Synthesis of Novel Sugar Amino Acids by Curtius Rearrangement

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Sugar amino acids (SAAs) bearing an amine group on the anomeric position are a challenging class of SAAs to synthesize due to the inherent instability of glycosylamines. We developed a novel synthetic strategy towards both furanoid and pyranoid δ-SAAs of this type, based on a Curtius rearrangement. The latter reaction, which is known to proceed with retention of configuration, was performed on carboxylic acids derived from the oxidation of glycosidic primary hydroxyls. Leu-enkephalin analogs were prepared by replacing the Gly-Gly moiety in the parent Leu-enkephalin pentapeptide with the furanoid and pyranoid δ-SAA.

Introduction

Sugar amino acids (SAAs) − carbohydrate derivatives bearing a carboxylic acid and an amino functionality − have attracted considerable interest in recent years as peptide and carbohydrate mimetics. The ease of peptide bond formation together with the potential structural diversity in the carbohydrate core of SAAs has stimulated the design and synthesis of an array of novel SAAs as well as their implementation in different fields of research.[1] With the objective of stabilizing the secondary structure of pharmacologically active peptides SAAs have been utilized as conformationally restricted peptide isosters. For instance, several research groups have synthesized linear Leu-enkephalin analogs as well as cyclic somatostatin and RGD peptides containing a single SAA residue at predesigned positions.[2] Moreover, oligomers assembled from multiple SAA building blocks have found interest as oligosaccharide mimetics with enhanced metabolic and conformational stability.[3] In line with these efforts, we and others are exploring the development of new potential host molecules by the construction of cyclic oligomers composed of SAAs.[4]

To broaden the scope of SAAs as versatile building blocks for drug discovery and material sciences the design, synthesis and application of novel SAAs, varying in the number, nature and stereochemistry of the functional groups, is imperative. In this respect our attention was attracted to δ-SAAs having an amino function at the anomeric position of the carbohydrate core. The challenge to synthesize this type of SAAs and their application in peptide synthesis is associated with the inherent lability of glycosylamines, which are prone to mutarotate, hydrolyze or undergo Amadori rearrangement.[5] Several research groups have disclosed synthetic strategies towards α-, β- and γ-SAAs having an anomeric amine.[6] One of the most common methods used for the introduction of an amine at the anomeric position is the reaction of a reducing sugar with ammonia or ammonium hydrogen carbonate.[7] This reaction was successfully applied by Danilov et al. in the synthesis of amino-mannuronic acid.[6e] Glycosylamines have also been prepared by reduction of glycosylazides, obtained from the glycosyl halide or acetate by nucleophilic displacement. For example, Fleet and Dondoni employed this reaction in the synthesis of furanoid and pyranoid α-SAAs.[6a,6b] The azide route was also adopted by Kessler and co-workers for the construction of the γ-SAA glucuronosylamine, which was subsequently incorporated into a somatostatin analog.[2c] An alternative approach towards glucuronosylamine, based on the acidic ring opening of an α-oxazoline, was developed in our laboratory.[3e] The γ-SAA obtained via this approach was used for the preparation of a backbone-modified phytoalexin elicitor heptasaccharide analog.

Here we wish to report the synthesis of the novel furanoid (**I**) and pyranoid δ-SAA (**II**), bearing an amine group at the anomeric position (see Figure 1). The key steps in the synthetic protocol involve oxidation of the primary hydroxyl function of the respective carbohydrate precursors to the carboxylate, followed by Curtius rearrangement, which is known to proceed with retention of configuration.[8] The use of the δ-SAAs in peptide synthesis is demonstrated by incorporating SAAs **I** and **II** in Leu-enkephalin analogs, chosen as representative examples of a short

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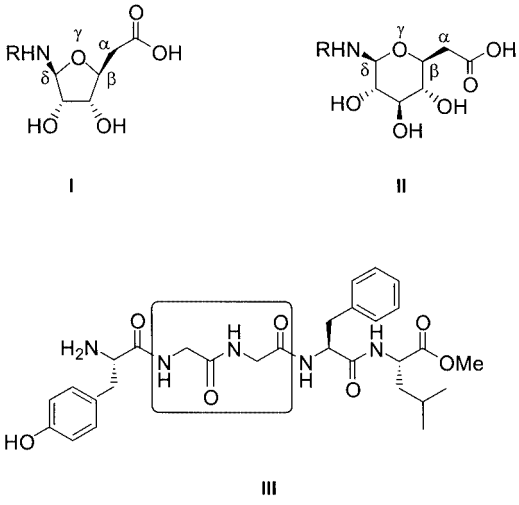
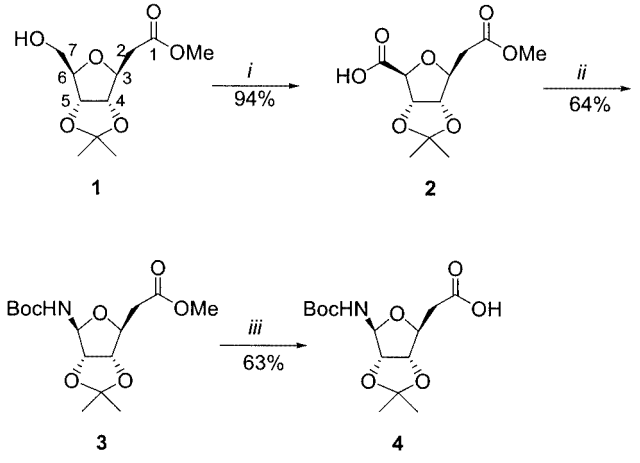


Figure 1. Furanoid δ-SAA **I**, pyranoid δ-SAA **II** and Leu-enkephalin **III**

peptide. The flexible Gly-Gly dipeptide in the original Leu-enkephalin pentapeptide (**III** in Figure 1) serves as a spacer, and replacement of this dipeptide by different SAAs has been previously reported.[2c,2d]

Results and Discussion

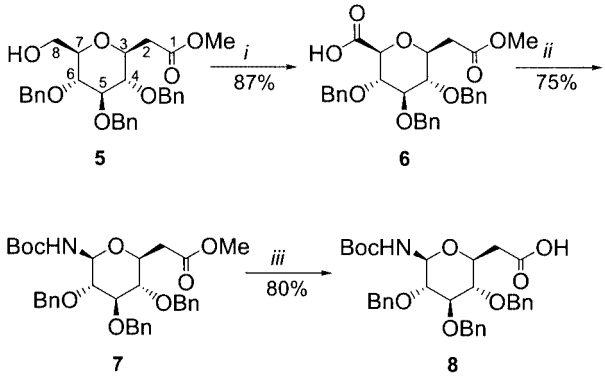
The synthesis of furanoid δ-SAA **4** commences with TEMPO oxidation of the primary alcohol group in **1**, which has been previously synthesized in our laboratory,[4a,4d] to afford carboxylic acid **2** in good yield (see Scheme 1).



Scheme 1. Synthesis of furanoid SAA; reagents and conditions: (*i*) *cat*. TEMPO, NaOCl, $NaHCO_3$, NaCl, KBr, $(nBu)_4NCl$, DCM/H_2O, 30 min, 0 °C; (*ii*) DPPA, Et_3N, *t*BuOH, 80 °C, 16 h; (*iii*) LiOH, H_2O_2, THF, 0 °C, 2 h

Curtius rearrangement was effected by treatment of **2** with diphenylphosphorazidate (DPPA) and Et_3N in *t*BuOH at 80 °C under anhydrous conditions.[9] The intermediate isocyanate was trapped with *t*BuOH to give the Boc-protected SAA **3** as a single isomer in 64%. The 3,6-*cis* relationship in **3** was ascertained by the medium H^3/H^6 cross peak observed in a NOESY spectrum. Saponification of the methyl ester under standard basic conditions (1 M aq. NaOH) led to epimerization at the C3 position.[10] To our satisfaction, deprotection of **3** using LiOOH proceeded without the undesired epimerization to give furanoid SAA **4** in 63% yield together with 26% of recovered starting material.

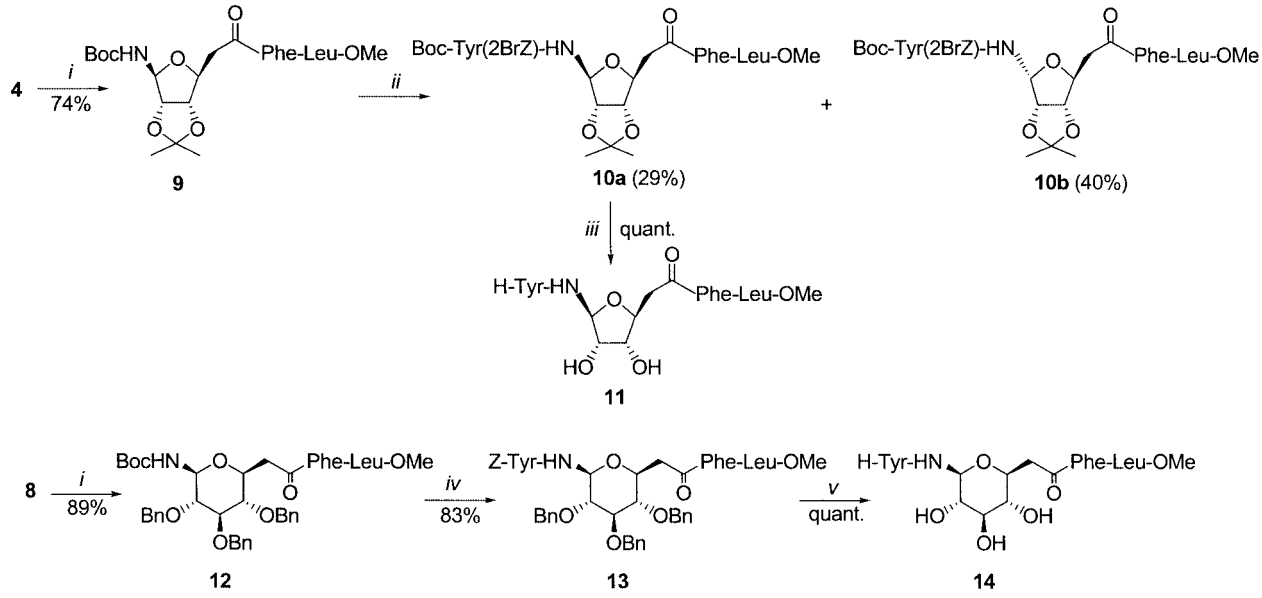
In a similar sequence of reactions, the pyranoid dipeptide isoster **8** was obtained starting from the known[4d] methyl 3,7-anhydro-4,5,6-tri-*O*-benzyl-2-deoxy-D-*gulo*-D-*glycero*-octonate (**5**) as depicted in Scheme 2. In contrast to the transformation of **1** into **2**, TEMPO oxidation of **5** did not afford the desired acid. Fortunately, treatment of **5** with a large excess of pyridinium dichromate (PDC) did furnish **6** in 87%, Curtius rearrangement of which under the aforementioned conditions gave the Boc-protected amine **7** in 75%. Ensuing saponification of the methyl ester in **7** in the presence of NaOH proceeded without racemization to provide the partially protected SAA **8**.



Scheme 2. Synthesis of pyranoid SAA; reagents and conditions: (*i*) PDC (10 equiv.), DMF, 16 h; (*ii*) DPPA, Et_3N, *t*BuOH, 80 °C, 16 h; (*iii*) NaOH, dioxane, 3 h

Having both δ-SAAs **4** and **8** in hand, attention was focused on the solution-phase synthesis of Leu-enkephalin analogs **11** and **14**. Condensation of **4** with HCl·H-Phe-Leu-OMe in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine (NMM) provided **9** in 74% yield (see Scheme 3). Acidolysis (50% TFA/DCM) of the Boc group in **9**, followed by condensation of the resulting free amine with Boc-Tyr(2BrZ)-OH (EDC/HOBt/NMM), led to the isolation of two products. The minor product was identified by NMR spectroscopy as the expected tetrapeptide **10a**, having the 3,6-*cis* relationship in the SAA residue. The major product proved to be the isomer **10b**, in which the amine substituent on the SAA core has anomerized to give the 3,6-*trans* configuration.

The *trans* configuration was established from the presence of an H^2/H^6 cross peak in the NOESY spectrum in combination with the large upfield shift (6 ppm) of C6 in the ^{13}C NMR spectrum (see Figure 2). Most likely, mutarotation of the anomeric amine already takes place during the acidic Boc-removal step.[5a,7b]



Scheme 3. Synthesis of Leu-enkephalin analogs **11** and **14**; reagents and conditions: (*i*) HCl·H-Phe-Leu-OMe, EDC·HCl, HOBt, NMM, 16 h; (*ii*) a) 50%TFA/DCM, 5 min; b) Boc-Tyr(2BrZ)-OH, EDC·HCl, HOBt, NMM, 16 h; (*iii*) a) $Pd(OH)_2$/C, MeOH, 5 h; b) 5% H_2O/TFA, 3 h; (*iv*) a) 50%TFA/DCM, 5 min; b) Z-Tyr-OH, EDC·HCl, HOBt, NMM, 16 h; (*v*) Pd/C, H_2, MeOH, 16 h

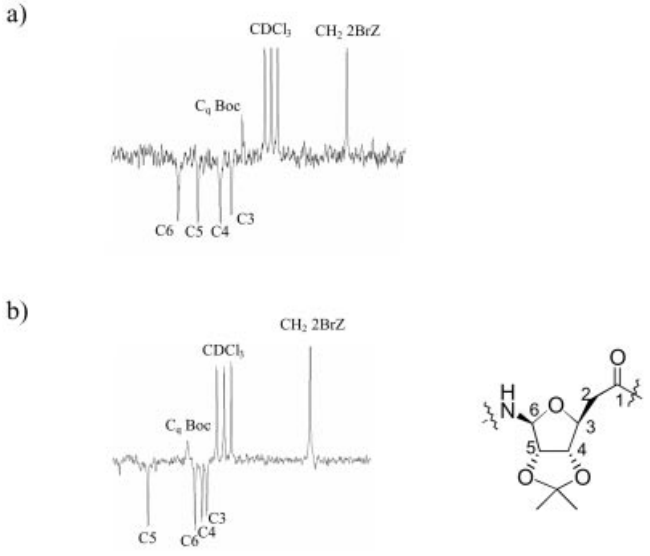


Figure 2. Parts of the ^{13}C NMR spectra of **10a** (a) and **10b** (b) recorded in $CDCl_3$ at 50 MHz

Deprotection of the 3,6-*cis* isomer **10a**, i.e. Pd^{II} catalyzed hydrogenation of the 2-bromobenzyloxycarbonyl (2BrZ) group, followed by acidolysis of the Boc and isopropylidene protective groups, proceeded uneventfully, affording the desired Leu-enkephalin analog **11** in good yield. The structure of **11** was ascertained by LCMS and NMR spectroscopy.

The synthesis of Leu-enkephalin analog **14**, having the pyranoid δ-SAA **II** instead of the Gly-Gly dipeptide, was accomplished in a similar sequence of transformations. Condensation of **8** with HCl·H-Phe-Leu-OMe (EDC/HOBt) gave dipeptide **12** in 89% yield. After removal of the Boc group in **12** (50% TFA/DCM), the resulting free amine was condensed with Z-Tyr-OH to furnish the protected tetrapeptide **13** in 83%. No anomerization was observed during this sequence of reactions judging by the $J_{6,7}$ of 9.2 Hz as observed in the ^1H NMR spectrum. Finally, removal of the Z- and benzyl groups was accomplished by hydrogenation with palladium on charcoal, yielding H-Tyr-SAA-Phe-Leu-OMe **14** quantitatively. The structure of **14** was unambiguously established by NMR spectroscopy and LCMS.

Compounds **11** and **14** were tested for their affinity for human μ, δ and κ opioid receptors using transfected hamster ovary cells. The SAA-containing compounds were not able to displace radioligands from their binding sites at concentrations as high as 10 μM.[11]

In conclusion, two new SAAs, having an amine group directly attached to the carbohydrate core, were successfully synthesized by application of a Curtius rearrangement to readily available carbohydrate building blocks. Future studies will focus on the conformational analysis of **11** and **14** in order to determine the structural basis for this loss of activity. Furthermore, SAAs **4** and **8** can be used for the incorporation into other biologically active sequences (i.e. RGD peptides, protein farnesyl transferase inhibitor[12]) as well as for the construction of cyclodextrin analogs.

Experimental Section

General Procedures and Materials: ^1H and ^{13}C NMR spectra were recorded with a Jeol JNM-FX-200 (200/50.1 MHz), a Bruker WM-300 (300/75.1 MHz) or a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard. All given ^{13}C NMR spectra are proton decoupled. Mass spectra were recorded with PE/SCIEX API 165 with an electron-spray interface. Column chromatography was performed on Fluka silica gel 60 (0.04−0.063 mm). TLC analysis was conducted on precoated DC sheets (Merck silica gel 60 F_{254}) with detection by UV absorption (254 nm) where applicable and

by spraying with 20% H_2SO_4 in ethanol, a ninhydrin solution or ammonium molybdate (25 g/L) and ceric ammonium sulfate (10 g/L), followed by charring at about 150 °C. Reactions were run at ambient temperature, unless stated otherwise. Reactions that require anhydrous conditions were stirred under an atmosphere of argon or nitrogen. Acetone (Acros, p.a.), dichloroethane (DCE; Biosolve, HPLC grade), DMF (Baker, p.a.), toluene (Biosolve, p.a.), 1,4-dioxane (Baker, p.a.), pyridine (Baker, p.a.) and dichloromethane (DCM; Baker, p.a.) were stored over molecular sieves (4 Å). Acetonitrile (Biosolve, p.a.) and MeOH (Biosolve, p.a.) were stored over molecular sieves (3 Å). Triethylamine (Acros) was boiled under reflux for 3 h with CaH_2, distilled and stored over KOH. Diphenylphosphorazidate (DPPA) was purchased from Aldrich and stored at +4 °C. Trifluoroacetic acid (TFA, Biosolve), 2,2,6,6-tetramethyl-4-piperidin-1-oxyl (TEMPO, Acros), 10% palladium on charcoal (Aldrich), 20% palladium(II) hydroxide on carbon (Janssen), 1-hydroxybenzotriazole (HOBt, Neosystem), *N*-[3-(dimethylamino)propyl]-*N*′-3-ethylcarbodiimide hydrochloride (EDC, Aldrich) and pyridinium dichromate (PDC, Aldrich) were used as received.

1-Methyl 3,6-Anhydro-2-deoxy-4,5-*O*-isopropylidene-D-*allo*-heptarate (2): Compound **1** (1.62 g, 6.24 mmol), TEMPO (0.015 g, 0.094 mmol, 0.015 equiv.), KBr (0.059 g, 0.50 mmol) and TBACl (0.094 g, 0.34 mmol) were dissolved in a mixture of DCM (40 mL) and aq. $NaHCO_3$ (1 M, 12.5 mL). The reaction mixture was cooled to 0 °C and a solution of NaOCl (13%, 12.5 mL), sat. NaCl (12.5 mL) and aq. $NaHCO_3$ (6.25 mL) was added dropwise over a period of 30 min. After stirring the resulting mixture for another 30 min at 0 °C it was washed with DCM (3 × 50 mL). The aqueous phase was acidified to pH 2 with 1 N HCl and extracted with DCM (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to afford crude acid **2** in 94% yield (1.51 g). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.01 (dd, *J* = 2.9 Hz, *J* = 6.8 Hz, 1 H, H5), 4.63 (dd, *J* = 2.9 Hz, *J* = 6.6 Hz, 1 H, H4), 4.60 (d, *J* = 2.9 Hz, 1 H, H6), 4.50 (dt, *J* = 2.9 Hz, *J* = 5.9 Hz, 1 H, H3), 3.73 (s, 3 H, OMe), 2.71 (dd, *J* = 2.9 Hz, *J* = 5.9 Hz, 2 H, 2 × H2), 1.56, 1.36 (2 × s, 6 H, 2 × CH_3 isopropylidene) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.45 (C6), 171.12 (C1), 114.14 (Cq isopropylidene), 83.91, 83.64, 83.09, 82.31 (C3, C4, C5, C6), 51.89 (OMe), 37.43 (C2), 26.73, 25.03 (2 × CH_3 isopropylidene).

Methyl 3,6-Anhydro-6-(*tert*-butoxycarbonylamino)-2,6-dideoxy-4,5-*O*-isopropylidene-D-a*llo*-hexonoate (3): Compound **2** (0.894 g, 3.44 mmol) was coevaporated with toluene (3 × 20 mL), and dissolved in *t*BuOH (20 mL). Crushed molecular sieves (4Å) were added and the reaction mixture was stirred for 30 min under an argon atmosphere. Et_3N (0.46 mL, 3.44 mmol) and DPPA (0.74 mL, 3.44 mmol) were added and the reaction mixture was heated under reflux. After 16 h the solution was filtered and concentrated, the residue was applied to a silica gel column. Elution with EtOAc/light petroleum (0:1 → 1:1, v/v) gave compound **3** in a 64% yield (0.89 g) as a white solid. ^{1}H NMR, COSY, NOESY (300 MHz, $CDCl_3$): δ = 5.59 (br. s, 1 H, HN), 5.34 (s, 1 H, H6), 4.71 (s, 2 H, H4, H5), 4.37 (t, *J* = 5.8 Hz, 1 H, H3), 3.71 (s, 3 H, OMe), 2.72 (dd, *J* = 3.6 Hz, *J* = 5.8 Hz, 2 H, 2 × H2), 1.53, 1.33 (2 × s, 6 H, 2 × CH_3 isopropylidene), 1.45 (s, 9 H, *tert*-Bu Boc) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.28 (C1), 154.45 [C(O) Boc), 113.69 (Cq isopropylidene), 88.68, 85.01, 83.49, 81.06 (C3, C4, C5, C6), 51.71 (OMe), 38.21 (C2), 28.15 (*tert*-Bu Boc), 26.87, 25.20 (2 × CH_3 isopropylidene). ES-MS: *m*/*z* = 332.1 $[M + H]^+$, 354.1 $[M + Na]^+$, 685.5 $[2M + Na]^+$.

3,6-Anhydro-6-(*tert*-butoxycarbonylamino)-2,6-dideoxy-4,5-*O*-isopropylidene-D-*allo*-hexonic Acid (4): A mixture of compound **3** (0.90 g, 2.8 mmol) in THF/H_2O_2 (25 mL, 2:1, v/v) was cooled to 0 °C. Aq. LiOH (6.72 mL of a 1.25 M solution) was slowly added to the solution. After stirring the reaction mixture for 3 h it was acidified to pH 2 with 1 N HCl and aq. Na_2SO_3 (10 mL) was added. The aqueous phase was extracted with EtOAc (3 × 30 mL), the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (eluent: EtOAc/light petroleum, 1:1 → 1:0, v/v, containing 0.5% AcOH) furnished compound **4** as a white solid in 63% yield (0.59 g) together with 26% of recovered starting material (0.23 g). ^{1}H NMR (200 MHz, MeOD): δ = 5.26 (d, *J* = 1.5 Hz, 1 H, H6), 4.62 (s, 2 H, H4, H5), 4.33 (dt, *J* = 1.5 Hz, *J* = 6.9 Hz, 1 H, H3), 2.61 (dd, *J* = 1.5 Hz, *J* = 7.0 Hz, 2 H, 2 × H2), 1.49, 1.31 (2 × s, 6 H, 2 × CH_3 isopropylidene), 1.45 (s, 9 H, *tert*-Bu Boc) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 174.08 (C1), 114.61 (Cq isopropylidene), 89.36, 85.61, 85.20, 82.56 (C3, C4, C5, C6), 39.76 (C2), 28.63 (*tert*-Bu Boc), 27.22, 25.39 (2 × CH_3 isopropylidene).

Boc-SAA-(isopropylidene)-Phe-Leu-OMe (9): A solution of Boc-Phe-Leu-OMe (0.29 g, 0.74 mmol) in DCM/TFA (6 mL, 1:1, v/v) was stirred for 5 min and subsequently concentrated. The residue was coevaporated with toluene (3 × 3 mL) and dissolved in DMF (5 mL), SAA **4** (0.23 g, 0.74 mmol) was added and the resulting mixture was cooled to 0 °C. HOBt (0.12 g, 0.89 mmol) and EDC·HCl (0.17 g, 0.89 mmol) were added and the reaction mixture was neutralized with NMM. After stirring for 16 h at ambient temperature the reaction mixture was concentrated and the residue redissolved in EtOAc (15 mL). The organic phase was washed with H_2O (10 mL), aq. $NaHCO_3$ (2 × 10 mL), H_2O (10 mL), $KHSO_4$ (2 × 10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (eluent: EtOAc/light petroleum, 0:1 → 2:1, v/v) afforded compound **9** as a white solid in 74% yield (0.32 g). ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.33−7.17 (m, 5 H, H_{arom} Phe), 6.76 (d, *J* = 7.7 Hz, 1 H, HN-Phe), 6.12 (d, 1 H, HN-Leu), 5.44 (dd, *J* = 3.3 Hz, *J* = 9.5 Hz, 1 H, HN-SAA), 4.64−4.48 (m, 5 H, Hα-Leu, H3,H4, H5, H6), 4.20 (q, *J* = 3.7 Hz, 1 H, Hα-Phe,), 3.68 (s, 3 H, OMe), 3.03 (d, *J* = 7.3 Hz, 2 H, 2 × Hβ-Phe,), 2.68 (dd, $J_{2a,3}$ = 4.4 Hz, $J_{2a,2b}$ = 15.0 Hz 1 H, H2a), 2.48 (dd, $J_{2b,3}$ = 4.0 Hz, $J_{2a,2b}$ = 14.6 Hz, 1 H, H2b), 1.58−1.43 (m, 3 H, 2 × Hβ-Leu, Hγ-Leu),1.53, 1.29 (2 × s, 6 H, 2 × CH_3 isopropylidene), 1.47 (s, 9 H, *tert*-Bu Boc), 0.91 (2 × d, *J* = 5.5 Hz, 6 H, 6 × Hδ-Leu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.63, 171.48, 170.06 [C(O)Leu, C(O) Phe, C(O) SAA), 154.96 [C(O) Boc), 136, 13 (Cq Phe), 129.12, 128.49, 126.91 (C_{arom} Phe), 114.09 (Cq isopropylidene), 87.98, 84.00, 81.76, 80.48 (C3, C4, C5, C6), 80.12 (Cq Boc), 54.74, 52.17, 50.74 (Cα-Phe, Cα-Leu, OMe), 41.34, 39.19, 38.10 (C2, Cβ-Phe, Cβ-Leu), 28.24 (*tert*-Bu Boc), 27.12, 25.26 (2 × CH_3 isopropylidene), 24.48 (Cγ-Leu), 22.75, 21.54 (2 × Cδ-Leu).

Boc-Tyr-(2BrZ)-SAA-(isopropylidene)-Phe-Leu-OMe (10): Compound **9** (0.12 g, 0.21 mmol) was dissolved in TFA/DCM (2 mL, 1:1, v/v) and the resulting mixture was stirred for 5 min. Toluene (10 mL) was added, the solution was concentrated and the residue was subsequently coevaporated with toluene (3 × 5 mL). The residue was dissolved in DMF (2 mL), Boc-Tyr -(2BrZ)-OH (0.11 g, 0.23 mmol) was added and the reaction mixture was cooled to 0 °C. HOBt (0.034 g, 0.25 mmol) and EDC·HCl (0.048 g, 0.25 mmol) were added and the reaction mixture was neutralized with NMM. After 16 h TLC analysis showed complete disappearance of the starting materials and the solvent was removed under reduced pressure. The residue was taken up in EtOAc (10 mL) and washed

with H_2O (5 mL), aq. $NaHCO_3$ (2 × 5 mL), H_2O (2 × 5 mL), $KHSO_4$ (2 × 5 mL) and brine (2 × 5 mL), the organic phase was dried ($MgSO_4$) and concentrated. The crude product was applied to a silica gel column and eluted with EtOAc/light petroleum (1:9 → 8:2, v/v) to give a higher running product **10a** in 29% yield (0.059 g) and a lower running product **10b** in 40% yield (0.084 g).
10a: ^{1}H NMR, COSY, NOESY, (600 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 8.1 Hz, 1 H, HN-SAA), 7.60 (d, *J* = 8.0 Hz, 1 H, H_{arom}-2BrZ), 7.49 (d, *J* = 7.6 Hz, 1 H, H_{arom}-2BrZ), 7.35 (t, *J* = 7.4 Hz, 2 H, H_{arom}-Phe), 7.21 (m, 9 H, H_{arom}-Phe, H_{arom},-Tyr, H_{arom}-2BrZ), 6.98 (d, *J* = 7.0 Hz, 1 H, HN-Phe), 6.67 (d, *J* = 6.5 Hz, 1 H, HN-Leu), 5.59 (d, *J* = 6.4 Hz, 1 H, H6), 5.46 (d, *J* = 7.7 Hz, 1 H, HN-Tyr), 5.35 (s, 2 H, CH_2 2BrZ), 4.62 (m, 1 H, Hα-Phe), 4.46 (m, 2 H, H4, Hα-Leu), 4.43 (m, 1 H, Hα-Tyr), 4.28 (br. s, 2 H, H3, H5), 3.68 (s, 3 H, OMe), 3.12−2.96 (m, 4 H, 2 × Hβ-Phe, 2 × Hβ-Tyr), 2.55 (dd, $J_{2a,3}$ = 4.2 Hz, $J_{2a,2b}$ = 14.0 Hz, 1 H, H2a), 2.34 (dd, $J_{2b,3}$ = 4.3 Hz, $J_{2a,2b}$ = 14.7 Hz, 1 H, H2b), 1.56−136 (m, 3 H, Hβ-Leu, 2 × Hγ-Leu), 0. 90, 0.89 (2 × s, 6 H, 6 × Hδ-Leu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.62, 172.04, 171. 89, 169.98 (4 × C(O), Tyr, SAA, Phe, Leu), 155.45 [C(O) Boc), 153.36[C(O) 2BrZ),149.93 (Cζ-Tyr), 136.13, 134.71 (Cγ-Tyr, C1−2BrZ, Cγ-Phe), 132.92, 130.49, 130.16, 130.04, 129.19, 128.55, 127.61, 127.00, 120.88 (13 × CH-$_{arom}$, Tyr, 2BrZ, Phe), 123.37 (C2(q) 2BrZ), 113.88 (Cq isopropylidene), 86.22 (C6), 84.28 (C5), 82.03 (C4), 80.97 (C3), 79.88 (Cq Boc), 69.54 (CH_2 2BrZ), 55.86, 55.04, 52.38, 50.86 (Cα-Tyr, Cα-Phe, Cα-Leu, OMe), 41.31, 39.49, 38.52, 38.06 (Cβ-Tyr, Cβ-Phe, Cβ-Leu, C2), 28.21 (*tert*-Bu Boc), 27.05, 25.24 (2 × CH_3 isopropylidene), 24.51 (Cγ-Leu), 22.81, 21.57 (2 × Cδ-Leu). ES-MS: *m*/*z* = 969.5 $[M + H]^+$, 991.4 $[M + Na]^+$.
10b: ^{1}H NMR, COSY, ROESY, HMQC-COSY (600 MHz, $CDCl_3$): δ = 7.58 (d, 1 H, H_{arom}, *J* = 8.0 Hz), 7.47 (dd, *J* = 7.5 Hz, *J* = 0.8 Hz, 2 H, H_{arom}), 7.32 (t, *J* = 7.4 Hz, 2 H, H_{arom}), 7.26−7.09 (m, 9 H, H_{arom}), 7.04 (d, *J* = 8.3 Hz, 1 H, HN-SAA), 7.00 (dd, *J* = 8.0 Hz, *J* = 4.0 Hz, 1 H, HN-Phe), 6.71 (d, *J* = 7.8 Hz, 1 H, HN-Leu), 5.64 (dd, *J* = 3.7 Hz, *J* = 8.7 Hz, 1 H, H6), 5.33 (s, 2 H, CH_2 2BrZ), 5.16 (d, *J* = 8.2 Hz, 1 H, HN-Tyr), 4.66 (q, *J* = 7.5 Hz, 1 H, Hα-Phe), 4.62 (m, 2 H, H4, H5), 4.53 (m, 1 H, Hα-Leu), 4.40 (m, 1 H, Hα-Tyr), 4.20 (br. s, 1 H, H3), 3.65 (s, 3 H, OMe), 3.08 (dd, $J_{\alpha,\beta}$ = 6.6, $J_{\beta,\beta}$ = 14.0 Hz, 1 H, Hβ-Phe), 3.01 (dd, $J_{\alpha,\beta}$ = 7.2, $J_{\beta,,\beta}$ 13.9 Hz, 1 H, Hβ-Phe), 2.56 (dd, $J_{\alpha,\beta}$ = 9.9, $J_{\beta,,\beta}$ 14.9 Hz, 1 H, Hβ-Tyr), 2.48 (dd, $J_{\alpha,\beta}$ = 4.5, $J_{\beta,,\beta}$ 14.9 Hz, 1 H, Hβ-Tyr), 2.43 (dd, $J_{2a,3}$ = 7.2 Hz, $J_{2a,2b}$ = 15.1 Hz, 1 H, H2a), 2.36 (dd, $J_{2b,3}$ = 5.8 Hz, $J_{2a,2b}$ = 15.1 Hz, 1 H, H2b), 1.58−1.49 (m, 2 H, Hβ-Leu), 1.41 (s, 3 H, CH_3 isopropylidene), 1.37 (s, 9 H, *tert*-Bu Boc), 1.34 (m, 1 H, Hγ-Leu), 1.27 (s, 3 H, CH_3 isopropylidene), 0.87 (d, *J* = 5.8 Hz, 6 H, Hδ-Leu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.86, 171.510, 170.711, 169.546 (4 × C(O), Tyr, SAA, Phe, Leu), 155.15 [C(O) Boc), 153.24 [C(O) 2BrZ), 149.89 (Cζ-Tyr), 136.56, 134.45, 134.13 (Cγ-Tyr, C1 2BrZ, Cγ-Phe,), 132.84, 130.36, 130.34, 130.09, 129.95, 129.29, 128.41, 127.55, 126.80, 120.98 (13 × CH $_{arom}$, Phe, Tyr, 2BrZ), 126.75 (C2 2BrZ), 113.60 (Cq isopropylidene), 83.65 (C5), 79.56 (C6), 78.96 (C4), 78.53 (C3), 69.51 (CH_2 2BrZ), 54.67 (Cα-Phe), 52.22 (OMe), 50.82 (Cα-Leu), 50.72 (Cα-Tyr), 41.09 (Cβ-Phe), 38.01, 37.93 (Cβ-Leu, C2), 36.06 (Cβ-Tyr), 28.17 (*tert*-Bu Boc), 26.13, 24.85 (2 × CH_3 isopropylidene), 24.59 (Cγ-Leu), 22.65, 21.77 (2 × Cδ-Leu). ES-MS: *m*/*z* = 969.5 $[M + H]^+$, 991.4 $[M + Na]^+$.

Tyr-SAA-Phe-Leu-OMe (11): Fully protected **10a** (0.015 g, 0.016 mmol) was dissolved in MeOH (1 mL), and the solution was degassed. $Pd(OH)_2$ (4 mg) was added and the resulting reaction mixture was stirred under an atmosphere of hydrogen gas. After 5 h the catalyst was removed by filtration through glass fiber (GF/2A, Whatman). The solvent was removed in vacuo and the residue was taken up in a mixture of TFA/*p*-cresol/H_2O (2 mL, 90/5/5, v/v/v) and stirred for 3 h. After addition of toluene (5 mL) the solution was concentrated. The crude tetramer was applied on a silica gel column and eluted with DCM/MeOH (1:0 → 9:1, v/v) to give pure **11** in quantitative yield (0.010 g). $[\alpha]_D^{20}$ = −0.28 (*c* = 0.3, MeOH). ^{1}H NMR, COSY, ROESY, HMQC-COSY (600 MHz, [D_6]acetone): δ = 7.79 (d, *J* = 8.0 Hz, 1 H, HN-Leu), 7.63 (d, *J* = 7.9 Hz, 1 H, HN-Phe), 7.25 (m, 4 H, H_{arom} Phe, Tyr), 7.18 (m, 1 H, H_{arom} Phe), 7.10 (d, *J* = 8.4 Hz, 2 H, H_{arom} Phe), 6.76 (d, *J* = 8.4 Hz, 2 H, H_{arom} Tyr), 4.81 (t, *J* = 4.6 Hz, 1 H, H5), 4.77 (m, 2 H, H6, Hα-Phe), 4.52 (m, 1Hm Hα-Leu), 4.39 (t, *J* = 5.5 Hz, 1 H, H4), 4.09 (dt, *J* = 5.7 Hz, *J* = 7.0 Hz, 1 H, H3), 3.66 (s, 3 H, OMe), 3.65 (m, 1 H, Hα-Tyr), 3.15 (dd, $J_{\alpha,\beta}$ = 5.8, $J_{\beta,\beta}$ = 14.0 Hz, 1 H, Hβ-Phe), 3.01 (dd, $J_{\alpha,\beta}$ = 4.0, $J_{\beta,\beta}$ = 14.4 Hz, 1 H, Hβ-Tyr), 2.98 (dd, $J_{\alpha,\beta}$ = 7.5, $J_{\beta,\beta}$ = 13.6 Hz, 1 H, Hβ-Phe), 2.80 (dd, $J_{\alpha,\beta}$ = 7.7, $J_{\beta,\beta}$ = 14.3 Hz, 1 H, Hβ-Tyr), 2.69 (dd, $J_{2a,3}$ = 7.6 Hz, $J_{2a,2b}$ = 14.6 Hz, 1 H, H2a), 2.55 (dd, $J_{2b,3}$ = 5.0 Hz, $J_{2a,2b}$ = 14.7 Hz, 1 H, H2b), 1.68 (m, 1 H, Hγ-Leu), 1.59 (m, 2 H, 2 × Hβ-Leu), 0.90, 0.89 (2 × d, *J* = 6.7 Hz, *J* = 6.7 Hz, 6 H, 2 × Hδ-Leu) ppm. ^{13}C NMR, HMQC-COSY (150 MHz, [D_6]acetone): δ = 176.23, 173.53, 171.83, 171.38 (4 × C(O), Tyr, SAA, Phe, Leu), 156.85 (Cq $_{arom}$, Cζ-Tyr), 138.26, 129.37 (2 × Cq $_{arom}$, Cγ-Tyr, Cγ-Phe), 131.20, 130.31, 130.26, 130.21, 129.00, 127.25 (7 × CH $_{arom}$, Phe, Tyr), 115.92 (2 × CH $_{arom}$, Tyr), 89.88 (C6), 80.40 (C3), 74.21 (C4), 72.57 (C5), 60.10 (Cα-Tyr), 55.23 (Cα-Phe), 52.35 (OMe), 51.47 (Cα-Leu), 41.38 (Cβ-Leu), 40.06 (C2), 38.50 (Cβ-Phe), 36.88 (Cβ-Tyr), 25.29 (Cγ-Leu), 23.17, 21.92 (2 × Cδ-Leu). LCMS: R_t 13.44 (5 → 75% ACN/H_2O, v/v), *m*/*z* = 615.5 $[M + H]^+$. HRMS: calcd. for $C_{31}H_{41}N_4O_9$ [M + H] 615.3030; found 615.2933.

1-Methyl 3,7-Anhydro-4,5,6-tri-*O*-benzyl-2-deoxy-D-*gulo*-D-*glycero*-octarate (6): Compound **5** (0.72 g, 1.43 mmol) was coevaporated with toluene (3 × 5 mL) and subsequently dissolved in DMF (10 mL). Crushed molecular sieves (4 Å) were added and the reaction mixture was cooled to 0 °C under an argon atmosphere. PDC (2.69 g, 7.15 mmol) was added and the reaction mixture was stirred for 16 h at ambient temperature. EtOAc (25 mL) was added, the resulting suspension was filtered through hyflo and concentrated under reduced pressure. Purification by column chromatography (eluent: EtOAc/light petroleum, 2:8 → 8:2, v/v, containing 0.5% AcOH) afforded the acid **6** in 87% yield (0.65 g). ^{1}H NMR (200 MHz, $CDCl_3$) :δ = 7.29 (s, 15 H, H $_{arom}$ 3 × Bn), 4.92−4.57 (m, 6 H, 3 × CH_2 Bn), 3.99−3.33 (m,5 H, H3, H4, H5, H6, H7), 3.58 (s, 3 H, OMe), 2.73 (dd, $J_{2a,3}$ = 3.7 Hz, $J_{2a,2b}$ = 16.1 Hz, 1 H, H2a), 2.49 (dd, $J_{2b,3}$ = 8.0 Hz, $J_{2a,2b}$ = 15.3 Hz, 1 H, H2b) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.25 (C8), 171.16 (C1), 138.01, 137.56, 137.44 (3 × Cq Bn), 128.31, 128.04, 127.76, 127.61 (CH $_{arom}$ 3 × Bn), 85.85, 80.27, 79.82, 77.85, 75.73 (C3, C4, C5, C6, C7), 75.39−74.91 (3 × CH_2 Bn), 51.74 (OMe), 36.91 (C2).

Methyl 3,7-Anhydro-4,5,6-tri-*O*-benzyl-7-(*tert*-butoxycarbonylamino)-2-deoxy-D-*gulo*-D-*glycero*-heptonate (7): After coevaporation with toluene (3 × 10 mL) compound **6** (1.11 g, 2.14 mmol) was dissolved in *t*BuOH (20 mL). Crushed molecular sieves (4 Å) were added and the reaction mixture was stirred under an argon atmosphere for 30 min. DPPA (0.46 mL, 2.14 mmol) and Et_3N (0.28 mL, 2.14 mmol) were added and the resulting mixture was heated under reflux. After 16 h TLC analysis revealed completion of the reaction and the solution was filtered and concentrated. The crude SAA was purified by silica gel column chromatography (eluent: EtOAc/light petroleum, 0:1 → 3:7, v/v) to give the expected product **7** in a yield of 75% (0.84 g) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.31 (s, 15 H, H $_{arom}$ 3 × Bn), 4.96−4.59 (m, 7 H, 3 × CH_2 Bn, H7), 3.78 (m, 2 H, H5, H6), 3.59 (s, 3 H,

OMe), 3.30 (m, 2 H, H3, H4), 2.71 (dd, $J_{2a,3}$ = 4.4 Hz, $J_{2a,2b}$ = 15.0 Hz, 1 H, H2a), 2.43 (dd, $J_{2b,3}$ = 7.3 Hz, $J_{2a,2b}$ = 15.0 Hz, 1 H, H2b), 1.44 (s, 9 H, *tert*-Bu Boc) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.67 (C1), 154.48 [C(O) Boc), 138.04, 137.68 (3 × C_q Bn), 128.22, 128.00, 127.58 (CH_{arom} 3 × Bn), 85.61, 80.70, 73.06 (C3, C4, C5, C6, C7), 75.42, 74.70 (3 × CH_2 Bn), 51.44 (OMe), 37.06 (C2), 27.99 (*tert*-Bu Boc).

3,7-Anhydro-4,5,6-tri-*O*-benzyl-7-(*tert*-butoxycarbonylamino)-2-deoxy-D-*gulo*-D-*glycero*-heptonic Acid (8): Compound **7** (0.95 g, 1.61 mmol) was dissolved in dioxane (10 mL) and aqueous 1 M NaOH (1.77 mL) was added. The resulting mixture was stirred for 3 h after which TLC analysis revealed complete consumption of the starting material. The reaction mixture was acidified with 1 M HCl and extracted with EtOAc (3 × 20 mL). The combined organic phases were dried ($MgSO_4$), concentrated and the residue applied to a silica gel column. The pure product **8** was obtained in 80% yield (0.74 g) by eluting with EtOAc/PE (2:8 → 8:2, v/v) containing 0.5% AcOH. ^{1}H NMR (200 MHz., MeOD): δ = 7.26 (s, 15 H, H_{arom} 3 × Bn), 4.88−4.62 (m, 7 H, 3 × CH_2 Bn, H7), 3.67 (m, 2 H, H5, H6), 3.46−3.29 (m, 2 H, H3, H4), 2.69 (dd, $J_{2a,3}$ = 3.7 Hz, $J_{2a,2b}$ = 15.3 Hz, 1 H, H2a), 2.38 (dd, $J_{2b,3}$ = 7.3 Hz, $J_{2a,2b}$ = 15.0 Hz, 1 H, H2b), 1.44 (s, *tert*-Bu Boc) ppm. ^{13}C NMR (50 MHz, MeOD): δ = 171.32 (C1), 157.20 [C(O) Boc), 139.49, 139, 16 (3 × C_q Bn), 128.22, 128.00, 127.58 (CH_{arom} 3 × Bn), 86.73, 82.54, 81.69, 74.50 (C3, C4, C5, C6, C7), 76.41, 75.84 (3 × CH_2 Bn), 38.17 (C2), 28.65 (*tert*-Bu Boc). ES-MS: *m*/*z* = 578.7 [M + H]$^+$, 600.4 [M + Na]$^+$.

Boc-SAA-(Bn$_3$)-Phe-Leu-OMe (12): The Boc protected SAA **8** (0.58 g, 1.01 mmol) was coupled to HCl·H-Phe-Leu-OMe (0.40 g, 1.01 mmol) as described for **9**. The crude product was purified by column chromatography (eluent: MeOH/DCM, 0:1 → 5:95, v/v) to give **12** in 89% yield (0.77 g) as a white solid. ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.31−7.18 (m, 20 H, H_{arom}, 3 × Bn, Phe), 6.84 (d, *J* = 7.9 Hz, 1 H, HN-Phe), 6.59 (d, *J* = 7.9 Hz, 1 H, HN-Leu), 5.39 (d, *J* = 8.7 Hz, 1 H, HN-SAA), 4.92, 4.69 (m, 8 H, 3 × CH_2Bn, H7, Hα-Phe), 4.60 (m, 1 H, Hα-Leu), 3.67 (m, 4 H, CH_3 OMe, H6), 3.54 (m, 1 H, H3), 3.39 (q, *J* = 9.5 Hz, 2 H, H4, H5), 3.18 (dd, $J_{\alpha,\beta}$ = 7.5, $J_{\beta,\beta}$ = 14.0 Hz, 1 H, Hβ-Phe), 3.08 (dd, $J_{\alpha,\beta}$ = 6.1, $J_{\beta,\beta}$ = 13.8 Hz, 1 H, Hβ-Phe), 2.62 (dd, $J_{2a,3}$ = 3.0 Hz, $J_{2a,2b}$ = 15.3 Hz, 1 H, H2a), 2.46 (dd, $J_{2b,3}$ = 6.5 Hz, $J_{2a,2b}$ 15.3 Hz, 1 H, H2b), 1.60−1.48 (m, 3 H, 2 × Hβ-Leu, Hγ-Leu), 1.43 (s, 9 H, *tert*-Bu Boc), 0.87 (t, *J* = 6.2 Hz, 6 H, 6 × Hδ-Leu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.31, 170.59, 170.22 (3 × C(O), SAA, Phe, Leu), 154.88 [C(O) Boc), 138.20, 137.74, 136.98 (3 × C_q Bn, C_q Phe), 129.37−126.76 (CH_{arom} 3 × Bn, Phe), 85. 58, 81.46, 79.94, 73.09 (C3, C4, C5, C6, C7), 81.37 (C_q Boc), 75.61, 75.12 (3 × CH_2 Bn), 54.16, 52.22, 50.59 (Cα-Phe, Cα-Leu, OMe), 41.58, 37.88, 36.94 (Cβ-Phe, Cβ-Leu, C2), 28.21 (*tert*-Bu Boc), 24.63 (Cγ-Leu), 22.72, 21.87 (2 × Cδ-Leu).

Z-Tyr-SAA-(Bn$_3$)-Phe-Leu-OMe (13): Trimer **12** (0.44 g, 0.52 mmol) was coupled to Z-Tyr-OH (0.16 g, 0.52 mmol) as described for compound **10**. Pure **13** was obtained in 83% yield (0.45 g) by column chromatography using MeOH/DCM (0:1 → 5:95, v/v) as eluent. ^{1}H NMR, COSY (300 MHz, $CDCl_3$): δ = 7.29−6.92 (m, 23 H, 15 × H_{arom} Bn, 5 × H_{arom} Phe, 2 × H_{arom} Tyr, HN-Phe), 6.69 (m, 3 H, 2 × H_{arom} Tyr, HN-Leu), 5.49 (m, 1 H, HN-Tyr), 5.08−4.51 (m, 9 H, 3 × CH_2 Bn, Hα-Phe, Hα-Leu, H7), 3.69 (m, 1 H, OMe, H6), 3.63 (s, 3 H, OMe), 3.39 (m, 1 H, H3), 3.29 (m, 2 H, H4, H5), 3.09−2.85 (m, 4 H, 2 × Hβ-Tyr, 2 × Hβ-Phe), 2.39 (dd, 1 H, H2a), 2.19 (dd, 1 H, H2b), 1.50 (m, 1 H, Hγ-Leu), 1.25 (m, 2 H, 2 × Hβ-Leu), 0.88 (s, 6 H, 6 × Hδ-Leu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$/MeOD): δ = 172.86, 171.34, 170.28 (4 × C(O), Tyr, SAA, Phe, Leu), 155.94, 155.39 [C(O) Z, Cζ-Tyr), 137.92, 137.56, 137.47, 136.55, 136.23 (3 × C_q Bn, C_q Z, Cγ-Phe, Cγ-Tyr), 131.68−126.07 (5 × CH_{arom} Z, 2 × CH_{arom} Tyr, 15 × CH_{arom} Bn, 5 × CH_{arom} Phe),115.42, 115.15 (2 × CH_{arom} Tyr), 85.28, 80.12, 79.21, 72.94 (C3, C4, C5, C6, C7) 75.57, 74.97, 74.73 (3 × CH_2 Bn), 66.60 (CH_2 Z), 54.13, 53.92, 52.04, 50.68 (Cα-Tyr, Cα-Phe, Cα-Leu, OMe), 40.61, 37.91, 37.54, 37.03 (Cβ-Tyr, Cβ-Phe, Cβ-Leu, C2), 24.48 (Cγ-Leu), 22.41, 21.44 (2 × Cδ-Leu). ESI-MS: *m*/*z* = 1049.6 [M + H]$^+$, 1071.8 [M + Na]$^+$, 1087.7 [M + K]$^+$.

Tyr-SAA-Phe-Leu-OMe (14): Fully protected **13** (0.45 g, 0.43 mmol) was dissolved in MeOH and the resulting solution was degassed. Pd/C (0.01 g) was added and after degassing for a second time the reaction was stirred under an atmosphere of H_2. After TLC indicated the complete conversion of the starting material to a lower running product, the solution was filtered through Glass Fiber (GF/2A, Whatman). The filtrate was concentrated and the residue purified by column chromatography (Eluent: DCM/MeOH, 1:0 → 9:1, v/v). to afford **14** quantitatively (0.28 g). $[\alpha]_D^{20}$ = −0.32 (*c* = 0.2, MeOH). ^{1}H NMR, COSY, ROESY, HMQC-COSY (600 MHz, [D$_6$]acetone): δ = 7.71 (d, *J* = 8.1 Hz, 1 H, HN-Leu), 7.51 (d, *J* = 8.2 Hz, 1 H, HN-Phe), 7.24 (m, 4 H, H_{arom} Phe), 7.16 (m, 1 H, H_{arom} Phe, Tyr), 7.12 (d, *J* = 8.4 Hz, 2 H, H_{arom} Phe), 6.75 (d, 2 H, H_{arom} Tyr), 4.76 (m, 1 H, Hα-Phe), 4.52 (m, 2 H, Hα-Leu, H6), 4.40 (d, *J* = 9.2 Hz, 1 H, H7), 3.70 (m, 1 H, H3), 3.67 (s, 3 H, OMe), 3.61 (dd, *J* = 4.1 Hz, *J* = 8.3 Hz, 1 H, Hα-Tyr), 3.42 (t, *J* = 8.9 Hz, 1 H, H5), 3.39 (t, *J* = 8.9 Hz, 1 H, H4), 3.11 (dd, $J_{\alpha,\beta}$ = 5.8, $J_{\beta,\beta}$ = 13.9 Hz, 1 H, Hβ-Phe), 3.08 (dd, $J_{\alpha,\beta}$ = 4.0, $J_{\beta,\beta}$ = 14.5 Hz, 1 H, Hβ-Tyr), 2.95 (dd, $J_{\alpha,\beta}$ = 8.2, $J_{\beta,\beta}$ = 13.9 Hz, 1 H, Hβ-Phe), 2.78 (dd, $J_{\alpha,\beta}$ = 8.4, $J_{\beta,\beta}$ = 14.4 Hz, 1 H, Hβ-Tyr), 2.63 (dd, $J_{2a,3}$ = 2.7 Hz, $J_{2a,2b}$ = 15.4 Hz, 1 H, H2a), 2.45 (dd, $J_{2b,3}$ = 7.3 Hz, $J_{2a,2b}$ = 15.3 Hz, 1 H, H2b), 1.71 (m, 1 H, Hγ-Tyr), 1.60 (m, 2 H, 2 × Hβ-Leu), 0.90 (d, *J* = 6.6 Hz, 3 H, Hδ-Leu), 0.88 (d, *J* = 6.5 Hz, 3 H, Hδ-Leu) ppm. ^{13}C NMR, HMQC-COSY (150 MHz, [D$_6$]acetone): δ = 176.24, 173.80, 171.92, 170.85 [C(O), Tyr, SAA, Phe, Leu), 156.78 (Cζ-Tyr), 138.37, 129.78 (2 × C_q, Cγ-Tyr, Cγ-Phe), 131.07, 130.26, 128.99, 128.91, 127.20 (7 × CH_{arom}, Phe, Tyr), 115.90 (2 × CH_{arom} Tyr), 84.79 (C7), 78.89 (C5), 75.70 (C3), 73.60 (C4), 69.56 (C6), 60.24 (Cα-Tyr), 55.18 (Cα-Phe), 52.41 (OMe), 51.35 (Cα-Leu), 41.44 (Cβ-Leu), 39.13 (C2), 38.53 (Cβ-Phe), 36.87 (Cβ-Tyr), 25.28 (Cγ-Leu), 23.22, 21.90 (2 × Cδ-Leu). LC-MS: R_t 12.85 (5−75% ACN/H_2O, v/v), *m*/*z* = 645.4 [M + H]$^+$. HRMS: calcd. for $C_{32}H_{43}N_4O_{10}$ [M + H] 645.3136; found 645.3129.

Urea 15: Compound **2** (0.16 g, 0.6 mmol) was dissolved in *t*BuOH (5 mL), then Et_3N (0.043 mL, 0.6 mmol) and DPPA (0.13 mL, 0.6 mmol) were added and the reaction mixture was heated under reflux. After 16 h TLC analysis revealed complete disappearance of the starting material. The solution was filtered and concentrated, the residue was applied to a silica gel column. Elution with EtOAc/light petroleum (0:1 → 1:1, v/v) gave compound **15** in 35% yield (0.10 g) as a white solid. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 84.31, 83.73, 83.52, 82.49, 81.12 (2 × C3, 2 × C4, 2 × C5, 2 × C6), 51.80 (2 × OMe), 38.27, 37.85 (2 × C2), 26.93, 25.20 (4 × CH_3 isopropylidene). ESI-MS: *m*/*z* = 489.4 [M + H]$^+$, 511.4 [M + Na]$^+$.

Biological Assay: Competitive radio-ligand binding assays were performed with Chinese hamster ovary (CHO) cells stably transfected with human μ, δ or κ receptors. Cells were harvested 72 h following plating in 50 mM Tris buffer (pH, 7.4) containing 10 mM EGTA, 5 mM $MgCl_2$ and homogenized using a Dounce homogenizer. The homogenate was then centrifuged (11000 × g)

for 15 min at 4 °C. The pellet was resuspended in 50 mM Tris buffer (pH 7.4) with 100 mM NaCl to yield a protein concentration of 800 μg/mL. Incubations were performed for 90 min at room temperature with 1 nM [^{3}H] DAMGO, [^{3}H] DPDPE or [^{3}H] U69,593 for μ, δ or κ receptors, respectively. The concentrations of tested drugs ranged from 10^{-10} to 10^{-5} M. Binding assays were carried out in mixtures containing 40 μg membrane protein, 10 mM EGTA, 5 mM $MgCl_2$ and 0.1% bovine albumin in a final volume of 250 μL. Nonspecific binding was determined in the presence of 1 μM naloxone. The reactions were terminated by rapid filtration through Whatman GF/B glass fiber filters using a Brandel cell harvester, then washed with 3 mL 50 mM Tris buffer. The filters were presoaked for at least 2 h in 3% polyethylenimine to decrease nonspecific binding. The filter disks were then placed in vials with 3 mL of Econosafe scintillation fluid and counted in Beckman LS 6500 scintillation counter. Ki values were derived from nonlinear regression analysis of competition curves using GraphPad software.

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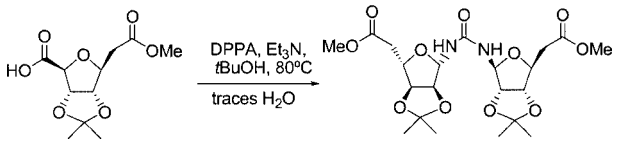

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