Synthesis and structure of a bis-glycosylated hexa-β-peptide

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Dedicated to Professor Richard R. Schmidt on the occasion of his 78th anniversary

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Abstract
The bis-glycosylated hexapeptide β-Ala-β-Ala-(β-D-Glc)-L-Asp-β-Ala-β-Ala-(β-D-Gal)-L-Asp (**14**) is prepared by fragment condensation of Fmoc-β-Ala-β-Ala-(β-D-GlcAc$_4$)-L-Asp-OH (**11**) and H_2N-β-Ala-β-Ala-(β-D-GalAc$_4$)-L-Asp-O^{t}Bu (**12**) under optimized conditions with the HBTU/HOBt reagent followed by deprotection of the intermediate fully protected hexapeptide. Hexapeptide **14** is shown to adopt a random coil structure in solution.

Keywords: β-Peptide, glycopeptides, glycosides, saccharide mimics, conformation

Introduction

Specific interactions of proteins with complex carbohydrate structures associated with cell surfaces play a major role in many biologically important mechanisms such as, for example, cell–cell recognition, signal transduction, infection and inflammation mechanisms, and immunological processes. Different glycosylation patterns and post-translational modifications of carbohydrate structures of glycoproteins are also responsible for their heterogeneity and biological properties.[1-4] Cells can be physically and biologically distinguished through their surface carbohydrate patterns. This is an important medicinal aspect with regard to specific tumor markers on cell surfaces which often consist of distinct complex oligosaccharide structures.[5] Therefore, studying carbohydrate–protein interactions at a molecular level provides a deeper understanding of fundamental biological regulation mechanisms and opens the gate for novel analytical tools or to manipulate such specific processes for therapeutic purposes. Unfortunately, isolation of pure complex oligosaccharides from natural sources in order to study carbohydrate–protein interaction in detail is a rather difficult venture owing to the micro-heterogeneity of naturally occurring saccharides. Synthetic oligosaccharides, on the other hand,

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provide for sufficient amounts of pure material for this purpose. However, the chemical synthesis of complex oligosaccharides is still a laborious and often a difficult task, although significant achievements in this field had been accomplished in the past decades. Thus, novel approaches for the efficient preparation of well-defined saccharide-containing structures which can mimic the interaction between a specific protein and its natural saccharide ligand are highly desirable.[6-9]
For the construction of mimics for complex oligosaccharides, we follow a concept in which simple glycosyl amino acid building blocks are used for the efficient combinatorial preparation of fully glycosylated peptide (glycopeptoid) libraries of the type shown in Figure 1 which, in turn, can bind to carbohydrate-recognizing proteins (Figure. 1).[10-14]

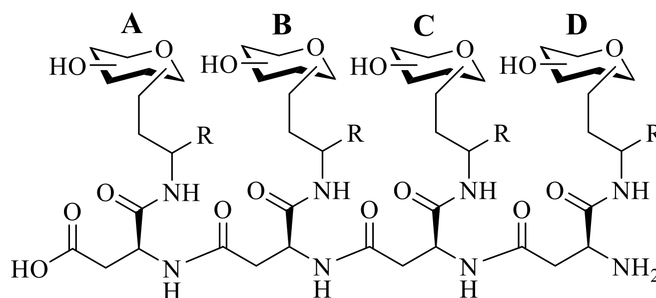

Figure 1. Glycopeptoids constructed out of monosaccharides **A-D** bound through spacers (R=H, alkyl, aryl) to a β-peptide Asp backbone as oligosaccharide mimics.

In order to study further the influence the β-peptide backbone of the aforementioned glycopeptoids may have on the binding characteristics of the respective glycopeptides, we anticipated to prepare a series of bis-glycosylated hexa-peptides with backbones of different flexibility. Here, we describe the chemical synthesis of the β-hexapeptide β-Ala-β-Ala-L-Asp-β-Ala-β-Ala-L-Asp bearing a D-galactoside and a D-glucoside unit at the Asp moieties.

Results and Discussion

For the preparation of the hexapeptide β-Ala-β-Ala-L-Asp-β-Ala-β-Ala-L-Asp we chose a blockwise approach. Therefore we first prepared suitably protected glycosylated tri-peptide blocks β-Ala-β-Ala-(D-Glc or D-Gal)-L-Asp which allowed for simple condensation to give target hexapeptide. Starting from known *t*-butyl 3-(benzyloxycarbonylamino)propionate[15] (**1**), catalytic hydrogenolysis afforded crude **2** which was conjugated without further purification with pentafluorophenyl 3-(fluorenylmethoxycarbonylamino)propionate[16] (**3**) to give dipeptide **4** in 68% yield. Next, the *t*-butyl ester of **4** was hydrolyzed to give **5** in quantitative yield. The latter dipeptide was sufficiently pure for the next step.

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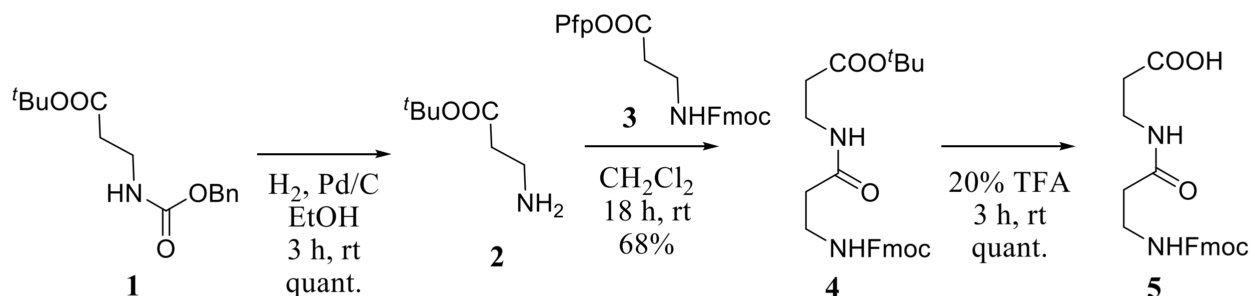
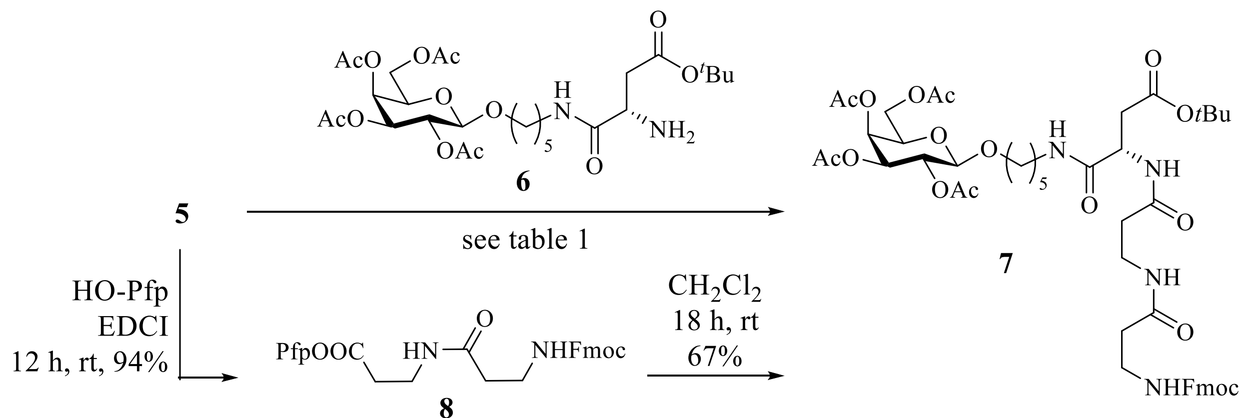


Scheme 1. Synthesis of Fmoc-protected dipeptide Fmoc-β-Ala-β-Ala **5**.

For the preparation of the glycosylated tripeptide block Fmoc-β-Ala-β-Ala-(β-D-Gal)-Asp-O*t*-Bu **7**, dipeptide **5** was condensed with the previously described galactosyl building block **6**[10] under various conditions (Scheme 2). Table 1 summarizes the conditions and the yields of tripeptide **7**.



Scheme 2. Conjugation of dipeptides **5** and **8** with galactosyl amino acid derivative **6** to give tripeptide **7** (see also Table 1).

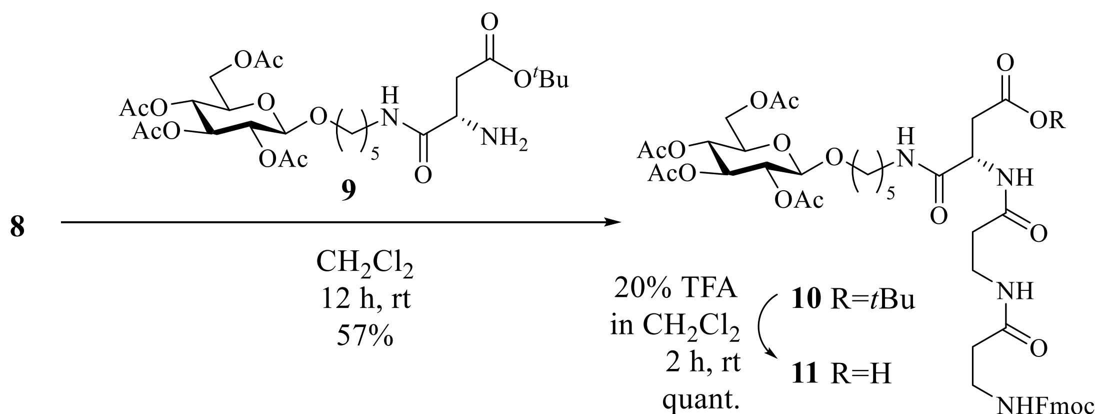
The best yield of tripeptide **7** was obtained with PyBOP, HOBt (Table 1, entry 2). All other condensation reagents gave only low to medium yields. The low yields were due to the formation of several unidentified byproducts during the condensations. Previously, galactosyl amino acid building block **6** could be condensed with pentafluorophenyl-activated amino acid derivatives in high yield.[10] Therefore, dipeptide **5** was also converted into the corresponding pentafluorophenyl ester **8** in 95% yield. However, EDCI[17] was used for this step instead of dicyclohexyl carbodiimide (DCC) as described in the original synthesis[16] because higher yields were obtained with EDCI. The activated ester **8** reacted smoothly with **6** to afford galactosylated tripeptide **7** in 67% yield. Here, work up and chromatographic purification was also facilitated because no by-products which were difficult to separate were formed. Likewise, coupling of dipeptide pentafluorophenyl ester **8** with previously prepared glucosylated asparaginic acid derivative **9**[10]

gave tripeptide **10** in 57% yield. The latter was further converted into acid **11** by hydrolyzing the *t*-butyl ester in **10**.

Table 1. Condensation of dipeptide **5** with galactosyl amino acid derivative **6** under various reaction conditions

Entry	Activating Reagent[a]	Conditions	Yield **7**
1	EDCI, HOBt	DMF, 1 h 0°C then 18 h rt	12%
2	PyBOP, HOBt	DMF, 5 h rt	54%
3	TBTU, HOBt	DMF, 4 h rt	34%
4	HBTU, HOBt	DMF, 4 h rt	44%

[a] EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride[17]; HOBt: 1-hydroxybenzotriazole; PyBOP: benzotriazol-1-yloxy-tri(pyrrolidino)phosphonium hexafluorophosphate[18]; TBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate[19]; HBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate.[19]



Scheme 3. Conjugation of dipeptide **8** with glucose building block **9** followed by partial deprotection to give glucosylated tripeptides **10** and **11**.

Next, peptide **7** was converted into tripeptide **12** by removing the Fmoc group. Tripeptides **11** and **12** were then coupled under various conditions to give the corresponding galactose- and glucose-containing hexapeptide **13** which finally afforded the free hexapeptide **14** in 71% yield upon complete deprotection (Scheme 4). Table 2 summarizes the conditions for the coupling of **11** and **12** with various standard peptide coupling reagents.

Using EDCI and HOBt as coupling reagent for condensing **11** and **12** did not result in a reaction at all, even under prolonged reaction time (Table 2, entry 1). Raising the temperature above room temperature turned out to be disadvantageous because it only resulted in the

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formation of unidentified decomposition products which could not be removed. EDCI was also a less efficient coupling reagent for the condensation of **5** and **6** (*cf* Table 1). Highest yields (61%) of hexapeptide **13** were obtained with the HBTU/HOBt reagent (Table 2, entry 4).

Table 2. Condensation of tripeptide **11** with tripeptide **12** under various reaction conditions

Entry	Activating Reagent[a]	Conditions	Yield **13**
1	EDCI, HOBt	DMF, 1 h 0°C then 24 h rt	-[b]
2	PyBOP, HOBt	DMF, 2 h rt	56%
3	TBTU, HOBt	DMF, 2 h rt	22%
4	HBTU, HOBt	DMF, 2 h rt	61%

[a] EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride[17]; HOBt: 1-hydroxybenzotriazole; PyBOP: benzotriazol-1-yloxy-tri(pyrrolidino)phosphonium hexafluorophosphate[18]; TBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate[19]; HBTU: *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate.[19] [b] No reaction

Complete removal of the protecting groups in hexapeptide **13** was achieved by applying a two step deprotection process. First, the *t*-butyl ester in **13** was hydrolysed. Next, the Fmoc and actely groups of the crude intermediate were concurrently removed with aqueous ammonia solution to afford the free glucose- and galactose-containing hexapeptide **14** in 71% yield.

For the determination of the secondary structure of **14** in aqueous solution, the CD spectrum and TOCSY and NOESY NMR spectra were measured. The CD spectrum shows a minimum at 202 nm with an ellipticity of -3.5 degree and a maximum at 190 nm with an ellipticity of 8.0 degree. This is indicative for a beta sheet or a random coil secondary structure of hexapeptide **14**. However, an unambiguous assignment of the conformation is not possible through the CD spectrum alone.[20-23] Likewise, the chemical shift of the α-H of the Asp moieties of **14** at 5.52 ppm indicates a random coil structure for the hexapeptide but is also not significant enough to decide whether **14** adopts a random coil or beta sheet structure.[24,25] The TOCSY and NOESY NMR spectra of **14** (Figure 2) show only TOCSY cross peaks but no NOESY cross peaks. Thus, there are only intraresidual long range couplings present but no interresidual ones which proves that **14** adopts a random coil structure.

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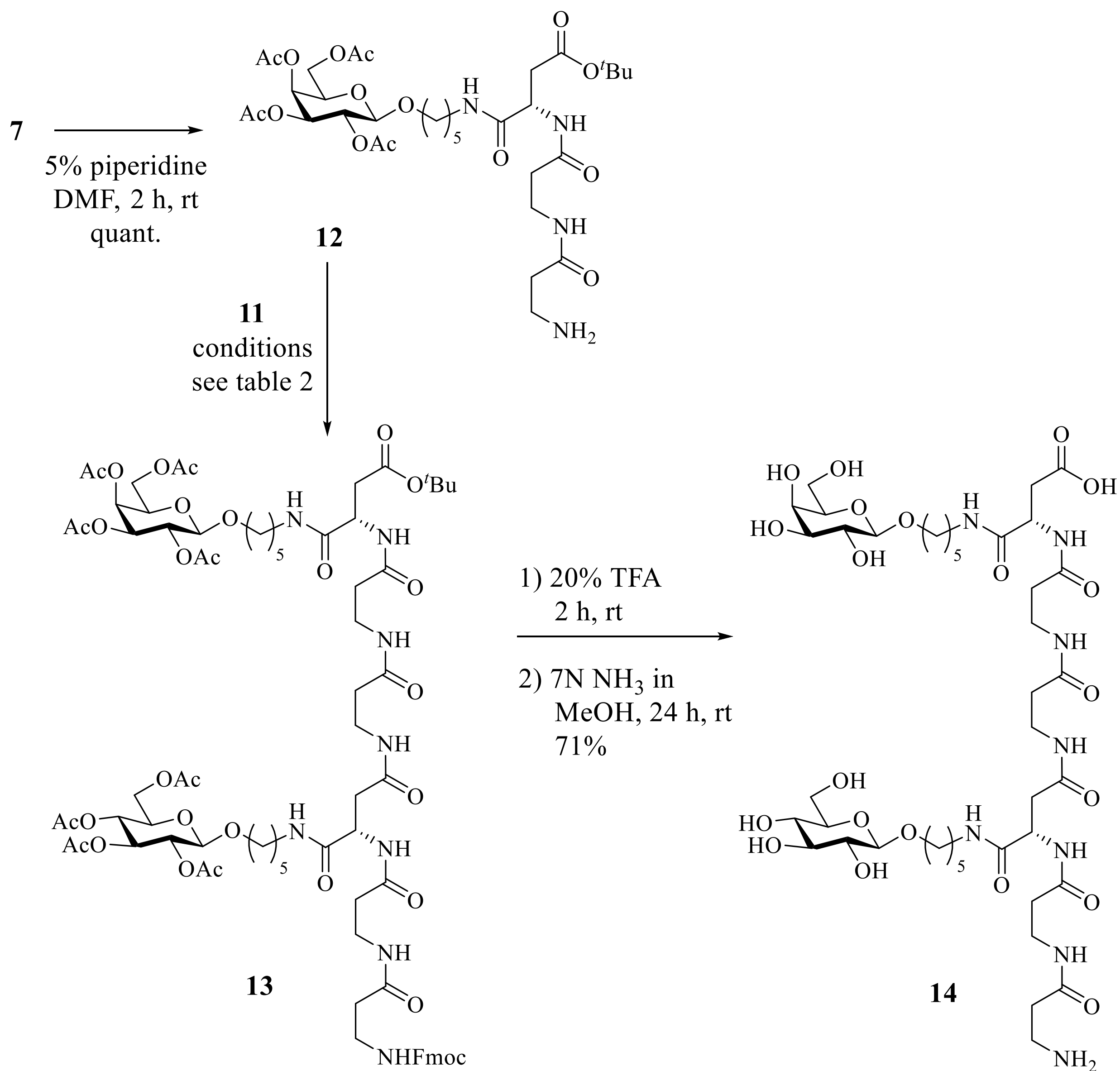

Scheme 4. Coupling of tripeptides **11** and **12** to afford hexapeptide **13**, and full deprotection of the latter to give hexapeptide **14** containing a glucose and a galactose moiety at the Asp residues.

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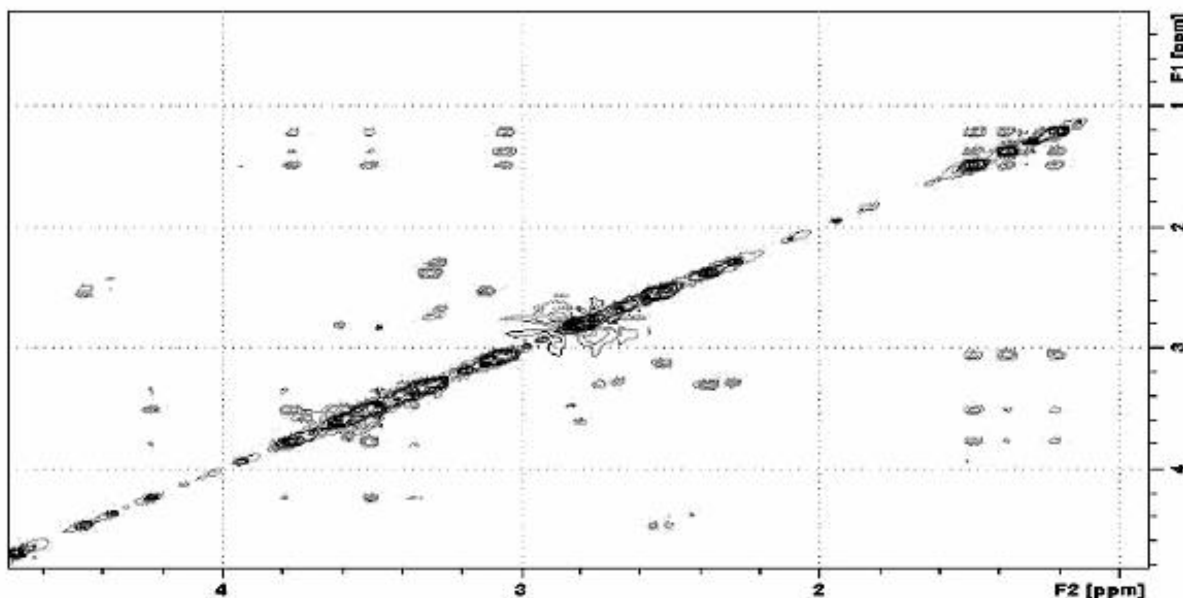

Figure 2. Superposition of the TOCSY and NOESY NMR spectra of **14** in 9:1 D_2O/H_2O; all cross peaks above and below the diagonal belong solely to the TOCSY spectrum; all signals of the NOESY spectrum are located in the diagonal.

Conclusions

We have developed an efficient synthetic strategy for the preparation of the bis-glycosylated hexapeptide β-Ala-β-Ala-(β-D-Glc)-L-Asp-β-Ala-β-Ala-(β-D-Gal)-L-Asp **14** and showed this hexapeptide to adopt a random coil conformation in aqueous solution. The synthetic strategy will now be further applied to the chemical synthesis of similar glycosylated hexapeptides containing conformationally more restricted β-amino acids instead of β-alanine, like for example (1*R*,2*R*)-2-aminocyclohexane- and cyclopentanecarboxylic acids. Studies toward the conformation and binding of these glycosylated hexapeptides to lectins will be published elsewhere.

Experimental Section

General. All solvents were dried and distilled prior to their use. Reactions were performed under Ar and monitored by TLC on Polygram Sil G/UV silica gel plates from Macherey & Nagel. Detection was affected by charring with H_2SO_4 (5% in EtOH) or by inspection of the TLC plates under UV light. NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz for proton spectra and 100 MHz for carbon spectra. Tetramethylsilane was used as the internal standard. Signal assignments were confirmed through HH-, CH-COSY, HMBC and HSQC experiments. Conformations were deducted from NOESY and TOCSY experiments and CD

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spectra, measured on a Jasco J-720 circular dichroism spectrometer using a 1 mm cell. FAB MS was performed on a Finnigan MAT TSQ 70 spectrometer. HRFD MS was performed on a Bruker FT-ICR spectrometer. Elemental analyses were performed on a Hekatech Euro EA 3000 CHN analyzer. Optical rotations were measured with a Perkin-Elmer Polarimeter 341. Preparative chromatography was performed on silica gel (0.032-0.063 mm) from Macherey & Nagel using different mixtures of solvents as eluents.

***t*-Butyl 3-aminopropionat**e (**2**). A suspension of *t*-butyl 3-(benzyloxycarbonylamino)propionate[15] (**1**) (1.39 g, 5.0 mmol) and Pd on charcoal (10%, 50 mg) in ethanol (20 mL) was stirred at room temperature under an atmosphere of hydrogen for 3 h. The mixture was filtered through a layer of Celite. Concentration of the filtrate gave crude **2** (0.79 g, 100%) which was used for the next step without further purification.

***t*-Butyl 3-[3-[[(9*H*-fluoren-9-yl)methyloxycarbonyl]amino]propanamido]propionate** (**4**). Pentafluorophenyl 3-[[(9*H*-fluoren-9-yl)methyloxy]carbonylamino]propionate[16] (**3**) (1.77 g, 5.0 mmol) was added at room temperature to stirred solution of **2** (0.79 g, 5.0 mmol) in CH_2Cl_2 (25 mL), and stirring was continued for 18 h. Concentration of the solution and chromatography of the residue with *n*-hexane/ethyl acetate (1:1) afforded **4** (1.49 g, 68%); mp 126 °C (*n*-hexane / ethyl acetate); ^{1}H NMR ($CDCl_3$): δ 7.69 (d, 2 H, Fmoc), 7.52 (d, 2 H, Fmoc), 7.32 (t, 2 H, Fmoc), 7.24 (t, 2 H, Fmoc), 4.29 (d, 2 H, CH_2-Fmoc) 4.14 (m, 1 H, CH-Fmoc), 4.06 (m, 2 H, CH_2), 3.42 (m, 2 H, CH_2), 2.36 (m, 4 H, CH_2), 1.38 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 174.0 (CO-NH), 172.1 (*C*O-$C(CH_3)_3$), 159.3 (CO-NH), 146.7, 144.1, 130.4, 129.8, 127.9, 122.7 (Fmoc), 84.0 ($C(CH_3)_3$), 69.5 (Fmoc-CH_2), 50.0 (*C*H_2-CONH), 39.8 (NH-*C*H_2), 38.7 (*C*H-*C*H_2-CO), 37.8 (NH-*C*H_2-*C*H_2), 37.8 (*C*H_2-*C*H_2-CO), 30.9 ($C(CH_3)_3$); FAB-MS calculated for $C_{25}H_{30}N_2O_5$: 438.2 $[M]^+$, found: 439.1 $[M+H]^+$, 461.1 $[M+Na]^+$; Anal. calculated for $C_{25}H_{30}N_2O_5$ (438.52): C 68.47, H 6.90, N 6.39, found: C 68.57, H 6.99, N 6.25.

3-[3-[[(9*H*-Fluoren-9-yl)methoxy]carbonylamino]propanamido]propionic acid (**5**). A solution of **4** (0.74 g, 1.7 mmol) and trifluoroacetic acid (4 mL) in chloroform (20 mL) was stirred at room temperature for 3 h. The solution was concentrated and toluene (3 × 20 mL) was co-evaporated to give crude **5** (0.65 g, 100%) which was used for the next step without further purification.

Fmoc-β-Ala-β-Ala-(β-D-Ac4Gal)-L-Asp-O*t*Bu (**7**). (a) EDCI[17] (191.7 mg, 1.0 mmol) was added at 0 °C to a stirred solution of **5** (0.61 g, 1.0 mmol) and HOBt (135.1 mg, 1.0 mmol) in DMF (20 mL). After stirring was continued at room temperature for 1 h, *t*-butyl α-[(5-aminopentyl)-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-L-asparaginate[10] (**6**) (0.65 g, 1.0 mmol) and *N*-methylmorpholine (450 μL) was added and stirring continued for 18 h. The mixture was poured into water (500 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered and concentrated. Chromatography of the residue with *C*H_2Cl_2/EtOH (19:1) afforded **7** (0.11 g, 12%); $[\alpha]_D^{20}$=-4.5 (*c*=0.65, $CHCl_3$); 1H NMR ($CDCl_3$): δ 7.69 (d, 2 H, Fmoc), 7.53 (d, 2 H, Fmoc), 7.30 (t, 2 H, Fmoc), 7.23 (m, 7H, Fmoc), 6.89 (d, 1H, NH-CO), 6.61 (bs, 1H, NH-Ala), 6.43 (bs, 1H, NH-Ala), 5.82 (1H, bs, NH-Ala), 5.31 (m, 1H, H-4), 5.21 (m,

1H, H-2, $J_{H1,H2}$ 8.1 Hz), 4.93 (dd, 1H, H-3, $J_{H2,H3}$ 10.6 Hz), 4.65 (b, 1H, CH-Asp), 4.34 (m, 1H, H-1), 4.28 (m, 2H, *C*H_2-Fmoc), 4.06 (m, 3 H, H-6a,b, CH-Fmoc), 3.81 (m, 2 H, O-*C*H_2, H-5), 3.42 (m, 1 H, O-*C*H_2), 3.38 (m, 4 H, NH-*C*H_2), 3.13 (m, 2 H, NH-*C*H_2), 2.71 (dd, 1 H, *C*H_2-Asp), 2.55 (dd, 1 H, -CH_2-Asp), 2.34 (s, 4H, CH_2-Ala), 2.07, 1.98, 1.97, 1.91 (s, 12 H, CH_3), 1.48 (m, 2 H, CH_2-CH_2-NH), 1.40 (m, 2 H, O-*C*H_2-*C*H_2), 1.36 (s, 9 H, CH_3), 1.26 (m, 2 H, *C*H_2); ^{13}C NMR ($CDCl_3$): δ = 175.9, 171.6, 171.5, 171.3, 170.4, 170.2, 170.1, 169.7, 156.5 (CO), 143.9, 141.2, 127.6, 127.0, 125.1, 119.9 (Fmoc), 101.3 (C-1), 81.9 (C(CH_3)$_3$), 70.8 (C-5), 70.6 (C-3), 69.9 (O-*C*H_2), 68.9 (C-2), 66.9 (*C*H_2-Fmoc), 61.6 (C-4), 49.9 (CH-Asp), 47.6 (CH-Fmoc), 39.8 (*C*H_2-NH), 37.2 (*C*H_2-Asp), 36.2 (NH-*C*H_2), 35.9 (*C*H_2), 35.6 (*C*H_2), 29.5 (*C*H_2), 29.4 (*C*H_2), 28.4 (C(CH_3)$_3$), 23.5 (*C*H_2), 20.8, 20.7, 20.6, 20.5 (CH_3); FAB-MS calculated for $C_{48}H_{64}N_4O_{17}$: 968.4 $[M]^+$; found: 969.3 $[M+H]^+$; FT-ICR-MS: calculated for $[M+Na]^+$: 991.41587 $[M+Na]^+$, found: 991.41707 $[M+Na]^+$.

(b) A solution of **6** (0.65 g, 1.0 mmol) in DMF (5 mL) was added at room temperature to a solution of **5** (0.38 g, 1.0 mmol), PyBOP[18] (0.52 g, 1.0 mmol), HOBt (135.1 mg, 1.0 mmol) and ethyl-diisopropylamine (258,4 mg, 2.0 mmol) in DMF (20 mL), and the mixture was stirred for 5 h. Work up as described above afforded **7** (0.52 g, 54%).

(c) A solution of **6** (0.65 g, 1.0 mmol) in DMF (5 mL) was added at room temperature to a solution of **5** (0.38 g, 1.0 mmol), TBTU[19] (0.32 g, 1.0 mmol), HOBt (135.1 mg, 1.0 mmol) and ethyl-diisopropylamine (258,4 mg, 2.0 mmol) in DMF (20 mL), and the mixture was stirred for 4 h. Work up as described above afforded **7** (0.33 g, 34%).

(d) A solution of **6** (0.65 g, 1.0 mmol) in DMF (5 mL) was added at room temperature to a solution of **5** (0.38 g, 1.0 mmol), HBTU[19] (0.38 g, 1.0 mmol), HOBt (135.1 mg, 1.0 mmol) and ethyl-diisopropylamine (258,4 mg, 2.0 mmol) in DMF (20 mL), and the mixture was stirred for 4 h. Work up as described above afforded **7** (0.43 g, 44%).

(e) A solution of **8** (0.61 g, 1.1 mmol) and **6** (0.65 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 18 h. Concentration of the solution and chromatography of the residue with *C*H_2Cl_2/EtOH (19:1) afforded **7** (0.64 g, 67%).

Pentafluorophenyl 3-[3-[[(9*H*-fluoren-9-yl)methoxy]carbonylamino]propanamido]propionate (**8**). A solution of **5** (0.65 g, 1.7 mmol), pentafluorophenol (0.35 g, 1.9 mmol) and dicyclohexyl carbodiimide (0.39 g, 1.9 mmol) in ethyl acetate (50 mL) was stirred at room temperature for 12 h. Filtration of the resulting mixture, concentration of the filtrate and chromatography of the residue with *n*-hexane/ethyl acetate (1:1) afforded **8** (0.88 g, 94%); ^{1}H NMR ($CDCl_3$): δ 7.68 (d, 2 H, Fmoc), 7.51 (d, 2 H, Fmoc), 7.31 (t, 2 H, Fmoc), 7.22 (t, 2 H, Fmoc), 5.99 (bs, 1 H, NH), 5.36 (bs, 1 H, NH), 4.30 (d, 2 H, CH_2), 4.13 (m, 1 H, CH), 3.57 (d, 2 H, CH_2), 3.43 (d, 2 H, CH_2), 2.87 (t, 2 H, CH_2), 2.36 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$): δ 172.8 (CO), 158.1 (O-CO), 144.4, 144.3, 141.7, 128.1, 127.4, 125.5, 120.3 (Fmoc, Ph), 67.1 (CH_2), 47.6 (CH_2), 37.3 (CH_2), 36.3 (CH_2), 34.3 (CH_2), 33.8 (CH_2); FAB-MS calculated for $C_{27}H_{21}F_5N_2O_5$: 548.1 $[M]^+$; found: 549.0 $[M+H]^+$, 571.0 $[M+Na]^+$; Anal. calculated for $C_{27}H_{21}F_5N_2O_5$ (548.46): C 59.13, H 3.86, N 5.11, C 58.89, H 3.57, N 5.01.

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Fmoc-β-Ala-β-Ala-(β-D-Ac$_4$Glc)-L-Asp-O^{t}Bu (**10**). A solution of **8** (0.61 g, 1.1 mmol) and *t*-butyl α-[(5-aminopentyl)-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl]-L-asparaginate[10] **9** (0.65 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 12 h. Concentration of the solution and chromatography of the residue with CH_2Cl_2/EtOH (19:1) afforded **10** (0.55 g, 57%); $[\alpha]_D^{20}$=-12.5 (*c*=0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.73 (d, 2 H, Fmoc), 7.57 (d, 2 H, Fmoc), 7.36 (t, 2 H, Fmoc) , 7.27 (t, 2H, Fmoc), 7.00 (d, 1 H, NH), 6.68 (bs, 1 H, NH), 6.43 (bs, 1 H, NH), 5.70 (bs, 1 H, NH), 5.16 (m, 1 H, H-3, $J_{H2,H3}$ 9.6 Hz), 5.05 (dd, 1 H, H-4, $J_{H4,H5}$ 9.8 Hz) 4.93 (m, 1 H, H-2, $J_{H1,H2}$ 8.1 Hz), 4.68 (bs, 1 H, CH), 4.41 (d, 1 H, H-1) 4.33 (m, 2 H, CH_2), 4.20 (m, 2 H, H-6a, CH-Fmoc), 4.08 (m, 1 H, H-6b), 3.81 (m, 1 H, O-CH_2), 3.64 (m, 2 H, H-5, O-CH_2), 3.45 (m, 4 H, CH_2), 3.16 (m, 2 H, CH_2), 2.75 (dd, 1 H, CH_2), 2.57 (dd, 1 H, CH_2), 2.33 (s, 4 H, CH_2), 2.04, 2.01, 1.98, 1.97 (s, 12 H, CH_3), 1.51 (m, 2 H, CH_2), 1.41 (m, 2 H, CH_2), 1.39 (s, 9 H, CH_3), 1.29 (m, 2 H, CH_2); ^{13}C NMR ($CDCl_3$): δ 171.5, 171.3, 170.7, 170.3, 169.6, 169.4, 161.2, 156.5 (CO), 143.9, 143.7, 141.2, 127.6, 127.0, 125.2, 119.9 (Fmoc, Ph), 100.8 (C-1), 81.7 ($C(CH_3)_3$), 72.7 (C-3), 71.7 (C-5), 71.3 (C-2), 69.9 (O*C*H_2), 68.4 (C-4), 66.7 (CH_2), 61.9 (C-6), 49.4 (CH-Asp), 47.2 (CH-Fmoc), 39.4 (CH_2), 37.3 (CH_2-NH), 37.2 (NH-CH_2), 36.2 (NH-CH_2), 36.0 (CH_2), 35.7 (CH_2), 29.0 (CH_2), 28.9(CH_2), 28.1 ($C(CH_3)_3$), 23.1 (CH_2), 20.7, 20.6, 20.6, 20.5 (CH_3); FAB-MS calculated for $C_{48}H_{64}N_4O_{17}$: 968.4 $[M]^+$, found: 969.2 $[M+H]^+$; FT-ICR-MS: calculated for $[M+Na]^+$: 991.41587, found: 991.41540.

Fmoc-β-Ala-β-Ala-(β-D-Ac$_4$Glc)-L-Asp-OH (**11**). A solution of **10** (50 mg, 50 μmol) and trifluoroacetic acid (1 mL) in chloroform (10 mL) was stirred at room temperature for 2 h. The solution was concentrated and toluene (3 × 10 mL) was co-evaporated to give crude **11** (45 mg, 100%) which was used for the next step without further purification.

H_2N-β-Ala-β-Ala-(β-D-Ac$_4$Gal)-L-Asp-O^{t}Bu (**12**). A solution of **7** (50 mg, 50 μmol) and piperidine (0.5 mL) in DMF (10 mL) were stirred at room temperature for 2 h. The solution was concentrated and co-evaporated with toluene (3 × 10 mL) to afford crude **12** (40 mg, 100%) which was used for the next step without further purification.

Fmoc-β-Ala-β-Ala-(β-D-Ac$_4$Glc)-L-Asp-β-Ala-β-Ala-(β-D-Ac$_4$Gal)-L-Asp-O^{t}Bu (**13**). (a) A solution of **12** (40 mg, 50 μmol) in DMF (5 mL) was added at room temperature to a solution of **11** (45 mg, 50 μmol), PyBOP[18] (26 mg, 50 μmol), HOBt (7 mg, 50 μmol) and ethyl-diisopropylamine (19 mg, 0.1 mmol) in DMF (5 mL), and the mixture was stirred for 2 h. Work up as described above for compound **7** afforded **13** (46 mg, 56%); $[\alpha]_D^{20}$=+1.7 (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.92 (m, 1 H, NH), 7.73 (m, 1 H, NH), 7.54 (d, 2H, Fmoc), 7.40 (d, 2 H, Fmoc), 7.17 (t, 2 H, Fmoc), 7.08 (t, 2 H, Fmoc), 5.91 (m, 1 H, NH), 5.17 (s, 2 H, CH), 4.93 (m, 2 H, H-$3_{Gal,Glc}$, $J_{H2,H3}$=9.6 Hz), 4.83 (m, 3 H, H-$4_{Gal,Glc}$, H-2_{Gal}), 4.72 (t, 1 H, H-2_{Glc}), 4.47 (m, 2 H, *C*H_2), 4.28 (d, 1 H, H-1_{Glc}, $J_{H1,H2}$=7.8 Hz), 4.02 (m, 4 H, H-1_{Gal}, H-6a,$b_{Gal,Glc}$, CHAsp), 3.94 (m, 4 H, H-6a,$b_{Gal,Glc}$, H-$5_{Gal,Glc}$), 3.75 (m, 2 H, O*C*H_2), 3.66 (m, 2 H, O*C*H_2), 3.24 (m, 8 H, *C*H_2), 2.97 (m, 4 H, *C*H_2), 2.39 (m, 4 H, *C*H_2), 2.21 (m, 6 H, *C*H_2), 1.94, 1.90, 1.87, 1.85, 1.83, 1.82, 1.81, 1.79, 1.75, (s, 24 H, CH_3), 1.34 (m, 6 H, *C*H_2), 1.19 (s, 9 H, $C(CH_3)_3$), 1.11 (m, 6 H,*C*H_2); ^{13}C NMR ($CDCl_3$): δ = 170.7, 170.4, 170.3, 170.2, 169.5, 165.5, 164.5, 164.3 (CO), 145.1, 143.5, 127.4, 126.8, 124.1, 119.6 (Fmoc, Ph), 100.8 (C-1_{Gal}), 100.3 (C-1_{Glc}), 81.4 ($C(CH_3)_3$),

74.2 (C-5_{Gal}), 72.6 (C-3_{Glc}), 71.3(C-3_{Gal}), 71.1(C-5_{Glc}), 70.7 (C-2_{Gal}), 70.2 (C-2_{Glc}), 69.6 (O*C*H_2), 68.7 (C-4_{Gal}), 68.1(C-4_{Glc}), 62.0 (C-6_{Gal}), 61.7(C-6_{Glc}), 60.9 (*C*H_2), 50.2 (CH), 48.7 (CH), 46.8 (CH), 39.1 (*C*H_2NH), 35.6, 35.5, 35.4, 35.4 (*C*H_2), 32.8, 32.7, 32.0, 31.5 (*C*H_2NH), 28.7 (*C*H_2), 28.5 (*C*H_2), 27.4 ($C(CH_3)_3$), 22.7(*C*H_2), 21.9, 21.2, 20.3, 20.2, 20.1, 20.1, 20.3 (CH_3); FAB-MS calculated for $C_{77}H_{108}N_8O_{31}$: 1640.7 $[M]^+$, found: 1641.5 $[M+H]^+$; FT-ICR-MS: calculated for $[M+2Na]^{2+}$: 843.34525, found: 843.34425.

(b) A solution of **12** (40 mg, 50 μmol) in DMF (5 mL) was added at room temperature to a solution of **11** (45 mg, 50 μmol), TBTU[19] (16 mg, 50 μmol), HOBt (7 mg, 50 μmol) and ethyl-diisopropylamine (19 mg, 0.1 mmol) in DMF (20 mL), and the mixture was stirred for 42h. Work up as described above for compound **7** afforded **13** (18 mg, 22%).

(c) A solution of **12** (40 mg, 50 μmol) in DMF (5 mL) was added at room temperature to a solution of **11** (45 mg, 50 μmol), HBTU[19] (19 mg, 50 μmol), HOBt (7 mg, 50 μmol) and ethyl-diisopropylamine (19 mg, 0.1 mmol) in DMF (20 mL), and the mixture was stirred for 42h. Work up as described above for compound **7** afforded **13** (49 mg, 61%).

H_2N-β-Ala-β-Ala-(β-D-Glc)-L-Asp-β-Ala-β-Ala-(β-D-Gal)-L-Asp-OH (**14**). A solution of **13** (30 mg, 18 μmol) and trifluoroacetic acid (0.5 mL) in chloroform (5 mL) was stirred at room temperature for 6 h, concentrated and co-evaporated with toluene (3 × 10 mL). The residue was dissolved in 7N methanolic NH_3 solution (10 mL), stirred at room temperature for 24 h and concentrated. The residue was dissolved in H_2O (10 mL) and extracted with diethyl ether (2 × 5 mL). Lyophilization of the aqueous phase and chromatography of the residue on Biogel with water afforded **14** (13 mg, 71%); $[\alpha]_D^{20}$=-2.7 (c = 0.15, H_2O); ^{1}H NMR (H_2O/D_2O 9:1): δ 8.29 (m, 1 H, NH), 7.96 (m, 3 H, NH), 7.60 (m, 2 H, NH), 6.87 (m, H, NH), 4.52 (d, 1 H, CHAsp), 4.40 (t, 1 H, H-3_{Glc}), 4.25 (d, 1 H, H-4_{Gal}), 3.94 (m, 1 H, CH), 3.79 (m, 4 H, H-4_{Glc}, H-2_{Gal}, H-2_{Glc}, H-3_{Gal}), 3.65 (m, 6 H, H-$1_{Gal,Glc}$, H-6a,$b_{Gal,Glc}$) 3.54 (m, 6 H, *C*H_2), 3.48 (bs, 6 H, *C*H_2, OH), 3.37-3.21 (m, 8 H, H-$5_{Gal,Glc}$, OH) , 3.12 (m, 4 H, OCH_2), 2.77 (m, 4 H, CH_2), 2.58-2.53 (m, 8 H, NH), 2.38 (m, 4H,CH_2), 1.49 (m, 4 H, CH_2), 1.37 (m, 4 H, CH_2), 1.21 (m, 4 H, CH_2); ^{13}C NMR (H_2O/D_2O 9:1): δ=174.6, 173.9, 172.9, 167.0, 153.3 (CO), 101.5 (C-1_{Gal}), 100.1 (C-1_{Glc}), 78.2, 75.9, 73.7 (C-2,3,$5_{Gal,Glc}$), 71.6 (OCH_2), 69.5 (C-$4_{Gal,Glc}$), 61.8 (C-$6_{Gal,Glc}$), 54.3 (CH), 52.1 (CH), 44.2 (CH_2), 44.1 (CH_2), 40.1 (CH_2NH), 36.8 (CH_2), 36.6 (CH_2), 35.6 (CH_2), 35.5(CH_2) , 32.9 (CH_2NH), 30.8 (CH_2NH), 30.6 (CH_2NH), 29.1 (CH_2NH), 28.8 (CH_2), 27.9 (CH_2), 23.1 (CH_2); FAB-MS calculated for $C_{42}H_{74}N_8O_{21}$: 1026.5 $[M]^+$, found: 1049.8 $[M+Na]^+$; FT-ICR-MS calculated for $[M+H]^+$: 1027.5041, found: 1027.5070.

Structure

CD spectra were measured for solutions of **14** (0.5 mg/ml) in 0.1 N phosphate buffer pH 7.5.

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