74 g, 83%) as a white foam. $[\alpha]_D^{22}=-8$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.76 (d, $J=$ 7.6 Hz, 2H, Fmoc), 7.55 (d, $J=7.1$ Hz, 2H, Fmoc), 7.36 (t, $J=7.4$ Hz, 2H, Fmoc), 7.26 (t, $J=7.2$ Hz, 2H, Fmoc), 7.04 (d, $J=7.8$ Hz, 2H, Tyr-H$^{\delta}$), 7.01 (d, $J=8.2$ Hz, 2H, Tyr-H$^{\delta}$), 6.68 (d, $J=8.0$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, $J=8.1$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.61 (s, 1H, Mtr-ArH), 4.42 (t, $J=7.2$ Hz, 1H, Tyr-H$^{\alpha}$), 4.35 (dd, $J=8.3$, 5.1 Hz, 1H, Arg-H$^{\alpha}$), 4.31 (m, 2H, 1×Tyr-H$^{\alpha}$+1×Fmoc), 4.16 (m, 2H, Fmoc), 3.78 (s, 3H, Mtr-OMe), 3.12 (br s, 2H, Arg-H$^{\delta}$), 2.94 (m, 3H, Tyr-H$^{\beta}$), 2.75 (dd, $J=13.8$, 9.9 Hz, 1H, Tyr-H$^{\beta}$), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 3H, Mtr-Me), 2.08 (s, 3H, Mtr-Me), 1.74 (m, 1H, Arg-H$^{\beta}$), 1.58 (m, 1H, Arg-H$^{\beta}$), 1.48 (m, 2H, Arg-H$^{\gamma}$), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{53}H_{63}N_6O_{11}S$ (M+H): 991.4276; found 991.4250.

4.3.10. H-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (10c). The Fmoc group of **13** (1.60 g, 1.61 mmol) was removed using the same method as in the synthesis of **10a**. The product was purified using flash chromatography (EtOAc/MeOH 10:1+2% Me_2NEt, $R_f=0.21$) to give **10c** (1.02 g, 83%) as a white foam. $[\alpha]_D^{22}=-10$ (*c* 0.5, MeOH); ^{1}H NMR (300 MHz, MeOH-d$_4$) δ 7.03 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\delta}$), 7.02 (d, $J=$ 8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.71 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.68 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.65 (s, 1H, Mtr-ArH), 4.44 (t, $J=7.2$ Hz, 1H, Tyr-H$^{\alpha}$), 4.37 (dd, $J=8.1$ Hz, $J=5.7$ Hz, 1H, Arg-H$^{\alpha}$), 3.82 (s, 3H, Mtr-OMe), 3.67 (dd, $J=8.1$, 5.4 Hz, 1H, Tyr-H$^{\alpha}$), 3.12 (br t, $J=4.5$ Hz, 2H, Arg-H$^{\delta}$), 2.92 (m, 3H, Tyr-H$^{\beta}$), 2.72 (dd, $J=14.0$, 8.0 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.61 (s, 3H, Mtr-Me), 2.12 (s, 3H, Mtr-Me), 1.73 (m, 1H, Arg-H$^{\beta}$), 1.59 (m, 1H, Arg-H$^{\beta}$), 1.48 (m, 2H, Arg-H$^{\gamma}$), 1.37 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{38}H_{53}N_6O_9S$ (M+H): 769.3595; found 769.3610.

4.4. Synthesis of the macrocycles 1a–c

4.4.1. Fmoc-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (14a). Compound **7** (1.60 g, 2.56 mmol) was dissolved in acetone (380 mL) and the mixture was cooled to 0 °C. Jones's reagent (4 M, 25.6 mL, prepared by dissolving 12.0 g CrO_3 and 6.9 mL concd H_2SO_4 in 23.1 mL water) was added. The solution was stirred at room temperature for 1.5 h and then quenched by addition of MeOH (100 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 (2×200 mL), dried over Na_2SO_4 and evaporated. The crude oxidation product was dissolved in THF (45 mL) and H-Tyr-Tyr-Tyr-O^{t}Bu **10a** (1.44 g, 2.56 mmol), HOBt (0.346 g, 2.56 mmol), EDC·HCl (0.515 g, 2.69 mmol), and *N*-methylmorpholine (0.56 mL, 5.12 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 2:3, R_f=0.10) to give **14a** (1.37 g, 45%). as a white amorphous solid $[\alpha]^{22}{}_D=-5$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.22 (s, 1H, Tyr-OH), 9.13 (s, 1H, Tyr-OH), 9.12 (s, 1H, Tyr-OH), 8.29 (d, *J*=7.3 Hz, 1H, NH), 8.12 (d, *J*=8.2 Hz, 1H, NH), 7.97 (d, *J*=8.2 Hz, 1H, NH), 7.83 (d, *J*=6.7 Hz, 2H, Fmoc), 7.79 (d, *J*=7.9 Hz, 2H, Bz-*o*), 7.75 (d, *J*=7.2 Hz, 2H, Bz-*o*), 7.62 (m, 3H, Bz-*p*+NH), 7.51 (d, *J*=7.6 Hz, 1H, Fmoc), 7.42 (t, *J*=7.7 Hz, 4H, Bz-*m*), 7.38 (d, *J*=8.8 Hz, 1H, Fmoc), 7.34 (td, *J*=7.5, 3.4 Hz, 2H, Fmoc), 7.15 (m, 2H, Fmoc), 7.01 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.95 (d, *J*=8.5 Hz, 2H, Tyr-H$^\delta$), 6.90 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.67 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.59 (d, *J*=8.4 Hz, 2H, Tyr-H$^\varepsilon$), 6.55 (d, *J*=8.4 Hz, 2H, Tyr-H$^\varepsilon$), 5.50 (t, *J*=9.9 Hz, 1H, SAA-H^3), 5.33 (t, *J*=9.6 Hz, 1H, SAA-H^4), 4.68 (t, *J*=8.3 Hz, 1H, SAA-H^1), 4.43 (m, 2H, 2×Tyr-H$^\alpha$), 4.27 (m, 3H, 1×Tyr-H$^\alpha$+SAA-H^5+1×Fmoc-H), 4.15 (dd, *J*=10.6, 6.9 Hz, 1H, Fmoc), 4.02 (t, *J*=6.5 Hz, 1H, Fmoc), 3.73 (q, *J*=9.2 Hz, SAA-H^2), 3.42 (s, 3H, OMe), 2.78 (m, 5H, 5×Tyr-H$^\beta$), 2.58 (dd, *J*=14.7, 8.1 Hz, 1H, Tyr-H$^\beta$), 1.31 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{67}H_{66}N_4O_{16}Na$ (M+Na): 1205.4372; found 1205.4388.

4.4.2. H-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (15a). Compound **14a** (400 mg, 0.338 mmol) was dissolved in THF (10 mL) and *N*-(2-mercaptoethyl)aminomethyl polystyrene (2.0 mmol/g, 1.69 g) and DBU (76 μL, 0.507 mmol) were added. After stirring the mixture for 6 h, the solid phase was filtered off and washed with THF (2×8 mL) and MeOH (2×8 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 **15a** (306 mg, 94%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-13$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.92 (d, *J*=7.8 Hz, 2H, Bz-*o*), 7.84 (d, *J*=7.8 Hz, 2H, Bz-*o*), 7.50 (m, 2H, Bz-*p*), 7.35 (m, 4H, Bz-*m*), 6.99 (d, *J*=8.5 Hz, 4H, Tyr-H$^\delta$), 6.89 (d, *J*=8.5 Hz, 2H, Tyr-H$^\delta$), 6.69 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.68 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 6.61 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 5.50 (t, *J*=9.8 Hz, 1H, SAA-H^3), 5.38 (t, *J*=9.6 Hz, 1H, SAA-H^4), 4.42 (m, 4H, SAA-H^1+3×Tyr-H$^\alpha$), 4.20 (d, *J*=9.8 Hz, 1H, SAA-H^5), 3.56 (s, 3H, OMe), 3.02 (dd, *J*=10.1, 8.06 Hz, 1H, SAA-H^2), 2.86 (m, 5H, 5×Tyr-H$^\beta$), 2.66 (dd, *J*=13.7, 7.9 Hz, 1H, Tyr-H$^\beta$), 1.34 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{52}H_{56}N_4O_{14}Na$ (M+Na): 983.3690; found 983.3687.

4.4.3. Fmoc-SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (17a). Compound **14a** (313 mg, 0.265 mmol) was dissolved in CH_2Cl_2 (4 mL) and Et_3SiH (105 μL, 0.66 mmol) and TFA (2 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude free acid was dissolved in THF (3 mL) and **15a** (255 mg, 0.265 mmol), HOBt (35.8 mg, 0.265 mmol), and DIC (50 μL, 0.32 μmol) were added. The mixture was stirred for 16 h and then concentrated. The residue was dissolved in MeOH and impregnated on silica. The product was purified using flash chromatography (CH_2Cl_2/MeOH 10:1, R_f=0.27) followed by size-exclusion chromatography to give **17a** (347 mg, 65%) as a white amorphous solid. $[\alpha]_D^{22}=-8$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.20 (s, 1H, Tyr-OH), 9.10 (s, 2H, 2×Tyr-OH), 9.07 (s, 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 8.33 (d, *J*=8.8 Hz, 1H, NH), 8.27 (d, *J*=7.3 Hz, 1H, NH), 8.11 (d, *J*=8.7 Hz, 1H, NH), 7.93 (m, 4H, 4×NH), 7.74 (m, 10H, Bz-*o*+2×Fmoc-H), 7.55 (m, 5H, Bz-*p*+NH), 7.39 (m, 12H, Bz-*m*+4×Fmoc-H), 7.12 (m, 2H, Fmoc), 6.99 (d, *J*=8.4 Hz, 2H, Tyr-H$^\delta$), 6.90 (m, 6H, Tyr-H$^\delta$), 6.78 (d, *J*=9.8 Hz, 2H, Tyr-H$^\delta$), 6.75 (d, *J*=9.1 Hz, 2H, Tyr-H$^\delta$), 6.65 (d, *J*=8.3 Hz, 2H, Tyr-H$^\varepsilon$), 6.51 (m, 10H, Tyr-H$^\varepsilon$), 5.51 (t, *J*=9.8 Hz, 1H, SAA-H^3), 5.46 (t, *J*=9.9 Hz, 1H, SAA-H^3), 5.33 (t, *J*=9.7 Hz, 1H, SAA-H^4), 5.28 (t, *J*=9.9 Hz, 1H, SAA-H^4), 4.71 (d, *J*=8.3 Hz, 1H, SAA-H^1), 4.64 (d, *J*=7.8 Hz, 1H, SAA-H^1), 4.31 (m, 9H, 2×SAA-H^5+1×Fmoc-H+6×Tyr-H$^\alpha$), 4.11 (m, 2H, SAA-H2+1×Fmoc), 3.99 (t, *J*=6.4 Hz, 1H, Fmoc), 3.69 (q, *J*=9.1 Hz, 1H, SAA-H^2), 3.39 (s, 3H, OMe), 3.37 (s, 3H, OMe), 2.82–2.20 (m, 12H, Tyr-H$^\beta$), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{115}H_{112}N_8O_{29}Na$ (M+Na): 2091.7433; found 2091.7444.

4.4.4. H-SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$-O^{t}Bu (18a). The title compound was prepared from compound **17a** (334 mg, 0.161 mmol) using the method described in the synthesis of **15a** to give **18a** (285 mg, 95%) as a yellowish amorphous solid. $[\alpha]_D^{21}=-11$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.86 (m, 8H, Bz-*o*), 7.49 (m, 4H, Bz-*p*), 7.35 (m, 8H, Bz-*m*), 6.97 (m, 6H, Tyr-H$^\delta$), 6.90 (d, *J*=8.7 Hz, 2H, Tyr-H$^\delta$), 6.81 (d, *J*=8.8 Hz, 2H, Tyr-H$^\delta$), 6.79 (d, *J*=8.9 Hz, 2H, Tyr-H$^\delta$), 6.69 (d, *J*=8.3 Hz, 6H, Tyr-H$^\varepsilon$), 6.61 (d, *J*=8.5 Hz, 4H, Tyr-H$^\varepsilon$), 6.57 (d, *J*=8.5 Hz, 2H, Tyr-H$^\varepsilon$), 5.73 (t, *J*=10.0 Hz, SAA-H^3), 5.42 (m, 3H, SAA-H^3+2×SAA-H^4), 4.77 (d, *J*=8.3 Hz, 1H, SAA-H^1), 4.50–4.30 ppm (m, 7H, SAA-H^1+6×Tyr-H$^\alpha$), 4.26 (d, *J*=10.0 Hz, 1H, SAA-H^5), 4.15 (dd, *J*=10.6, 8.4 Hz, 1H, SAA-H^2), 4.12 (d, *J*=9.9 Hz, 1H, SAA-H^5), 3.56 (s, 3H, OMe), 3.48 (s, 3H, OMe), 3.04 (dd, *J*=10.2, 8.0 Hz, 1H, SAA-H^2), 2.95-2.40 (m, 12H, Tyr-H$^\beta$), 1.34 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{100}H_{102}N_8O_{27}Na$ (M+Na): 1869.6752; found 1869.6736.

4.4.5. Cyclo[SAA(di-*O*-Bz)-Tyr$_3$-SAA(di-*O*-Bz)-Tyr$_3$] (19a). Compound **18a** (37.6 mg, 20.3 μmol) was dissolved in CH_2Cl_2 (1.6 mL) and Et_3SiH (8.1 μL, 51 μmol) and TFA (0.8 mL) were added. The mixture was stirred for 4 h and coevaporated with toluene. The crude product was dissolved in THF (20 mL) and DIPEA (10 μL, 61 μmol) and HAPyU (10.6 mg, 24.4 μmol) were added. The mixture was stirred for 1 h at room temperature and then evaporated. The product was purified by flash chromatography (CH_2Cl_2/MeOH 6:1, R_f=0.29) followed by size-exclusion chromatography to give **19a** (13.3 mg, 37%) as a white amorphous solid. $[\alpha]_D^{23}=-6$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz, 150 °C) δ 8.47 (s, 2H, Tyr-OH), 8.41 (s, 2H, Tyr-OH), 8.34 (s, 2H, Tyr-OH), 7.86 (d, *J*=8.4 Hz, 2H, NH), 7.80 (t, *J*=7.2 Hz, 8H, Bz-*o*), 7.47 (m, 4H, Bz-*p*), 7.41 (d, *J*=8.0 Hz, 2H, NH), 7.34 (m, 10H, Bz-*m*+2×NH), 7.05 (d, *J*=8.3 Hz, 2H, NH), 6.89 (d, *J*=8.5 Hz, 4H, Tyr-H$^\delta$), 6.83 (d, *J*=8.4 Hz, 4H, Tyr-H$^\delta$), 6.79 (d, *J*=8.3 Hz, 4H, Tyr-H$^{\delta}$), 6.66 (d, J=8.5 Hz, 4H, Tyr-H$^{\varepsilon}$), 6.60 (d, J=8.4 Hz, 4H, Tyr-H$^{\varepsilon}$), 6.52 (d, J=8.5 Hz, 4H, Tyr-H$^{\varepsilon}$), 5.85 (t, J=10.0 Hz, 2H, SAA-H^{3}), 5.66 (t, J=9.6 Hz, 2H, SAA-H^{4}), 5.10 (d, J=8.0 Hz, 2H, SAA-H^{1}), 4.49 (d, J=9.7 Hz, 2H, SAA-H^{5}), 4.35 (m, 4H, Tyr-H$^{\alpha}$), 4.13 (q, J=8.4 Hz, 2H, SAA-H^{2}), 4.01 (q, J=7.9 Hz, 2H, Tyr-H$^{\alpha}$), 3.48 (s, 6H, OMe), 2.9–2.6 (m, 12H, Tyr-H$^{\beta}$); HRMS (FAB) calcd for $C_{96}H_{92}N_8O_{26}Na$ (M+Na): 1795.6020; found 1795.6010.

4.4.6. Cyclo(SAA-Tyr$_3$-SAA-Tyr$_3$) (1a). Compound **19a** (89.1 mg, 50.3 μmol) was dissolved in MeOH (18 mL) and NaOMe/MeOH (1 M, 180 μL) was added. The solution was stirred for 18 h, then neutralised with AcOH and evaporated. The residue was purified using size-exclusion chromatography on a short column to afford **1a** (41.7 mg, 61%) as a white amorphous solid. $[\alpha]_D^{21}=-15$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 6.97 (m, 12H, Tyr-H$^{\delta}$), 6.70 (m, 12H, Tyr-H$^{\varepsilon}$), 4.75 (d, J=8.5 Hz, 2H, SAA-H^{1}), 4.52 (dd, J=9.4, 5.1 Hz, 2H, Tyr-H$^{\alpha}$), 4.41 (dd, J=8.5, 4.7 Hz, 2H, Tyr-H$^{\alpha}$), 4.29 (t, J=6.8 Hz, 2H, Tyr-H$^{\alpha}$), 3.82 (d, J=9.7 Hz, 2H, SAA-H^{5}), 3.78 (t, J=9.4 Hz, 2H, SAA-H^{3}), 3.41 (t, J=9.4 Hz, 2H, SAA-H^{4}), 3.33 (s, 6H, OMe), ~3.3 (SAA-H^{2}, obscured by solvent signal), 3.05–2.80 (m, 10H, Tyr-H$^{\beta}$), 2.54 (dd, J=14.1, 9.4 Hz, 2H, Tyr-H$^{\beta}$); HRMS (FAB) calcd for $C_{68}H_{76}N_8O_{22}Na$ (M+Na): 1379.4972; found 1379.4955.

4.4.7. Fmoc-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (14b). The title compound was prepared from **7** (1.09 g, 1.74 mmol) and **10b** (1.08 g, 1.74 mmol) using the method described in the synthesis of **14a**. The product was purified with flash chromatography (toluene/EtOAc 1:1, R_f=0.13) to give **14b** (1.39 g, 64%) as a white amorphous solid. $[\alpha]_D^{22}=-12$ (c 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.19 (s, 1H, Tyr-OH), 9.11 (s, 1H, Tyr-OH), 8.19 (d, J=7.0 Hz, 1H, NH), 8.15 (d, J=7.9 Hz, 1H, NH), 8.14 (d, J=8.0 Hz, 1H, NH), 7.80 (d, J=7.8 Hz, 2H, Fmoc), 7.75 (d, J=7.3 Hz, 2H, Bz-o), 7.68 (d, J=7.3 Hz, 2H, Bz-o), 7.52 (m, 4H, Bz-p+1×Fmoc-H+1×NH), 7.35 (m, 12H, 1×Fmoc-H+Bz-m+Bzl), 7.12 (m, 2H, Fmoc), 6.98 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.94 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.64 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.57 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.46 (t, J=9.9 Hz, 1H, SAA-H^{3}), 5.32 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.04 (s, 2H, Bzl), 4.65 (d, J=8.3 Hz, 1H, SAA-H^{1}), 4.44 (dd, J=12.5, 7.5 Hz, 1H, Tyr-H$^{\alpha}$), 4.31 (d, J=10.0 Hz, 1H, SAA-H^{5}), 4.22 (m, 3H, Tyr-H$^{\alpha}$+Glu-H$^{\alpha}$+1×Fmoc-H), 4.13 (dd, J=10.7, 6.9 Hz, 1H, Fmoc), 3.99 (t, J=6.7 Hz, 1H, Fmoc), 3.71 (q, J=9.3 Hz, 1H, SAA-H^{2}), 3.40 (s, 3H, OMe), 2.81 (m, 3H, 3×Tyr-H$^{\beta}$), 2.71 (dd, J=14.2, 7.9 Hz, 1H, Tyr-H$^{\beta}$), 2.19 (m, 2H, Glu-H$^{\gamma}$), 1.81 (m, 1H, Glu-H$^{\beta}$), 1.64 (m, 1H, Glu-H$^{\beta}$), 1.26 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{70}H_{70}N_4O_{17}Na$ (M+Na): 1261.4634; found 1261.4623.

4.4.8. H-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (15b). The title compound was prepared from **14b** (720 mg, 0.581 mmol) using the method described in the synthesis of **15a** to give **15b** (534 mg, 90%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-24$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.91 (d, J=7.9 Hz, 2H, Bz-o), 7.81 (d, J=8.5 Hz, 2H, Bz-o), 7.50 (m, 2H, Bz-p), 7.32 (m, 9H, Bz-m+Bzl), 7.01 (d, J=8.4 Hz, 4H, Tyr-H$^{\delta}$), 6.69 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.67 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.48 (t, J=9.6 Hz, 1H, SAA-H^{3}), 5.40 (t, J=9.5 Hz, 1H, SAA-H^{4}), 5.08 (s, 2H, Bzl), 4.44 (d, J=8.0 Hz, 1H, SAA-H^{1}), 4.49 (t, J=6.6, 1H, Tyr-H$^{\alpha}$), 4.40 (t, J=6.9 Hz, 1H, Tyr-H$^{\alpha}$), 4.25 (m, 2H, SAA-H^{5}+Glu-H$^{\alpha}$), 3.55 (s, 3H, OMe), 2.94 (m, 5H, SAA-H^{2}+Tyr-H$^{\beta}$), 2.26 (m, 2H, Glu-H$^{\gamma}$), 1.95 (m, 1H, Glu-H$^{\beta}$), 1.77 (m, 1H, Glu-H$^{\beta}$), 1.39 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{55}H_{60}N_4O_{15}Na$ (M+Na): 1039.3953; found 1039.3960.

4.4.9. Fmoc-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (17b). The title compound was prepared from **14b** (604 mg, 0.487 mmol) and **15b** (496 mg, 0.487 mmol) using the method described in the synthesis of **17a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 15:1, R_f=0.18) followed by size-exclusion chromatography to give **17b** (767 mg, 72%) as a white amorphous solid. $[\alpha]_D^{22}=+4$ (c 0.5, DMSO); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.23 (s, 1H, Tyr-OH), 9.16 (s, 1H, Tyr-OH), 9.13 (s, 1H, Tyr-OH), 9.10 (s, 1H, Tyr-OH), 8.36 (d, J=8.5 Hz, 1H, NH), 8.25 (d, J=7.1 Hz, 1H, NH), 8.20 (m, 2H, 2×NH), 8.14 (d, J=7.7 Hz, 1H, NH), 8.09 (d, J=7.3 Hz, 1H, NH), 7.85 (m, 3H, 2×Fmoc-H+NH), 7.77 (m, 4H, Bz-o), 7.70 (m, 4H, Bz-o), 7.55 (m, 6H, 4×Bz-p+1×NH+1×Fmoc-H), 7.37 (m, 21H, Bz-m+3×Fmoc-H+2×Bzl), 7.15 (m, 2H, Fmoc), 7.01 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.97 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.97 (d, J=8.6 Hz, 2H, Tyr-H$^{\delta}$), 6.77 (d, J=8.4 Hz, 2H, Tyr-H$^{\delta}$), 6.66 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.60 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.59 (d, J=8.1 Hz, 2H, Tyr-H$^{\varepsilon}$), 6.47 (d, J=8.4 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.51 (t, J=9.8 Hz, 1H, SAA-H^{3}), 5.47 (t, J=10.1 Hz, 1H, SAA-H^{3}), 5.37 (t, J=9.8 Hz, 1H, SAA-H^{4}), 5.31 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.06 (s, 2H, Bzl), 5.05 (s, 2H, Bzl), 4.71 (d, J=8.2 Hz, 1H, SAA-H^{1}), 4.66 (d, J=8.2 Hz, 1H, SAA-H^{1}), 4.44 (m, 3H, SAA-H^{5}+2×Tyr-H$^{\alpha}$), 4.29 (m, 5H, SAA-H^{5}+2×Tyr-H$^{\alpha}$+1×Glu-H$^{\alpha}$+1×Fmoc-H), 4.13 (m, 3H, SAA-H^{2}+1×Glu-H$^{\alpha}$+1×Fmoc-H), 4.02 (t, J=6.5 Hz, 1H, Fmoc), 3.71 (q, J=9.4 Hz, 1H, SAA-H^{2}), 3.41 (s, 3H, OMe), 3.38 (s, 3H, OMe), 2.75 (m, 7H, 7×Tyr-H$^{\beta}$), 2.56 (dd, J=15.3, 11.2 Hz, 1H, Tyr-H$^{\beta}$), 2.23 (t, J=8.0 Hz, 2H, Glu-H$^{\gamma}$), 2.10 (m, 2H, Glu-H$^{\gamma}$), 1.83 (m, 1H, Glu-H$^{\beta}$), 1.70 (m, 2H, Glu-H$^{\beta}$), 1.56 (m, 1H, Glu-H$^{\beta}$), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{121}H_{120}N_8O_{31}Na$ (M+Na): 2203.7957; found 2203.7947.

4.4.10. H-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-O^{t}Bu (18b). The title compound was prepared from **17b** (676 mg, 0.310 mmol) using the method described in the synthesis of **15a** to give **18b** (599 mg, 99%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-23$ (c 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 400 MHz) δ 7.72 (d, J=8.5 Hz, 2H, Bz-o), 7.67 (d, J=8.4 Hz, 2H, Bz-o), 7.63 (d, J=8.5 Hz, 2H, Bz-o), 7.58 (d, J=8.4 Hz, 2H, Bz-o), 7.28 (m, 4H, Bz-p), 7.12 (m, 18H, Bz-m+Bzl), 6.81 (m, 6H, Tyr-H$^{\delta}$), 6.63 (d, J=8.5 Hz, 2H, Tyr-H$^{\delta}$), 6.48 (m, 6H, Tyr-H$^{\varepsilon}$), 6.33 (d, J=8.5 Hz, 2H, Tyr-H$^{\varepsilon}$), 5.51 (t, J=10.0 Hz, 1H, SAA-H^{3}), 5.29 (t, J=9.8 Hz, 1H, SAA-H^{3}), 5.24 (t, J=9.7 Hz, 1H, SAA-H^{4}), 5.20 (t, J=9.6 Hz, 1H, SAA-H^{4}), 4.87 (s, 4H, Bzl), 4.55 (d, J=8.4 Hz, 1H, SAA-H^{1}), 4.30 (t, J=6.5 Hz, 1H, Tyr-H$^{\alpha}$), 4.23 (m, 3H, SAA-H^{1}+2×Tyr-H$^{\alpha}$), 4.16 (dd, J=9.1 Hz, J=5.0 Hz, Tyr-H$^{\alpha}$), 4.06 (m, 3H, 2×SAA-H^{5}+Glu-H$^{\alpha}$), 3.93 (m, 2H, SAA-H^{2}+Glu-H$^{\alpha}$), 3.34 (s, 3H, OMe), 3.25 (s, 3H, OMe), 2.83 (dd, $J=10.1$ Hz, $J=8.0$ Hz, 1H, SAA-H^2), 2.72 (m, 6H, 6×Tyr-H^β), 2.55 (dd, $J=14.2$, 5.0 Hz, 1H, Tyr-H^β), 2.26 (dd, $J=13.9$, 9.4 Hz, 1H, Tyr-H^β), 2.07 (m, 2H, 2×Glu-H^γ), 1.94 (m, 2H, 2×Glu-H^γ), 1.72 (m, 1H, Glu-H^β), 1.57 (m, 3H, 3×Glu-H^β), 1.14 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{106}H_{110}N_8O_{29}Na$ (M+Na): 1981.7276; found 1981.7268.

4.4.11. Cyclo[SAA(di-OBz)-Tyr-Glu(OBzl)-Tyr-SAA-(di-OBz)-Tyr-Glu(OBzl)-Tyr] (19b). The title compound was prepared from **18b** (314 mg, 0.166 mmol) using the method described in the synthesis of compound **19a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 12:1, $R_f=0.25$) followed by size-exclusion chromatography to give **19b** (166 mg, 53%) as a white foam. $[\alpha]_D^{22}=-3$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz, 120 °C) δ 8.49 (br s, 2H, Tyr-OMe), 8.33 (br s, 2H, Tyr-OMe), 7.80 (m, 10H, Bz-*o*+2×NH), 7.48 (m, 6H, Bz-*p*+2×NH), 7.32 (m, 18H, Bz-*m*+Bzl), 7.05 (d, $J=7.8$ Hz, 2H, NH), 6.99 (d, $J=8.4$ Hz, 4H, Tyr-H^δ), 6.81 (d, $J=8.4$ Hz, 4H, Tyr-H^δ), 6.68 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 6.50 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 5.78 (t, $J=9.7$ Hz, 2H, SAA-H^3), 5.60 (t, $J=9.4$ Hz, 2H, SAA-H^4), 5.09 (s, 4H, Bzl), 5.05 (d, $J=8.1$ Hz, 2H, SAA-H^1), 4.46 (d, $J=9.8$ Hz, 2H, SAA-H^5), 4.43 (dd, $J=14.9$, 7.7 Hz, 2H, Tyr-H^α), 4.34 (m, 2H, Tyr-H^α), 4.15 (dd, $J=10.0$, 8.6 Hz, 2H, SAA-H^2), 3.91 (dd, $J=7.7$, 6.6 Hz, 2H, Glu-H^α), 3.48 (s, 6H, OMe), 3.00 (m, 4H, Tyr-H^β), 2.87 (dd, $J=14.4$, 5.1 Hz, 2H, Tyr-H^β), 2.63 (dd, $J=14.4$, 8.3 Hz, 2H, Tyr-H^β), 2.22 (m, 4H, Glu-H^γ), 1.90 (m, 4H, Glu-H^β); HRMS (FAB) calcd for $C_{102}H_{100}N_8O_{28}$ (M+H): 1907.6545; found 1907.6549.

4.4.12. Cyclo[SAA-Tyr-Glu-Tyr-SAA-Tyr-Glu-Tyr] (1b). Palladium black (75 mg) was suspended in MeOH containing 5% formic acid (3 mL). Compound **19b** (149 mg, 78.8 μmol) was dissolved in MeOH containing 5% formic acid (9 mL) and added to the suspension. After 20 min, the catalyst was filtered off (caution: catalyst may catch fire when filtered to dryness), toluene (5 mL) was added, and the mixture was evaporated. The residue was dissolved in MeOH (30 mL) and NaOMe/MeOH (1 M, 450 μL) was added. The solution was stirred for 22 h, then neutralised with AcOH, and evaporated. The residue was dissolved in water and applied to a C_{18} cartridge. The column was washed with water and the compound was eluted with 30% MeOH in water to afford **1b** (82.3 mg, 81%) as a fluffy white powder after lyophilization. $[\alpha]_D^{22}=-31$ (*c* 0.2, water); ^{1}H NMR (D_2O, 400 MHz) δ 7.03 (d, $J=8.4$ Hz, 8H, Tyr-H^δ), 6.78 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 6.69 (d, $J=8.4$ Hz, 4H, Tyr-H^ε), 4.58 (dd, $J=10.0$, 4.6 Hz, 2H, Tyr-H^α), 4.51 (t, $J=6.2$ Hz, 2H, Tyr-H^α), 4.45 (d, $J=8.7$ Hz, 2H, SAA-H^1), 3.86 (t, $J=7.1$ Hz, 2H, Glu-H^α), 3.68 (d, $J=10.0$ Hz, 2H, SAA-H^5), 3.66 (t, $J=9.4$ Hz, 2H, SAA-H^2), 3.40 (t, $J=9.5$ Hz, 2H, SAA-H^3), 3.32 (s, 6H, OMe), 3.20 (m, 4H, 2×SAA-H^4+2×Tyr-H^β), 2.92 (m, 4H, Tyr-H^β), 2.16 (m, 4H, 2×Tyr-H^β+2×Glu-H^β), 2.03 (m, 2H, Glu-H^β), 1.88 (m, 4H, Glu-H^γ); HRMS (FAB) calcd for $C_{60}H_{72}N_8O_{24}Na$ (M+Na):1311.4557; found 1311.4581.

4.4.13. Fmoc-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (14c). The title compound was prepared from **7** (809 mg, 1.30 mmol) and **10c** (998 mg, 1.30 mmol) using the method described in the synthesis of **14a**. The product was purified with flash chromatography (toluene/MeOH 7:1, $R_f=0.21$) followed by size-exclusion chromatography to give **14c** (837 mg, 1.39 g, 46%) as a white foam. $[\alpha]_D^{24}=-5$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.20 (s, 1H, Tyr-OH), 9.12 (s, 1H, Tyr-OH), 8.15 (m, 2H, NH), 8.11 (d, $J=8.2$ Hz, 1H, NH), 7.82 (d, $J=7.0$ Hz, 2H, Fmoc), 7.77 (d, $J=7.3$ Hz, 2H, Bz-o), 7.73 (d, $J=7.3$ Hz, 2H, Bz-*o*), 7.55 (m, 4H, Bz-*p*+1×Fmoc-H+1×NH), 7.37 (m, 7H, 3×Fmoc-H+Bz-*m*), 7.15 (m, 2H, Fmoc), 7.00 (d, $J=8.4$ Hz, 2H, Tyr-H^δ), 6.97 (d, $J=8.5$ Hz, 2H, Tyr-H^δ), 6.66 (s, 1H, Mtr-ArH), 6.66 (d, $J=8.4$ Hz, 2H, Tyr-H^ε), 6.59 (d, $J=8.3$ Hz, 2H, Tyr-H^ε), 5.49 (t, $J=9.9$ Hz, 1H, SAA-H^3), 5.34 (t, $J=9.7$ Hz, 1H, SAA-H^4), 4.68 (d, $J=8.3$ Hz, 1H, SAA-H^1), 4.46 (dd, $J=12.5$, 7.6 Hz, 1H, Tyr-H^α), 4.35 (d, $J=9.9$ Hz, 1H, SAA-H^5), 4.25 (m, 3H, Arg-H^α+Tyr-H^α+1×Fmoc-H), 4.15 (dd, $J=10.7$ Hz, $J=6.9$ Hz, 1H, Fmoc), 4.02 (t, $J=6.7$ Hz, 1H, Fmoc), 3.76 (s, 3H, Mtr-OMe), 3.73 (q, $J=9.3$ Hz, 1H, SAA-H^2), 3.42 (s, 3H, SAA-OMe), 2.92 (br m, 2H, Arg-H^δ), 2.81 (m, 3H, Tyr-H^β), 2.73 (dd, $J=14.3$, 7.7 Hz, 1H, Tyr-H^β), 2.59 (s, 3H, Mtr-Me), ∼2.50 (Mtr-Me, obscured by solvent signal), 2.03 (s, 3H, Mtr-Me), 1.52 (m, 1H, Arg-H^β), 1.28 (m, 3H, 1×Arg-H^β+2×Arg-H^γ), 1.28 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{74}H_{81}N_7O_{18}SNa$ (M+Na): 1410.5257; found 1410.5261.

4.4.14. H-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (15c). The title compound was prepared from **14c** (400 mg, 0.288 mmol) using the method described in the synthesis of **15a** to give **15c** (334 mg, 99%) as a yellowish foam. $[\alpha]_D^{24}=-14$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 300 MHz) δ 7.91 (d, $J=8.5$ Hz, 2H, Bz-*o*) 7.79 (d, $J=8.5$ Hz, 2H, Bz-*o*), 7.53 (t, $J=7.5$ Hz, 1H, Bz-*p*), 7.45 (t, $J=7.4$ Hz, 1H, Bz-*p*), 7.38 (t, $J=7.6$ Hz, 2H, Bz-*m*), 7.29 (t, $J=7.7$ Hz, 2H, Bz-*m*), 7.01 (d, $J=8.4$ Hz, 4H, Tyr-H^δ), 6.69 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 6.67 (d, $J=8.5$ Hz, 2H, Tyr-H^ε), 6.63 (s, 1H, Mtr-ArH), 5.48 (t, $J=9.6$ Hz, 1H, SAA-H^3), 5.41 (t, $J=9.7$ Hz, 1H, SAA-H^4), 4.46 (t, $J=6.7$ Hz, 1H, Tyr-H^α), 4.46 (d, $J=8.0$ Hz, 1H, SAA-H^1), 4.39 (d, $J=7.2$ Hz, 1H, Tyr-H^α), 4.26 (d, $J=9.5$ Hz, 1H, SAA-H^5), 4.19 (dd, $J=8.3$ Hz, $J=5.5$ Hz, 1H, Arg-H^α), 3.79 (s, 3H, Mtr-OMe), 3.56 (s, 3H, SAA-OMe), 3.01 (m, 3H, SAA-H^2+Tyr-H^δ), 2.89 (m, 4H, Tyr-H^β), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 3H, Mtr-Me), 2.09 (s, 3H, Mtr-Me), 1.61 (m, 1H, Arg-H^β), 1.35 (m, 3H, 1×Arg-H^β+2×Arg-H^γ), 1.35 (s, 9H, O^{t}Bu); HRMS (FAB) calcd for $C_{59}H_{71}N_7O_{16}SNa$ (M+Na): 1188.4576; found 1188.4596.

4.4.15. Fmoc-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (17c). The title compound was prepared from **14c** (315 mg, 0.227 mmol) and **15c** (265 mg, 0.227 mmol) using the method described in the synthesis of **17a**. The product was purified with flash chromatography (CH_2Cl_2/MeOH 12:1, $R_f=0.29$) followed by size-exclusion chromatography to give **17c** (271 mg, 48%) as a white foam. $[\alpha]_D^{22}=+7$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 9.21 (s. 1H, Tyr-OH), 9.13 (s. 1H, Tyr-OH), 9.10 (s. 1H, Tyr-OH), 9.06 (s, 1H, Tyr-OH), 8.35 (d, $J=8.8$ Hz, 1H, NH), 8.16 (m, 3H, 3×NH), 8.06 (d, $J=8.2$ Hz, 1H, NH), 7.82 (d, $J=7.5$ Hz, 2H, Fmoc), 7.78 (m, 5H, 4×Bz-*o*+1×NH), 7.72 (m, 4H, 4×Bz-*o*), 7.55 (m, 6H, 4×Bz-*p*+1×Fmoc-H+1×NH), 7.40 (m, 11H, 3×Fmoc-H+8×Bz-*m*), 7.15 (m, 2H, Fmoc), 7.01 (d, $J=8.5$ Hz, 2H, Tyr-H^δ), 6.97 (d, $J=8.6$ Hz, 2H, Tyr-H^δ), 6.97

(d, $J=8.6$ Hz, 2H, Tyr-H$^{\delta}$), 6.75 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\delta}$), 6.66 (m, 4H, Tyr-H$^{\varepsilon}$+2×Mtr-ArH), 6.60 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.59 (d, $J=8.1$ Hz, 2H, Tyr-H$^{\varepsilon}$), 6.47 (d, $J=8.4$ Hz, 2H, Tyr-H$^{\varepsilon}$), 5.52 (t, $J=9.8$ Hz, 1H, SAA-H^{3}), 5.48 (t, $J=9.7$ Hz, 1H, SAA-H^{3}), 5.38 (t, $J=9.7$ Hz, 1H, SAA-H^{4}), 5.33 (t, $J=9.6$ Hz, 1H, SAA-H^{4}), 4.72 (d, $J=8.4$ Hz, 1H, SAA-H^{1}), 4.68 (d, $J=8.2$ Hz, 1H, SAA-H^{1}), 4.46 (m, 2H, 2×Tyr-H$^{\alpha}$), 4.42 (d, $J=10.0$ Hz, 1H, SAA-H^{5}), 4.35 (m, 2H, SAA-H^{5}+TyrH$^{\alpha}$), 4.26 (m, 3H, Arg-H$^{\alpha}$+Tyr-H$^{\alpha}$+1×Fmoc-H), 4.15 (m, 1H, Fmoc), 4.08 (m, 2H, SAA-H^{2}+Arg-H$^{\alpha}$), 4.02 (t, $J=6.7$ Hz, 1H, Fmoc), 3.77 (s, 3H, Mtr-OMe), 3.76 (s, 3H, Mtr-OMe), 3.72 (q, $J=9.7$ Hz, 1H, SAA-H^{2}), 3.42 (s, 3H, SAA-OMe), 3.41 (s, 3H, SAA-OMe), 2.86 (m, 11H, 4×Arg-H$^{\delta}$+7×Tyr-H$^{\beta}$), 2.59 (s, 6H, Mtr-Me), ~2.50 (2×Mtr-Me, obscured by solvent signal), 2.25 (br dd, $J=12.8$, 8.7 Hz, 1H, Tyr-H$^{\beta}$), 2.03 (s, 3H, Mtr-Me), 2.01 (s, 3H, Mtr-Me), 1.53 (m, 1H, Arg-H$^{\beta}$), 1.43 (m, 1H, Arg-H$^{\beta}$), 1.29 (s, 9H, O^{t}Bu), 1.28 (m, 6H, 2×Arg-H$^{\beta}$+4×Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{129}H_{142}N_{14}O_{33}S_2Na$ (M+Na): 2501.9203; found 2501.9209.

4.4.16. H-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-O^{t}Bu (18c). The title compound was prepared from **17c** (291 mg, 0.117 mmol) using the method described in the synthesis of **15a** to give **18c** (254 mg, 96%) as a yellowish amorphous solid. $[\alpha]_D^{22}=-16$ (*c* 0.5, MeOH); ^{1}H NMR (MeOH-d$_4$, 400 MHz) δ 7.91 (d, $J=8.5$ Hz, 2H, Bz-*o*) 7.85 (d, $J=8.6$ Hz, 2H, Bz-*o*), 7.82 (d, $J=8.5$ Hz, 2H, Bz-*o*), 7.77 (d, $J=8.5$ Hz, 2H, Bz-*o*), 7.47 (m, 4H, Bz-*p*), 7.31 (m, 8H, Bz-*m*), 7.00 (m, 6H, Tyr-H$^{\delta}$), 6.85 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\delta}$), 6.67 (m, 6H, Tyr-H$^{\varepsilon}$), 6.62 (s, 1H, Mtr-ArH), 6.61 (s, 1H, Mtr-ArH), 6.56 (d, $J=8.5$ Hz, 2H, Tyr-H$^{\varepsilon}$), 5.74 (t, $J=9.9$ Hz, 1H, SAA-H^{3}), 5.47 (t, $J=9.7$ Hz, 1H, SAA-H^{3}), 5.42 (t, $J=9.6$ Hz, 1H, SAA-H^{4}), 5.40 (t, $J=9.6$ Hz, 1H, SAA-H^{4}), ~4.81 (SAA-H^{1}, partially obscured by solvent signal), 4.46 (t, $J=6.7$ Hz, 1H, Tyr-H$^{\alpha}$), 4.45 (d, $J=8.1$ Hz, 1H, SAA-H^{1}), 4.40 (m, 3H, 3×Tyr-H$^{\alpha}$), 4.33 (d, $J=9.8$ Hz, 1H, SAA-H^{5}), 4.25 (d, $J=9.7$ Hz, 1H, SAA-H^{5}), 4.21 (dd, $J=8.4$, 5.2 Hz, 1H, Arg-H$^{\alpha}$), 4.14 (m, 2H, Arg-H$^{\alpha}$+SAA-H^{2}), 3.78 (s, 3H, Mtr-OMe), 3.77 (s, 3H, Mtr-OMe), 3.55 (s, 3H, SAA-OMe), 3.47 (s, 3H, SAA-OMe), 3.02 (m, 3H, SAA-H^{2}+Arg-H$^{\delta}$), 2.92 (m, 8H, 2×Arg-H$^{\delta}$+6×Tyr-H$^{\beta}$), 2.77 (dd, $J=14.0$, 5.1 Hz, 1H, Tyr-H$^{\beta}$), 2.66 (s, 3H, Mtr-Me), 2.65 (s, 3H, Mtr-Me), 2.59 (s, 6H, 2×Mtr-Me), 2.51 (dd, $J=14.5$ Hz, $J=9.7$ Hz, 1H, Tyr-H$^{\beta}$), 2.08 (s, 3H, Mtr-Me), 2.06 (s, 3H, Mtr-Me), 1.63 (m, 1H, Arg-H$^{\beta}$), 1.54 (m, 1H, Arg-H$^{\beta}$), 1.44 (m, 1H, Arg-H$^{\beta}$), 1.35 (m, 12H, 1×Arg-H$^{\beta}$+2×Arg-H$^{\gamma}$+O^{t}Bu), 1.19 (m, 2H, Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{114}H_{132}N_{14}O_{31}S_2Na$ (M+Na): 2279.8522; found 2279.8530.

4.4.17. Cyclo[SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr-SAA(di-OBz)-Tyr-Arg(Mtr)-Tyr] (19c). The title compound was prepared from **18c** (89.3 mg, 39.5 μmol) using the method described in the synthesis of compound **19a**. The product was purified with flash chromatography (CH_2Cl_2:MeOH 8:1, $R_f=0.32$) followed by size-exclusion chromatography to give **19c** (29.3 mg, 34%) as a white amorphous solid. $[\alpha]_D^{24}=-5$ (*c* 0.5, MeOH); HRMS (FAB) calcd for $C_{110}H_{122}N_{14}O_{30}S_2Na$ (M+Na): 2205.7790; found 2205.7795. A resolved NMR spectrum could not be obtained.

4.4.18. Cyclo(SAA-Tyr-Arg-Tyr-SAA-Tyr-Arg-Tyr) (1c). Compound **19c** (25.5 mg, 11.7 μmol) was dissolved in TFA containing 5% thioanisole (2.5 mL). After 24 h, toluene (2.5 mL) was added and the mixture was evaporated. The residue was dissolved in MeOH (5 mL) and NaOMe/MeOH (1 M, 75 μL) was added. After 24 h, the mixture was acidified with AcOH and evaporated. The product was purified using preparative HPLC (C_{18} column, 15→20% B in A over 60 min, A: H_2O +0.1% TFA, B: CH_3CN +0.1% TFA, $t_R=10$ min) to afford **1c** (9.3 mg, 59%) as a fluffy white powder after lyophilization. $[\alpha]_D^{22}=-15$ (*c* 0.2, MeOH); ^{1}H NMR (D_2O, 400 MHz) δ 7.03 (d, $J=8.4$ Hz, 4H, Tyr-H$^{\delta}$), 7.00 (d, $J=8.2$ Hz, 4H, Tyr$^{\delta}$), 6.77 (d, $J=8.3$ Hz, 4H, Tyr$^{\varepsilon}$), 6.68 (d, $J=7.8$ Hz, 4H, Tyr$^{\varepsilon}$), 4.57 (m, 4H, 4×Tyr-H$^{\alpha}$, partially obscured by solvent signal), 4.43 (d, $J=8.6$ Hz, 2H, SAA-H^{1}), 3.81 (t, $J=7.0$ Hz, 2H, Arg-H$^{\alpha}$), 3.66 (m, 4H, SAA-H^{5}+SAA-H^{2}), 3.40 (t, $J=9.8$ Hz, 2H, SAA-H^{3}), 3.32 (s, 6H, OMe), 3.18 (t, $J=9.5$ Hz, SAA-H^{4}), 3.11 (dd, $J=15.1$, 7.4 Hz, 2H, Tyr-H$^{\beta}$), 2.96 (m, 8H, 4×Tyr-H$^{\beta}$+4×Arg-H$^{\delta}$), 2.32 (dd, $J=12.3$, 11.6 Hz, 2H, Tyr-H$^{\beta}$), 1.66 (m, 2H, Arg-H$^{\beta}$), 1.52 (m, 2H, Arg-H$^{\beta}$), 1.27 (m, 2H, Arg-H$^{\gamma}$), 1.16 (m, 2H, Arg-H$^{\gamma}$); HRMS (FAB) calcd for $C_{62}H_{82}N_{14}O_{20}Na$ (M+Na): 1365.5728; found 1365.5729.

4.5. NMR titrations

All experiments were performed at 400 MHz in a deuterated phosphate buffer (100 mM phosphate, pH 7.2). In the titration experiments, a stock solution with 0.5 mM receptor concentration was prepared. The ligand to be titrated was dissolved in a portion of the stock solution to give a solution with 0.5 mM receptor and 100 mM ligand. These two solutions were mixed in different proportions to give a series of solutions with 0.5 mM receptor and ligand concentrations up to 80 mM. ^{1}H NMR experiments were performed on the solutions and the chemical shifts of receptor signals were fitted to a 1:1 binding isotherm using non-linear regression.[31] Acidic and basic ligands were used as sodium salts and hydrochloride salts, respectively.

Acknowledgements

We thank the Swedish Research Council, the Swedish Strategic Research Foundation, and the program 'Glycoconjugates in Biological Systems' sponsored by the Swedish Strategic Research Foundation for financial support.

References and notes

1. Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155–3184.
2. Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514.
3. Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230–253.
4. Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421–435.

5. Peri, F.; Cipolla, L.; Forni, E.; La Ferla, B.; Nicotra, F. *Chemtracts Org. Chem.* **2001**, *14*, 481–499.
6. Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189–8196.
7. Chakraborty, T. K.; Srinivasu, P.; Bikshapathy, E.; Nagaraj, R.; Vairamani, M.; Kiran Kumar, S.; Kunwar, A. C. *J. Org. Chem.* **2003**, *68*, 6257–6263.
8. van Well, R. M.; Marinelli, L.; Erkelens, K.; van der Marel, G. A.; Lavecchia, A.; Overkleeft, H. S.; van Boom, J. H.; Kessler, H.; Overhand, M. *Eur. J. Org. Chem.* **2003**, 2303–2313.
9. Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156–10167.
10. van Well, R. M.; Overkleeft, H. S.; Overhand, M.; Vang Carstenen, E.; van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **2000**, *41*, 9331–9335.
11. Lohof, E.; Planker, E.; Mang, C.; Burkhart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2761–2763.
12. Gruner, S. A. W.; Kéri, G.; Schwab, R.; Venetianer, A.; Kessler, H. *Org. Lett.* **2001**, *3*, 3723–3725.
13. Stöckle, M.; Voll, G.; Günther, R.; Lohof, E.; Locardi, E.; Sibylle, G.; Kessler, H. *Org. Lett.* **2002**, *4*, 2501–2504.
14. Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. *Chem. Eur. J.* **2002**, *8*, 4366–4376.
15. van Well, R. M.; Overkleeft, H. S.; van der Marel, G. A.; Bruss, D.; Thibault, G.; de Groot, P. G.; van Boom, J. H.; Overhand, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 331–334.
16. Irvine, J. C.; McNicoll, D.; Hynd, A. *J. Chem. Soc.* **1911**, *11*, 250–261.
17. Yamasaki, T.; Kubota, Y.; Tsuchiya, T.; Umezawa, S. *Bull. Chem. Soc. Jpn* **1976**, *49*, 3190–3192.
18. Onodera, K.; Kitaoka, S.; Ochiai, H. *J. Org. Chem.* **1962**, *27*, 156–159.
19. Fugedi P.; Csaba P. U.S. Patent 5,849,709, 1998; *Chem. Abstr.*, **1997**, *126*, 60,291.
20. Mehta, A.; Jaouhari, R.; Benson, T. J.; Douglas, K. T. *Tetrahedron Lett.* **1992**, *33*, 5441–5444.
21. Sheppeck, J. E., II; Kar, H.; Hong, H. *Tetrahedron Lett.* **2000**, *41*, 5329–5333.
22. Quibell, M.; Packman, L. C.; Johnson, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1219–1225.
23. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 442–445.
24. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.
25. Bienert, M.; Henklein, P.; Beyermann, M.; Carpino, L. A. In *Synthesis of Peptides and Peptidomimetics*, 4th ed.; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Houben–Weyl Methods of Organic Chemistry; Georg Thieme: Stuttgart, 2002; Vol. E22a, Chapter 3.8, p 562.
26. Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831–8838.
27. Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695–698.
28. ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442–3444.
29. Fujino, M.; Wakimasu, M.; Kitada, C. *Chem. Pharm. Bull.* **1981**, *29*, 2825–2831.
30. Horton, D.; Turner, W. N. *J. Org. Chem.* **1965**, *30*, 3387–3394.
31. Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1990; pp 123–143.