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Synthesis of an Fmoc-Asn-heptasaccharide building block and its application to chemoenzymatic glycopeptide synthesis

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ABSTRACT

The Fmoc-protected heptasaccharide asparagine building block β-GlcNAc-(1→2)-α-Man-(1→3)-[β-GlcNAc-(1→2)-α-Man-(1→6)]β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-(Fmoc)Asn was obtained by chemical synthesis. Two flexible strategies were developed with optimized conditions for the simultaneous debenzylation of the sugar and the amino acid part. The heptasaccharide asparagine building block is a partial structure of many glycoproteins and can be used for glycopeptide synthesis in solution and on the solid phase. In this work the heptasaccharide asparagine was elongated in solution to an Fmoc-glycopentapeptide methylester. After chemical cleavage of the Fmoc group the methylester was removed enzymatically by chymotrypsin. The use of β-(1→4)-galactosyltransferase and α-(2→6)-sialyltransferase in the presence of alkaline phosphatase allowed the efficient transfer of four sugar units to the acceptor resulting in an undecasaccharide glycopentapeptide.

1. Introduction

With the growing demand for human recombinant glycoprotein therapeutics the synthesis of defined glycoforms of these valuable compounds is receiving an increasing interest. Due to the inherent heterogeneity of expressed glycoproteins and the lack of appropriate high-resolution separation methods, chemical synthesis[1–3] has advanced in recent years to become a viable option for the preparation of pure glycoforms. However, the synthesis of a glycoprotein is not a straightforward task and relies on many factors, notably the given protein sequence and the number and kind of glycosylation sites.[4] In particular, N-glycoproteins have challenged scientists and a few examples have been successfully synthesized recently.[5–10] Currently, the most flexible approach to synthetic N-glycoproteins appears to be based on native chemical ligation,[11] which typically requires the synthesis of glycopeptides and glycopeptide thioesters on the solid phase. In principle these glycopeptides can be assembled either by a late introduction of the glycosyl amine following the Lansbury protocol[12] or by an incorporation of an Fmoc-protected glycosyl amino acid.[13] Here we wish to report the synthesis of the Fmoc-Asn building block **1**, which is suitable for the synthesis of complex-type N-glycopeptides in solution[14] and on the solid phase[15] (Scheme 1). This glycosyl amino acid contains a deprotected biantennary N-glycan heptasaccharide, which was elongated in solution to a heptasaccharide glycopentapeptide. Enzymatic oligosaccharide extension[16] by β-(1→4)-galactosyltransferase and α-(2→6)-sialyltransferase gave the corresponding glycopeptide **A** bearing a biantennary N-glycan undecasaccharide.

2. Results and discussion

The synthesis of N-glycopeptides or N-glycopeptide thioesters with the complexity of compound **A** or **B** was demonstrated by utilizing building block **1**.[14,15] The Fmoc-protected glycosyl amino acid **1** was obtained from the chemically synthesized heptasaccharide azide **2**[17–20] following two routes, which were optimized and differ from our initial report.[14] An alternative approach[21] to compound **1** was established using a glycopeptide isolated from egg yolk.[22]

The heptasaccharide **2** can be synthesized according to the double regio- and stereoselective glycosylation approach[23] and subsequent removal of the base-labile protecting groups.[18] Thus heptasaccharide **2** retains an anomeric azide and four benzyl ethers. This protection pattern requires the selective reduction of the azide moiety and coupling to an amino acid prior to the debenzylation of the sugar part.[17] A selective debenzylation in the presence of an azide would be desirable; however, for oligosaccharides no promising method has been established. Due to the susceptibility of the Fmoc group to catalytic hydrogenation[24] the introduction of an Fmoc-Asp-OBzl residue was a priori discarded and an *N*-benzyloxycarbonyl group was employed for the aspartic acid derivatives **3** and **4**. A subsequent global debenzylation of the heptasaccharide-Asn conjugates was envisioned followed by the installation of the Fmoc moiety.

Prior to the attachment of Z-Asp-OBzl **3**, heptasaccharide azide **2** (Scheme 2) was reduced in methanol using 1,3-propanedithiol

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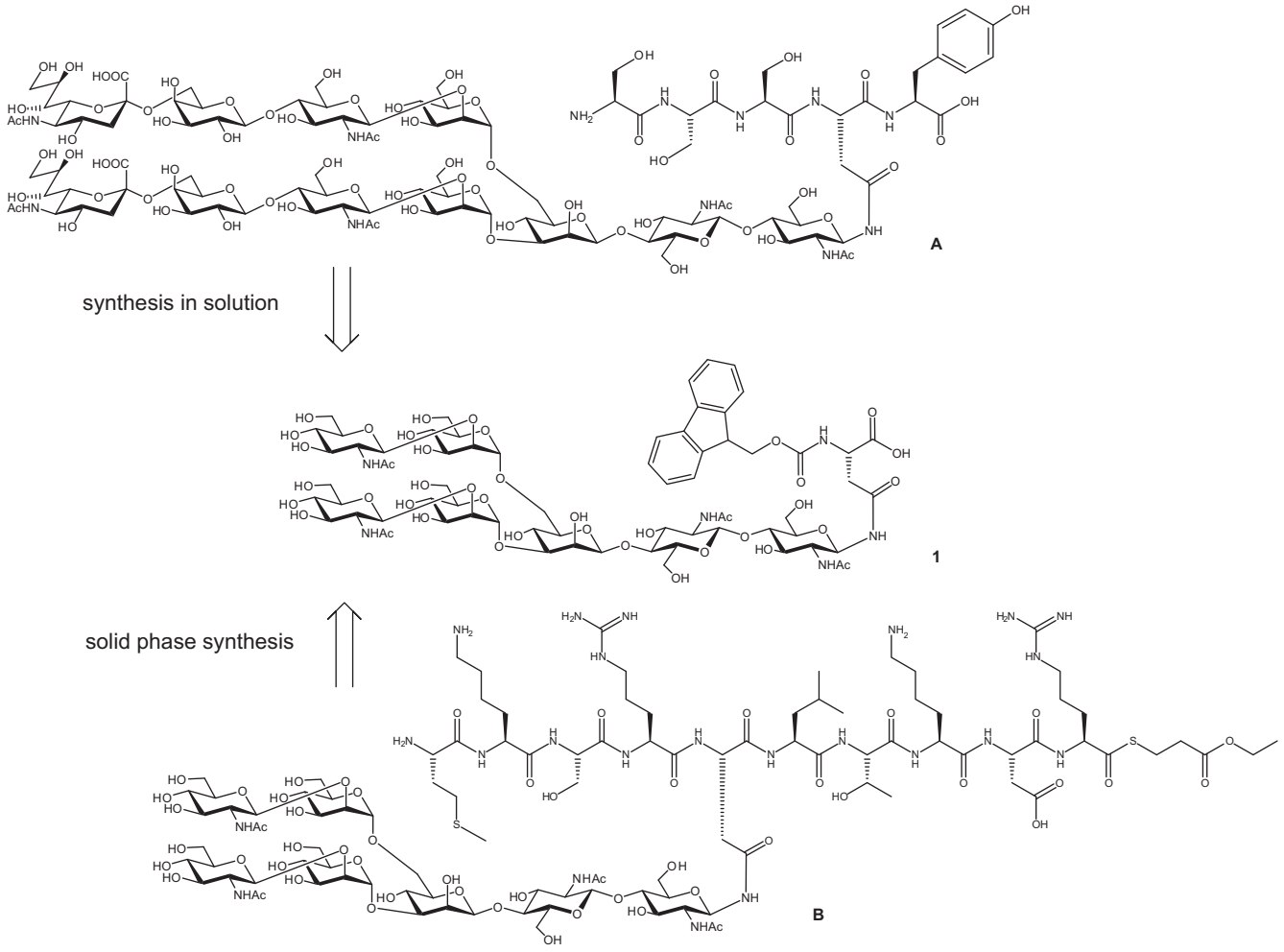


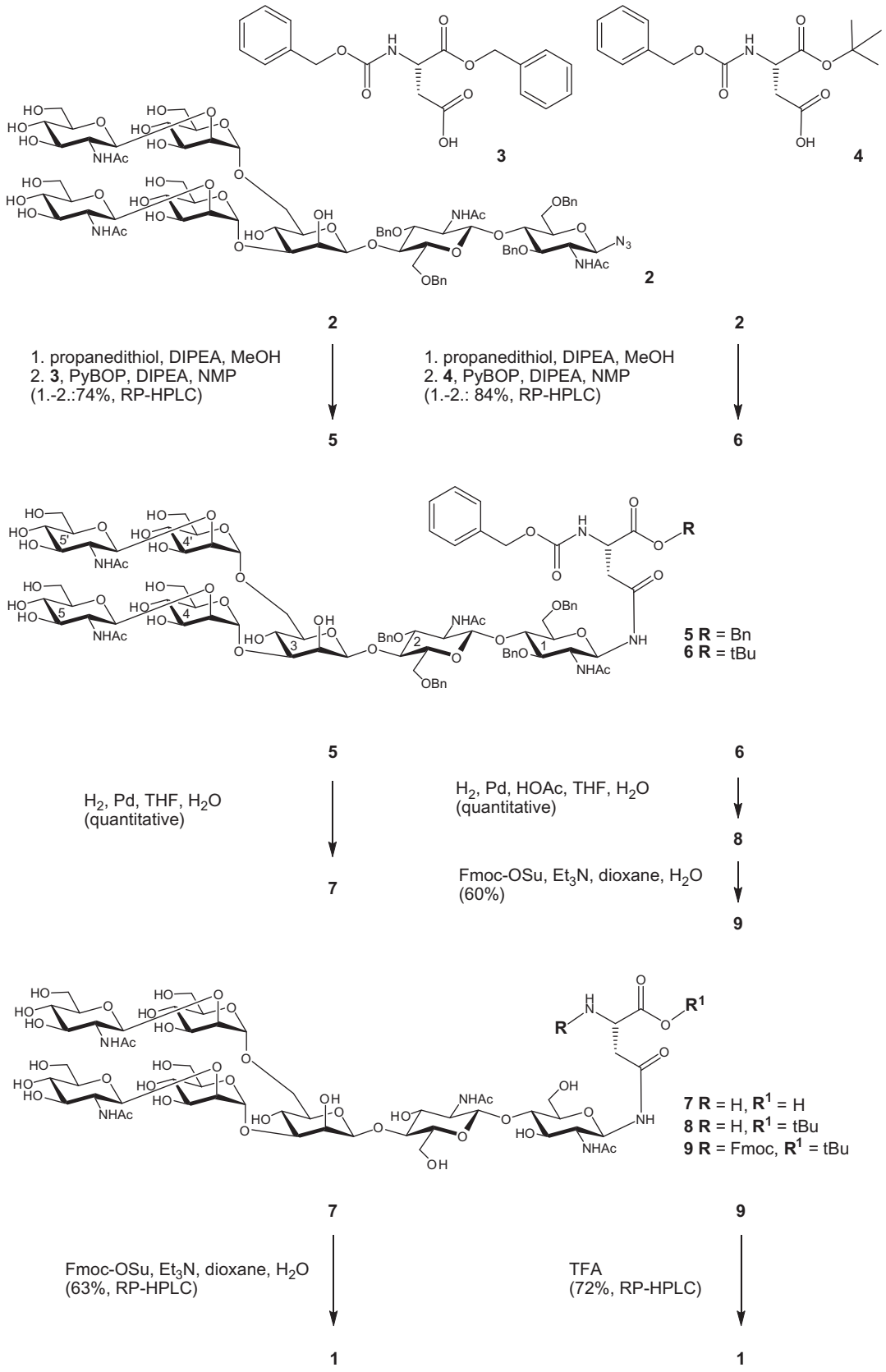
Scheme 1.

and DIPEA,[25] which ensures basic conditions, thus preventing anomerization of the glycosyl amine. Special attention is needed when coupling the aspartic acid side chain with the glycosyl amine. The unprotected hydroxyl groups of the sugar moiety are susceptible to acylation, therefore only a minimal excess of amino acid should be used. Additionally, an appropriate solvent is required to properly dissolve both the polar heptasaccharide and the hydrophobic aspartic acid derivative **3**. In our initial report, the coupling of Z-Asp-OBzl (**3**) to the glycosyl amine was accomplished via an intermediate pentafluorophenyl ester.[26] A more convenient protocol employs the in situ activation of the aspartic acid side chain using PyBOP (benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate). After a short preactivation, 4 equiv of **3** gave full acylation of the glycosyl amine in NMP (*N*-methylpyrrolidone) within 10 min. The majority of the reagents were separated from the resulting glycosyl amino acid **5** by solid-phase extraction (SPE), and subsequently **5** was purified by RP-HPLC. A preliminary isolation of **5** by SPE permitted faster HPLC purification, furnishing pure **5** in 74% yield. Long coupling times and excess amino acid are undesirable, as random esterification occurred at one of the hydroxyl functions. The monoacylated by-products, approximately 6% overall yield, were separated by SPE in a fraction eluting after **5**.

The hydrogenolysis of compound **5** required special conditions and the choice of the solvent was fundamental for the outcome of the reaction. Initially, PdO-hydrate was used as a catalyst in methanol containing 10% of acetic acid. After hydrogenolysis of **5**, two by-products were found along with the fully unprotected glycosyl amino acid **7**. Electrospray-ionization mass spectrometry indicated that the methyl ester **7b** of **7** had formed (m/z +CH_3) along with an aspartimide **7a** (m/z –H_2O). Succinimide formation of aspartic acid derivatives is a known problem in peptide synthesis,[27,28] and β-benzyl esters of aspartic acid undergo both acid- and base-catalyzed ring closure.[29] Nevertheless, aspartimide formation from a glycosyl asparagine α-benzyl ester has not been described so far. It is likely that the acetic acid renders the benzyl ester susceptible to transesterification and to intramolecular nucleophilic attack by the nitrogen on the β-amide. The formation of a glycosylated aspartimide after activation of the α-carboxylate was reported.[30,31]

To overcome the side reactions during hydrogenolysis different solvent systems were tested. Among these, a mixture of THF and water proved to be the most effective. No acid was required and both components were non-nucleophilic. Indeed, in this solvent the side products found in methanol–acetic acid were not generated. Hence, further purification was not necessary and the palladium catalyst was simply filtered off after hydrogenation to afford **7** nearly quantitatively. The N-terminus of **7** was protected using Fmoc-OSu and triethylamine, without affecting either the hydroxyl or the carboxyl functions.[32] The target building block **1** was isolated by solid-phase extraction removing unreacted Fmoc-OSu and further purified by RP-HPLC.

A second approach to the glycosyl amino acid **1** was conducted using Z-Asp-O*t*Bu **4**. The *tert*-butyl ester is stable to hydrogenation and should reduce the risk for both transesterification and succinimide formation. Furthermore a *tert*-butyl ester should allow for a selective acetylation of the complete carbohydrate part at a



Scheme 2.

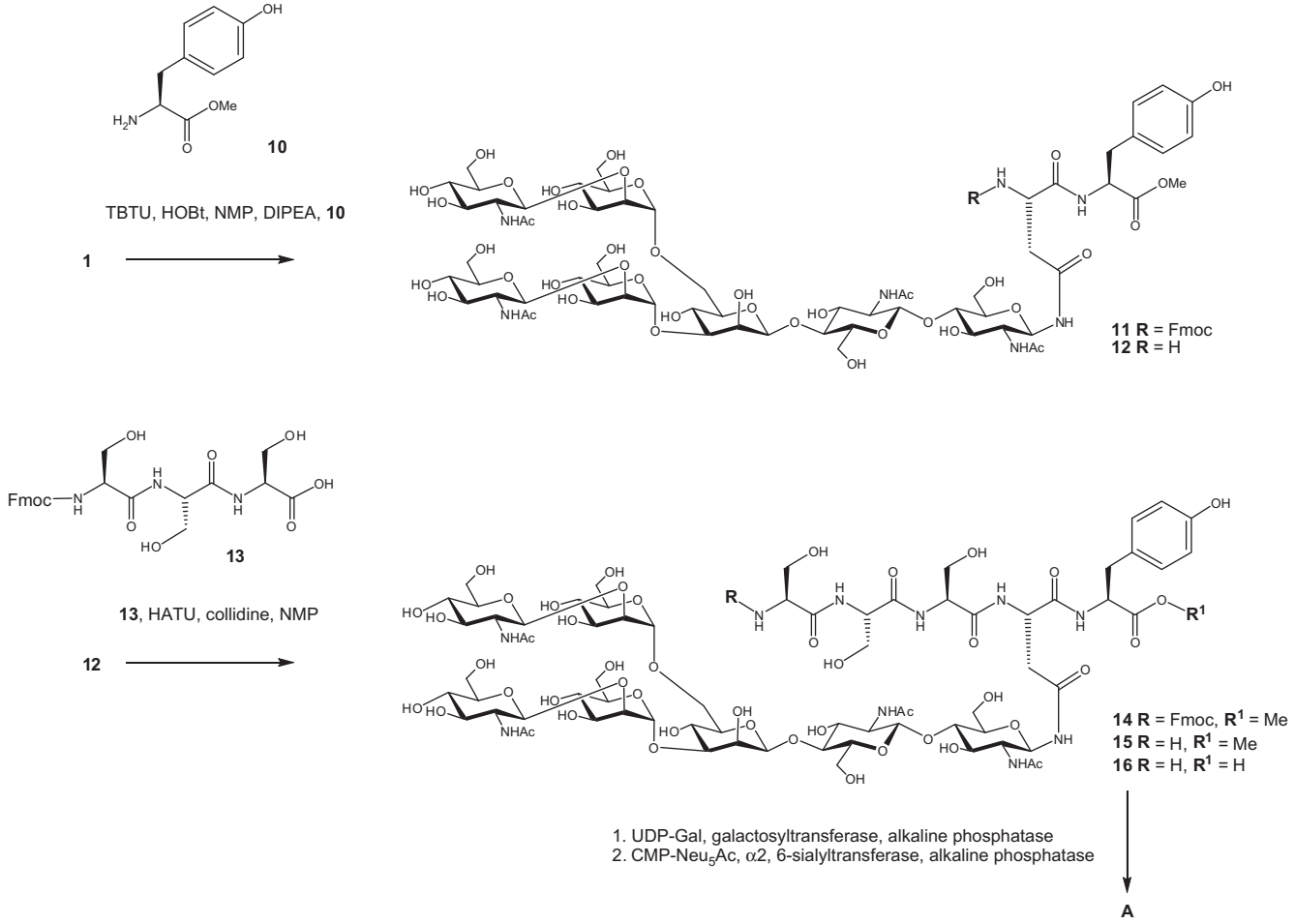
later stage (e.g., compound **9**) by preventing unwanted activation of the α-carboxyl group via a mixed anhydride. For this approach, Z-Asp-O*t*Bu **4** was liberated from the corresponding dicyclohexylammonium salt. Azide **2** was reduced as described above and subsequently coupled with **4** preactivated with PyBOP in NMP. Again, the coupling was complete within a few minutes. It

is worth mentioning that if water was added before solvent removal, only 2% of over acylated product was isolated after solid-phase extraction. In the coupling experiments where the excess of activated amino acid was not hydrolyzed in situ, up to 10% of the glycosyl amino acid had undergone unwanted esterification by the aspartic acid at one hydroxyl group, mainly during concentration of the coupling solution.

Compound **6** can be hydrogenated more safely than the corresponding benzyl ester **5**. No aspartimide formation could be detected when **6** was dissolved in methanol–acetic acid, whereas the benzyl ester **5** gave rise to ring closure under the same conditions. Unexpectedly, hydrogenolysis of **6** in THF–water proceeded much slower than **5**. While the Z group was quickly cleaved, the benzyl ethers remained intact even after prolonged hydrogenation, as confirmed by mass spectrometry. It can be assumed that the liberated amino group interferes with the palladium catalyst, whereas in the case of **7** the amino group is protonated in the zwitterionic amino acid. Fully debenzylated **8** was finally obtained after addition of 5% acetic acid to the THF–water hydrogenation solvent. The *tert*-butyl ester of **8** was completely stable under these conditions and the amino group was subsequently masked with an Fmoc function to furnish **9**.

Compound **9** is a versatile intermediate, which is amenable to O-acetylation without side reactions, because both the C- and the N-terminus are blocked.[33] Additionally, the *tert*-butyl and the Fmoc protective groups are orthogonal, providing greater flexibility than the strategy mentioned above. However, hydroxyl protection of the glycosyl amino acid[33] was not required because **1** could be successfully coupled on a solid support.[15] The *tert*-butyl ester was finally cleaved with TFA and Fmoc heptasaccharide asparagine **1** was obtained in 72% yield after RP-HPLC. The structure was identical to the batch synthesized via **5**, as confirmed by NMR, MS and retention times on HPLC.

With the Fmoc-heptasaccharide-Asn building block **1** in hand the reactivity in peptide couplings was probed on the partial sequence 21–25 of bovine ribonuclease. After activation of **1** using TBTU–HOBt (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate–1-hydroxybenzotriazole) in NMP the coupling to tyrosine methyl ester **10** was complete within 10 min. The resulting glycopeptide **11** was isolated in 74% after RP-HPLC. Removal of the Fmoc group was achieved in NMP containing 10% of piperidine. Notably the Fmoc-deprotection required a reaction time of 30 min indicating a steric hindrance of the substrate. Under these conditions the methyl ester was not affected and the intermediate glycopeptide-amine **12** was isolated by RP-HPLC. The segment coupling of **12** with the Fmoc-protected serine tripeptide **13** required some optimization. A test reaction indicated that the activation of **13** with TBTU–HOBt gave only slow coupling with **12**. When the stronger coupling reagent HATU was employed in the presence of collidine[34] the formation of the glycopentapeptide proceeded to completion within 10 min and the protected glycopeptide **14** was obtained in 70% yield after HPLC purification. The HPLC peak for **14** showed a satellite peak (10–15%), which was efficiently removed during purification indicating that some racemization[35] might have occurred during the segment coupling. Despite the unprotected hydroxyl groups of the heptasaccharide part only the amino group was found to react in a chemoselective manner (Scheme 3).



Scheme 3.

Prior to the envisioned enzymatic elongation of the sugar part the Fmoc group and the methyl ester were removed because these functions tend to be unstable in the presence of free amino groups found in proteins. At first, the Fmoc group was cleaved in NMP containing 5% of piperidine followed by enzymatic hydrolysis of the methyl ester with the protease chymotrypsin without intermediate purification. In contrast to the alkaline hydrolysis of methyl esters[36] their enzymatic hydrolysis proceeds without racemization. Chymotrypsin is selective for the cleavage of peptides and peptide esters after aromatic amino acids or leucine residues.[37] In the peptide sequence of intermediate **15**, only one cleavage site is present at the C-terminal methyl ester, which allows for an aqueous reaction medium and extended reaction times. Generally, proteases convert the more reactive esters faster than the corresponding amides. In the case of additional cleavage sites within the peptide sequence, the amidase activity of a protease can be greatly reduced by conducting the reaction in the presence of an organic cosolvent (e.g., 50% DMF) thus ensuring selective hydrolysis of the ester over the amide.[38] After complete chemical and enzymatic deprotection, the free glycopeptide **16** was isolated by gel filtration in 96% yield.

The stepwise elongation of the terminal GlcNAc moieties of the heptasaccharide part of **16** was accomplished by enzymatic sugar transfer[39–42] in a one-pot manner.[16] Dual galactosylation was achieved by using bovine β-(1→4)-galactosyltransferase, UDP-galactose, and the reactivity-enhancing alkaline phosphatase.[16] According to TLC analysis the reaction had already proceeded to completion within 2 h at 37 °C. The subsequent dual α-(2→6)-sialylation was carried out by adding α-(2→6)-sialyltransferase and CMP-sialic acid to the reaction mixture. However, this conversion required longer incubation times and a second addition of enzyme and sugar nucleotide. In the subsequent separation of the reaction mixture by gel filtration, only 3% of monosialylated nonasaccharide was found indicating that the enzymatic galactosylation was nearly quantitative. The desired undecasaccharide-glycopentapeptide **A** was obtained in 91% yield, which corresponds to an average yield per enzymatic sugar transfer of over 97%. The purity of the final glycopeptide **A** can be viewed in the 500 MHz ^{1}H NMR spectrum (Scheme 4). The assignments of the ^{1}H and ^{13}C resonances of the synthetic building blocks and glycopeptides were carried out by 2D-NMR methods[43] including TOCSY, NOESY, HMQC, HMQC–COSY, HMQC–DEPT,[44] and HMQC–TOCSY.

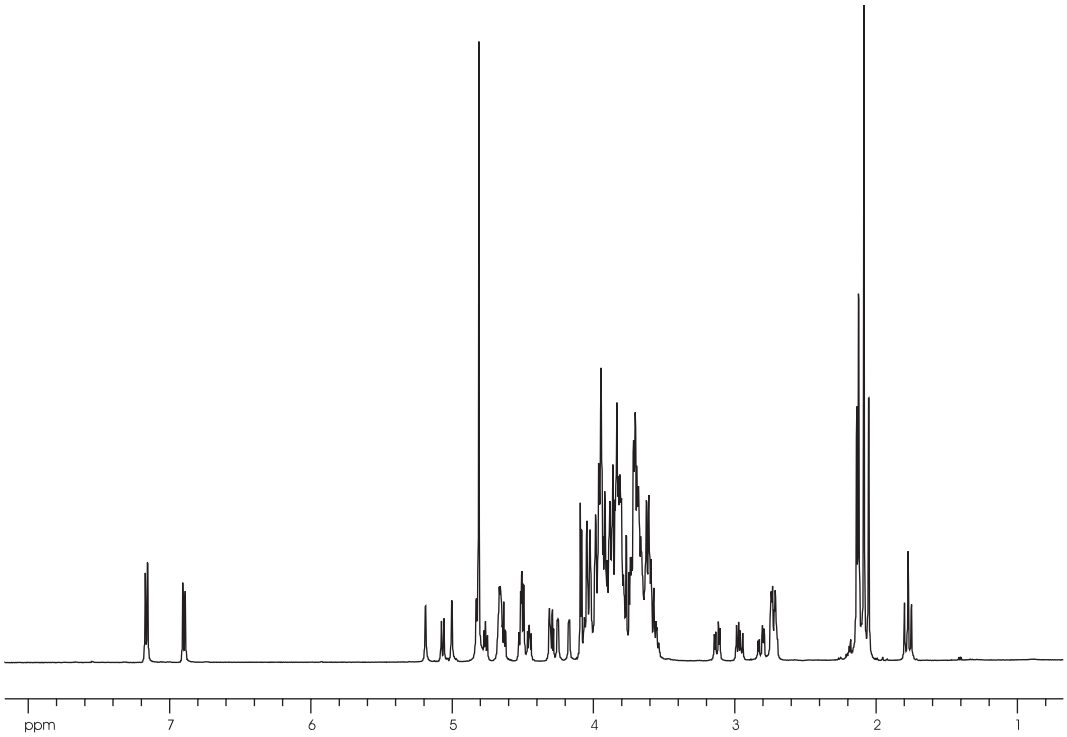
In summary, we have optimized the attachment of aspartic acid onto partially protected synthetic N-glycans. These glycoconjugates were deprotected and converted to their Fmoc derivatives. The glycosyl amino acid **1** can be applied either to solid-phase synthesis or to the solution synthesis leading to glycopeptides in good yields and high purity. Efficient methods for the generation of complex glycopeptides and derivatives thereof are a prerequisite for the further development of chemical synthesis of homogeneous glycoproteins.

3. Experimental

3.1. General methods

TLC was performed on silica gel plates (60-F254; E. Merck, Darmstadt) and visualized by spraying with 0.5 M H_2SO_4 in EtOH containing 0.1% orcinol. UDP-galactose, cytidine-5′-monophospho-*N*-acetylneuraminic acid, bovine β-(1→4)-galactosyltransferase (E.C. 2.4.1.22.), α-(2→6)-sialyltransferase (E.C. 2.4.99.1.), and bovine serum albumin were obtained from Sigma Chemical Co. Calf intestinal alkaline phosphatase (molecular biology grade) (E.C. 3.1.3.1.) was obtained from Boehringer Mannheim. Amino acids, peptides, and other reagents were purchased from Bachem (Läufelfingen, Switzerland). PyBOP was purchased from Iris Biotech (Marktredwitz, Germany).

For size exclusion chromatography a Pharmacia Hi Load Superdex 30 column (600 × 16 mm) was used on a Pharmacia LKB gradient system 2249 equipped with a Pharmacia LKB Detector VWM 2141 (Freiburg, Germany). Analytical and preparative HPLC was performed on either a Pharmacia Äkta Purifier 10 or a Pharmacia Äkta Basic device.



Scheme 4. 500 MHz-^{1}H NMR of **A** in D_2O.

Solid-Phase Extraction (SPE) was performed with Waters Sep-Pak Vac C18 cartridges. NMR spectra were recorded on Bruker AMX 500 and DRX 500 instruments. For spectra recorded in D_2O the HOD signal (4.81 ppm) was used as a reference. ESI-TOF mass spectra were recorded on a Micromass LCT instrument coupled to an Agilent 1100 HPLC. MALDI-TOF mass spectra were recorded on a Voyager Biospectrometry workstation (Vestec/Perseptive) MALDI-TOF mass spectrometer, using 2,5-dihydroxybenzoic acid (DHB) as a matrix. Specific rotation was recorded on a Perkin–Elmer Polarimeter 241.

3.2. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)}-N^2-benzyloxycarbonyl-L-asparagine benzyl ester 5

Heptasaccharide **2** (294 mg, 173 μmol, 1 equiv) was dissolved in freshly distilled MeOH (9 mL) and ethyldiisopropylamine (DIPEA) (305 μL, 1.75 mmol) was added. The reaction flask was purged with argon and 1,3-propanedithiol (915 μl, 9.11 mmol) was added. The solution was stirred for 2 h at room temperature (TLC: isopropanol–1 M NH_4OAc 4:1), concentrated in vacuo, and the resulting glycosyl amine was dried under high vacuum. Amino acid **3** (256 mg, 716 μmol, 4 equiv) and PyBOP (373 mg, 716 μmol) were dissolved in NMP (4 mL) and treated with DIPEA (245 μL, 1.41 mmol). After 10 min the preactivation mixture was added to the glycosyl amine and the solution was stirred for 30 min at room temperature. The mixture was concentrated under high vacuum and the residue was isolated by solid-phase extraction (one 10 g cartridge), in order to remove the majority of the reagents. The solid was dissolved in acetonitrile–water (100 mL, 1:4) and loaded onto the cartridge. Impurities were washed off with acetonitrile–water (200 mL, 30% acetonitrile), and **5** was eluted with acetonitrile–water (200 mL, 2:3). The fractions containing the product were pooled, lyophilized, and further purified by RP-HPLC (column: Macherey-Nagel Nucleogel RP 100-10 (250 × 21 mm), eluent: 32% acetonitrile–water, flow rate: 8 mL/min) to afford **5** (268 mg, 77%). $[\alpha]_D^{23}$ −0.3 (*c* 0.5, MeOH); R_f glycosyl amine = 0.51 (isopropanol–1 M NH_4OAc 4:1); R_f **5** = 0.77 (isopropanol–1 M NH_4OAc 4:1); ^{1}H NMR (500 MHz, [D_6] DMSO): δ = 8.44 (d, $J_{NH,1}$ = 8.8 Hz, 1H, γNH Asn), 7.99 (d, $J_{NH,2}$ = 7.1 Hz, 1H, NH-2^2), 7.83 (d, $J_{NH,2}$ = 8.9 Hz, 1H, NH-2^1), 7.58 (m, 3H, NH-2^5, NH-2$^{5'}$, NH-urethane), 7.37–7.18 (m, 20H, Ar), 5.11, 5,06 (2d, J_{gem} = 12.8 Hz, 2H, CH_2O), 5.05–4.94 (m, 11H, H-1^1, H-1^4, OH-3^5, OH-3$^{5'}$, OH-4^3, OH-4^5, OH-4$^{5'}$, CH_2O), 4.75 (d, $J_{OH,4}$ = 4.8 Hz, 1H, OH-4^4), 4.71 (d, $J_{OH,4}$ = 4.8 Hz, 1H, OH-4$^{4'}$), 4.66 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.62–4.40 (m, 12H, αCH Asn, H-1^2, H-1^3, OH-2^3, OH-6^4, OH-6^5, OH-6$^{5'}$, CH_2O), 4.36 (d, $J_{1,2}$ = 8.1 Hz, 1H, H-1^5), 4.31 (d, J_{gem} = 12.2 Hz, 1H, CH_2O), 4.23 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1$^{5'}$), 4.19 (m, 1H, OH-6$^{4'}$), 3.99–3.94 (m, 2H, H-2^3, OH-3^4), 3.90 (d, $J_{OH,3}$ = 8.0 Hz, 1H, OH-3$^{4'}$), 3.88–3.83 (m, 2H, H-2^4, H-4^1), 3.78–3.21 (m, 34H, H-2^1, H-2^2, H-2$^{4'}$, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-5^1, H-5^2, H-5^4, H-5$^{4'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 3.14–3.02 (m, 5H, H-4^5, H-4$^{5'}$, H-5^3, H-5^5, H-5$^{5'}$), 2.67 (dd, J_{gem} = 16.1 Hz, J_{vic} = 5.0 Hz, 1H, βCHa Asn), 2.50 (dd, J_{vic} = 7.2 Hz, 1H, βCHb Asn), 1.80, 1.77, 1.70 (3s, 12H, NAc); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.2 (C═O ester), 169.7, 169.5, 169.4, 169.3 (C═O NAc), 155.8 (C═O urethane), 139.2, 138.5, 138.4, 136.7, 135.9 (C-i Ar), 128.3–126.9 (C-Ar), 101.5 (C-1$^{5'}$), 101.1 (C-1^5), 100.2 (C-1^3), 99.8 (C-1^4), 99.6 (C-1^2), 97.6 (C-1$^{4'}$), 81.5 (C-3^3), 81.4 (C-3^1), 80.8 (C-3^2), 79.2 (C-2$^{4'}$), 78.8 (C-2^4), 78.5 (C-1^1), 77.0 (C-5^5), 76.9 (C-5$^{5'}$), 76.1 (C-5^1), 75.9 (C-4^2), 75.5 (C-5^3), 74.6 (C-4^1), 74.4 (C-5^2), 74.2 (C-5^4), 73.9 (C-3^5, C-3$^{5'}$), 73.8 (CH_2O), 73.6 (C-5$^{4'}$), 73.2, 72.2, 71.8 (CH_2O), 70.5 (C-4$^{5'}$), 70.3 (C-4^5), 69.9 (C-3^4, C-3$^{4'}$), 69.1 (C-2^3), 68.7 (C-6^2), 68.3 (C-6^1), 67.6 (C-4^4), 67.1 (C-4$^{4'}$), 66.0 (CH_2O, C-6^3), 65.6 (CH_2O), 64.9 (C-4^3), 61.5 (C-6^4), 61.1 (C-6$^{4'}$), 61.0 (C-6^5), 60.8 (C-6$^{5'}$), 55.6 (C-2^5, C-2$^{5'}$), 55.3 (C-2^2), 53.2 (C-2^1), 50.4 (αC Asn), 36.8 (βC Asn), 23.2, 22.9, 22.7 (NAc); ESI-MS (*m/z*) calcd for $C_{97}H_{126}N_6O_{40}$ $[M]^+$: 2014.80; found: 2037.81 $[M+Na]^+$, 1030.41 $[M+2Na]^{2+}$.

3.3. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-L-asparagine 7

Protected glycosyl amino acid **5** (129 mg, 64.0 μmol) and PdO-hydrate (190 mg) were suspended in THF–water (10 mL, 1:1). The reaction flask was purged with argon and the mixture was stirred vigorously under a hydrogen atmosphere for 41 h (TLC: isopropanol–1 M NH_4OAc 2:1). The solvent was removed in vacuo and after dilution with water, the catalyst was filtered off through a syringe filter unit (Millipore, IC Millex-LG, 25 mm external diameter, 0.2 μm pore size). The clear solution was lyophilized affording crude **7** (89.8 mg, 98%), which was used in the next step without purification. $[\alpha]_D^{23}$ −0.3 (*c* 1, H_2O); R_f **5** = 0.91 (isopropanol–1 M NH_4OAc 2:1); R_f **7** = 0.27 (isopropanol–1 M NH_4OAc 2:1); ^{1}H NMR (500 MHz, D_2O): δ = 5.17 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 5.12 (d, $J_{1,2}$ = 9.7 Hz, 1H, H-1^1), 4.97 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.82 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.66 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1^2), 4.60 (m, 2H, H-1^5, H-1$^{5'}$), 4.30 (dd, $J_{2,3}$ = 1.9 Hz, 1H, H-2^3), 4.24 (dd, $J_{2,3}$ = 1.9 Hz, 1H, H-2^4), 4.16 (dd, $J_{2,3}$ = 1.9 Hz, 1H, H-2$^{4'}$), 4.03–3.45 (m, 40H, αCH Asn, H-2^1, H-2^2, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-4^5, H-4$^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-5$^{4'}$, H-5^5, H-5$^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.92 (dd, J_{gem} = 16.6 Hz, J_{vic} = 4.1 Hz, 1H, βCHa Asn), 2.79 (dd, J_{vic} = 7.3 Hz, 1H, βCHb Asn), 2.13, 2.10, 2.06 (3s, 12H, NAc); ^{13}CNMR (125 MHz, D_2O, DMSO-d_6 as internal standard): δ = 177.0, 176.4, 176.3, 176.2, 174.8 (C═O), 102.8 (C-1^2), 102.0 (C-1^3), 101.2 (C-1^5, C-1$^{5'}$), 101.1 (C-1^4), 98.6 (C-1$^{4'}$), 82.0 (C-3^3), 81.1 (C-4^2), 80.4 (C-4^1), 79.7 (C-1^1), 78.1 (C-2^4), 78.0 (C-2$^{4'}$), 77.8 (C-5^1), 77.4 (C-5^5, C-5$^{5'}$), 76.0 (C-5^2), 75.9 (C-5^3), 75.1 (C-5^4), 75.0, 74.9 (C-3^5, C-3$^{5'}$), 74.44 (C-5$^{4'}$), 74.39 (C-3^1), 73.6 (C-3^2), 71.8 (C-2^3), 71.5 (C-4^5, C-4$^{5'}$), 71.1 (C-3$^{4'}$), 71.0 (C-3^4), 68.93 (C-4$^{4'}$), 68.90 (C-4^4), 67.4 (C-6^3), 67.3 (C-4^3), 63.3, 63.2 (C-6^4, C-6$^{4'}$), 62.2 (C-6^5, C-6$^{5'}$), 61.54 (C-6^2), 61.48 (C-6^1), 56.9 (C-2^5, C-2$^{5'}$), 56.5 (C-2^2), 55.2 (C-2^1), 53.2 (αC Asn), 38.5 (βC Asn), 23.9, 23.8, 23.7 (NAc); ESI-MS (*m/z*) calcd for $C_{54}H_{90}N_6O_{38}$ $[M]^+$: 1430.53; found: 1431.45 $[M+H]^+$, 1453.44 $[M+Na]^+$. Aspartimide by-product **7a**: ESI-MS (*m/z*) calcd for $C_{54}H_{88}N_6O_{37}$ $[M]^+$: 1412.52; found: 1435.44 $[M+Na]^+$. Methyl ester by-product **7b**: ESI-MS (*m/z*) calcd for $C_{55}H_{92}N_6O_{38}$ $[M]^+$: 1430.53; found: 1445.60 $[M+H]^+$, 1467.57 $[M+Na]^+$.

3.4. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(9-fluorenylmethoxycarbonyl)-L-asparagine 1

The glycosyl amino acid **7** (90.2 mg, 63.0 μmol) was dissolved in water (9 mL) and treated with TEA (18 μL, 129 μmol). A solution of Fmoc-OSu (70.0 mg, 207 μmol) in dioxane (6 mL) was added and the reaction mixture was allowed to stir for 2 h at room temperature (TLC: isopropanol–1 M NH_4OAc 2:1). Subsequently, a second

portion of Fmoc-OSu (19.5 mg, 57.8 μmol) and TEA (10 μL, 71.7 μmol) were added. After 4 h the reaction was quenched with acetic acid (24 μL, pH 5) and lyophilized. The residue was diluted with water (100 mL), centrifuged, and the supernatant was loaded onto a 5-g Waters RP cartridge in order to remove the majority of the reagents by solid-phase extraction. Impurities were washed off with water (100 mL) and **1** was eluted with acetonitrile–water (100 mL, 1:9). The fractions containing the product were pooled, lyophilized, and further purified by RP-HPLC (column: YMC-Pack ODS S-5 μm (250x20 mm), gradient: 27–55% acetonitrile (0.1% trifluoroacetic acid), flow rate: 9.5 mL/min) to afford **1** (65.6 mg, 63%). $[\alpha]_D^{23}$ −3.7 (*c* 0.3, H_2O); R_f = 0.75 (isopropanol–1 M NH_4OAc 2:1); ^1H NMR (500 MHz, D_2O, DMSO-d_6 as internal standard): δ = 7.7–7.3 (m, 8H, Fmoc), 4.93 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 4.81 (d, $J_{1,2}$ = 9.5 Hz, 1H, H-1^1), 4.73 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.56 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.40–4.35 (m, 3H, H-1^2, H-1^5, H-1$^{5'}$), 4.28 (m, 2H, CH_2O), 4.10 (m, 1H, H-9 Fmoc), 4.05 (m, 1H, H-2^3), 3.99 (m, 1H, H-2^4), 3.91 (m, 1H, H-2$^{4'}$), 3.77–3.20 (m, 40H, αCH Asn, H-2^1, H-2^2, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-4^5, H-4$^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-5$^{4'}$, H-5^5, H-5$^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.54 (dd, J_{gem} = 15.2 Hz, J_{vic} = 2.6 Hz, 1H, βCHa Asn), 2.37 (dd, J_{vic} = 8.7 Hz, 1H, βCHb Asn), 1.88, 1.86 (2s, 12H, NAc); ^{13}C NMR (125 MHz, D_2O, DMSO-d_6 as internal standard): δ = 176.2, 176.0, 174.8 (C=O), 159.1 (C=O urethane), 145.4, 142.5, 129.7, 129.1, 126.8, 121.8 (C-Ar), 102.9 (C-1^2), 102.0 (C-1^3), 101.2 (C-1^4, C-1^5, C-1$^{5'}$), 98.6 (C-1$^{4'}$), 82.0 (C-3^3), 81.1 (C-4^2), 80.3 (C-4^1), 79.8 (C-1^1), 78.02 (C-2^4), 77.95 (C-2$^{4'}$), 77.7 (C-5^1), 77.4 (C-5^5, C-5$^{5'}$), 75.9 (C-5^2, C-5^3), 75.1 (C-5^4), 75.95, 74.85 (C-3^5, C-3$^{5'}$), 74.4 (C-5$^{4'}$), 74.3 (C-3^1), 73.5 (C-3^2), 71.7 (C-2^3), 71.5 (C-4^5, C-4$^{5'}$), 71.0 (C-3^4, C-3$^{4'}$), 68.9 (C-4^4, C-4$^{4'}$), 68.1 (CH_2O), 67.4 (C-6^3), 67.2 (C-4^3), 63.3, 63.2 (C-6^4, C-6$^{4'}$), 62.2 (C-6^5, C-6$^{5'}$), 61.5 (C-6^2), 61.3 (C-6^1), 56.9 (C-2^5, C-2$^{5'}$), 56.5 (C-2^2), 55.4 (C-2^1), 54.0 (αC Asn), 48.4 (C-9 Fmoc), 39.9 (βC Asn), 23.9, 23.8, 23.6 (NAc); ESI-MS (*m*/*z*) calcd for $C_{69}H_{100}N_6O_{40}$ $[M]^+$: 1652.60; found: 1675.49 $[M+Na]^+$, 1691.42 $[M+K]^+$, 849.25 $[M+2Na]^{2+}$.

3.5. N^2-Benzyloxycarbonyl-L-aspartic acid α-*tert*-butyl ester 4

N^2-Benzyloxycarbonyl-L-aspartic acid α-*tert*-butyl ester dicyclohexylamine salt (Bachem) (5 g, 9.91 mmol) was dissolved in CH_2Cl_2 (300 mL), washed with 5% $KHSO_4$ (3×) and water, dried over $MgSO_4$, filtered, and concentrated in vacuo affording **4** (3.17 g, 99%). R_f = 0.62 (CH_2Cl_2–MeOH–HOAc 90:8:2); ESI-MS (*m*/*z*) calcd for $C_{16}H_{21}NO_6$ $[M]^+$: 323.14; found: positive mode 345.90 $[M+Na]^+$, negative mode 322.12 $[M-H]^-$, 645.20 $[2M-H]^-$.

3.6. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-man->nopyranosyl-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)}-N^2-benzyloxycarbonyl-L-asparagine *tert*-butyl ester 6

Heptasaccharide azide **2** (289 mg, 170 μmol, 1 equiv) was dissolved in freshly distilled MeOH (10 mL) and ethyldiisopropylamine (300 μL, 1.72 mmol) was added. The reaction flask was purged with argon and 1,3-propanedithiol (868 μmol, 8.65 mmol) was subsequently added. The reaction mixture was stirred for 2 h at room temperature (TLC: isopropanol–1 M NH_4OAc 4:1). The solvent was removed in vacuo and the resulting glycosyl amine was dried under high vacuum. Amino acid **4** (330 mg, 1.02 mmol, 6 equiv) and PyBOP (531 mg, 1.02 μmol) were dissolved in NMP (5 mL) and treated with ethyldiisopropylamine (350 μL 2.01 mmol). After 10 min, the preactivation mixture was added to the glycosyl amine and the solution was stirred for 2 h at room temperature. Water (10 mL) was added to hydrolyze excess amino acid active ester and the reaction mixture was concentrated under high vacuum. The crude product was purified by solid-phase extraction (one 10-g cartridge): the residue was dissolved in acetonitrile–water (100 mL, 1:9) and loaded onto the cartridge. Impurities were washed off with acetonitrile–water (200 mL, 30% acetonitrile) and **6** was eluted with acetonitrile–water (200 mL, 2:3). The fractions containing the product were pooled and lyophilized affording **6** (289 mg, 85.9%). $[\alpha]_D^{23}$ −1.7 (*c* 1.3, MeOH); R_f glycosyl amine = 0.51 (isopropanol–1 M NH_4OAc 4:1); R_f **6** = 0.72 (isopropanol–1 M NH_4OAc 4:1); ^1H NMR (500 MHz, DMSO-d_6): δ = 8.38 (d, $J_{NH,1}$ = 8.6 Hz, 1H, γNH Asn), 8.00 (d, $J_{NH,2}$ = 7.1 Hz, 1H, NH-2^2), 7.93 (d, $J_{NH,2}$ = 8.8 Hz, 1H, NH-2^1), 7.59 (m, 3H, NH-2^5, NH-2$^{5'}$, NH-urethane), 7.37–7.18 (m, 25H, Ar), 5.02–4.87 (m, 11H, H-1^1, H-1^4, OH-3^5, OH-3$^{5'}$, OH-4^3, OH-4^5, OH-4$^{5'}$, CH_2O), 4.77 (d, $J_{OH,4}$ = 4.8 Hz, 1H, OH-4^4), 4.72 (d, $J_{OH,4}$ = 4.8 Hz, 1H, OH-4$^{4'}$), 4.65 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.62–4.44 (m, 12H, αCH Asn, H-1^2, H-1^3, OH-2^3, OH-6^4, OH-6^5, OH-6$^{5'}$, CH_2O), 4.36 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1^5), 4.34 (d, J_{gem} = 10.1 Hz, 1H, CH_2O), 4.23 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1$^{5'}$), 4.19 (m, 1H, OH-6$^{4'}$), 3.99–3.94 (m, 2H, H-2^3, OH-3^4), 3.93 (d, $J_{OH,3}$ = 7.5 Hz, 1H, OH-3$^{4'}$), 3.88–3.83 (m, 2H, H-2^4, H-4^1), 3.78–3.21 (m, 34H, H-2^1, H-2^2, H-2$^{4'}$, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-5^1, H-5^2, H-5^4, H-5$^{4'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 3.14–3.02 (m, 5H, H-4^5, H-4$^{5'}$, H-5^3, H-5^5, H-5$^{5'}$), 2.67 (dd, J_{gem} = 16.1 Hz, J_{vic} = 5.0 Hz, 1H, βCHa Asn), 2.50 (dd, J_{vic} = 7.2 Hz, 1H, βCHb Asn), 1.80, 1.77, 1.72 (3s, 12H, NAc), 1.34 (1s, 9H, *t*Bu); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 170.5 (C=O ester), 169.7, 169.5, 169.4, 169.3 (C=O NAc), 155.8 (C=O urethane), 139.2, 138.5, 138.4, 136.9, (C-i Ar), 128.3–126.9 (C-Ar), 101.5 (C-1$^{5'}$), 101.2 (C-1^5), 100.2 (C-1^3), 99.9 (C-1^4, C-1^2), 97.6 (C-1$^{4'}$), 81.5 (C-3^3), 81.4 (C-3^1), 80.7 (C-3^2), 79.3 (C-2$^{4'}$), 78.8 (C-2^4), 78.4 (C-1^1), 77.0 (C-5^5), 76.9 (C-5$^{5'}$), 76.1 (C-5^1), 75.9 (C-4^2), 75.5 (C-5^3), 74.6 (C-4^1), 74.4 (C-5^2), 74.2 (C-5^4), 73.9 (C-3^5, C-3$^{5'}$), 73.8 (CH_2O), 73.6 (C-5$^{4'}$), 73.2, 72.2, 71.8 (CH_2O), 70.5 (C-4$^{5'}$), 70.3 (C-4^5), 69.9 (C-3^4, C-3$^{4'}$), 69.1 (C-2^3), 68.7 (C-6^2), 68.3 (C-6^1), 67.6 (C-4^4), 67.0 (C-4$^{4'}$), 65.9 (C-q *t*Bu), 65.4 (CH_2O, C-6^3), 64.9 (C-4^3), 61.5 (C-6^4), 61.1 (C-6$^{4'}$), 61.0 (C-6^5), 60.8 (C-6$^{5'}$), 55.6 (C-2^2, C-2^5, C-2$^{5'}$), 53.2 (C-2^1,) 50.9 (αC Asn), 37.0 (βC Asn), 27.5 (CH_3 *t*Bu), 23.2, 22.9, 22.7 (NAc); ESI-MS (*m*/*z*) calcd for $C_{94}H_{128}N_6O_{40}$ $[M]^+$: 1980.82; found: 1981.90 $[M+H]^+$, 991.39 $[M+2H]^{2+}$.

3.7. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-L-asparagine *tert*-butyl ester 8

Protected glycosyl amino acid **6** (288 mg, 145 μmol) and PdO-hydrate (453 mg) were suspended in THF–water–HOAc (11 mL, 10:10:1). The reaction flask was purged with argon and the mixture was stirred vigorously under a hydrogen atmosphere for 3 days (TLC: isopropanol–1 M NH_4OAc 2:1). THF was removed in vacuo and after dilution with water (100 mL) the catalyst was filtered off through a syringe filter unit (Millipore, IC Millex-LG, 25 mm external diameter, 0.2 μm pore size). The clear solution was lyophilized affording crude **8** (211.7 mg, 97.9%), which was used in the next step without purification. $[\alpha]_D^{23}$ +1.8 (*c* 2.5, H_2O); R_f **6** = 0.83 (isopropanol–1 M NH_4OAc 2:1); R_f **8** = 0.51 (isopropanol–1 M NH_4OAc 2:1); ^1H NMR (500 MHz, D_2O, DMSO-d_6 as internal standard): δ = 5.17 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 5.12 (d, $J_{1,2}$ = 9.8 Hz, 1H, H-1^1), 4.97 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.82 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.66 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^2), 4.60 (m, 2H,

H-1^5, H-1$^{5'}$), 4.30 (dd, $J_{2,3}$ = 1.9 Hz, 1H, H-2^3), 4.24 (dd, $J_{2,3}$ = 1.7 Hz, 1H, H-2^4), 4.15 (dd, $J_{2,3}$ = 1.7 Hz, 1H, H-2$^{4'}$), 4.03–3.45 (m, 40H, αCH Asn, H-2^1, H-2^2, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-4^5, H-4$^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-5$^{4'}$, H-5^5, H-5$^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 3.11 (dd, J_{gem} = 17.5 Hz, J_{vic} = 4.9 Hz, 1H, βCHa Asn), 2.97 (dd, J_{vic} = 4.9 Hz, 1H, βCHb Asn), 2.17, 2.10, 2.06 (3s, 12H, NAc), 1.53 (1s, 9H, *t*Bu); ^{13}C NMR (125 MHz, D_2O, DMSO-d_6 as internal standard): δ = 176.3, 176.1, 173.0, 169.4 (C═O), 102.8 (C-1^2), 101.9 (C-1^3), 101.1 (C-1^5, C-1$^{5'}$, C-1^4), 98.5 (C-1$^{4'}$), 81.9 (C-3^3), 81.1 (C-4^2), 80.2 (C-4^1), 79.7 (C-1^1), 78.0 (C-2^4), 77.8 (C-2$^{4'}$), 77.6 (C-5^1), 77.3 (C-5^5, C-5$^{5'}$), 75.9 (C-5^2), 75.8 (C-5^3), 75.1 (C-5^4), 74.9, 74.8 (C-3^5, C-3$^{5'}$), 74.4 (C-5$^{4'}$), 74.2 (C-3^1), 73.5 (C-3^2), 71.7 (C-2^3), 71.4 (C-4^5, C-4$^{5'}$), 71.0 (C-3$^{4'}$), 70.9 (C-3^4), 68.9 (C-4$^{4'}$), 68.8 (C-4^4), 67.4 (C-6^3), 67.2 (C-4^3), 63.2, 63.1 (C-6^4, C-6$^{4'}$), 62.1 (C-6^5, C-6$^{5'}$, C-q *t*Bu), 61.5 (C-6^2), 61.4 (C-6^1), 56.8 (C-2^5, C-2$^{5'}$), 56.4 (C-2^2), 55.2 (C-2^1), 52.3 (αC Asn), 36.5 (βC Asn), 28.5 (CH_3 *t*Bu), 23.9, 23.7, 23.6 (NAc); ESI-MS (*m*/*z*) calcd for $C_{58}H_{98}N_6O_{38}$ $[M]^+$: 1486.59; found: positive mode 1487.49 $[M+H]^+$, 1509.52 $[M+Na]^+$, negative mode 1485.46 $[M-H]^-$.

3.8. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(9-fluorenylmethoxycarbonyl)-L-asparagine *tert*-butyl ester 9

Glycosyl amino acid **8** (189 mg, 127 μmol) was dissolved in water (18 mL) and treated with triethylamine (56 μL, 402 μmol). A solution of Fmoc-OSu (181 mg, 537 μmol) in dioxane (16 mL) was added. The reaction mixture was allowed to stir for 90 min at room temperature (TLC: isopropanol–1 M NH_4OAc 2:1), the reaction was subsequently quenched with acetic acid (50 μl, pH 5), and the reaction mixture was lyophilized. The residue was diluted with water (50 mL), centrifuged, and the supernatant was loaded onto a 5-g RP cartridge in order to remove the majority of the reagents by solid-phase extraction. Impurities were washed off with acetonitrile–water (100 mL, 2:8) and **9** was eluted with acetonitrile–water (100 mL, 3:7). The fractions containing the product were pooled and lyophilized affording **9** (130 mg, 59.8%). $[\alpha]_D^{23}$ −5.5 (*c* 1.1, H_2O); R_f = 0.78 (isopropanol–1 M NH_4OAc 2:1); ^{1}H NMR (500 MHz, D_2O, DMSO-d_6 as internal standard): δ = 7.4–6.6 (m, 8H, Fmoc), 4.93 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 4.81 (d, $J_{1,2}$ = 9.5 Hz, 1H, H-1^1), 4.73 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.56 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.40–4.35 (m, 3H, H-1^2, H-1^5, H-1$^{5'}$), 4.28 (m, 2H, CH_2O), 4.16 (m, 1H, H-9 Fmoc), 4.05 (m, 1H, H-2^3), 3.99 (m, 1H, H-2^4), 3.91 (m, 1H, H-2$^{4'}$), 3.77–3.20 (m, 40H, αCH Asn, H-2^1, H-2^2, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-4^5, H-4$^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-5$^{4'}$, H-5^5, H-5$^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.50 (dd, J_{gem} = 15.2 Hz, J_{vic} = 2.6 Hz, 1H, βCHa Asn), 2.30 (dd, J_{vic} = 8.7 Hz, 1H, βCHb Asn), 1.86, 1.73 (2s, 12H, NAc), 1.01 (1s, 9H, *t*Bu); ^{13}C NMR (125 MHz, D_2O, DMSO-d_6 as internal standard): δ = 176.2, 176.0, 174.8 (C═O), 159.1 (C═O urethane), 145.4, 142.2, 129.7, 129.1, 126.8, 121.8 (C-Ar), 102.9 (C-1^2), 102.0 (C-1^3), 101.2 (C-1^4, C-1^5, C-1$^{5'}$), 98.6 (C-1$^{4'}$), 82.0 (C-3^3), 81.1 (C-4^2), 80.3 (C-4^1), 79.8 (C-1^1), 78.07 (C-2^4), 77.93 (C-2$^{4'}$), 77.7 (C-5^1), 77.4 (C-5^5, C-5$^{5'}$), 76.0 (C-5^2, C-5^3), 75.1 (C-5^4), 74.99, 74.87 (C-3^5, C-3$^{5'}$), 74.4 (C-3^1, C-5$^{4'}$), 73.6 (C-3^2), 71.8 (C-2^3), 71.5 (C-4^5, C-4$^{5'}$), 71.1 (C-3^4, C-3$^{4'}$), 68.9 (C-4^4, C-4$^{4'}$), 68.1 (CH_2O), 67.4 (C-6^3), 67.2 (C-4^3), 63.4 (C-q *t*Bu), 63.3, 63.2 (C-6^4, C-6$^{4'}$), 62.2 (C-6^5, C-6^5), 61.6 (C-6^2), 61.3 (C-6^1), 56.9 (C-2^5, C-2$^{5'}$), 56.5 (C-2^2), 55.4 (C-2^1), 54.0 (αC Asn), 48.4 (C-9 Fmoc), 39.9 (βC Asn), 28.7 (CH_3 *t*Bu), 23.9, 23.8, 23.6 (NAc). ESI-MS (*m*/*z*) calcd for $C_{73}H_{108}N_6O_{40}$ $[M]^+$: 1708.66; found: 1709.58 $[M+H]^+$, 855.34 $[M+2H]^{2+}$.

3.9. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(9-fluorenylmethoxycarbonyl)-L-asparagine 1

Glycosyl amino acid **9** (108 mg, 63.2 μmol) was treated with TFA (8 mL). The reaction mixture was allowed to stir for 5 min at room temperature (TLC: isopropanol–1 M NH_4OAc 2:1), subsequently the TFA was removed in vacuo and the solid was dried under high vacuum. The residue was diluted with water, lyophilized, and purified by RP-HPLC (column: YMC-Pack ODS S-5 μm (250 × 20 mm), gradient: 27–55% acetonitrile (0.1% trifluoroacetic acid), flow rate: 9.5 mL/min) affording **1** (75.3 mg, 72.1%).

3.10. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(9-fluorenylmethoxycarbonyl)-L-asparaginyl-L-tyrosine-methylester 11

Glycosyl amino acid **1** (16 mg, 9.68 μmol), L-tyrosine methylester hydrochloride (4 mg, 17.3 μmol), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (5.9 mg, 18.4 μmol), and HOBt (2.8 mg, 18 μmol) were dissolved in NMP (450 μL). The coupling was started by addition of a mixture of ethyldiisopropylamine (10.7 μL, 61 μmol) and NMP (100 μL). After complete reaction (5–10 min) (TLC: isopropanol–1 M NH_4OAc 2:1) the mixture was dried in vacuum and the residue was purified by RP-HPLC (column: Macherey-Nagel Nucleogel RP 100-8 (300 × 7.7 mm), gradient: 25–42 % acetonitrile, flow rate: 2 mL/min) and lyophilized to afford **11** (13.1 mg, 73.9%). $[\alpha]_D^{23}$ −10.0 (*c* 0.2, H_2O); R_f = 0.73 (isopropanol–1 M NH_4OAc 2:1); ^{1}H NMR (500 MHz, D_2O, acetone-d_6 (20% v/v) as internal standard): δ = 7.62–7.10 (m, 8H, Fmoc), 6.78, 6.56 (2 m, 4H, Tyr), 5.0 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 4.87 (d, $J_{1,2}$ = 9.4 Hz, 1H, H-1^1), 4.80 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1$^{4'}$), 4.63 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.46 (d, $J_{1,2}$ = 6.8 Hz, 1H, H-1^2), 4.44–4.40 (m, 3H, αCH Tyr, H-1^5, H-1$^{5'}$), 4.36 (m, 1H, αCH Asn), 4.22 (m, 1H, CH_2O), 4.13 (m, 2H, CH_2O, H-2^3), 4.05 (m, 1H, H-2^4), 3.97 (m, 2H, H-2$^{4'}$, H-9 Fmoc), 3.83–3.24 (m, 42H, CH_3O, H-2^1, H-2^2, H-2^5, H-2$^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-3$^{4'}$, H-3^5, H-3$^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-4$^{4'}$, H-4^5, H-4$^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-5$^{4'}$, H-5^5, H-5$^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.85–2.68 (m, 2H, βCHab Tyr), 2.60–2.38 (m, 2H, βCHab Asn), 1.93, 1.91 (2s, 12H, NAc). ^{13}C NMR (125 MHz, D_2O, acetone-d_6 as internal standard): δ = 174.8, 174.7, 173.2, 172.4 (C═O), 155.1 (C-p Tyr), 143.8, 141.2 (C-Ar), 130.7 (C-o Tyr), 128.3, 127.8, 127.6, 125.4, 120.4 (C-Ar), 115.7 (C-m Tyr), 101.7 (C-1^2), 100.8 (C-1^3), 100.0 (C-1^5, C-1$^{5'}$), 99.9 (C-1^4), 97.3 (C-1$^{4'}$), 80.8 (C-3^3), 79.9 (C-4^2), 79.1 (C-4^1), 78.6 (C-1^1), 76.8 (C-2^4), 76.7 (C-2$^{4'}$), 76.5 (C-5^1), 76.2 (C-5^5, C-5$^{5'}$), 74.7 (C-5^2, C-5^3), 73.9 (C-5^4), 73.7, 73.6 (C-3^5, C-3$^{5'}$), 73.2 (C-5$^{4'}$), 73.0 (C-3^1), 72.3 (C-3^2), 70.5 (C-2^3), 70.3 (C-4^5, C-4$^{5'}$), 69.8 (C-3^4, C-3$^{4'}$), 67.7 (C-4^4, C-4$^{4'}$), 67.1 (CH_2O), 66.0 (C-4^3, C-6^3), 62.0 (C-6^4), 61.9 (C-6$^{4'}$), 61.0 (C-6^5, C-6$^{5'}$), 60.3 (C-6^2), 60.1 (C-6^1), 55.7 (C-2^5, C-2$^{5'}$), 55.2 (C-2^2), 54.4 (αC Tyr), 54.1 (C-2^1), 53.0 (CH_3O), 51.5 (αC Asn), 47.0 (C-9 Fmoc), 37.3 (βC Asn), 36.0 (βC Tyr), 22.7, 22.6, 22.4 (NAc); MALDI-TOF-MS (*m*/*z*) calcd for $C_{79}H_{111}N_7O_{42}$ $[M]^+$: 1829.67; found: 1853.77 $[M+Na]^+$.

3.11. *N*-(9-Fluorenylmethoxycarbonyl)-L-seryl-L-seryl-L-serine ethyldiisopropylammonium salt 13

Commercially available L-seryl-L-seryl-L-serine (Bachem) (40 mg, 143 μmol) was dissolved in water (1.2 mL) in the presence of ethyldiisopropylamine (20 μL). A solution of Fmoc-OSu (80 mg, 237 μmol) in acetonitrile (1 mL) was added followed by pH adjustment to a value of 8.5 with ethyldiisopropylamine. After 2.5d the solution was evaporated, taken up in water (20 mL), and loaded onto two Sep Pak cartridges. The salts were eluted with water (20 mL). The desired peptide **13** was eluted with 10% and 30% acetonitrile (20 mL each), lyophilized, and used for the coupling with **12**. R_f **13** = 0.62 (isopropanol–1 M NH_4OAc 4:1). ^1H NMR (500 MHz, D_2O, acetone-d_6 as internal standard): δ = 7.95–7.46 (m, 8H, Fmoc), 4.64–4.60 (m, 1H, αCH SerA), 4.53–4.50 (m, 2H, CH_2O Fmoc), 4.44 (m, 3H, αCH SerB,C, H-9 Fmoc) 4.03–3.90 (m, 6H, βCH_2 SerA,B,C), 3.84–3.77 (m, 2H, CH *i*Pr), 3.31–3.26 (m, 2H, CH_2 Et), 1.45–1.41 (m, 15H, CH_3); ^{13}C NMR (125 MHz, D_2O, acetone-d_6 as internal standard): δ = 143.9, 141.2, 128.4, 127.8, 125.5, 120.4 (C-Ar), 67.2 (CH_2O Fmoc), 62.1 (βC SerB), 61.7 (βC SerC), 61.5 (βC SerA), 56.8 (αC SerB,C), 54.6 α(C SerA), 54.6 (CH *i*Pr), 47.0 (C-9 Fmoc), 42.7 (CH_2 Et), 18.0, 16.6 (CH_3 *i*Pr), 12.4 (CH_3 Et), Ser A, B, C were not assigned to the peptide sequence.

3.12. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-L-asparaginyl-L-tyrosine-methylester 12

Fmoc-glycopeptide **11** (13 mg, 7.1 μmol) was dissolved in NMP (400 μL) and deprotected by addition of piperidine (40 μL). After 1 h (TLC: isopropanol–1 M NH_4OAc 2:1) the volatiles were evaporated in vacuum and the remainder was purified by RP-HPLC (column: Macherey-Nagel Nucleogel RP 100-8 (300 × 7.7 mm), gradient: 2–50 % acetonitrile, flow rate: 2 mL/min). The fractions containing the desired product were lyophilized and afforded **12** (9.9 mg, 86.7%), which was used for the synthesis of **14**. R_f = 0.48 (isopropanol–1 M NH_4OAc 2:1); $C_{64}H_{101}N_7O_{40}$.

3.13. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(9-fluorenylmethoxycarbonyl-L-seryl-L-seryl-L-seryl)-L-asparaginyl-L-tyrosine-methylester 14

Deprotected glycopeptide **12** (9.9 mg, 6.2 μmol), Fmoc-Ser-Ser-Ser-OH × Et*N*iPr_2 **13** (10 mg, 15.9 μmol), and O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate (HATU) (6 mg, 15.8 μmol) were dissolved in NMP (500 μL). 2,4,6-Collidine (2.1 μL, 15.9 μmol) dissolved in NMP (10 μl) was added. The reaction was complete after 5–10 min (TLC: isopropanol–1 M NH_4OAc 2:1) and the reaction mixture was evaporated in vacuum and purified by RP-HPLC (column: Macherey-Nagel Nucleogel RP 100-8 (300 × 7.7 mm), gradient: 20–40% acetonitrile, flow: 2 mL/min). The fractions containing the product were lyophilized affording **14** (9.0 mg, 69.9 %). $[\alpha]_D^{23}$ −0.3 (*c* 0.15, MeOH–H_2O 5:1); R_f = 0.68 (isopropanol–1 M NH_4OAc 2:1); ^1H NMR (500 MHz, D_2O, acetone-d_6 (25% v/v) as internal standard): δ = 7.70–7.20 (m, 8H, Fmoc), 6.88, 6.43 (2 m, 4H, Tyr), 5.0 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 4.86 (d, $J_{1,2}$ = 9.6 Hz, 1H, H-1^1), 4.81 (d, $J_{1,2}$ <1.0 Hz, 1H, H-$1^{4'}$), 4.63 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.60 (m, 1H, αCH Asn), 4.47 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^2), 4.43 (2d, $J_{1,2}$ = 8.2 Hz, 2H, H-1^5, H-$1^{5'}$), 4.40 (m, 1H, αCH Tyr), 4.36 (m, 1H, αCH SerC), 4.30 (m, 1H, αCH SerB), 4.25 (d, J_{vic} = 6.0 Hz, 2H, CH_2O Fmoc), 4.17 (m, 1H, αCH SerA), 4.14 (m, 1H, H-2^3), 4.09 (t, 1H, H-9 Fmoc), 4.06 (m, 1H, H-2^4), 3.97 (m, 1H, H-$2^{4'}$), 3.83–3.27 (m, 48H, CH_3O, βCH_2 SerA,B,C, H-2^1, H-2^2, H-2^5, H-$2^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-$3^{4'}$, H-3^5, H-$3^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-$4^{4'}$, H-4^5, H-$4^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-$5^{4'}$, H-5^5, H-$5^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.85 (dd, J_{gem} = 14.0 Hz, J_{vic} = 6.5 Hz, 1H, βCHa Tyr), 2.79 (dd, J_{vic} = 7.5 Hz, 1H, βCHb Tyr), 2.63 (dd, J_{gem} = 15.7 Hz, J_{vic} = 4.4 Hz, 1H, βCHa Asn), 2.53 (dd, J_{vic} = 6.5 Hz, 1H, βCHb Asn), 1.93, 1.92 , 1.84 (3s, 12H, NAc); ^{13}C NMR (125 MHz, D_2O, acetone-d_6 as internal standard): δ = 174.7, 174.5, 173.2, 172.7, 172.4, 172.0, 171.7, 171.3 (C=O), 155.2 (C-p Tyr), 143.9, 141.3 (C-Ar), 130.8 (C-o Tyr), 128.4, 128.0, 127.8, 125.5, 120.4 (C-Ar), 115.7 (C-m Tyr), 101.7 (C-1^2), 100.8 (C-1^3), 100.0 (C-1^5, C-$1^{5'}$), 99.9 (C-1^4), 97.4 (C-$1^{4'}$), 80.8 (C-3^3), 79.9 (C-4^2), 79.3 (C-4^1), 78.7 (C-1^1), 76.9 (C-2^4), 76.8 (C-$2^{4'}$), 76.5 (C-5^1), 76.3 (C-5^5, C-$5^{5'}$), 74.8 (C-5^2, C-5^3), 73.9 (C-5^4), 73.8, 73.7 (C-3^5, C-$3^{5'}$), 73.2 (C-$5^{4'}$), 73.1 (C-3^1), 72.3 (C-3^2), 70.5 (C-2^3), 70.4 (C-4^5, C-$4^{5'}$), 69.9 (C-3^4, C-$3^{4'}$), 67.7 (C-4^4, C-$4^{4'}$), 67.3 (CH_2O), 66.2 (C-6^3), 66.1 (C-4^3), 62.1, 62.0 (C-6^4, C-$6^{4'}$), 61.8 (βC SerA), 61.3 (βC SerB,C), 61.0 (C-6^5, C-$6^{5'}$), 60.3 (C-6^2), 60.2 (C-6^1), 57.1 (αC SerA), 56.1 α(C SerB), 56.0 (αC SerC), 55.7 (C-2^5, C-$2^{5'}$), 55.2 (C-2^2), 54.8 (αC Tyr), 54.1 (C-2^1), 52.9 (CH_3O), 50.2 (αC Asn), 47.0 (C-9 Fmoc), 36.9 (βC Asn), 36.2 (βC Tyr), 22.7, 22.6, 22.4 (NAc), Ser A, B, C were not assigned to the peptide sequence; MALDI-TOF-MS (*m/z*) calcd for $C_{88}H_{126}N_{10}O_{48}$ $[M]^+$: 2090.77; found: 2114.6 $[M+Na]^+$.

3.14. N^4-{*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-O-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(L-seryl-L-seryl-L-seryl)-L-asparaginyl-L-tyrosine 16

Fmoc glycopeptide methylester **12** (5.4 mg, 2.58 μmol) **14** was dissolved in NMP (200 μL) and piperidine (10 μL) was added. After 1 h (TLC: isopropanol–1 M NH_4OAc 2:1) the volatiles were removed in vacuum. The remainder was taken up in water (600 μL) and chymotrypsin (0.8 mg dissolved in 300 μL of water) was added. After 1 h (TLC: isopropanol–1 M NH_4OAc 2:1) the mixture was centrifuged. The supernatant was concentrated in vacuum to a volume of 400 μL and purified by gel filtration (column: Pharmacia Hi Load Superdex 30 (600 × 16 mm) eluent: 100 mM NH_4HCO_3, flow rate 750 μL/min) and lyophilized to afford **16** (4.6 mg, 96.0%). $[\alpha]_D^{23}$ −5.6 (*c* 0.5, H_2O); R_f **15** = 0.44 (isopropanol–1 M NH_4OAc 2:1); R_f **16** = 0.32 (isopropanol–1 M NH_4OAc 2:1); ^1H NMR (500 MHz, D_2O, DMSO-d_6 as internal standard): δ = 6.91 (d, J_{vic} = 8.5 Hz, 2H, H-o Tyr), 6.63 (d, 2H, H-m Tyr), 4.92 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^4), 4.81 (d, $J_{1,2}$ = 9.6 Hz, 1H, H-1^1), 4.72 (d, $J_{1,2}$ <1.0 Hz, 1H, H-$1^{4'}$), 4.57 (d, $J_{1,2}$ <1.0 Hz, 1H, H-1^3), 4.51 (t, J_{vic} = 6.5 Hz, 1H, αCH Asn), 4.41 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^2), 4.36 (t, J_{vic} = 5.3 Hz, 1H, αCH SerA), 4.35 (2d, $J_{1,2}$ = 8.6 Hz, 2H, H-1^5, H-$1^{5'}$), 4.27 (t, J_{vic} = 5.4 Hz, 1H, αCH SerB), 4.15 (dd, J_{vic} = 7.1, J_{vic} = 5.4 Hz, 1H, αCH Tyr), 4.05 (m, 1H, H-2^3), 3.98 (m, 1H, H-2^4), 3.90 (m, 1H, H-$2^{4'}$), 3.78–3.22 (m, 46H, αCH SerC, βCH_2 SerA,B,C, H-2^1, H-2^2, H-2^5, H-$2^{5'}$, H-3^1, H-3^2, H-3^3, H-3^4, H-$3^{4'}$, H-3^5, H-$3^{5'}$, H-4^1, H-4^2, H-4^3, H-4^4, H-$4^{4'}$, H-4^5, H-$4^{5'}$, H-5^1, H-5^2, H-5^3, H-5^4, H-$5^{4'}$, H-5^5, H-$5^{5'}$, H-6ab^1, H-6ab^2, H-6ab^3, H-6ab^4, H-6ab$^{4'}$, H-6ab^5, H-6ab$^{5'}$), 2.86 (dd, J_{gem} = 14.0 Hz, J_{vic} = 4.9 Hz, 1H, βCHa Tyr), 2.69 (dd, J_{vic} = 7.5 Hz, 1H, βCHb Tyr), 2.56 (dd, J_{gem} = 16.1 Hz, J_{vic} = 5.4 Hz, 1H, βCHa Asn), 2.45 (dd, J_{vic} = 7.4 Hz, 1H, βCHb Asn), 1.88, 1.85 , 1.80 (3s, 12H, NAc); ^{13}C NMR (125 MHz, D_2O, DMSO-d_6 as internal standard): δ = 176.1, 176.0, 174.0, 172.9, 172.5, 172.0 (C=O), 155.7 (C-p Tyr), 132.3 (C-o Tyr), 130.8 (C-i Tyr), 116.8 (C-m Tyr), 102.8 (C-1^2), 102.0 (C-1^3), 101.2 (C-1^5, C-$1^{5'}$),

101.1 (C-1^4), 98.5 (C-$1^{4'}$), 82.0 (C-3^3), 81.1 (C-4^2), 80.3 (C-4^1), 79.7 (C-1^1), 78.0 (C-2^4), 77.9 (C-$2^{4'}$), 77.7 (C-5^1), 77.4 (C-5^5, C-$5^{5'}$), 75.9 (C-5^2, C-5^3), 75.1 (C-5^4), 74. 9, 74.8 (C-3^5, C-$3^{5'}$), 74.5 (C-$5^{4'}$), 74.3 (C-3^1), 73.5 (C-3^2), 71.7 (C-2^3), 71.5 (C-4^5, C-$4^{5'}$), 71.0 (C-3^4, C-$3^{4'}$), 68.9 (C-4^4, C-$4^{4'}$), 67.4 (C-6^3), 67.2 (C-4^3), 63.2 (C-6^4 , C-$6^{4'}$, βC SerC), 62.6 (βC SerA, B), 62.2 (C-6^5, C-$6^{5'}$), 61.52 (C-6^2), 61.45 (C-6^1), 57.9 (αC Tyr), 57.0 (αC SerA), 56.9 (αC SerB, C-2^5, C-$2^{5'}$), 56.7 (αC SerC), 56.6 (C-2^2), 55.2 (C-2^1), 51.7 (αC Asn), 38.22 (βC Tyr), 38.16 (βC Asn), 23.9, 23.8, 23.7 (NAc), Ser A, B, C were not assigned to the peptide sequence; MALDI-TOF-MS (*m/z*) calcd for $C_{72}H_{114}N_{10}O_{46}$ $[M]^+$: 1854.7; found: 1879.5 $[M+Na]^+$.

3.15. N^4-{*O*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→6)-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[(5-acetamido-3,5-dideoxy-D-gly-cero-α-D-galacto-non-2-ulopyranosylonic acid)-(2→6)-β-DD-galactopyranosyl-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)}-N^2-(L-seryl-L-seryl-L-seryl)-L-asparaginyl-L-tyrosine A

Glycopeptide **16** (4.6 mg, 2.48 μmol) was dissolved in 20 mM sodium cacodylate buffer (pH 7.4, 1400 μL) containing bovine serum albumin (1.0 mg), NaN_3 (2.5 μmol), $MnCl_2$ (1.4 μmol), uridine-5′-diphosphogalactose (5.6 mg, 8.4 μmol), alkaline phosphatase (E.C. 3.1.3.1, 6 U), and GlcNAc-β-(1→4)-galactosyltransferase (E.C. 2.4.1.22, 120 mU). The reaction mixture was incubated for 24 h at 37 °C maintaining a pH value of 7.0 by addition of 1 M NaOH. After complete reaction (TLC, 2:1 isopropanol–1 M NH_4OAc) cytidine-5′-monophospho-N-acetylneuraminic acid (4.8 mg, 6.2 μmol) and β-galactoside-α-(2→6)-sialyltransferase (E.C. 2.4.99.1, 25 mU) were added. Incubation was continued for 24 h at 37 °C (pH 7.0) followed by another addition of cytidine-5′-monophospho-N-acetylneuraminic acid (4.8 mg, 6.2 μmol) and β-galactoside-α-(2→6)-sialyltransferase (25 mU). After 24 h of reaction time, the precipitate was removed by centrifugation. The supernatant was concentrated to 400 μL and purified by gel filtration (column: Pharmacia Hi Load Superdex 30 (600 × 16 mm) mobile phase: 100 mM NH_4HCO_3, flow rate: 750 μL/min). The positive fractions were collected and lyophilized to afford **A** (6.2 mg, 91.1%). R_f digalactoside = 0.22 (isopropanol–1 M NH_4OAc 2:1); R_f **A** = 0.16 (isopropanol–1 M NH_4OAc 2:1).

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