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Note

Synthesis of a *galacto*-configured C-ketoside-based γ-sugar-amino acid and its use in peptide coupling reactions

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Abstract—γ-Sugar-amino acid analogues in the form of C-ketosides can be prepared in 5–6 steps starting from D-galactono-1,5-lactone. The key step in the synthesis is the trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted C-glycosylation of 2-deoxy-3-ulopyranosonates with trimethylsilyl cyanide. Hydrogenation of the resulting β-cyano esters provides C-ketoside-based γ-sugar-amino acids that serve as building blocks for the synthesis of unnatural neoglycopeptides.

Keywords: Glycoconjugates; C-Ketosides; Neoglycopeptides; Sugar-amino acids; Glycomimetics

Molecular recognition of carbohydrates by proteins plays an important role in many intracellular and intercellular processes, such as the trafficking of proteins, bacterial and viral infection, normal cell differentiation, tumour progression and metastasis.[1] Besides these biological functions, carbohydrates have also been recognized as highly functionalized synthetic scaffolds, which if modified can demonstrate interesting properties.[2] In particular, the introduction of an amino and a carboxylic acid function into the carbohydrate platform, leading to glycosamino acids (GAAs), sugar-amino acids (SAAs) and sugar-amino acid hybrids (SAAHs), has been pursued by several groups.[3,4] GAAs, SAAs and SAAHs can easily undergo oligomerization to 'saccharide peptide hybrids', 'glycopeptoids' or 'glycotides' via well-developed peptide chemistry and represent ideal building blocks for combinatorial synthesis.[3b,5]

As part of our ongoing studies on carbohydrate–protein interactions, we became interested in the design and synthesis of novel *galacto*-configured unnatural glycopeptides **1** and **2** (Scheme 1). Scaffolds **1** and **2** are designed to probe the binding-site periphery of galactose binding proteins[6,7] using the appendages $AA_1,\ldots,AA_n$ and $AA_{21},\ldots,AA_{2n}$ (AA = amino acid), which may be hydrophobic, hydrophilic, neutral or charged. The unchanged carbohydrate core encompassed by C_3–C_8 in **1** and **2** may allow unperturbed binding of the carbohydrate moiety to the protein, which often takes place via the non-reducing end of the sugar.[7] In addition, many sugar binding proteins (with the exception of glycosidases) recognize both monosaccharidic α- and β-glycosides.[8] The synthesis of scaffolds **1** and **2** requires access to unprotected C-ketoside based γ-amino acid building blocks **3** and **4**. Here we describe the synthesis of compounds **3** and **4** and their use in peptide coupling reactions. A preliminary account without experimental details on the synthesis of unprotected sugar-fused GABA analogues was recently communicated.[9]

The synthesis of compound **3** is outlined in Scheme 2. The commercially available lactone **5** was converted into ketose **6**[13] on treatment with excess *tert*-butylacetate enolate (4 equiv) at −78 °C in 90% yield as previously described.[14] Addition of a second enolate was not observed. The *tert*-butyl group was cleaved with trifluoroacetic acid in dichloromethane and the free acid was esterified to either the methyl or benzyl ester (Cs_2CO_3, MeI or BnBr) to afford the ketoses **7** and **8**

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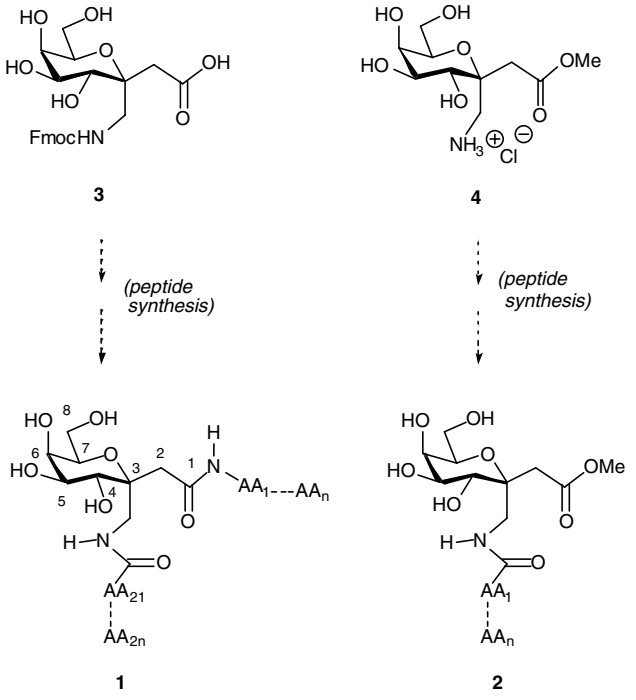
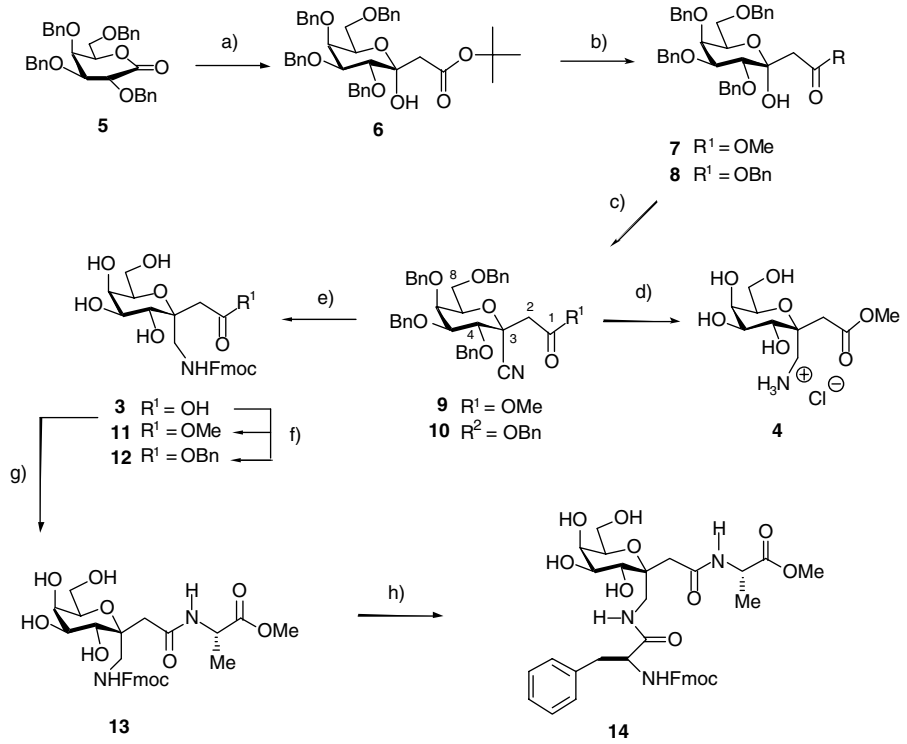


Scheme 1. General structure of C-ketoside based glycopeptides **1** and **2** from γ-SAA-building blocks **3** and **4**.

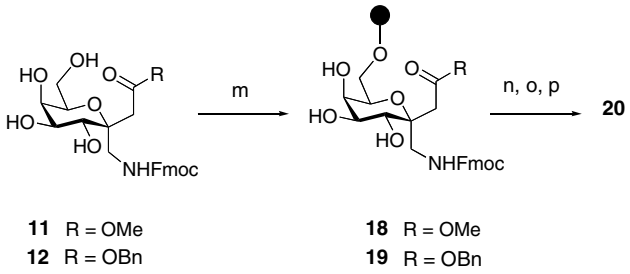
in yields averaging 75%. The cyano function was incorporated into the acetals **7** and **8** by trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted C-glycosylation with trimethylsilyl cyanide (TMSCN) in acetonitrile to yield the C-ketosides **9** and **10** in 75% and 66% yields, respectively. Analysis of the product mixture after a reaction time of 36 h revealed in both cases the presence of unreacted starting material (approximately 20%). Increasing the reaction time, further addition of reagents (promoter and nucleophile) or changing the solvent (CH_2Cl_2) did not improve the yields. The stereochemistry of the cyano compounds **9** and **10** was established by measuring the heteronuclear $^3J_{C,H}$ coupling constants between the cyano carbon and H-4 as well as between C-2 and H-4 as previously reported.[9] In summary, the observed coupling constants $^3J_{C\text{-}2,H\text{-}4}$ <4 Hz and $^3J_{CN,H\text{-}4}$ >7 Hz for compounds **9** and **10** require the axial orientation of the cyano group.[10] Subsequently, the cyano compound **10** was hydrogenated using Pearlman's catalyst in acidified (HCl) methanol for 48 h and the generated amino function was protected as 9-fluorenylmethoxycarbamate by treatment with Fmoc pentafluorophenylester (Fmoc-OPfp) in an acetone/water mixture. Sugar-γ-amino acid **3** was isolated in 42% yield. To demonstrate the use of



Scheme 2. Synthesis of glycotripeptide **14** incorporating a γ-SAA. Reagents and conditions: (a) $LiCH_2COOt$-Bu (4 equiv), THF, −78 °C to rt, 1 h, 85–95%; (b) 50% TFA in CH_2Cl_2 then Cs_2CO_3 (1.3 equiv), MeI or BnBr (3 equiv), DMF, rt, 1 h, 70–80%; (c) TMSOTf (3 equiv), TMSCN (5 equiv), CH_3CN, 0 °C to rt, 36 h, 66–75%; (d) C/Pd(OH)$_2$, H_2, HCl (48–60 h), 80–90%; (e) then Fmoc-OPfp (4 equiv), H_2O/acetone 1:2, $NaHCO_3$ (1.2 equiv), 42%; (f) $Cs_2(CO_3)$ (1.3 equiv), BnBr (3 equiv) or MeI (2 equiv), DMF, rt, 64% or 50%; (g) **3**, H_2N-Ala-OMe (3.5 equiv), DCC (5 equiv), HOBt (3.5 equiv), DMF, 6 h, 72%; (h) 40% morpholine in DMF, 1 h, then Fmoc-OPfp (3 equiv), HOBt (3 equiv), DMF, 6 h, 92%.

sugar-γ-amino acid building block **3** in peptide coupling reactions we subjected it to a double peptide coupling cycle. Initially, **3** was coupled to alanine (DCC, $Cl^-NH_3^+$-Ala-OMe, HOBt, DMF, 48 h) to afford glycodipeptide **13** in 72% yield (Scheme 2). Removal of the Fmoc group (morpholine, DMF, 1 h) followed by additional peptide coupling using activated phenylalanine (Fmoc-Phe-OPfp, HOBt, DMF, 14 h) yielded the glycotripeptide **14** in 92% isolated yield (Scheme 2). Similar coupling of **4** with phenylalanine (Fmoc-Phe-OPfp) gave dipeptide **15** in 65% isolated yield along with a small amount (~5%) of the corresponding lactam (Scheme 3).

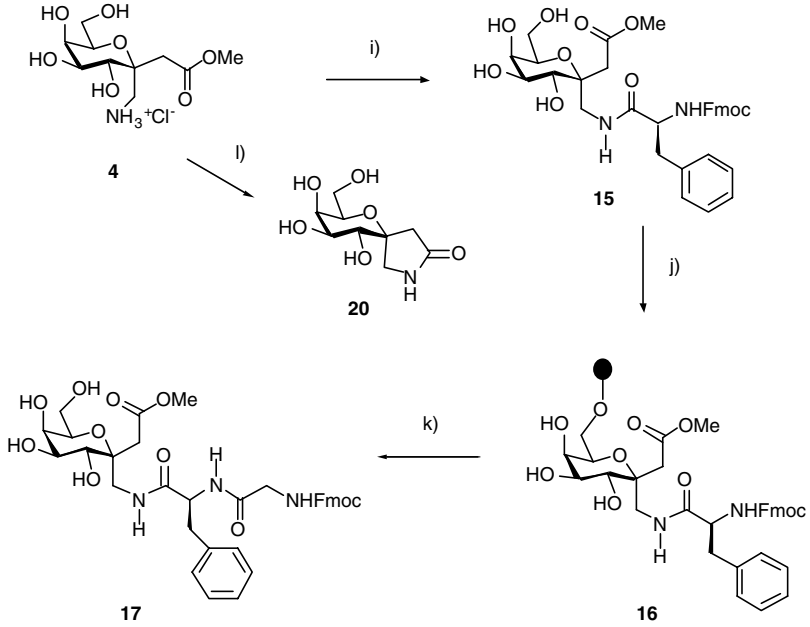
Encouraged by our solution-phase results, we immobilized the dipeptide **15** on the Merrifield trityl-chloride resin via the carbohydrate scaffold[11] and coupled the resin-bound dipeptide **16** to glycine (Fmoc-Gly-OPfp, DMF). After cleavage from the resin (1% TFA, 10 min), we obtained glycotripeptide **17** as the only product in 60% isolated yield (Scheme 3). Attempts to use the corresponding immobilized (trityl resin) sugar-γ-amino esters **18** and **19** as building blocks for additional peptide couplings failed (Scheme 4).[12] This failure was attributed to a very efficient intramolecular cyclization because removal of the Fmoc group of resin-bound **11** and **12** (25% morpholine in DMF) and subsequent peptide coupling provided lactam **20** in quantitative yield after resin cleavage (Scheme 4). Similarly, peptide coupling of **4** to Pfp-activated phenylalanine using basic



Scheme 4. Attempted solid-phase glycopeptide synthesis of sugar-γ-amino esters **11** and **12**. Reagents and conditions: (m) trityl-chloride resin (1.3 equiv), DIEA (3 equiv), DMF, 2 h then quenching by the addition of $CH_3OH/DIEA/CH_2Cl_2$ 1:2:17, 5 min; (n) 50% morpholine in DMF, 30 min; (o) Fmoc-Gly-OPfp (3 equiv), HOBt, DIEA (3 equiv); (p) TFA (3%) in CH_2Cl_2, 10 min.

conditions (diisopropylethylamine (DIEA)) produced lactam **20** in quantitative yield (Scheme 3).

In summary, we have demonstrated the suitability of sugar-γ-amino acids **3** and sugar-γ-amino ester **4** as building blocks for unnatural glycopeptide synthesis. Amino acid **3** can be incorporated into peptides via the usual C→N fashion without lactam formation. Amino ester **4** can be elongated into glycopeptides with minimal lactam formation under neutral conditions. However, basic conditions result in intramolecular lactam formation. In addition, immobilization of the sugar-γ-peptides on Merrifield-modified trityl resin via the unprotected carbohydrate scaffold may be an attractive strategy for the combinatorial synthesis of neoglycopeptides libraries



Scheme 3. Solid-phase glycopeptide synthesis via immobilization of the carbohydrate scaffold. Reagents and conditions: (i) Fmoc-Phe-OPfp (3 equiv), $NaHCO_3$ (1.3 equiv), H_2O/acetone 3:1, 13 h, 65%; (j) trityl-chloride resin (1.3 equiv), DIEA (3 equiv), DMF, 2 h then quenching by the addition of $CH_3OH/DIEA/CH_2Cl_2$ 1:2:17, 5 min; (k) 50% morpholine in DMF, 1 h then Fmoc-Gly-OPfp (3 equiv), DMF then TFA (3%) in CH_2Cl_2, 10 min, 60%; (l) Fmoc-Phe-OPfp (3 equiv), DIEA (2 equiv), DMF, quant.

and may find future use in the synthesis of conformationally constrained cyclic neoglycopeptides.

1. Experimental

1.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 360 or 500 MHz for ^1H and at 100 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ [δ_H 7.26, δ_C (centre of triplet) 77.0] or to CH_3OH [δ_H 73.35, δ_C (centre of septet) 49.0] or to acetone as the internal standard (D_2O). TLC was performed on E. Merck Silica Gel 60 F254 with detection by charring with 8% H_2SO_4 acid. Silica gel (0.040–0.063 mm) was used for column chromatography. Lactone **5** was purchased from Toronto Research Chemicals. Trityl resin was purchased from Novabiochem.

1.2. Methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-3-octulopyranosid)onate (7)

Acetal **6**[13] (1.00 g, 1.52 mmol) was dissolved in a 1:1 mixture containing CH_2Cl_2 and trifluoroacetic acid (25 mL) and stirred for 2 h at 0 °C. Toluene (30 mL) was added and the mixture was concentrated under reduced pressure and codistilled with toluene (2 × 10 mL). The residue was dissolved in dry DMF (30 mL) and caesium carbonate (743 mg, 2.28 mmol) and methyl iodide (284 μL, 4.56 mmol) were added. The mixture was stirred for 4 h. The reaction flask was opened and left in the fumehood overnight to remove excess of methyl iodide. The residue was concentrated under reduced pressure, and H_2O (40 mL) and CH_2Cl_2 (40 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatographic purification using 5% EtOAc in toluene as eluant gave **7** (766 mg, 82%) as a colourless oil. ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 20H, aromatics), 5.43 (s br, 1H, OH), 4.95 (d, 1H, J_{gem} = 11.5 Hz, OCH_2Ph), 4.91 (d, 1H, J_{gem} = 11.6 Hz, OCH_2Ph), 4.74 (ABq, 2H, J_{gem} = 11.7 Hz, OCH_2Ph), 4.65 (d, 1H, J_{gem} = 11.5 Hz, OCH_2Ph), 4.58 (d, 1H, J_{gem} = 11.5 Hz, OCH_2Ph), 4.41 (ABq, 2H, J_{gem} = 11.8 Hz, OCH_2Ph), 4.15 (m, 1H, H-7), 4.08 (dd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 2.8 Hz, H-5), 4.00 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.75 (d, 1H, H-4), 3.60, (s, 3H, OCH_3), 3.59 (dd, 1H, $J_{7,8a}$ = 7.7 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8), 3.45 (dd, 1H, $J_{7,8b}$ = 5.6 Hz, H-8b), 2.75 (d, 1H, $J_{2a,2b}$ = 15.6 Hz, H-2a), 2.33 (d, 1H, H-2b). The signal at 5.43 disappeared by addition of D_2O; ESIMS *m*/*z* calcd for $C_{37}H_{41}O_8$ $[M+H]^+$: 613.28014. Found 613.28033.

1.3. Benzyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-3-octulopyranosid)onate (8)

Acetal **6**[13] (1.07 g, 1.63 mmol) was dissolved in a (1:1) mixture containing CH_2Cl_2 and trifluoroacetic acid (25 mL) and stirred for 2 h at 0 °C. Toluene (30 mL) was added and the mixture was concentrated under reduced pressure and codistilled with toluene (2 × 10 mL). The residue was dissolved in dry DMF (30 mL), and caesium carbonate (743 mg, 2.28 mmol) and benzyl bromide (542 μL, 4.56 mmol) were added. The mixture was stirred for 2 h. The residue was concentrated under reduced pressure, and H_2O (40 mL) and CH_2Cl_2 (40 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layer was dried (Na_2SO_4) and concentrated. Chromatographic purification using 5% EtOAc in toluene as eluant gave **8** (809 mg, 72%) as a colourless oil. ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 25H, aromatic), 5.24 (s br, 1H, OH), 5.08 (ABq, 2H, J_{gem} = 12,3 Hz, OCH_2Ph), 5.00 (d, 1H, J_{gem} = 11.4 Hz, OCH_2Ph), 4.95 (d, 1H, J_{gem} = 11.5 Hz, OCH_2Ph), 4.77 (ABq, 2H, J_{gem} = 11.7 Hz, OCH_2Ph), 4.67 (d, 1H, J_{gem} = 11.4 Hz, OCH_2Ph), 4.61 (d, 1H, J_{gem} = 11.5 Hz, OCH_2Ph), 4.44 (ABq, 2H, J_{gem} = 11,8 Hz, OCH_2Ph), 4.17 (m, 1H, H-7), 4.10 (dd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,7}$ = 2.7 Hz, H-5), 4.00 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.82 (d, 1H, H-4), 3.60 (dd, 1H, $J_{7,8a}$ = 6.2 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a). 3.43 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 2.87 (d, 1H, $J_{2a,2b}$ = 14.6 Hz, H-2a), 2.43 (d, 1H, H-2b). The signal at 5.24 disappeared by addition of D_2O (15 μL); ESIMS *m*/*z* calcd for $C_{43}H_{44}O_8Na$ $[M+Na]^+$: 711.29338. Found 711.29395.

1.4. Methyl (3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-3-cyano-2-deoxy-D-*glycero*-L-*manno*-oct)onate (9)

Acetal **7** (20 mg, 32.6 μmol) was dissolved under an inert argon atmosphere in dry acetonitrile (1 mL). TMSCN (13 μL, 97.5 μmol) and TMSOTf (19 μL, 98 μmol) were added at 0 °C. The temperature was raised to rt and the mixture was stirred for an additional 8 h. CH_2Cl_2 (2 mL) and satd aq $NaHCO_3$ (2 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL) and the combined organic layer was dried (Na_2SO_4), concentrated and chromatographically purified using 5% EtOAc in toluene as the eluant. Compound **9** (15 mg, 75%) was obtained as a colourless syrup. $[\alpha]_D$ +37 (*c* 1.5, $CDCl_3$); ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 20H, aromatic), 5.05 (d, 1H, J_{gem} = 11.7 Hz, OCH_2Ph), 4.90 (d, 1H, J_{gem} = 11.3 Hz, OCH_2Ph), 4.71 (ABq, 2H, J_{gem} = 11.4 Hz, OCH_2Ph), 4.70 (d, 1H, J_{gem} = 11.7 Hz, OCH_2Ph), 4.55 (d, 1H, J_{gem} = 11.3 Hz, OCH_2Ph), 4.44 (ABq, 2H, J_{gem} = 11.8 Hz, OCH_2Ph), 4.07–4.03 (m, 2H, H-6, H-7), 4.01 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.92 (dd, 1H, $J_{4,5}$ = 2.6 Hz), 3.59 (dd, 1H, $J_{7,8a}$ = 8.0 Hz, $J_{8a,8b}$ = 9.3 Hz, H-8a), 3.57 (s,

3H, OCH_3), 3.53 (dd, 1H, $J_{7,8b} = 5.5$ Hz, H-8b), 2.94 (d, 1H, $J_{2a,2b} = 15.2$ Hz, H-2a), 2.64 (d, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 176.86 (C=O), 138.51, 137.79 × 2, 137.58, 128.60–127.66 (C–H, aromatic), 116.52 (CN), 81.94, 76.63, 76.19, 75.37, 75.18, 74.85, 74.49, 73.43, 72.80, 67.72, 52.04, 41.21; HMBC ($CDCl_3$): $J_{H4,CN} = 7.3$ Hz, $J_{H4,C2} = 3.6$ Hz; ESIMS *m/z* calcd for $C_{38}H_{40}NO_7$ $[M+H]^+$: 622.28047. Found 622.28095. Anal. Calcd for $C_{38}H_{39}NO_7$: C, 73.41; H, 6.32; N, 2.25. Found: C, 73.19; H, 6.20; N, 2.30.

1.5. Benzyl (3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-3-cyano-2-deoxy-D-*glycero*-L-*manno*-oct)onate (10)

Acetal **7** (99.7 mg, 144.8 μmol) was dissolved under an argon atmosphere in dry acetonitrile (1 mL). TMSCN (58 μL, 435 μmol) and TMSOTf (19 μL, 98 μmol) were added at 0 °C. The temperature was raised to rt and the mixture was stirred for an additional 6 h. CH_2Cl_2 (6 mL) and satd aq $NaHCO_3$ (4 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic layer were dried (Na_2SO_4), concentrated and chromatographically purified using 3% EtOAc in toluene as the eluant. Compound **10** (67 mg, 66%) was obtained as a white powder $[\alpha]_D$ +33 (*c* 1.1, $CDCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.20 (m, 25H, aromatic), 5.06 (ABq, 2H, $J_{gem} = 12.4$ Hz, OCH_2Ph), 5.0 (d, 1H, $J_{gem} = 12.6$ Hz, OCH_2Ph), 4.90 (d, 1H, $J_{gem} = 11.3$ Hz, OCH_2Ph), 4.71 (ABq, 2H, $J_{gem} = 11.5$ Hz, OCH_2Ph), 4.70 (d, 1H, $J_{gem} = 11.6$ Hz, OCH_2Ph), 4.55 (d, 1H, $J_{gem} = 11.6$ Hz, OCH_2Ph), 4.43 (ABq, 2H, $J_{gem} = 11.8$ Hz, OCH_2Ph), 4.07–4.02 (m, 2H, H-6, H-7), 4.03 (d, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.92 (dd, 1H, $J_{5,6} = 2.7$ Hz, H-5), 3.56 (dd, 1H, $J_{7,8b} = 7.9$ Hz, $J_{8a,8b} = 9.2$ Hz, H-8a), 3.45 (dd, 1H, $J_{7,8b} = 5.2$ Hz, H-8b), 3.00 (d, 1H, $J_{2a,2b} = 15.1$ Hz, H-2a), 2.70 (d, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 116.3 (CN); HMBC ($CDCl_3$); $J_{H4,CN} = 8.5$ Hz, $J_{H4,C2} = 3.8$ Hz; ESIMS *m/z* calcd for $C_{44}H_{44}NO_7$ $[M+H]^+$: 698.31177. Found 698.31213. Anal. Calcd for $C_{44}H_{44}NO_7$: C, 75.73; H, 6.21; N, 2.01. Found: C, 75.52; H, 6.09; N, 2.03.

1.6. 3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-9-fluorenylmethoxycarbonyl))-D-*glycero*-L-*gluco*-octonic acid (3)

Compound **10** (100 mg, 143.0 μmol), hydrochloric acid (200 μmol) and Pearlman's catalyst ($Pd(OH)_2$/C, 85 mg) were added to dry CH_3OH (15 mL) and hydrogenated (1 atm) for 48 h. The residue was filtered through a Millipore filter and concentrated under reduced pressure. The residue was dissolved in a mixture of water (5 mL) and acetone (10 mL), sodium bicarbonate powder (61 mg) and Fmoc-pentafluorophenylester (Fmoc-OPfp, 450 mg) were added. After stirring the reaction for 12 h, formic acid (0.5 mL) was added. The reaction was stirred for 10 min. Then water (10 mL) was added and EtOAc (3 × 10 mL) was used to extract the product. Product **3** was purified with an EtOAc equilibrated column using EtOAc/CH_3OH/H_2O, 12:2:1 as the eluant. The product **3** (72 mg, 42%) was obtained as an oil. ^{1}H NMR (1:1, CD_3OD/$CDCl_3$): δ 7.78 (d, 2H, $J = 7.0$ Hz, Fmoc), 7.65 (d, 2H, $J = 7$ Hz, Fmoc), 7.40–7.26 (m, 4H, Fmoc), 4.36 (d, 2H, $J = 6.9$ Hz, OCH_2, Fmoc), 4.20 (t, 1H, $J = 6.9$ Hz, CHCH$_2$, Fmoc), 4.15 (d, 1H, $J_{4,5} = 10.0$ Hz, H-4), 3.82 (d, 1H, $J_{5,6} = 2.8$ Hz, H-6), 3.78–3.53 (m, 6H), 2.70 (d, 1H, $J_{2a,2b} = 15.1$ Hz, H-2a), 2.62 (d, 1H, H-2b); ESIMS *m/z* calcd for $C_{24}H_{27}NO_9Na$ $[M+Na]^+$: 496.15835. Found 496.15869.

1.7. Methyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl-(*N*-9-fluorenylmethoxycarbonyl))-D-*glycero*-L-*manno*-oct]onate (11)

Compound **3** (55 mg, 182.3 μmol), CH_3I (23 μL, 364 μmol) and sodium bicarbonate (30.6 mg, 364.6 μmol) were stirred in DMF for 4 h. The solvent was removed under reduced pressure redissolved in CH_3OH (10 mL) and filtered over Celite. The residue was purified with a gradient silica chromatography using 9–12.5% CH_3OH in ethyl acetate as eluant. Product **11** (57 mg, 64%) was obtained as a white powder. ^{1}H NMR (CD_3OD): δ 7.78 (d, 2H, $J = 7.0$ Hz, Fmoc), 7.65 (d, 2H, $J = 7.0$ Hz, Fmoc), 7.40–7.26 (m, 4H, Fmoc), 4.36 (d, 2H, $J = 6.9$ Hz, OCH_2, Fmoc), 4.20 (t, 1H, $J = 6.9$ Hz, CHCH$_2$, Fmoc), 4.15 (d, 1H, $J_{4,5} = 10.0$ Hz, H-4), 3.82 (d, 1H, $J_{5,6} = 2.8$ Hz, H-6), 3.78–3.53 (m, 9H including s at 3.64), 2.70 (d, 1H, $J_{2a,2b} = 15.1$ Hz, H-2a), 2.62 (d, 1H, H-2b); ESIMS *m/z* calcd for $C_{25}H_{30}NO_9$ $[M+H]^+$: 488.19205. Found 488.19187.

1.8. Benzyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-9-fluorenylmethoxycarbonyl))-D-*glycero*-L-*manno*-oct]onate (12)

Compound **3** (8 mg, 17 μmol), benzyl bromide (50 μL, 170 μmol) and sodium bicarbonate (16.8 mg, 0.20 mmol) were dissolved in DMF (1 mL) and stirred for 4 h. Filtration (Celite) and chromatographic purification with a gradient silica gel chromatography using 9–12.5% CH_3OH in EtOAc as eluant gave product **12** (5.2 mg, 50%) as a white powder. ^{1}H NMR (CD_3OD): δ 7.78 (d, 2H, $J = 7.0$ Hz, Fmoc), 7.65 (d, 2H, $J = 7.0$ Hz, Fmoc), 7.40–7.24 (m, 9H, Fmoc, Ph), 5.10 (ABq, 2H, $J_{gem} = 12.0$ Hz, OCH_2Ph), 4.35 (d, 2H, $J = 6.9$ Hz, OCH_2, Fmoc), 4.19 (t, 1H, $J = 6.9$ Hz, CHCH$_2$, Fmoc), 4.18 (d, 1H, $J_{4,5} = 9.0$ Hz, H-4), 3.83 (d, 1H, $J_{5,6} = 2.7$ Hz, H-6), 3.78–3.53 (m, 6H), 2.74 (d, 1H, $J_{2a,2b} = 14.6$ Hz, H-2a), 2.66 (d, 1H, H-2b); ESIMS *m/z* calcd for $C_{31}H_{33}NO_9Na$ $[M+Na]^+$: 586.20530. Found 586.20579.

1.9. 3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-9-fluorenylmethoxycarbonyl))-D-*glycero*-L-*manno*-octon-(L-Ala-OCH_3)amide (13)

Acid **3** (6 mg, 12.7 μmol), *N*,*N*′-1,3 dicyclohexylcarbodiimide (16 mg, 78 μmol), alanine methyl ester hydrochloride (12 mg, 85 μmol) and hydroxybenzotriazole (3 mg, 22 μmol) were dissolved in DMF (1.5 mL) and stirred for 48 h. The urea precipitate was filtered (Celite) and the residue was concentrated under reduced pressure. The residue was chromatographed twice using 10% CH_3OH in EtOAc as the eluant. Product **13** (5 mg, 72%) was obtained as a white powder. ^{1}H NMR (7:1, $CDCl_3/CD_3OD$): δ 7.81–7.75 (d br, 1H, $J = 7$ Hz, NH(Ala)), 7.70 (d, 2H, $J = 7.6$ Hz, Fmoc), 7.53 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.33 (t, 2H, $J = 7.6$ Hz, Fmoc), 7.23 (t, 2H, $J = 7.6$ Hz, Fmoc), 6.10–6.00 (t br, 1H, $J \sim 6$ Hz, NH(Fmoc)), 4.15 (t, 1H, $J = 6.7$ Hz, Fmoc), 3.86 (d, 1H, $J_{5,6} = 3$ Hz, H-6), 3.84 (d, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.66 (s, 3H, OCH_3), 3.52 (dd, 1H, $J_{gem} = 14.8$ Hz, $J_{vic} = 6.0$ Hz, CH_2NH), 3.38 (dd, 1H, CH_2NH), 2.44 (d, 1H, $J_{2a,2b} = 15.1$ Hz, H-2a), 2.47 (d, 1H, H-2b), H-2b; 1.33 (d, 3H, CH_3 (Ala)); The signal at 7.81 and 6.10 disappeared under this conditions over a period of 2 days; MALDI-MS *m*/*z* calcd for $C_{28}H_{34}N_2O_{10}Na$ $[M+Na]^+$: 581.2. Found 581.3.

1.10. 3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-L-Phe-N^{α}(Fmoc)amide))-D-*glycero*-L-*manno*-octon-(L-Ala-OMe)amide (14)

The glycodipeptide **13** (5.2 mg, 9.1 μmol) was dissolved in DMF (1.5 mL). Morpholine (1 mL) was added and the reaction was stirred for 1 h at rt. The solvent was removed under reduced pressure and codistilled with toluene (2×3 mL). The residue was dissolved in dry DMF (1.5 mL), Fmoc-Phe-OPfp (14.9 mg, 27 μmol) and 1-hydroxybenzotriazole (3 mg, 22 μmol) were added. The reaction was stirred for 14 h. The solvent was removed under reduced pressure and the product **14** was purified by column chromatography using 9% CH_3OH in EtOAc. The product **14** (5.8 mg, 92%) was obtained as a white powder. ^{1}H NMR (CD_3OD): δ 7.75 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.58 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.37 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.28 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.25–7.14 (m, 5H, Phe), 4.37 (dd, 1H, $J = 6.0$ Hz, 9.0 Hz), 4.33–4.20 (m, 3H, H(α,Ala), OCH_2 (Fmoc)), 4.14 (t, 1H, CHCH_2 (Fmoc)), 4.00 (d, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.84 (d, 1H, $J_{5,6} = 3.1$ Hz, H-5), 3.75–3.68 (m, 2H), 3.66 (s, 3H, OCH_3), 3.65–3.42 (m, 4H), 3.12 (dd, 1H, $J_{gem} = 13.7$ Hz, $J_{vic} = 5.6$ Hz, CH_2(Phe)), 2.90 (dd, 1H, $J_{vic} = 10.2$ Hz, CH_2(Phe)), 2.50 (s, 2H, H-2a, H-2b), 1.28 (d, 3H, CH_3(Ala)); ^{13}C NMR (CD_3OD): δ 174.92, 174.47, 172.20 (×2), 145.26, 145.10, 142.54 (×2), 138.60, 130.39–120.87 (*C*–H aromatics), 79.56, 74.51, 72.45, 71.93, 71.07, 68.13, 63.29, 58.17, 52.77, 49.65, 48.33, 42.98, 38.81, 38.64, 17.26; ESIMS *m*/*z* calcd for $C_{37}H_{44}N_3O_{11}$ $[M+H]^+$: 706.29758. Found 706.29773.

1.11. Methyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl-(*N*-L-Phe-N^{α}(Fmoc)amide))-D-*glycero*-L-*manno*-oct]onate (15)

Cyano compound **9** (50 mg, 80 μmol), hydrochloric acid (110 μmol) and $Pd(OH)_2$/C (Pearlman's catalyst) were added to CH_3OH (8 mL) and hydrogenated for 60 h. The residue was filtered through a Millipore filter and concentrated under reduced pressure to yield **4** (23 mg, 90%), which was directly used for peptide coupling reactions. Compound **4** (23 mg, 76 μmol) sodium bicarbonate (13.5 mg, 160 μmol) and Fmoc-Phe-OPfp (126 mg, 227 μmol) were dissolved in a mixture of 20% H_2O in acetone (10 mL) and stirred for 13 h. The solvent was removed under reduced pressure and the residue was purified by chromatography using 7% CH_3OH in EtOAc as the eluant on a EtOAc equilibrated silica column. Product **15** (31.2 mg, 65%) was obtained as a white powder. ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.53 (d, 2H, $J = 7.4$ Hz, Fmoc), 7.33 (t, 2H, Fmoc), 7.23 (t, 2H, $J = 7.4$ Hz, Fmoc), 7.30–7.15 (m, 5H, Ph), 7.00 (t br, 1H, $J = 6.5$ Hz, N*H*, Fmoc), 6.00 (d br, 1H, N*H*, Phe), 4.37 (t, 2H, $J = 6.7$ Hz, CHCH_2, Fmoc), 4.10 (t, 1H, $J = 6.7$ Hz, C*H*CH_2, Fmoc), 4.12–4.08 (m, 1H, Hα(Phe)), 3.86 (d, 1H, $J_{4,5} = 9.6$ Hz, H-4), 3.78 (d, 1H, $J_{5,6} = 3$ Hz, H-6), 3.65–3.32 (m, 9H), 3.05–2.87 (m, 2H, CH_2Ph), 2.80–2.60 (s br, 4H, $4 \times$ OH), 2.50 (d, 1H, $J_{2a,2b} = 15.4$ Hz, H-2a), 2.37 (d, 1H, H-2b). The signals at 7.05, 6.10 and 2.80–2.60 disappeared by addition of D_2O; MALDI-MS *m*/*z* calcd for $C_{38}H_{41}N_2O_{10}Na$ $[M+Na]^+$: 657.2. Found 657.6.

1.12. Methyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl-(*N*-L-Phe-Gly-N^{α}(Fmoc)amide))-D-*glycero*-L-*manno*-oct]onate (17)

Compound **15** (46 mg, 76 μmol), tritylchloride resin (160 mg, capacity 950 μmol/g) were added to CH_2Cl_2 solution (2 mL) containing diisopropylethylamine (66 μL, 380 μmol). The mixture was stirred for 2 h while the thin layer chromatogram showed the complete disappearance of the starting material. The solvent was removed by filtration and the resin was washed with diisopropylethylamine/CH_3OH/CH_2Cl_2 (2:1:17, $3 \times$ 10 mL), DMF (2×5 mL) and CH_2Cl_2 (2×10 mL) before it was dried under vacuum overnight. A portion of this resin **16** (35 mg) was taken and suspended in a mixture of CH_2Cl_2/morpholine (1:1, 2 mL) for 2 h. The solvent was removed by filtration and the resin was washed with DMF (2×5 mL), CH_2Cl_2 (2×5 mL), CH_3OH (2×5 mL) and CH_2Cl_2 (2×5 mL) before it

was dried under vacuum overnight. The resin (30 mg), Fmoc-Gly-OPfp (23 mg, 49.6 μmol) and 1-hydroxybenzotriazole (3 mg, 22 μmol) were added to a solution of DMF (1.5 mL) and stirred for 7 h. The resin was washed with diisopropylethylamine/CH_3OH/CH_2Cl_2 (2:1:17, 3 × 5 mL), DMF (2 × 5 mL) and CH_2Cl_2 (3 × 5 mL) before it was dried under vacuum overnight. Cleavage of **16** from the resin was achieved using 1% TFA in CH_2Cl_2 for 5 min. After filtration and chromatographic purification using 9% CH_3OH in EtOAc, peptide **17** (6.8 mg, 60%) was obtained as a white powder. ^{1}H NMR (CD_3OD): δ 7.80 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.66 (d, 2H, $J = 7.4$ Hz, Fmoc), 7.38 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.31 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.27–7.10 (m, 5H, Ph), 4.65 (dd, 1H, $J = 5.5$ Hz, $J = 9.1$ Hz, Hα(Phe)), 4.35 (d, 2H, $J = 7.0$ Hz, CH_2 (Fmoc)), 4.21 (t, 1H, $J = 7.0$ Hz, Fmoc), 4.07 (d, 1H, $J_{4,5} = 9.9$ Hz, H-4), 3.78 (d, 1H, $J_{5,6} = 3.1$ Hz, H-6), 3.77–3.48 (m, 11H, including s at 3.6 (3H, OCH_3)), 3.17 (d, 1H, $J_{gem} = 13.7$ Hz, CH_2Ph), 2.93 (dd, 1H, CH_2Ph), 2.64 (d, 1H, $J_{2a,2b} = 14.8$ Hz, H-2a), 2.52 (d, 1H, H-2b); ESIMS m/z calcd for $C_{36}H_{42}N_3O_{11}$ $[M+H]^+$: 692.28499. Found 692.28478.

1.13. 3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl)-D-*glycero*-L-*manno*-octonic acid lactam (20)

^{1}H NMR (CD_3OD): δ 3.85 (d, 1H, $J_{5,6} = 3.0$ Hz, H-6), 3.75 (d, 1H, $J_{4,5} = 9.8$ Hz, H-4), 3.68–3.65 (m, 4H, H-7, H-8a, H-8b, CH_2N), 3.46 (d, 1H, CH_2N), 3.42 (dd, 1H, H-5), 2.78 (d, 1H, $J_{2a,2b} = 17.5$ Hz, H-2a), 2.37 (d, 1H, H-2b). MALDI-MS m/z calcd for $C_9H_{16}NO_6$ $[M+H]^+$: 234.1. Found 234.5.

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References

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97–130; (b) McAuliffe, J. C.; Hindsgaul, O. Carbohydrates in Medicine. In *Molecular and Cellular Glycobiology, Frontiers in Molecular Biology*; Fukuda, M., Hindsgaul, O., Eds.; Oxford University Press: Oxford, 2000; Vol. 10, pp 249–285.
2. (a) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Leahy, E. M.; Salvino, J.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengler, P. A.; Hamely, P.; Smith, A. B., III; Reisine, T.; Raynor, K.; Maechler, L.; Donaldson, C.; Vale, W.; Freidinger, R. M.; Cascieri, M. R.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550–12568; (b) Hirschmann, R.; Hynes, J. J.; Cichy-Knight, M. A.; van Rijn, R. D.; Sprengeler, P. A.; Spoors, P. G.; Shakespeare, W. C.; Pietranico-Cole, S.; Barbosa, J.; Liu, J., et al. *J. Med. Chem.* **1998**, *41*, 1382–1391; (c) Nicolaou, K. C.; Trujillo, J. L.; Chibale, K. *Tetrahedron* **1997**, *53*, 8751–8778; (d) Smith, A. B., III; Sasho, S.; Barwis, B. A.; Sprengeler, P.; Barbosa, J.; Hirschmann, R.; Cooperman, B. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3133–3141; (e) Dinh, T. Q.; Smith, C. D.; Du, X.; Armstrong, R. W. *J. Med. Chem.* **1998**, *41*, 981–985; (f) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2503–2505; (g) Opatz, T.; Kallus, C.; Wunberg, T.; Schmidt, W.; Henke, S.; Kunz, H. *Eur. J. Org. Chem.* **2003**, 1527–1536; (h) Streicher, B.; Wünsch, B. *Eur. J. Org. Chem.* **2001**, 115–120; (i) Hirschmann, R.; Ducry, L.; Smith, A. B., III. *J. Org. Chem.* **2000**, *65*, 8307–8316.
3. For a list of selected references: (a) Heyns, K. H.; Paulsen, H. *Chem. Ber.* **1955**, *88*, 188–195; (b) McDevitt, J. P.; Lansbury, P. T. *J. Am. Chem. Soc.* **1996**, *118*, 3818–3828; (c) Graf von Roedern, E.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156–10167; (d) Graf von Roedern, E.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 687–689; (e) Chakraborty, T. K.; Jayaprakash, S.; Diwan, P. V.; Nagaraj, R.; Jampani, S. R. B.; Kunwar, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 12962–12966; (f) 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*, 2761–2764; (g) Sofia, M. J.; Hunter, R.; Chan, T. Y.; Vaughan, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802–2803; (h) Ghosh, M.; Dulina, R. R.; Kakarla, R.; Sofia, M. J. *J. Org. Chem.* **2000**, *65*, 8387–8390; (i) Fuchs, T.; Schmidt, R. R. *Synthesis* **1998**, 753–756.
4. For recent reviews on sugar-amino acids see: (a) Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395–4421; (b) Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 231–253; (c) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514; (d) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421–435.
5. (a) Wessel, H. P.; Mitchell, C. M.; Lobato, C. M.; Schmid, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2712–2713; (b) Nicolaou, K. C.; Floerke, H.; Egan, M. G.; Barth, T.; Estevez, V. A. *Tetrahedron Lett.* **1995**, *36*, 1775–1778; (c) Kim, J. M.; Roy, R. *Carbohydr. Lett.* **1996**, *1*, 465–468.
6. (a) Lemieux, R. U.; Du, M.-H.; Spohr, U. *J. Am. Chem. Soc.* **1994**, *116*, 9803–9804; (b) Dietrich, H.; Schmidt, R. R. *Carbohydr. Res.* **1993**, *250*, 161–164; (c) Toogood, P. L.; Galliker, P. K.; Glick, G. D.; Knowles, J. R. *J. Med. Chem.* **1991**, *34*, 3140–3143.
7. Quiocho, F. A. *Annu. Rev. Biochem.* **1986**, *55*, 287–315.
8. Goldstein, I. J.; Poretz, R. D. In *The Lectins*; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic Press: London, 1986; p 35.
9. Schweizer, F.; Otter, A.; Hindsgaul, O. *Synlett* **2001**, 1743–1746.
10. Tavorska, I.; Taravel, F. R. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 15–61.
11. Lenzof, C. C. *Acc. Chem. Res.* **1978**, *11*, 327–333.
12. Deprotection (20% piperidine in DMF or 50% morpholine in DMF for 30 min) of the Fmoc-group in sugar-γ-amino esters **11** and **12** results in spontaneous lactam formation.
13. Orsini, F.; di Teodoro, E. *Tetrahedron: Asymmetry* **2003**, *14*, 2521–2528.
14. Lohse, A.; Schweizer, F.; Hindsgaul, O. *Comb. Chem. High Throughput Screening* **2002**, *5*, 389–394.