108. Solid-Phase Synthesis of a Sialyl-Tn-Glycoundecapeptide of the MUC1 Repeating Unit

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Dedicated to Professor *Dieter Seebach* on the occasion of his 60th birthday

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The synthesis of glycopeptides carrying tumour-associated antigens is of interest for cancer diagnosis and treatment. Here, a very efficient route to disaccharide threonine building block **8** is presented which allows the introduction of the sialyl-Tn antigen into a peptide. The syntheses of the undecapeptide and the sialyl-Tn-containing glycoundecapeptide, which are a part of the repeating unit of MUC1, were performed by solid-phase synthesis with an allylic anchor cleavable under neutral conditions. After detachment from the resin, the peptide and the glycopeptide are completely deprotected giving the target compounds **13** and **15**, respectively.

Introduction. – Mucins are a family of highly glycosylated glycoproteins that are found in mucus and on cell surfaces of epithelial cells [1] [2]. They all have a characteristic domain which consists of a variable number of tandem repeats. The repeating units contain many serine and threonine residues which often are *O*-glycosylated. In case of cancer, the expression of mucins is increased, and their carbohydrate side chains are altered due to incomplete glycosylation and early sialylation as was shown by several immunohistochemical studies [3]. As a consequence, the peptide is no longer covered by the carbohydrates and reveals new epitopes of the peptide which are tumour-specific [4]. Glycoconjugates which appear predominantly on cancer cells expose so-called tumour-associated antigens. These structures are used in the diagnosis and treatment of cancer. Monoclonal antibodies against the cancer-specific structures are used to indicate the presence of a tumour and could serve for targeting drugs to the tumour [1]. Semi-synthetic and synthetic tumour-associated antigens are being tested as vaccines against cancer [5]. Examples for tumour-associated carbohydrate antigens are the T-(*Thomson-Friedenreich*, β-Gal-(1 $\rightarrow$ 3)-α-GalNac1-*O*-Ser/Thr), Tn-(α-GalNAc1-*O*-Ser/Thr) and sialyl-Tn-antigen (α-NeuAc-(2 $\rightarrow$ 6)-α-GalNAc1-*O*-Ser/Thr).

Both, the peptide and the carbohydrate part of the glycopeptide, are important for producing a cellular as well as a humoral immune response. Therefore, it is of interest to synthesize tumour-related glycopeptides and apply them as the epitopes in cancer research. Several examples have been reported for the synthesis of a repeating unit [6] or of parts of repeating units of mucins [7] [8] with Tn-antigen, but none for a mucin glycopeptide with sialyl-Tn-antigen. Very recently, a solid-phase synthesis of a sialyl-Tn glycopeptide fragment of HIV gp 120 was described [9].

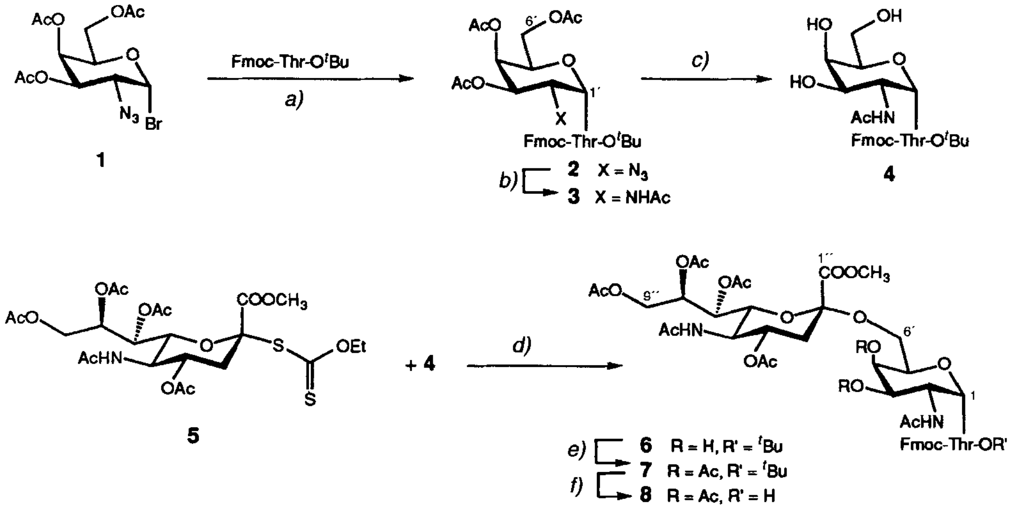
We here report the synthesis of an undecapeptide and a sialyl-Tn-glycoundecapeptide, which are part of the 20 amino acid repeating unit of MUC1 [10]. The chosen sequence contains the threonine which is the most preferred *O*-glycosylation site for *N*-acetylgalactosaminyl-transferase according to *in vitro* glycosylation experiments [11].

Results and Discussion. – To introduce the sialyl-Tn moiety into the glycopeptide, a sialyl-Tn-threonine building block had to be synthesized. This was achieved according to the preliminarily described strategy [12], but with the (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) *N*-protecting group which is suitable for solid-phase synthesis instead of the (benzyloxy)carbonyl group. In comparison to other published syntheses of the sialyl-Tn-antigen, this strategy is more straightforward. In one of these known syntheses, serine was coupled to the disaccharide to give the sialyl-Tn conjugate as a mixture of α-D and the undesired β-D-anomer. The protecting groups of serine, (benzyloxy)carbonyl and benzyl, could only be removed simultaneously [13]. Another synthesis used a neuraminic acid donor with a phenylthio group in position 3 to increase the stereoselectivity; a number of additional steps were necessary in this strategy [14]. In a more recent synthesis, $^{t}BuMe_2Si$ protecting groups within the galactosamine portion, which were used during the glycosylation of the threonine, had to be exchanged for the isopropylidene group prior to sialylation [9] [15].

In the synthesis described here, Fmoc-threonine *tert*-butyl ester (Fmoc-Thr-O^{t}Bu) was glycosylated between 0° and room temperature with the acetylated galactosyl bromide **1** which was activated by silver carbonate and silver perchlorate [16] to give a crude product (α-D/β-D ≈ 16:1; conversion 80%) (*Scheme 1*). The pure α-D-anomer **1** was isolated in a yield of 47% after purification by flash chromatography. After conversion of the azido group into the acetylamino group with thioacetic acid [17], the *O*-acetyl groups of **3** were removed in MeOH solution by careful addition of 1% NaOMe in MeOH so that the pH did not exceed 8.5 (wet pH paper). The base-sensitive Fmoc group was not affected by this reaction, and **4** was obtained in good yield. The acceptor **4** was sialylated with the sialyl xanthate **5** [18] and methylsulfenyl triflate as the promotor [19] which can be generated *in situ* from methylsulfenyl bromide and silver triflate [20] (*Scheme 1*). The reaction was successful if performed in CH_2Cl_2/MeCN at −62°. The reaction proceeded diastereoselectively (α-D/β-D 4:1) and with high regioselectivity. After purification by flash chromatography and reversed-phase HPLC, the α-D-disaccharide **6** was isolated in 32% yield. To obtain building block **8**, which can be used in solid-phase synthesis, the sialyl-Tn-threonine conjugate **6** was *O*-acetylated (→ **7**) and *C*-terminally deblocked by cleavage of the *tert*-butyl ester with CF_3COOH.

For the solid-phase synthesis, an aminomethyl-polystyrene (AMPS) resin modified with the HYCRON anchor [7] was used. The β-Ala-AMPS resin was functionalized with the anchor-amino acid conjugate **9** (*Scheme 2*), which was activated by *N,N'*-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazol (HOBt) in CH_2Cl_2 (→ **10**). The coupling reactions were performed in dimethlyformamide (DMF) with 4.2 equiv. of the Fmoc-amino acid activated by *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) [21], HOBt, and *N*-methylmorpholine (NMM). To minimize formation of diketopiperazine, the second amino acid was introduced as the Boc-protected derivative. After coupling and acidolytic removal of the Boc group, the ammonium salt of the resin-linked dipeptide was formed, which did not undergo intramolecular aminolysis to give the diketopiperazine. This change in the *N*-terminal protection illustrates an advantage of the allylic anchor, which is stable to both, acids and bases used

Scheme 1

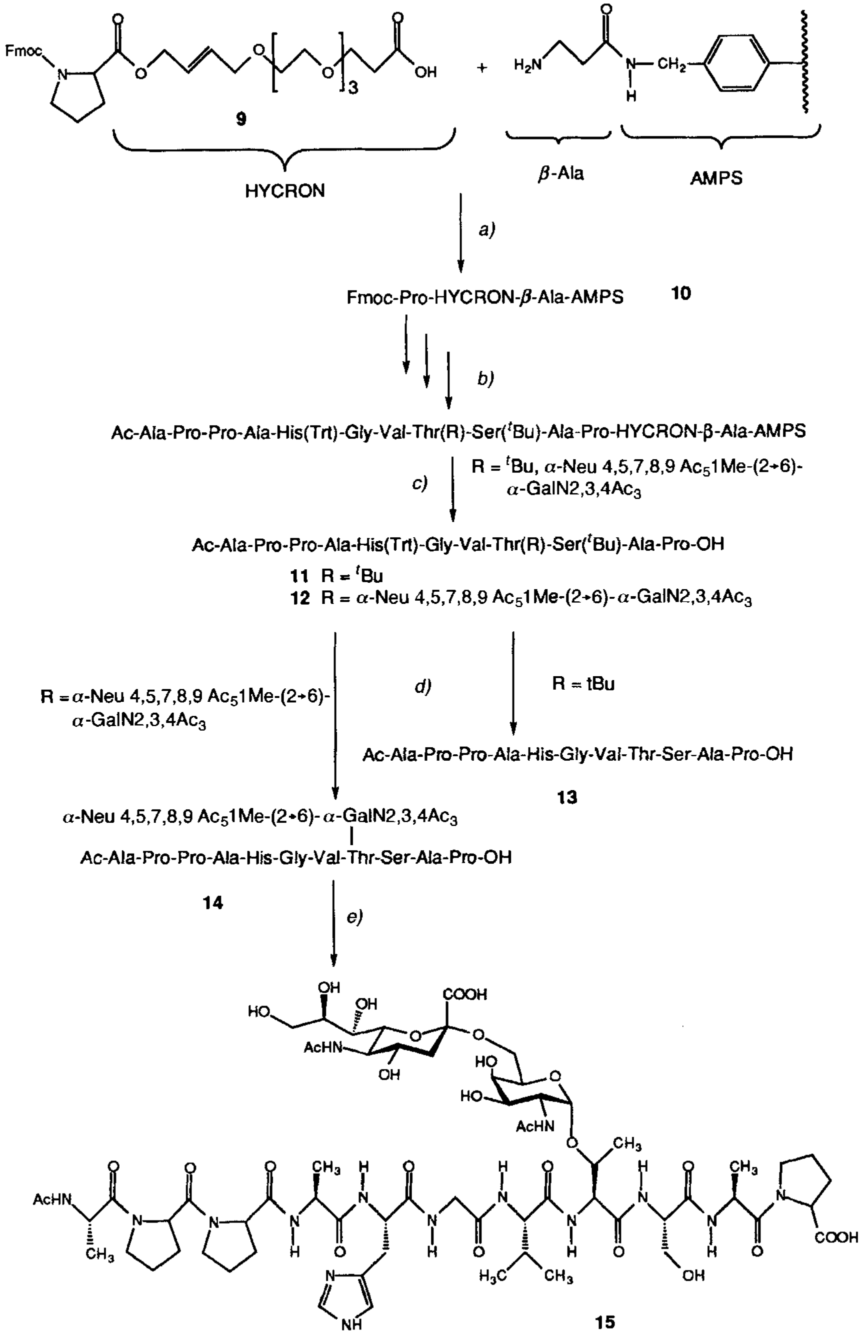


a) $AgClO_4/Ag_2CO_3$ 1:11, CH_2Cl_2/toluene 10:9, 47%. *b)* MeCOSH; 84%. *c)* MeOH, NaOMe, pH < 8.5, 1.5 h; 77%, 1.5 h; 77%. *d)* MeSBr, AgOTf, MeCN, CH_2Cl_2, −62°, 3 h 45 min; 32%. *e)* Ac_2O, pyridine; 88%. *f)* CF_3COOH, anisole 13:1, 1 h; quant.

in solid-phase syntheses. Further chain extensions were performed using Fmoc-amino acids. The cleavage of the Fmoc groups was carried out with morpholine/DMF 1:1 and was monitored by UV absorption of the fulvene-morpholine adduct. Each coupling reaction was followed by a capping with Ac_2O/pyridine 1:3. For washings, DMF was used after each step. No repeated acylation reactions were required. After the last coupling reaction, the Fmoc group was removed and the N-terminus was acetylated. The peptide or glycopeptide, respectively, was detached from the resin by allyl transfer to morpholine catalysed by $[Pd(PPh_3)_4]$ under neutral conditions [22]. The cleavage yield [1]) was 96% for peptide **11** and 98% for the glycopeptide **12** (*Scheme 2*). The synthesis of the glycopeptide was performed in close analogy to that of the peptide. In the third coupling step, the sialyl-Tn building block **8** was used, and the coupling time was extended to 15 h. The sialyl-Tn building block was also added in *ca.* 4-fold excess. Half of the excess of the glycosylated amino acid was recovered after chromatography. It is noteworthy that *Kihlberg et al.* [9] used only 1.05 equiv. of the sialyl-Tn building block for the solid-phase synthesis. This is obviously the reason for an incomplete coupling reaction. As a consequence these authors obtained the non-glycosylated terminated peptide as an undesired by-product. After cleavage from the resin, the still protected peptide **11** and glycopeptide **12** were purified by chromatography. The amino acid side-chain protecting groups *N*-trityl of His and *O-tert*-butyl of Ser and Thr were removed by treatment with anisole/ethyl methyl sulfide in CF_3COOH. After reversed-phase HPLC, **13** was obtained in an overall yield of 30% and **14** in an overall yield of 42% based on the initial loading of the starting amino acid L-proline onto the resin. The

[1]) Cleavage yield = $100\,[1 - (p_{pep}/p_{\beta\text{-Ala}})_a/(p_{pep}/p_{\beta\text{-Ala}})_b]$; p = capacity, a = after cleavage, b = before cleavage.

Scheme 2



a) DIC, HOBt, CH_2Cl_2. *b*) See text. *c*) $[Pd(PPh_3)_4]$, morpholine, DMF/DMSO 1:1. *d*) CF_3COOH, anisole, EtSMe. *e*) 1. Aq. NaOH, MeOH; 2. aq. NaOH.

protecting groups of the carbohydrate part of glycopeptide **14** were removed by treatment with aqueous NaOH in MeOH at a controlled pH of 10–11 (removal of the acetyl groups) and, subsequently, by cleavage of the neuraminic acid methyl ester with 5 mM aqueous NaOH at a carefully controlled pH of 11.5. No β-elimination was observed under these conditions. At any pH lower than 11, no hydrolysis of the methyl ester could be performed. The sialyl-Tn-containing glycoundecapeptide **15** was isolated in a yield of 76% after purification by reversed-phase HPLC and was characterized by high-field ^{1}H- and ^{13}C-NMR-spectra and by mass spectroscopy.

Experimental Part

General. DMF was purchased from *Roth*, Karlsruhe; CH_2Cl_2 for glycosylations was distilled from Pb/K alloy, CH_2Cl_2 for the solid-phase synthesis from P_2O_5, and MeCN from CaH_2. TBTU was purchased from *Richelieu Biotechnologies*, Montreal. Solvents were removed under reduced pressure at < 40° (bath). Org. solns. were dried with $MgSO_4$. TLC: *Silica gel 60 F_{254}* (*Merck*, Darmstadt), detection by UV light and by dipping the plate into a 1:1 mixture of 1M H_2SO_4 in EtOH and 3% 3-methoxyphenol soln. in EtOH followed by heating. Column chromatography: silica gel (particle size 0.063–0.200 mm) from *J. T. Baker* (Deventer), flash chromatography (FC) with silica gel (particle size 35–70 µm) from *Amicon* (Danvers). Reversed-phase HPLC: anal.: *Eurospher C8* column (5 µm, 250 × 4 mm; *Knauer*, Berlin), flow rate 1 ml min^{-1}, detection at 210 nm; prep.: *Eurospher C8* column (7 µm, 250 × 40 mm; *Knauer*, Berlin) flow rate 20 ml min^{-1}. Optical rotations: *Perkin-Elmer-241* polarimeter. NMR: *Bruker-AC-200* (^{1}H at 200 MHz, ^{13}C at 50.3 MHz) and *Bruker-AM-400* or *-ARX-400* spectrometer (^{1}H at 400 MHz, ^{13}C at 100.6 MHz); chemical shifts δ in ppm and J values in Hz. FAB-MS: *Finnigan-MAT-95* with glycerol or 3-nitrobenzyl alcohol (nba) as matrix and ionisation by caesium ions; assignments to the peptide fragments were performed according to the nomenclature suggested in [23]. Amino-acid analysis were performed by *Orpegen Pharma*, Heidelberg.

N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine tert-Butyl Ester (**2**). *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-L-threonine *tert*-butyl ester [24] (10 g, 25.2 mmol) and powdered 4-Å molecular sieves (*ca.* 10 g) were stirred in a mixture of dry toluene (70 ml) and dry CH_2Cl_2 (100 ml) for 1 h at r.t. under Ar and exclusion of light. After cooling the mixture to 0°, Ag_2CO_3 (7.6 g, 27.7 mmol) was added followed by $AgClO_4$ (672 mg, 3.24 mmol) dissolved in toluene (*ca.* 20 ml). The mixture was stirred for 30 min at 0°. A soln. of crude 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide (**1**) [25] (9.9 g, 25.2 mmol) in toluene (125 ml) and CH_2Cl_2 (125 ml) was added dropwise to the mixture. After stirring overnight at r.t., the mixture was diluted with CH_2Cl_2 and filtered through *Celite*. The filtrate was extracted twice with sat. $NaHCO_3$ soln. (200 ml) and twice with H_2O (200 ml). The solvents were removed, and the residue containing the crude product (α-D/β-D *ca.* 16:1) and Fmoc-Thr-O^{t}Bu (2 g) was purified by FC (light petroleum ether/AcOEt 6:1) pure α-D-diastereoisomer **2** (8.35 g, 47%). Colourless amorphous solid. $[\alpha]_D^{22} = +79.8$ ($c = 0.99$, $CHCl_3$). R_f 0.63 (light petroleum ether/AcOEt 3:2), 0.73 (light petroleum ether/AcOEt 1:1). The β-D-anomer was not obtained in a pure form. **2**: ^{1}H-NMR (200 MHz, $CDCl_3$): 7.78–7.25 (*m*, 8 H, Fmoc); 5.65 (*d*, $J = 9.5$, NH); 5.47 (*m*, H–C(4')); 5.34 (*dd*, $J(2',3') = 11.2$, $J(3',4') = 3.1$, H–C(3')); 5.10 (*d*, $J(1',2') = 3.6$, H–C(1')); 4.46–4.27 (*m*, H–C(5'), 2H–C(6'), H–C(9'') (Fmoc), H–C(2), H–C(3)); 4.09 (*d*, CH_2O (Fmoc)); 3.63 (*dd*, $J(1',2') = 3.6$, $J(2',3') = 11.2$, H–C(2')); 2.15, 2.07, 2.04 (3*s*, 3 Ac); 1.50 (*s*, *t*-Bu); 1.35 (*d*, $J(3,4) = 6.4$, Me(4)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.2, 169.9, 169.7, 169.1 (C=O); 156.8 (C=O (Fmoc)); 143.8, 141.2, 127.6, 127.0, 125.2, 119.9 (Fmoc); 99.1 (C(1')); 82.8 (Me_3C); 76.2 (C(2)); 68.0, 67.5, 67.1 (C(3'), C(4'), C(5')); 67.4 (CH_2O (Fmoc)); 61.7 (C(6')); 59.2, 57.7 (C(2'), C(2)); 47.1 (C(9'') (Fmoc)); 27.9 (Me_3C); 20.5 (*Me*CO); 18.8 (Me(4)); $J(1',2')$ and the δ of C(1') and Me(3) are significant of the α-D-configuration [26]. Anal. calc. for $C_{35}H_{42}N_4O_{12}$ (710.7): C 59.15, H 5.92, N 7.89; found: C 58.84, H 6.10, N 7.51.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-threonine tert-Butyl Ester (**3**). To **2** (6.42 g, 9.18 mmol), freshly distilled thioacetic acid (43.8 g, 41 ml) was added, and the mixture was stirred overnight under Ar at r.t. After the reagent was distilled off, the crude product was codistilled with toluene several times and purified by FC (light petroleum ether/AcOEt 1:1): **3** (5.54 g, 84%). Colourless foam. $[\alpha]_D^{22} = +63.4$ ($c = 1.03$, $CHCl_3$). R_f 0.41 (light petroleum ether/AcOEt 1:3). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.26–7.29 (*m*, 8 H, Fmoc); 6.00 (*d*, $J = 9.9$, NH); 5.59 (*d*, $J = 9.4$, NH); 5.36 (*d*, $J(4',5') < 0.8$, H–C(4')); 5.07 (*dd*, $J(2',3') = 11.3$, $J(3',4') = 2.9$, H–C(3')); 4.86 (*d*, $J(1',2') = 3.4$, H–C(1')); 4.59 (*ddd*, $J(1',2') = 3.5$, $J(2',3') = 11.0$, H–C(2')); 4.48–4.38 (*m*, 2 H), 4.26–4.17 (*m*, 4 H), 4.08–4.02 (*m*, 2 H; CH_2O

(Fmoc), H–C(9″) (Fmoc), H–C(5′), 2 H–C(6′), H–C(2), H–C(3)); 2.14, 2.01, 1.97 (*s*, MeCO); 1.43 (*s*, *t*-Bu); 1.30 (*d*, *J*(3,4) = 6.3, Me(4)). ^{13}C-NMR (50.3 MHz, $CDCl_3$): 170.8, 170.3, 170.2, 169.9 (C=O); 156.4 (C=O (Fmoc)); 143.6, 141.2, 127.7, 127.0, 125.0, 119.9 (Fmoc); 99.8 (C(1′)); 83.1 (Me_3C); 76.8 (C(3)); 68.6, 67.3 (C(3′), C(4′), C(5′)); 67.1 (CH_2O (Fmoc)); 62.1 (C(6′)); 58.9 (C(2)); 47.2, 47.1 (C(2′), C(9″) (Fmoc)); 28.0 (Me_3C); 23.1 (*Me*CON); 20.6, 20.5 (*Me*CO); 18.5 (Me(4)). Anal. calc. for $C_{37}H_{46}N_2O_{13}$ (726.8): C 61.16, H 6.34, N 3.86; found: C 61.28, H 6.28, N 3.96.

O-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-threonine tert-*Butyl Ester* (**4**). To a soln. of **3** (1 g, 1.38 mmol) in MeOH (30 ml), 1% NaOMe soln. was added until the pH was 8.5. After 1.5 h, *Dowex 50W X8* (H^+) was added to the mixture which was stirred for another hour at r.t. The ion exchanger was filtered off and the solvent evaporated. Purification of the product was achieved by FC (AcOEt/EtOH 20:1): **4** (636 mg, 75%). White amorphous solid. $[\alpha]_D^{22} = +40.2$ ($c = 1.00$, $CHCl_3$). R_f 0.61 (AcOEt/MeOH 3:1). ^{1}H-NMR (^{1}H,^{1}H-COSY; 400 MHz, CD_3OD): 7.84–7.32 (*m*, 8 H, Fmoc); 4.84 (*d*, *J*(1′,2′) = 3.9, H–C(1′)); 4.59–4.48 (*m*, *J* = 10.7), CH_2O (Fmoc); 4.37 (*m*, H–C(3)); 4.33–4.26 (*m*, H–C(2′), H–C(9″) (Fmoc)); 4.17 (*m*, H–C(2)); 3.93–3.89 (*m*, H–C(4′)); 3.93–3.89 (*m*, 1 H), 3.78–3.73 (*m*, 2 H; H–C(5′), 2 H–C(6′)); 3.70 (*dd*, *J*(2′,3′) = 10.9, *J*(3′,4′) = 2.8, H–C(3′)); 2.04 (*s*, MeCO); 1.47 (*s*, *t*-Bu); 1.28 (*d*, *J*(3,4) = 6.4, Me(4)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 173.2, 170.6 (C=O); 156.7 (C=O (Fmoc)); 143.8, 141.3, 128.3, 127.7, 127.1, 125.1, 120.0 (Fmoc); 99.8 (C(1′)); 82.9 (Me_3C); 76.3 (C(3)); 70.5, 79.3, 69.3 (C(3′), C(4′), C(5′)); 67.2 (CH_2O (Fmoc)); 62.0 (C(6′)); 59.2 (C(2)); 50.4 (C(2′)); 47.2 (C(9″) (Fmoc)); 28.0 (Me_3C); 23.1 (*Me*CO); 18.9 (Me(4)). Anal. calc. for $C_{31}H_{40}N_2O_{10} \cdot H_2O$ (618.7): C 60.19, H 6.80, N 4.53; found: C 60.27, H 6.69, N 4.40.

*O-{2-Acetamido-2-deoxy-6-O-[methyl(5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-*glycero*-α-D-galacto-non-2-ulopyranosyl)onate]-α-D-galactopyranosyl}-N-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-threonine* tert-*Butyl Ester* (**6**). A mixture of (**4**) (927 mg, 1.545 mmol), **5** (2.18 g, 3.657 mmol), powdered 3-Å molecular sieves (2 g), dry MeCN (40 ml), and dry CH_2Cl_2 (18 ml) was stirred for 1 h at r.t. under Ar. After addition of AgOTf (0.94 g, 3.657 mmol), the mixture was cooled to −62°. Within 10 min, cooled 1.6M methylsulfenyl bromide in 1,2-dichloroethane (2.29 ml) was added. The mixture was protected from light. The reaction was terminated after 3 h 45 min by addition of $(i\text{-}Pr)_2NH$ (0.73 ml). The mixture was stirred for 30 min at −62°, warmed to 0°, filtered through *Celite*, and washed with CH_2Cl_2. The solvent was evaporated and the yellow oil (4.05 g) purified by FC (light petroleum ether/AcOEt 1:1): 908 mg of anomeric-disaccharide mixture. The α-D and β-D-anomers were separated by reversed-phase HPLC ($MeCN/H_2O$ 45:55).

α-D-Anomer **6**: Yield 528 mg (32%). Colourless oil. $[\alpha]_D^{22} = +11.7$ ($c = 0.95$, $CHCl_3$). R_f 0.60 (AcOEt/EtOH 10:2), 0.40 (AcOEt/EtOH 10:1). ^{1}H-NMR (^{1}H,^{1}H-COSY; 400 MHz, CD_3OD): 7.86–7.35 (*m*, 8 H, Fmoc); 5.46–5.44 (*m*, H–C(8″)); 5.39 (*d*, *J*(6″,7″) = 2.0, *J*(7″,8″) = 8.6, H–C(7″)); 4.89 (*ddd*, *J*(4″,5″) = 11.5, H–C(4″), partly covered by the signal of CD_3OH); 4.82 (*d*, *J*(1′,2′) = 3.9, H–C(1′)); 4.64 (*dd*, *J* = 6.4, 10.7, (CH_2O (Fmoc)); 4.53 (*dd*, *J* = 6.1, 10.8, (CH_2O (Fmoc)); 4.39–4.27 (*m*, H_a–C(9″), H–C(9‴) (Fmoc), H–C(3), H–C(2′)); 4.21–4.12 (*m* (4.19 (*J*(5″,6″) = 10.8, *J*(6″,7″) = 2.1)), H–C(6″), H_b–C(9″)); 4.04–3.90 (*m*, H–C(5″), H–C(5′), H–C(4′), H_a–C(6″)); 3.88 (*s*, MeO); 3.68–3.59 (*m*, H–C(3′), H_b–C(6′)); 2.70 (*dd*, *J*(3″e,3″a) = 12.8, *J*(3″e,4″) = 4.6, H_e–C(3″)); 2.18, 2.12, 2.05, 2.03, 1.88 (5*s*, 6 MeCO); 1.90 (*t*, *J*(3″e,3″a) = 12.9, H_a–C(3″)); 1.49 (*s*, *t*-Bu); 1.27 (*d*, *J*(3,4) = 6.3, Me(4)). ^{13}C-NMR (100.6 MHz, CD_3OD): 173.8, 173.5, 172.5, 171.9, 171.6, 171.1, 169.4 (C=O); 159.0 (C=O (Fmoc)); 145.4, 145.1, 142.7, 128.8, 128.2, 126.1, 126.0, 121.0 (Fmoc); 100.7 (C(1′)); 100.0 (C(2′)); 83.5 (Me_3C); 76.7 (C(3)); 73.4, 71.2, 70.8, 70.0, 68.9 (C(3′), C(4′), C(5′), C(4″), C(6″), C(7″), C(8″)); 67.6 (CH_2O (Fmoc)); 65.0, 63.5 (C(6′), C(9″)); 60.9 (C(2)); 53.4, 50.9, 50.1 (C(2′), C(5″), MeO); 48.6 (C(9″) (Fmoc)); 38.7 (C(3″)); 28.4 (Me_3C); 23.2, 22.7, 21.2, 20.9, 20.7 (*Me*CO); 19.8 (Me(4)). FAB-MS (nba, pos.): 1096.4 (3.1, $[M + Na]^+$; calc. 1096.4), 1076.4 (6.1, $[M(2 \times {}^{13}C) + H]^+$; calc. 1076.4), 1075.3 (17.9, $[M(1 \times {}^{13}C) + H]^+$; calc. 1075.4), 1074.4 (36.2, $[M + H]^+$; calc. 1074.4), 678.0 (12.3, $[M(1 \times {}^{13}C) - (\text{Fmoc-Thr-}^t\text{Bu})]^+$; calc. 678.2), 677.1 (50.8, $[M - (\text{Fmoc-Thr-}^t\text{Bu})]^+$; calc. 677.2), 474.0 (11.3, $[\text{Neu2enAc}_5{}^1\text{Me} + H]^+$; calc. 474.2), 415.0 (16.7, $[\text{Neu2,5dienAc}_4{}^1\text{Me}(1 \times {}^{13}C)]^+$; calc. 415.1), 414.0 (74.5, $[\text{Neu2,5dienAc}_4{}^1\text{Me}]^+$; calc. 414.1). Anal. calc. for $C_{51}H_{67}N_3O_{22} \cdot H_2O$ (1092.1): C 56.09, H 6.37, N 3.85; found: C 56.18, H 6.47, N 3.77.

β-D-Anomer: Yield 124 mg (7.5%). Colourless oil. $[\alpha]_D^{22} = +16.3$ ($c = 1.00$, $CHCl_3$): R_f 0.60 (AcOEt/EtOH 10:2), 0.40 (AcOEt/EtOH 10:1). ^{1}H-NMR (^{1}H, ^{1}H-COSY, 400 MHz, CD_3OD): 7.86–7.34 (*m*, 8 H, Fmoc); 5.47 (*dd*, *J*(6″,7″) = 2.0, *J*(7″,8″) = 4.8, H–C(7″)); 5.37 (*m*, *J*(7″,8″) = 4.6, *J*(8″,9″a) = 2.4, *J*(8″,9″b) = 7.0, H–C(8″)); 5.27 (*ddd*, *J*(3″e,4″) = 4.9, *J*(4″,5″) = 10.9, H–C(4″)); 4.81 (*d*, *J* = 3.6, H–C(1′)); 4.79 (*dd*, *J*(8″,9″a) = 2.3, H_a–C(9″)); 4.62 (*dd*, *J* = 6.4, 10.8, (CH_2O (Fmoc)); 4.53 (*dd*, *J* = 6.1, 10.9, (CH_2O (Fmoc)); 4.33–4.27 (*m*, H–C(3), H–C(2′), H–C(9‴) (Fmoc), H–C(6″)); 4.19–4.14 (*m*, H–C(2)); 4.17 (*dd*, *J*(8″,9″b) = 6.9, *J*(9″a,9″b) = 12.4, H_b–C(9″)); 4.04–3.99 (*m*, H–C(4′), H–C(5′)); 4.01 (*t*, H–C(5″)); 3.84 (*s*, MeO); 3.75 (*dd*, H_a–C(6′)); 3.72 (*dd*, *J*(2′,3′) = 11.1, *J*(3′,4′) = 3.0, H–C(3′)); 3.55 (*dd*, *J*(5′,6′b) = 6.3, *J*(6′a,6′b) = 9.3, H_b–C(6′)); 2.53 (*dd*, *J*(3″e,3″a) = 12.9, *J*(3″e,4″) = 4.9, H_e–C(3″)); 2.15, 2.11, 2.05, 2.03, 2.02, 1.90 (6*s*, 6 MeCO);

1.87 (*t*, *J*(3″e,3″a) = 12.7, H_a–C(3″)); 1.48 (*s*, *t*-Bu); 1.26 (*d*, *J*(3,4) = 6.4, Me(4)). ^{13}C-NMR (100.6 MHz, CD_3OD): 173.8, 173.5, 172.5, 172.3, 171.9, 171.7, 171.1, 168.8 (C=O); 159.1 (C=O (Fmoc)); 145.4, 145.2, 142.7, 128.9, 128.2, 126.2, 126.0, 121.0 (Fmoc); 100.8 (C(1′)); 100.0 (C(2″)); 83.5 (Me_3C); 76.6 (C(3)); 72.9, 72.4, 70.4, 69.9, 69.8 (C(3′), C(4′), C(5′), C(4″), C(6″), C(7″), C(8″)); 67.6 (CH_2O (Fmoc)); 63.8, 63.6 (C(6′), C(9″)); 60.9 (C(2)); 53.4, 50.9, 50.3 (C(2′), C(5″), MeO); 48.7 (C(9‴) (Fmoc); 38.4 (C(3″)); 28.4 (*Me*$_3$C); 23.3, 22.7, 21.1, 20.8, 20.7, (*Me*CO); 19.9 (Me(4)); anomer assignments by the H–C(4″) signal and *J*(7″,8″) according to [27]. FAB-MS (nba, pos.): 1097.0 (5.4, $[M + Na]^+$; calc. 1096.4), 1077.1 (5.7, $[M(2 \times {}^{13}C) + H]^+$; calc. 1076.4), 1076.1 (16.1, $[M(1 \times {}^{13}C) + H]^+$; calc. 1075.4), 1075.2 (28.3, $[M + H]^+$; calc. 1074.4), 678.7 (20.7, $[M(1 \times {}^{13}C) -$ (Fmoc-Thr-^{t}Bu)$]^+$; calc. 678.2), 677.6 (69.8, [*M* – (Fmoc-Thr-^{t}Bu)]$^+$; calc. 677.2), 474.3 (14.6, [Neu2enAc$_5$-^{1}Me + H]$^+$, calc. 474.2), 415.1 (18.7, [Neu2,5dienAc$_4$^{1}Me(1 × ^{13}C)]$^+$; calc. 415.1), 414.1 (100, [Neu2,5dienAc$_4$-^{1}Me]$^+$; calc. 414.1). Anal. calc. for $C_{51}H_{67}N_3O_{22} \cdot H_2O$ (1092.1): C 56.09, H 6.37, N 3.85; found: C 55.70, H 6.49, N 3.68.

O-{*2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto*-non-2-ulopyranosyl)onate]-α-*D*-galactopyranosyl*}-N-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*L*-threonine* tert-*Butyl Ester* (**7**). A soln. of **6** (498 mg, 0.43 mmol) in pyridine (20 ml) was cooled to 0°, and then Ac_2O (10 ml) was added. After the mixture was stirred overnight at r.t., CH_2Cl_2 (200 ml) and ice were added. The soln. was washed twice with sat. $NaHCO_3$ soln. (20 ml) and twice with H_2O. The aq. phase was again extracted with CH_2Cl_2. The solvents were removed, and the residue was codistilled with toluene. The product was purified by FC (light petroleum ether/AcOEt 1:3): **7** (470 mg, 88%). Colourless oil. $[\alpha]_D^{22} = +27.7$ (*c* = 1.01, $CHCl_3$). R_f 0.62 (AcOEt/EtOH 10:2), 0.47 (AcOEt/EtOH 10:1). ^{1}H-NMR (400 MHz, CD_3OD): 7.88–7.35 (*m*, 8 H, Fmoc); 5.47–5.42 (*m*, H–C(8″)); 5.37 (*dd*, *J*(6″,7″) = 2.2, *J*(7″,8″) = 9.2, H–C(7″)); 5.05 (*dd*, *J*(2′,3′) = 11.5, *J*(3′,4′) = 3.1, H–C(3′)); 4.91 (*d*, *J* = 3.8, H–C(1′)); 4.86 (*ddd*, *J*(3″e,4″) = 4.6, *J*(4″,5″) = 12.0, H–C(4″)); 4.70 (*dd*, *J* = 6.1, 10.8, (CH_2O (Fmoc)); 4.52 (*dd*, *J* = 5.9, 10.8, (CH_2O (Fmoc)); 4.45 (*dd*, *J*(1′,2′) = 3.8, *J*(2′,3′) = 11.4, H–C(2′)); 4.38 (*m*, H–C(3)); 4.33 (*dd*, *J*(8″,9″a) = 2.6, *J*(9″a,9″b) = 12.6, H_a–C(9″)); 4.29 (*m*, H–C(9‴) (Fmoc)); 4.20–4.17 (*m*, H–C(2), H–C(5′), H_a–C(6′), H–C(6″)); 4.11 (*dd*, *J*(8″,9″b) = 5.3, *J*(9″a,9″b) = 12.4, H_b–C(9″)); 4.02 (*t*, H–C(5″)); 3.93 (*dd*, *J*(5′,6′b) = 6.8, *J*(6″a,6″b) = 10.0, H_b–C(6′)); 3.86 (*s*, MeO); 2.65 (*dd*, *J*(3″e,3″a) = 12.7, *J*(3″e,4″) = 4.6, H_e–C(3″)); 2.21, 2.16, 2.14, 2.05, 2.02, 1.98, 1.97, 1.88 (*s*, 8 MeCO); 1.88 (*t*, H_a–C(3″)); 1.48 (*s*, *t*-Bu); 1.27 (*d*, *J* = 6.4, Me(4)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 170.9, 170.5, 170.4, 170.2, 170.1, 169.7, 167.9 (C=O); 143.8, 141.4, 172.8, 127.1, 125.1, 120.0 (Fmoc); 100.1 (C(1′)); 98.6 (C(2″)); 83.1 (Me_3C); 76.7 (C(3)); 72.6, 69.1, 68.9, 68.6, 68.3, 67.8, 67.3 (C(3′), C(4′), C(5′), C(4″), C(6″), C(7″), C(8″)); 67.2 (CH_2O (Fmoc)); 63.5, 62.4 (C(6′), C(9″)); 59.2 (C(2)); 52.8 (MeO); 49.4, 47.4 (C(2′), C(5″)); 47.3 (C(9‴) (Fmoc)); 37.7 (C(3″)); 28.1 (*Me*$_3$C); 23.2, 23.1, 21.0, 20.8, 20.7 (*Me*CO); 18.6 (Me(4)). FAB-MS (nba, pos.): 1160.1 (5.7, $[M(1 \times {}^{13}C) + H]^+$; calc. 1159.4), 1159.1 (9.4, $[M + H]^+$; calc. 1158.4), 762.8 (14.6, $[M(1 \times {}^{13}C) -$ (Fmoc-Thr-^{t}Bu)$]^+$; calc. 762.3), 761.7 (40, [*M* – (Fmoc-Thr-^{t}Bu)]$^+$; calc. 761.3), 641.6 (8.6, [*M* – (Fmoc-Thr-^{t}Bu) – AcNH – AcO]$^+$, calc. 641.3), 414.4 (63.9, [Neu2,5dienAc$_4$^{1}Me]$^+$; calc. 414.1). Anal. calc. for $C_{55}H_{71}N_3O_{24} \cdot H_2O$ (1176.2): C 56.21, H 6.25, N 3.57; found: C 56.15, H 6.24, N 3.58.

O-{*2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto*-non-2-ulopyranosyl)onate]-α-*D*-galactopyranosyl*}-N-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*L*-threonine* (**8**). A soln. of **7** (730 mg, 0.63 mmol), anisole (1.4 ml), and CF_3COOH (18 ml) was stirred for 1 h at r.t. After removal of the CF_3COOH, the product was codistilled with toluene and then purified by FC (AcOEt/EtOH 4:1): **8** (696 mg, quant.). Colourless oil. $[\alpha]_D^{22} = +28.9$ (*c* = 1.00, MeOH), 0.65 (AcOEt/MeOH 1:1), (AcOEt/MeOH 2:1). ^{1}H-NMR (400 MHz, $CD_3OD/CDCl_3$ 6:1): 7.73–7.34 (*m*, 8 H, Fmoc); 5.45–5.42 (*m*, H–C(4′), H–C(8″)); 5.36 (*dd*, *J*(6″,7″) = 1.8, *J*(7″,8″) = 9.1, H–C(7″)); 5.10 (*dd*, *J*(2′,3′) = 11.5, *J*(3′,4′) = 2.9, H–C(3′)); 4.99 (*d*, *J*(1′,2′) = 3.6, H–C(1′)); 4.84 (*ddd*, *J*(3″e,4″) = 4.7, *J*(4″,5″) = 11.9, H–C(4″)); 4.63 (*dd*, *J* = 6.4, 10.6, CH_2O (Fmoc)); 4.45 (*dd*, *J* = 6.2, 10.6, CH_2O (Fmoc)); 4.41 (*dd*, *J*(1′,2′) = 3.6, *J*(2′,3′) = 11.5, H–C(2′)); 4.42 (*m*, H–C(3)); 4.31 (*dd*, *J*(8″,9″a = 2.3, *J*(9″a,9″b) 12.2, H_a–C(9″)); 4.22 (*t*, *J* = 6.7, H–C(5′)); 4.30–4.27 (*m*, 1 H), 4.17–4.14 (*m*, 3 H; H–C(9‴) (Fmoc), H–C(2), H–C(6″), H_a–C(6′)); 4.11 (*dd*, *J*(8″,9″b) = 5.1, *J*(9″a,9″b) = 12.5, H_b–C(9″)); 4.01 (*t*, H–C(5′)); 3.91 (*dd*, *J*(5′,6′b) = 6.7, *J*(6′a,6′b) = 10.0, H_b–C(6′)); 3.85 (*s*, MeO); 2.64 (*dd*, *J*(3″e,3″a) = 12.7, *J*(3″e,4″) = 4.6, H–C(3′)); 2.20, 2.15, 2.13, 2.04, 2.02, 2.00, 1.99, 1.87 (8*s*, 8 MeCO); 1.28 (*d*, *J*(3,4) = 6.0, Me(4)). ^{13}C-NMR (100.6 MHz, $CD_3OD/CDCl_3$ 6:1): 173.5, 173.3, 172.3, 172.1, 172.0, 171.7, 171.5, 171.4, 169.1 (C=O); 159.0 (C=O (Fmoc)); 145.2, 145.0, 142.6, 128.7, 128.1, 126.0, 125.9, 120.9 (Fmoc); 100.7 (C(1′)); 99.8 (C(2″)); 77.8 (C(3)); 73.2, 70.6, 69.8, 69.3, 69.2, 68.9, 68.5 (C(3′), C(4′), C(5′), C(4″), C(6″), C(7″), C(8″)); 67.5 (CH_2O (Fmoc)); 64.4, 63.4 (C(6′), C(9″)); 53.3 (MeO); 49.9 (C(5″)); 48.4 (C(9‴) (Fmoc)); 38.7 (C(3″)); 22.8, 22.7, 21.2, 20.9, 20.8, 20.7, 19.3 (*Me*CO); 16.6 (C(4)). FAB-MS (nba, neg): $C_{51}H_{63}N_3O_{24}$ (1102.1) 1102.5 (14.1, $[M(2 \times {}^{13}C) - H]^-$; calc. 1102.4), 1101.4 (31.0, $[M(1 \times {}^{13}C) - H]^-$; calc. 1101.4), 1100.4 (45.1, $[M - H]^-$; calc. 1100.4).

Solid-Phase Synthesis. The synthesis of the peptide and the glycopeptide was performed manually in a mechanically agitated reactor. Fmoc-Ser-OH and Fmoc-Thr-OH carried *tert*-butyl (^{t}Bu) and Fmoc-His-OH triphenylmethyl (Trt) protected side chains. After each step, the resin was washed 5 × with 20 ml of DMF or CH_2Cl_2.

Functionalization of the Resin with the Anchor-Amino Acid Conjugate **10**. Boc-*β*-Ala-AMPS (*ca.* 1.5 mmol *β*-Ala/g; 2.4 g, *ca.* 3.6 mmol) was shaken in CH_2Cl_2 (20 ml) and CF_3COOH (15 ml), for 45 min. Then the resin was neutralized with 2 ml of $(i\text{-}Pr)_2EtN$ in CH_2Cl_2 (20 ml) for 10 min, followed by addition of DMF (6.5 ml), **9** (6.94 g, 11.3 mmol) [7] dissolved in CH_2Cl_2 (22 ml), HOBt (2 g, 13 mmol), and DIC (2 ml, 1.64 g, 13 mmol). The mixture was shaken for 16 h. The amount of L-proline on the resin per gram resin (0.43 mmol Pro/g) was determined by amino-acid analysis. For the synthesis of peptide **13**, 0.82 g (0.349 mmol of Pro) of Fmoc-Pro-HYCRON-*β*-Ala-AMPS, and for the synthesis of glycopeptide **15**, 0.42 g (0.181 mmol of Pro) of Fmoc-Pro-HYCRON-*β*-Ala-AMPS were used. Fmoc groups were cleaved by treatment of the resin with DMF/morpholine 1:1 for 50 min. The removal of the Fmoc group was monitored by UV absorption at 280 nm. For the coupling reaction, a 4.2-fold excess of the Fmoc-amino acid (first coupling reaction: Boc-Ala), HOBt, TBTU, and a 8.5-fold excess of NMM and DMF (*ca.* 14 ml) were added to the resin, and the mixture was shaken for 4.5 h. Cleavage of the Boc protecting group was performed with CH_2Cl_2/CF_3COOH 1:1 for 50 min, followed by neutralization with $(i\text{-}Pr)_2EtN/CH_2Cl_2$ 1:10. Each coupling reaction was followed by capping of the unreacted amino functions with pyridine/Ac_2O 3:1. In the synthesis of the glycopeptide **15**, coupling of the sialyl-Tn-building block was performed with 8.46 mg (0.768 mmol) of **8**, 118 mg (0.768 mmol) of HOBt, 0.17 ml (1.536 mmol) of NMM, 247 mg (0.768 mmol) of TBTU, and DMF (14 ml) for 15 h. After the last coupling reaction, the Fmoc group was removed in the usual manner, and the N-terminus was acetylated with pyridine/Ac_2O 3:1 for 1.5 h. For the cleavage of the peptide or glycopeptide, the resin was dried thoroughly *in vacuo* and DMF/DMSO/morpholine (peptide: 8:8:1; glycopeptide: 7:7:0.6) was added and the mixture freed from O_2 by passing Ar through the suspension. A cat. amount of $[Pd(PPh_3)_4]$ was added, and the mixture was shaken for 18 h under Ar in the dark. The resin was filtered and washed with DMF. The combined solns. were evaporated, and the residue was dissolved in $CHCl_3$. This $CHCl_3$ soln. was washed twice with 0.5N HCl and twice with brine. The aq. phases were extracted twice with $CHCl_3$. The combined org. phase was evaporated and the resulting oil purified by chromatography ($CHCl_3$/EtOH).

N-*Acetyl-*L-*alanyl-*L-*prolyl-*L-*prolyl-*L-*alanyl-*L-*histidyl-glycyl-*L-*valyl-*L-*threonyl-*L-*seryl-*L-*alanyl-*L-*proline* (**13**). At r.t., anisole (0.2 ml), EtSMe (0.2 ml), and CF_3COOH (8 ml) were added to **11** (270 mg), and the mixture was stirred for 1.5 h. The soln. was concentrated and the residue crystallized by dropping it into Et_2O while stirring. The suspension was centrifuged to and the precipitate washed with Et_2O: **13** (119 mg, 30%; yield based on the initial amount of the starting L-proline on the resin after purification by reversed-phase HPLC(H_2O/MeCN 4:1) and lyophilization). HPLC (*A*: 0.1% CF_3 COOH in MeCN; *B*: 0.1% CF_3COOH/H_2O; → 100% *A* in *B* within 42 min); t_R 11.8 min. $[\alpha]_D^{22} = -142.5$, ($c = 0.9$, H_2O). ^{1}H-NMR (^{1}H,^{1}H-CPSY, 400 MHz, D_2O); 8.46 (*s*, 1 H, H−C(ε)(His)); 7.19 (*s*, 1 H, H−C(δ)(His)); 4.61–4.57 (*m*, 2 H, H−C(α)(pro,His)); 4.49, 4.43 (2*q*, *J*(α,β) = 7.0, 2 H, H−C(α(Ala)); 4.33 (*t*, *J*(α,β) = 5.3, 1 H, H−C(α)(Ser)); 4.31–4.30 (*m*, 1 H, H−C(α)(Thr)); 4.28–4.24 (*m*, 1 H, H−C(α(Pro))); 4.12–4.10 (*m*, 4 H, H−C(β)(Thr), H−C(α)(Ala, Pro, Val)); 3.86 (*q*, *J* = 16.3, 2 H, H−C(α)(Gly)); 3.71–3.48 (*m*, 8 H, H−C(β)(Ser) (3.81, (*d*, *J*(α,β) = 5.3)), H−C(δ)(Pro)); 3.16–3.08 (*m*, 2 H, H−C(β)(His)); 2.23–1.72 (*m*, 13 H, H−C(β)(Val) (2.11–2.07) H−C(β(Pro), H−C(γ)(Pro)); 1.87 (*s*, MeCO); 1.24, 1.21, 1.19, (*d*, 9 H, H−C(β)(Ala)); 1.08 (*d*, *J*(β,γ) = 6.3, 3 H,H−C(γ)(Thr)); 0.82 (*t*, 6 H, H−C(γ)(Val)). ^{13}C-NMR (100.6 MHz, D_2O): 174.8, 174.0, 173.7, 172.8, 172.2, 172.1, 171.8, 171.5, 171.5, 171.2, 170.7, 169.8 (C=O); 66.9 (C(β)(Thr)); 61.0 (C(β)(Ser)); 60.0, 59.5, 58.7 (C(α)(Pro)); 58.6, 55.2, 52.3 (C(α)(Val, Thr, His, Ser)); 49.5 (C(α)(Ala)); 47.6, 47.5, 47.4 (C(δ)(Pro)); 47.4 (C(α)(Ala)); 42.2 (C(α(Gly)); 30.0, (C(β)(Val)); 29.2, 27.9, 26.4, 24.6, 24.5, 24.4, 22.0, (C(β)(Pro,His), C(γ)(Pro)); 21.3 (*Me*CO); 18.6, 18.3, 17.5 (C(γ)(Thr, Val)); 16.3, 15.5, 15.2 (C(γ(Ala)). FAB-MS (glycerol/AcOH, pos.): 1048.7 (13.6, $[M(3 \times {}^{13}C)) + H]^+$; calc. 1048.5), 1047.7 (32.5, $[M(1 \times {}^{13}C) + H]^+$; calc. 1047.5), 1046.6 (52.0, $[M + H]^+$; calc. 1046.5). Anal. calc. for $C_{46}H_{71}N_{13}O_{15} \cdot H_2O$ (1136.1): C 48.59, H 7.13, N 16.02; found: C 48.42, H 7.14, N 15.91.

N-*Acetyl-*L-*alanyl-*L-*prolyl-*L-*prolyl-*L-*alanyl-*L-*histidyl-glycyl-*L-*valyl-O*^{3}*-{2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto*-non-2-ulopyranosyl)onate]-α-*D*-galactopyranoxyl}-*L*-threonyl-*L*-seryl-*L*-alanyl-*L*-proline* (**14**). As described for **13**, acidolysis of **12** (209 mg) with anisole (0.14 ml), EtSMe (0.1 ml), and CF_3COOH (5.5 ml) for 1 h 50 min at r.t. yielded **14** (137 mg, 42% based on the initial amount of the starting L-proline on the resin). HPLC (*A*: 0.1% CF_3COOH in MeCN; *B*: 0.1% aq. CF_3COO4; 1 → 100% *A* in *B* within 45 min): t_R 17.5 min. $[\alpha]_D^{22} = -58.6$ ($c = 1.00$, MeOH). R_f 0.26 ($CHCl_3$/MeOH/AcOH 8.8.1), 0.39 (BuOH/pyridine/AcOH/H_2O 4:1:1:2). ^{1}H-NMR (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY; 400 MHz, D_2O): 8.47 (br. *s*, 1 H, H−C(ε)(His)); 7.27 (br. *s*, 1 H, H−C(δ)(His)); 5.27 (*m*, H−C(4′), H−C(7″), H–C(8″)); 5.06 (*dd*, J(2′,3′) = 11.2, H–C(3′)); 5.01 (*d*, J(1′,2′) = 3.5, H–C(1′)); 4.79 (*ddd*, H–C(4″)); 4.70 (1 H, H–C(α)(His)); 4.60 (*dd*, 1 H, H–C(α)(Pro)); 4.53 (*d*, 1 H, H–C(α)(Thr)); 4.45 (*q*, 1 H, H–C(α)(Ala)); 4.36 (*dd*, 1 H, H–C(α)(Ser)); 4.29–4.06 (*m*, 11 H, H–C(α)(Ala), H–C(β)(Thr), H–C(α)(Pro), H_a–C(9″) (4.23), H–C(α)(Val) (4.21), H–C(2′) (4.18), H–C(5′) (4.15), H–C(α)(Ala, Pro) (4.12), H_b–C(9″) (4.10), H–C(6″) (4.09)); 3.87–3.65 (*m*, 13 H, H–C(α)(Gly) (3.85), H–C(5″) (3.79), H_a–C(6′) (3.76), MeO (3.74), H–C(β)(Ser) (3.70), H–C(δ)(Pro)); 3.52–3.53 (*m*, 2 H, H–C(δ)(Pro)); 3.36 (*dd*, 1 H, H_b–C(6′); 3.15, 3.09 (*m*, 2 H, H–C(β)(His)); 2.67 (*dd*, J(3″e,3″a) = 12.9, J(3″e,4″) = 4.4, H_e–C(3″)); 2.23–1.74 (*m*, 14 H, H–C(β)(Pro), H–C(γ)(Pro), H–C(β)(Val), H_a–C(3′) (1.83)); 2.14, 2.08, 2.05, 1.97, 1.92, 1.88, 1.80 (7*s*, 9MeCO); 1.28–1.20 (*m*, 12 H, H–C(β)(Ala) (1.27, H–C(β)(Ala), H–C(γ)(Thr)); 0.86 (*t*, 6 H, H–C(γ)(Val)). ^{13}C-NMR (100.6 MHz, D_2O): 174.9, 174.4, 174.2, 173.8, 173.4, 173.2, 173.0, 172.7, 172.5, 172.3, 172.2, 171.6, 171.1, 170.8, 168.8 (C=O); 99.3 (C(1′)); 98.9 (C(2″)); 77.9 (C(β)(Thr)); 71.9 (C(6″)); 69.6, 69.5 (C(3′), C(4″)); 68.7 (C(5′)); 68.4, 68.2, 67.7 (C(4′), C(7″), C(8″)); 63.4 (C(6′)); 62.4 (C(9″)); 61.6 (C(β)(Ser)); 60.4, 59.5, 58.9, 52.8, 49.8, 48.9, 47.9 (C(α)(Pro), C(α)(Val), C(α)(His), C(2′), C(5″), MeO, C(α)(Ala)); 57.3 (C(α)(Thr)); 55.4 (C(α(Ser)); 53.7 (C(α)(Pro)); 47.8, 47.7 (C(δ)(Pro)); 42.6 (C(α)(Gly)); 36.8 (C(3″)); 30.5 (C(β)(Val)); 29.5, 29.4, 28.2, 24.9, 24.8, 24.7, 24.6, (C(β)(Pro), C(γ)(Pro), C(β)(His)); 22.2, 20.2, 21.7, 20.7, 20.4, 20.3, 20.2, (*Me*CO), 18.6, 18.4, 18.8 (C(γ)(Thr, Val)), 16.6, 15.6, C(Cβ)(Ala)). FAB-MS (glycerol, pos.; $C_{78}H_{115}N_{15}O_{34}$ (1806.9)): 1807.3 (44, $[M + H]^+$, calc. 1806.8), 414.1 (24, $[Neu2,5dienAc_4{}^1Me]^+$; calc. 414.1), 185.1 (100, Y_2; calc. 185.1).

N-*Acetyl*-L-*alanyl*-L-*prolyl*-L-*prolyl*-L-*alanyl*-L-*histidyl-glycyl*-L-*valyl*-O^3-*[2-acetamido-2-deoxy-6*-O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulopyranosylonic acid)*-α-D-*galactopyranoxyl]*-L-*threonyl*-L-*seryl*-L-*alanyl*-L-*proline* (**15**). A soln. of **14** (45 mg, 24.9 μmol) in MeOH (16.5 ml) with 0.165 ml of aq. 1M NaOH was stirred for 2.5 h at r.t. The mixture was neutralized with 90 mM aq. AcOH (1.7 ml, pH 6) and evaporated. The residue was dissolved in 5 mM aq. NaOH (50 ml) was stirred for 3 h 45 min at r.t. With 2.65 ml of 90 mM aq. AcOH the soln. was neutralized to pH 6 and then the solvent distilled off. Purification by reversed-phase HPLC and lyophilization gave **15** (29 mg, 76%). t_R 9.6 min (*A*: MeCN; *B*; H_2O; 1 → 70% *A* in *B* within 45 min). $[\alpha]_D^{22} = -98.86$ (*c* 1.07, H_2O). R_f 0.08 (BuOH/pyridine/AcOH/H_2O 4:1:1:2). ^{1}H-NMR (^{1}H,^{1}H-COSY, 400 MHz, D_2O): 8.33 (br. *s*, 1 H, H–C(ε)(His)); 7.16 (br. *s*, 1 H, H–C(δ(His)); 4.84 (*d*, J(1′,2′ = 3.5, H–C(1′)); 4.61 (*dd*, 1 H, H–C(α(Pro)); 4.57 (*m*, 1 H, H–C(α(His)); 4.54 (*m*, 1 H, H–C(α(Thr)); 4.45 (*dd*, 1 H, H–C(α(Ala)); 4.40–4.37 (*m*, 2 H, H–C(α(Ser, Ala)); 4.27 (*dd*, 1 H, H–C(α(Pro)); 4.21–4.10 (*m*, 4 H, H–C(α)(Val) (4.30), H–C(β)(Thr) (4.27), H–C(α)(Ala) (4.23), H–C(α)(Pro) (4.22)); 3.99–3.90 (*m*, H–C(2′) (3.97) H_a–C(9″) (3.95)); 3.83–3.66 (*m*, H–C(8″) (3.81) H–C(β)(Ser) (3.74, 3.66) H–C(α)(Pro) (3.73, 3.66), H–C(3′) (3.72)); 3.60–3.45 (*m*, H–C(4″) (3.54), H–C(δ)(Pro) (3.52), H_e–C(9″) (3.46)); 3.13 (*m*, 2 H, H–C(β)(His)); 2.58 (*dd*, 1 H, J(3″e,3″a) = 12.3, J(3″e,4″) = 4.4, H_e–C(3′)); 2.24–1.76 (*m*, 13 H, H–C(β)(Pro), H–C(γ)(Pro), H–C(β)(Val) (2.00)); 1.92, 1.88 (*s*, 3MeCO); 1.54 (*t*, J(3″e,3″a) = 12.3, H_a–C(3″)); 1.29–1.20 (*m*, 12 H, H–C(β)(Ala) (1.28, 1.21, J(α,β) = 6.8), H–C(γ)(Thr)); 0.86 (*t*, J(β,γ) = 6.5, 6 H, H–C(δ)(Val)). ^{13}C-NMR (100.6 MHz, D_2O): 179.3, 175.1, 174.8, 174.0, 173.9, 173.7, 173.3, 172.8, 172.6, 172.2, 172.1, 171.2, 171.0, 170.5 (C=O); 100.4 (C(2″)); 99.2 (C(1′)); 77.1 (C(β)(Thr)); 72.6, 71.8, 70.1, 68.8, 68.4, 68.3, (C(3′), C(4′), C(5′), C(4″), C(6″), C(7″), C(8″)); 63.8, 62.8 (C(6′), C(9″)); 61.6 (C(β)(Ser)); 62.1, 60.2, 59.5, 58.7, 57.3, 55.0, 53.0, 52.0, 49.8, 49.7, 47.7, 47.6 (C(α)(Pro, Val, His), C(2′), C(5″), C(α)(Ala, Thr, Ser), C(δ)(Pro)); 42.5 (C(α)(Gly)); 40.2 (C(3″)); 30.2 (C(β)(Val)); 29.4, 29.3, 28.1, 27.4, 24.7, 24.6 (C(β)(Pro), C(γ)(Pro), C(β)(His)); 22.3, 22.1, 21.6 (MeCO); 18.5, 18.3, 17.8 (C(γ)(Thr, Val)); 16.5, 15.4, 15.1 (C(β)(Ala)). FAB-MS (glycerol, neg.; $C_{65}H_{101}N_{15}O_{28}$ (1540.6)): 1563.2 (13.3, $[M(2\times{}^{13}C) + Na - 2 H]^-$; calc. 1563.7), 1562.2 (31.6, $[M(1\times{}^{13}C) + Na - 2 H]^-$; calc. 1562.7), 1561.2 (41.5, $[M + Na - 2 H]^-$; calc. 1561.7), 1541.4 (11.0, $[M(2\times{}^{13}C) - H]^-$; calc. 1540.7), 1540.3 (23.9, $[M(1\times{}^{13}C) - H]^-$; calc. 1539.7), 1539.3 (35.4, $[M - H]^-$; calc. 1538.7), 493.4 (17.5 [α-NeuAc-(2 → 6)-α-GalNAc – H]$^-$; calc. 493.2), 372.1 (59.8, [Y_4-α-NeuAc-(2 → 6)-α-GalNAc]$^-$ calc. 372.2), 350.1 (43.9, A_4; calc. 350.2), 308.0 (42.3, B_3; calc. 308.2), 290.0 (59.7, [Neu2enAc – H]$^-$; calc. 290.1), 230.9 (12.1, [Neu2,5dien-H]$^-$; calc. 231.1), 169.8 (84.3, Z_2, calc. 170.1), 141.8 (66.4, X_1, calc. 142.1), 113.9 (99.0, B_1, Y_1; calc. 114.1), 112.8 (100, 'B_1, Y_1'; calc. 113.1).

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