# Biomimetic Synthesis of the Tumor-Associated (2,3)-Sialyl-T Antigen and Its Incorporation into Glycopeptide Antigens from the Mucins MUC1 and MUC4

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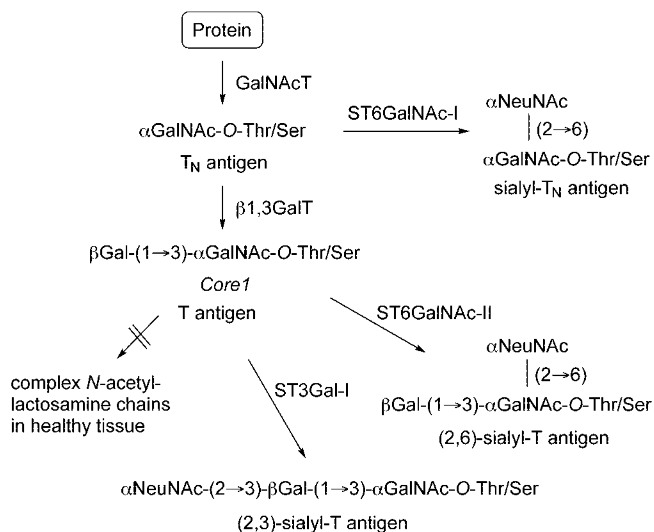
*Dedicated to Professor Joachim Engels on the occasion of his 60th birthday*

**Abstract:** Glycoproteins on epithelial tumor cells often exhibit aberrant glycosylation profiles. The incomplete formation of the glycan side chains resulting from a down-regulated glucosamine transfer and a premature sialylation results in additional peptide epitopes, which become accessible to the immune system in mucin-type glycoproteins. These cancer-specific structure alterations are considered to be a promising basis for selective immunological attack on tumor cells. Among the tumor-associated saccharide antigens, the (2,3)-sialyl-T antigen has been identified as the most abundant glycan, found in several different carcinoma cell lines. According to a linear biomimetic strategy, the (2,3)-sialyl-T antigen was synthesized by a stepwise glycan chain extension of a protected galactosamine-threonine precursor. For the construction of immunostimulating antigens combining both peptide and saccharide motifs, this antigen was incorporated into glycopeptide partial structures from the mucins MUC1 and MUC4 by sequential solid-phase synthesis.

**Keywords:** antigens • glycopeptides • MUC1 • MUC4 • solid-phase synthesis

## Introduction

Glycoproteins which are major constituents on epithelial cell surfaces are decisively involved in various important physiological processes such as cell adhesion or signal transduction.[1] In a number of cases, the carbohydrate portions of membrane glycoproteins are key elements in biological selectivity: carbohydrates in the blood group substances, for instance.[2] During glycoprotein biosynthesis, the post-translational *O*-glycosylation of the protein backbone proceeds in different compartments of the Golgi apparatus. It is initiated by the enzymatic coupling of *N*-acetyl-galactosamine to serine or threonine residues (see Scheme 1). As the glycoprotein passes through the Golgi, stepwise saccharide chain extension leads to the formation of complex, branched glycan structures. Galactosylation of the central *N*-acetyl-galactosamine moiety furnishes the *core 1* saccharide structure βGal-(1→3)-αGalNAc-*O*-Ser/Thr (also called T-antigen), which acts as a substrate for β1,6-GlcNAc transferases. The addition of β1,6-*N*-acetyl-glucosamine yields the *core 2* trisaccharide (βGal-(1→3)-[βGlcNAc-(1→6)-]αGalNAc), which can be further extended by the addition of polylactos-




Scheme 1. Glycopeptide biosynthesis in cancer cells. GalNAcT: UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyl transferase. β1,3GalT: *Core 1* β1,3-galactosyl transferase. C2GnT-I: *Core 2* β1,6-*N*-acetylglucosaminyl transferase-I. ST6GalNAc-I: CMP-Neu5Ac:GalNAc-R α2,6-sialyl transferase-I. ST6GalNAc-II: CMP-Neu5Ac:GalNAc-R α2,6-sialyl transferase-II. ST3Gal-I: CMP-Neu5Ac:Galβ1,3GalNAc-R α2,3-sialyl transferase-I.

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amine chains. Chain growth is usually terminated by the attachment of sialic acid or fucose or by sulfation.[3] Unlike in healthy cells, the activity of certain glycosyltransferases, in particular β1,6-GlcNAc transferase, is drastically down-regulated in epithelial tumor cells, whereas the activity of sialyltransferases is strongly increased, often resulting in a premature sialylation.[4] Consequently, glycoproteins in cancer cells carry cryptic, incomplete glycan side chains.[5] This results in the exposure of additional peptide epitopes, masked in normal cells, which hence become accessible to the immune system. The $T_N$, T, sialyl-$T_N$ and (2,3)-sialyl-T structures constitute important examples of such tumor-associated saccharide antigens (Scheme 1). Among these, the (2,3)-sialyl-T antigen has been identified as the most abundant glycan found in different tumor cell lines such as the breast cancer cell line T47D[6] and the gastric carcinoma cell lines HT-29 and K562.[7]

Cancer immunotherapy selectively targeting the tumor-associated glycoprotein structure alterations—deficient glycosylation and, thus, exposure of additional peptide epitopes—would certainly be attractive. In this context, glycopeptides containing partial structures of cancer-associated cell-surface glycoproteins combining both tumor-associated saccharide and peptide structural information are considered to be promising candidates for the construction of tumor-selective immunostimulating antigens. Mucin-type glycoproteins constitute suitable target molecules for the induction of tumor-selective immune responses.

Mucins are heavily *O*-glycosylated glycoproteins expressed on the surfaces of many types of epithelial cells.[8,9] Their main function is to provide lubrication, as well as protection from proteolytic degradation and invasion of microorganisms. Of particular interest are the mucins MUC1 and MUC4, which both exist in membrane-bound forms. The mucin MUC1,[10–14] which was the first mucin-type glycoprotein to be defined by murine monoclonal antibodies, is a ubiquitously distributed integral membrane glycoprotein found on epithelial cells in a variety of tissues. The extracellular domain consists of tandem repeats comprising 20 amino acids of the sequence HGVTSAPDTRPAPGSTAPPA (**1**), including five potential *O*-glycosylation sites. First isolated from tracheobronchial tissue in 1991,[15] the human mucin MUC4 contains the largest apoprotein of all known mucins.[16] Its heterodimeric structure consists of a mucin-characteristic α-chain and a membrane-associated β-chain, which are connected through the proteolytic cleavage region GDPH.[17] The extracellular α-chain, which contains a region rich in proline, threonine, and serine, is composed of a repetitive sequence of 16 amino acids (TSSASTGHATPLPVTD, **2**)**.** Both MUC1 and MUC4 adopt extended structures, and so are major components of the glycocalyx.

Abnormal expression of the mucins MUC1 and MUC4 has been observed in tumor cells of various tissues, including lung,[18] colon,[19] pancreatic,[20,21] ovarian, and breast cancers.[22,23] In addition, MUC4 is the only tumor-associated mucin exclusively occurring on pancreatic adenocarcinoma cells. Tumor-associated glycopeptide partial structures from the mucins MUC1 and MUC4[24] are therefore promising candidates for the development of tumor-selective vaccines.

## Results and Discussion

The tumor-associated (2,3)-sialyl-T antigen was synthesized by an efficient linear strategy, which mimicks the enzymatic glycan chain extension taking place in the Golgi apparatus in cancer cells,[4] and was subsequently incorporated into glycopeptide structures from the tandem repeat regions of the mucins MUC1 and MUC4. For this purpose, a suitably protected threonine derivative was first coupled with *N*-acetyl galactosamine. From this $T_N$ antigen-threonine conjugate, the (2,3)-sialyl-T building block was assembled by stepwise saccharide chain extension and utilized in solid-phase glycopeptide syntheses.[25–27] In contrast to other strategies reported earlier,[28–30] the complex sialic acid was introduced, as in nature, at a late stage of the synthesis.

**Synthesis of the (2,3)-sialyl-T threonine building block**: According to this biomimetic strategy, the (2,3)-sialyl-T threonine building block for solid-phase glycopeptide synthesis was prepared by starting from the preformed *N*-acetylgalactosamine threonine conjugate **3** (Scheme 2).[31,32] To provide a glycosyl acceptor suitable for galactosylation at the 3-OH position, **3** was converted into the corresponding benzylidene derivative **4** by de-*O*-acetylation and subsequent formation of the 4,6-benzylidene acetal. The de-*O*-acetylation was carefully carried out under Zemplén conditions[33] at pH 8.5,[31] followed by protection of the 4-OH and 6-OH groups with α,α-dimethoxytoluene and a catalytic amount of *p*-toluenesulfonic acid as reagents. The benzylidene derivative **4** was isolated in 44% yield (over two steps) when *N*,*N*-dimethyl formamide was used as the solvent in the second step and methanol was removed continuously from the reaction mixture at 50°C and reduced pressure (15 mbar). For the same reaction, the yield was increased to 75% (two steps) in acetonitrile as the solvent and at ambient temperature.[34]

The galactosylation of $T_N$-threonine conjugate **4** was one of the key steps in the synthesis of the sialyl-T threonine antigen. The reaction was optimized by comparing different glycosyl donors derived from 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl galactopyranose **5**[35] (Table 1). First, **5** was converted into the anomeric trichloroacetimidate α-**6**,[36–38] which was subjected to glycosylation by Schmidt's procedure.[39,40] The galactosylation of **4** with trichloroacetimidate α-**6** was carried out at −15°C by the use of catalytic amounts of trimethylsilyl triflate in 1,2-dichloroethane as the promoter. After purification by flash chromatography, a mixture of the desired product **7** and the corresponding orthoester **8** was isolated in a ratio of 1:2.7. Separation of the two components was achieved by semi-preparative RP-HPLC and gave the β-glycosylation product **7** in a yield of 21%, together with 58% of the orthoester **8**.

Second, treatment of galactose derivative **5** with a mixture of ethanethiol and boron trifluoride etherate in dichloromethane afforded ethylthio glycoside **9** in 63% yield (α/β ratio

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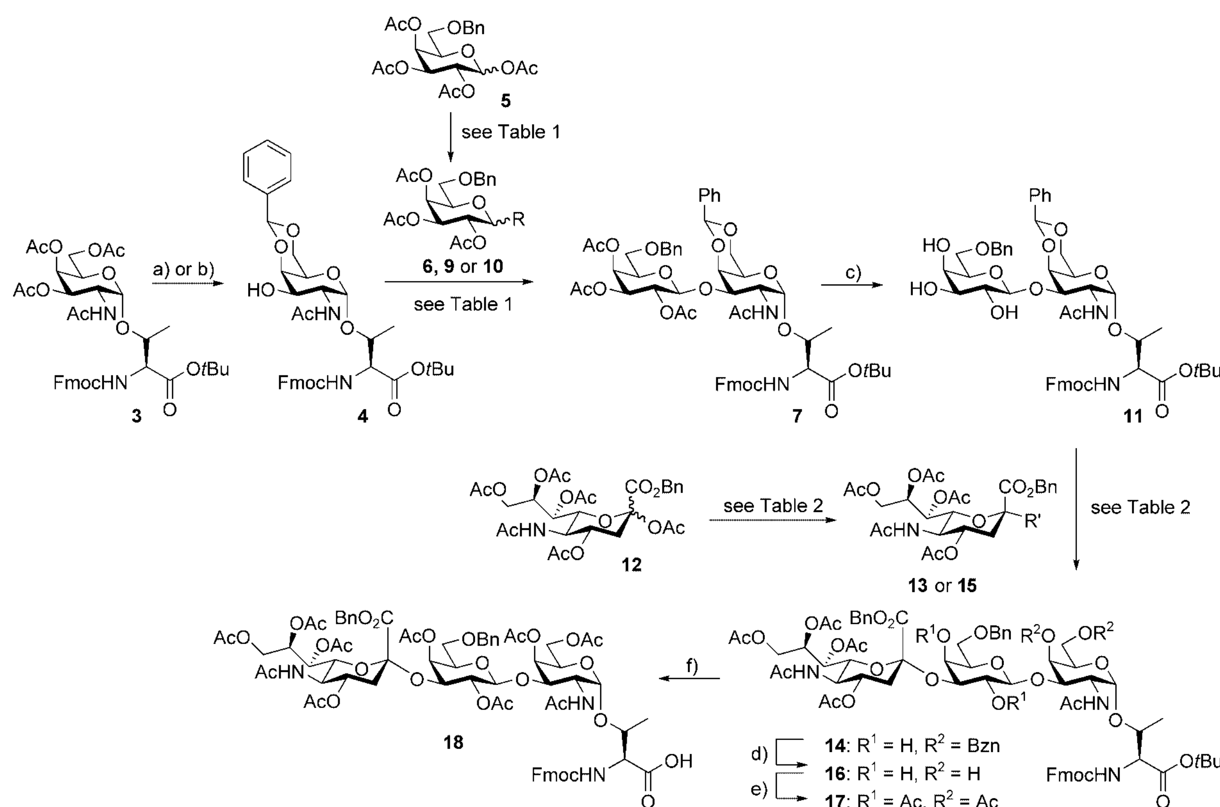


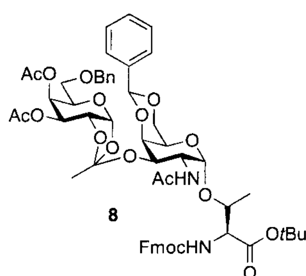

Scheme 2. Reagents and conditions: a) i) MeOH, NaOMe, pH 8.5, 3 h; ii) DMF, α,α-dimethoxytoluene, PTSA, 15 mbar, 50 °C, 2 h, 44 %. b) i) MeOH, NaOMe, pH 8.5, 3 h; ii) MeCN, α,α-dimethoxytoluene, PTSA, room temperature, 15 h, 75 %. c) MeOH, NaOMe, pH 8.5, 12 h, 62 %. d) AcOH (80 %), 80 °C, 1 h, 82 %. e) pyridine/$Ac_2O$ (2.3:1), DMAP, 6 h, 87 %. f) TFA, anisole, quant. DMAP = *N,N*-dimethylaminopyridine; DMF = dimethylformamide; DTBP = di-*tert*-butylpyridine; PTSA = *p*-toluenesulfonic acid; TFA = trifluoroacetic acid.

Table 1. Synthesis of glycosyl donors **6**, **9**, and **10** and subsequent galactosylation of $T_N$-threonine conjugate **4**.

5 —Conditions A→ **6**, **9** or **10** —Conditions B / Glycosylation of **4**→ **7**

| Compound | R | Conditions A | Conditions B | Yield of **7** [%] |
|---|---|---|---|---|
| α-**6** | $OC(=NH)CCl_3$ | 1. $H_2NNH_2$·AcOH, DMF, 83 %<br>2. $Cl_3CCN$, DBU, $CH_2Cl_2$, 53 % | cat. TMSOTf, MS 4 Å, $ClCH_2CH_2Cl$ | 21[a] |
| α,β-**9** | SEt | EtSH, $BF_3$·$Et_2O$, $CH_2Cl_2$, 63 % ($\alpha/\beta$=1.2:1) | cat. TfOH, NIS, MS 4 Å, $CH_2Cl_2$, 0 °C to 20 °C | 52 |
| α-**10** | Br | 33 % HBr in glacial acid, $CH_2Cl_2$, 0 °C, 49 % | AgOTf, MS 4 Å, $CH_2Cl_2$, −40 °C to +20 °C | 66 |
| α-**10** | Br | 33 % HBr in glacial acid, $CH_2Cl_2$, 0 °C, 49 % | $Hg(CN)_2$, MS 4 Å, $CH_3NO_2/CH_2Cl_2$, 0 °C to 20 °C | 93 |

[a] In addition, 58 % of the corresponding orthoester **8** were formed.




1.2:1). The glycosylation of $T_N$-threonine conjugate **4** with β-thio glycoside β-**9** was promoted with *N*-iodosuccinimide and catalytic amounts of trifluoromethanesulfonic acid in dichloromethane at 0 °C and resulted in the formation of 52 % of the β-glycosylation product **7** (Scheme 2).

Finally, different activation procedures for the glycosylation of $T_N$-threonine conjugate **4** were explored with galactosyl bromide α-**10** as donor, this compound also being obtained from galactose derivative **5**.[41] According to the classical procedure developed by Koenigs and Knorr,[42] the 3-β-galactosylation of **4** with α-**10** was carried out with two equivalents of silver triflate as the promoter at −40 °C in dichloromethane and gave the product **7** of the β-glycosylation reaction in 66 % yield.

Alternatively, the same glycosylation reaction was performed by Helferich's procedure[43] with mercury(II) cyanide for activation at ambient temperature and a mixture of nitromethane and dichloromethane as solvents. Under these

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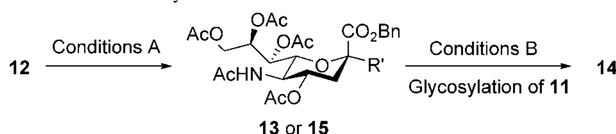
conditions, the desired β-product **7** was formed selectively in 93% yield, due to the neighboring group effect of the 2-acetoxy function of the galactosyl donor.

The de-*O*-acetylation of the disaccharide derivative **7**, without the Fmoc group being affected or β-elimination of the saccharide promoted, was achieved by careful, prolonged treatment with sodium methoxide in methanol at pH 8.5 and resulted in the formation of a 62% yield of the disaccharide acceptor **11**. The regio- and stereoselective glycosylation at the 3′-OH position of the galactose moiety of this compound with a sialic acid donor comprises another pivotal step in the synthesis of the sialyl-T threonine building block. For this type of sialylation, sialic acid thio donors have usually been employed in the Lewis antigen series.[44] Therefore, *N*-acetylneuraminic acid benzyl ester **12** was first converted into the corresponding ethylthio sialoside **13** in 85% yield. The regioselective 3′-sialylation of the disaccharide **11** with ethylthio donor **13** was carried out in acetonitrile at −37 °C in the presence of *N*-iodosuccinimide and catalytic amounts of trifluoromethanesulfonic acid, furnishing the trisaccharide derivative **14** as an anomeric mixture. Separation of the anomers by flash chromatography gave a 38% yield of the desired α product and a 24% yield of the corresponding β anomer (Table 2).

Table 2. Glycosylation of **11** with sialosyl donors **13** and **15**.




| Compound | R′ | Conditions A | Conditions B | Yield of **14** [%] |
|---|---|---|---|---|
| **13** | SEt | 1) EtSH, $BF_3{\cdot}Et_2O$, $CH_2Cl_2$, 85% | cat. TfOH, NIS, MS 3 Å, $CH_3CN$, −37 °C to −23 °C | 38[a] |
| **15** | S–C(=S)–OEt | 1) $CH_3COCl$ 2) KS(CS)OEt, EtOH, 49% | PhSCl, AgOTf, DTBP, MS 3 Å, $CH_3CN/CH_2Cl_2$, −68 °C | 49[b] |
| **15** | S–C(=S)–OEt | 1) $CH_3COCl$ 2) KS(CS)OEt, EtOH, 49% | MeSBr, AgOTf, MS 3 Å, $CH_3CN/CH_2Cl_2$, −68 °C | 58 |

[a] Plus 24% of the corresponding β anomer. [b] Plus 2% of the corresponding β anomer.

Besides thio sialosides, sialosyl xanthates[45] have also proven to be efficient glycosyl donors for sialylation reactions. Xanthate **15**[45,46] of the *N*-acetylneuraminic acid benzyl ester was prepared in a yield of 49% from **12** by intermediate formation of the anomeric chloride. The glycosylation of disaccharide conjugate **11** with sialosyl xanthate **15** was performed at low temperature (−68 °C) in a mixture of acetonitrile/dichloromethane (2:1) in order to favor the kinetically controlled stereoselective formation of the α-sialoside **14**. Activation of the xanthate with phenylsulfenyl triflate, which was generated in situ from phenylsulfenyl chloride and silver triflate,[47] and the use of di-*tert*-butylpyridine as a proton scavenger gave a 49% yield of the desired α2,3-sialyl-T threonine conjugate **14**.[48] Only a 2% yield of the corresponding β anomer was produced. Completely stereoselective α-sialylation was achieved in a yield of 58% when methylsulfenyl triflate, produced in situ from methylsulfenyl bromide and silver triflate,[49] was used as reagent for activation of xanthate **15**.[50]

For the conversion of trisaccharide **14** into a (2,3)-sialyl-T threonine conjugate suitable as a building block for sequential solid-phase glycopeptide synthesis, a number of protecting group manipulations were required. Acidolytic cleavage of the benzylidene acetal of **14** with 80% aqueous acetic acid proceeded at 80 °C in a yield of 82% without affecting the acid-labile *tert*-butyl ester to give **16**. The ensuing *O*-acetylation of the remaining hydroxy groups, including the sterically hindered 4′-OH, was performed with a mixture of pyridine and acetic acid catalyzed by *N*,*N*-(dimethylamino)-pyridine in a yield of 87%. Subsequent acidolysis of the *tert*-butyl ester of **17** with trifluoroacetic acid in dichloromethane and anisole as a cation scavenger quantitatively provided the Fmoc-(2,3)-sialyl-T threonine building block **18**.

**Solid-phase synthesis of a MUC1 glycopeptide containing the tumor-associated (2,3)-sialyl-T antigen**: The Fmoc-(2,3)-sialyl-T threonine building block **18** was incorporated in the sequential solid-phase synthesis of a glycododecapeptide derived from the tandem repeat sequence **1** of the epithelial mucin MUC1. The glycopeptide was assembled in an automated synthesizer by the Fmoc strategy, with side chain *O*-*tert*-butyl-protected threonine/serine and *N*-Pmc arginine building blocks. NovaSyn Tentagel coupled with the novel fluoride-labile PTMSEL linker[51,52] loaded with the start amino acid Fmoc-proline **19** (Scheme 3) was employed as the resin. The PTMSEL anchor can be cleaved by tetrabutylammonium fluoride trihydrate in dichloromethane under almost neutral conditions, thereby avoiding side reactions such as aspartimide rearrangements.

The first eight amino acids of the MUC1 sequence were coupled by the standard procedure. Piperidine in *N*-methylpyrrolidone (NMP) was used for the removal of the temporary Fmoc protecting group in each coupling cycle, followed by coupling with an excess (20 equiv) of the next Fmoc-amino acid activated by HBTU[53]/HOBt[54] and diisopropylethylamine (DIPEA) in *N*,*N*-dimethylformamide (DMF). Unreacted amino groups were capped after each cycle with acetic anhydride in the presence of DIPEA and catalytic amounts of HOBt in NMP. Subsequent coupling of only 1.4 equivalents of the valuable Fmoc-(2,3)-sialyl-T threonine building block **18** was carried out manually over a period of 4 h with use of HATU/HOAt[55] and *N*-methylmorpholine (NMM) in NMP for activation. The final two Fmoc-amino acids were again attached by the standard procedure, and the *N*-terminal

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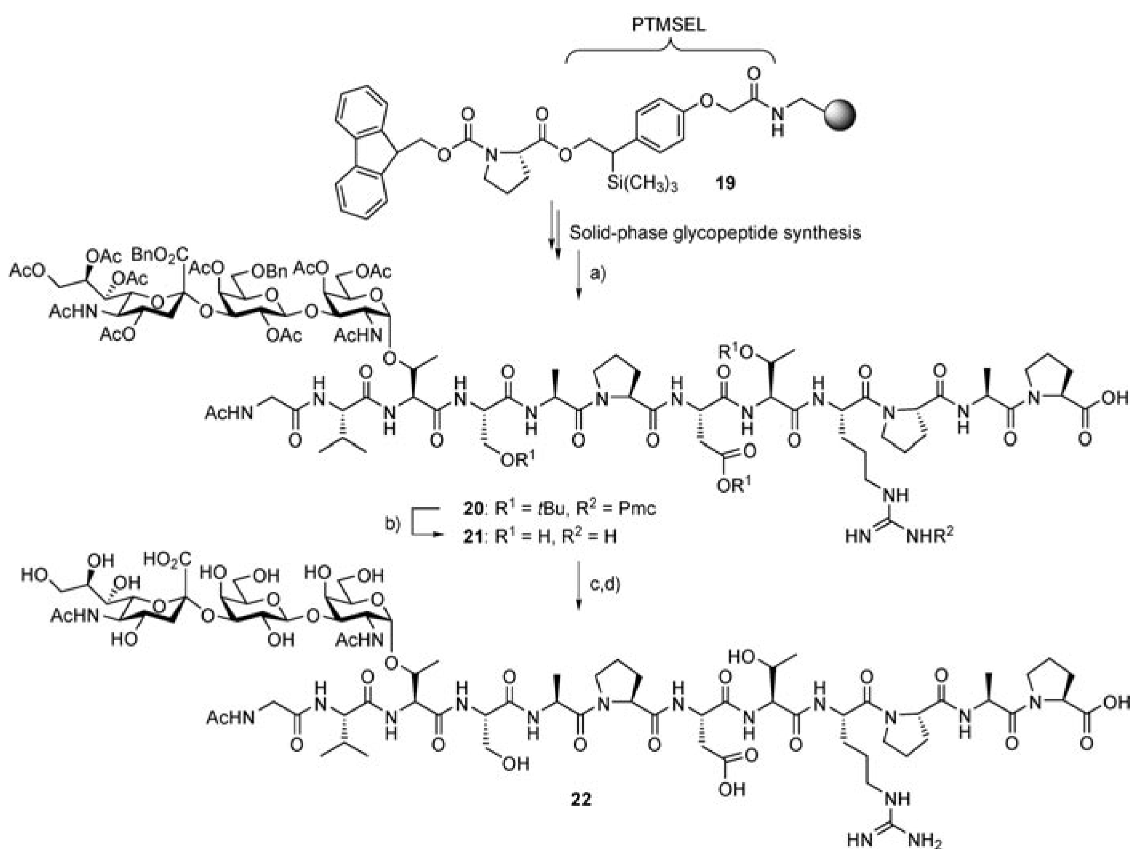


Scheme 3. Reagents and conditions: a) i) solid-phase glycopeptide synthesis (SPGS): Fmoc-removal (piperidine/NMP (20%)), coupling (1st–8th, 10th–11th: Fmoc-AA-OH (20 equiv), HBTU/HOBt/DIPEA, DMF, 9th: **18** (1.4 equiv), HATU/HOAt/NMM, DMF, 4 h), capping: $Ac_2O$, DIPEA, HOBt (4:1:0.12). ii) TBAF·3$H_2O$ (2×2.5 equiv), $CH_2Cl_2$, 45 min (each time), 39% (with respect to **19**). b) TFA/TIS/$H_2O$ (15:0.9:0.9), 2 h. c) $H_2$, Pd/C (10%), MeOH, 23 h. d) $NaOH_{aq}$ (5 mM), 59 h (56% over three steps). HATU = *O*-(7-aza-benzotriazole-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOAt = *N*-hydroxy-7-azabenzotriazole, HOBt = *N*-hydroxybenzotriazole; NMM = *N*-methylmorpholine; TBAF = tetrabutylammonium fluoride; TIS = triisopropylsilane; Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl-; PTMSEL = (2-phenyl-2-trimethylsilyl)ethyl-linker.

Fmoc group was exchanged for an acetyl group. Treatment with tetrabutylammonium fluoride in dichloromethane released the glycopeptide **20** from the resin without affecting the permanent protecting groups of the amino acid side chains and the glycan portion. After purification by semi-preparative RP-HPLC, the protected glycododecapeptide **20** was isolated in a yield of 39% (based on the loaded resin **19**).

Cleavage of the acid-labile protecting groups of the amino acid side chain functionalities with a mixture of trifluoroacetic acid, triisopropylsilane, and water furnished the partially deblocked glycopeptide **21**. For the simultaneous removal of the sialic acid benzyl ester and the galactose benzyl ether, **21** was dissolved in methanol and subjected to hydrogenolysis, with 10% palladium on activated charcoal as the catalyst. The final de-*O*-acetylation of the saccharide moiety was accomplished by prolonged treatment with 5 mM aqueous sodium hydroxide solution and carefully monitored by analytical RP-HPLC. Under these basic conditions (pH 11.5), neither β-elimination of the glycan nor epimerization of the amino acids were observed. The completely deblocked MUC1 glycododecapeptide **22** was obtained after purification by semi-preparative RP-HPLC in a yield of 56% (over three steps).

**Solid-phase synthesis of a (2,3)-sialyl-T glycopeptide derived from the tandem repeat sequence of the epithelial mucin MUC4**: The automated solid-phase synthesis of the MUC4 glycohexadecapeptide containing the (2,3)-sialyl-T antigen was performed starting from commercially available Tentagel S resin[56] coupled with the acid-labile Wang linker[57] loaded with Fmoc-aspartic acid β-*tert*-butyl ester **23** (Scheme 4). All Fmoc-amino acids were coupled in excess (20 equiv) by the standard Fmoc strategy procedure described above. *N*-Tritylimidazole was used for protection of the histidine side chain. The manual coupling of 1.3 equivalents of the Fmoc-(2,3)-sialyl-T threonine building block **18**, dissolved in NMP, was again activated by HATU/HOAt and NMM. After completion of the 16-amino acid MUC4 tandem repeat sequence **2**, the *N*-terminus of the glycopeptide was acetylated. Simultaneous detachment of the glycohexadecapeptide from the resin and cleavage of the acid-labile protecting groups on the aspartic acid, serine, threonine, and histidine side chains was achieved by treatment of the resin with a mixture of trifluoroacetic acid, triisopropylsilane, and water to give a 46% yield of the partially deprotected MUC4 glycopeptide **24** (yield based on loaded resin **23**, after semipreparative RP-HPLC).

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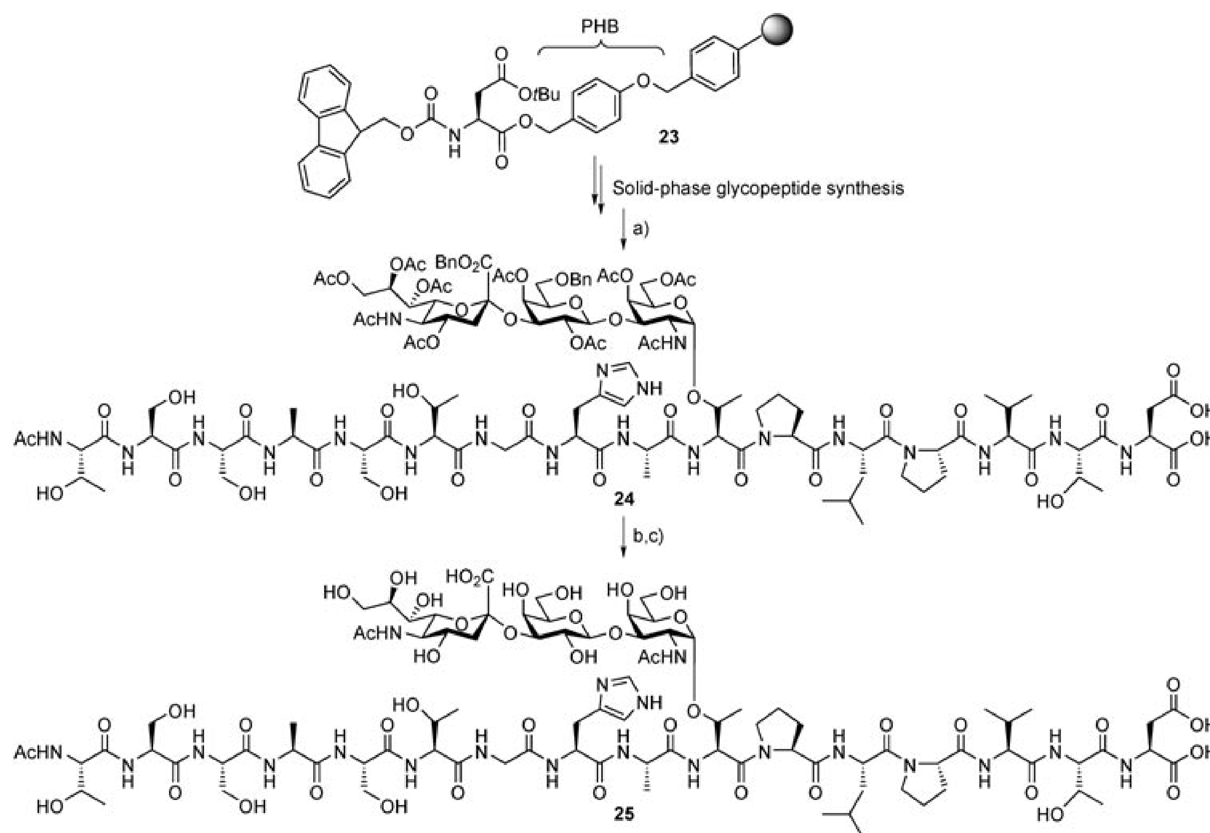


Scheme 4. Reagents and conditions: a) i) solid-phase glycopeptide synthesis (SPGS): Fmoc-removal (piperidine/NMP (20%)), coupling (1st–5th, 7th–15th.: Fmoc-AA-OH (20 equiv), HBTU/HOBt/DIPEA, DMF; 6th **18** (1.3 equiv), HATU/HOAt/NMM, DMF, 4 h), capping: $Ac_2O$, DIPEA, HOBt (4:1:0.12). ii) TFA/TIS/$H_2O$ (15:0.9:0.9), 2 h, 46% (with respect to **23**). b) $H_2$, Pd/C (10%), MeOH, 23 h. c) i) MeOH, NaOMe, pH 10, 48 h. ii) $NaOH_{aq}$ (5 mM), 36 h (64% over three steps).

Complete deprotection of the MUC4 glycopeptide **24** was accomplished by concomitant hydrogenolysis of the sialic acid benzyl ester and the galactose benzyl ether, followed by de-*O*-acetylation of the glycan moiety. The deacetylation was first carried out under Zemplén conditions[33] at pH 10. In this case, one *O*-acetyl group, presumably at the hindered 4′-position of the galactose portion, remained resistant to saponification, so additional treatment with aqueous sodium hydroxide at pH 11.5 as described above was necessary. After purification by semi-preparative RP-HPLC, the MUC4 glycohexadecapeptide **25** was isolated in a yield of 64% (over three steps).

## Conclusion

The sequential solid-phase synthesis of two different mucin-type glycopeptides has been accomplished with the use of a preformed Fmoc-(2,3)-sialyl-T threonine conjugate as a building block. For the preparation of the crucial glycosyl amino acid conjugate **18**, a novel, linear synthetic strategy based on the biosynthesis of the (2,3)-sialyl-T antigen has been established. In addition to an efficient selective protecting group strategy compatible with the sensitive structure of the *O*-glycosyl amino acid derivative, the key steps included the stereoselective β-galactosylation of a $T_N$ threonine derivative in a yield of 93% and the regio- and stereoselective α-sialylation of a disaccharide threonine precursor, which proceeded in yields of up to 58%. The obtained Fmoc-(2,3)-sialyl-T threonine building block **18** proved to be stable under the conditions applied during the standard Fmoc procedure for glycopeptide assembly, therefore constituting a versatile synthetic conjugate for the solid-phase synthesis of (2,3)-sialyl-T glycopeptides. By the use of different anchoring systems in automated solid-phase syntheses, the glycopeptides were assembled either in their completely protected or in partially deblocked forms, as is demonstrated for glycopeptides of the tandem repeat regions of MUC1 and MUC4.

Investigations on the immunological activity of the tumor-associated (2,3)-sialyl-T MUC1 and MUC4 glycopeptides **22** and **25** concerning their influence on the proliferation of peripheric blood lymphocytes are currently underway.[46] In addition, detailed conformational studies by NMR spectroscopy in aqueous solution are being undertaken.

## Experimental Section

**General**: Solvents for moisture-sensitive reactions (acetonitrile, nitromethane, methanol, dichloromethane) were distilled and dried by standard procedures. DMF (amine-free, for peptide synthesis) was purchased from

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Roth, acetic anhydride and pyridine in p.a. quality from ACROS. Reagents were purchased at the highest available commercial quality and were used without further purification unless outlined otherwise. Fmoc-protected amino acids were purchased from Novabiochem. As resins for solid-phase synthesis, aminomethylated TentaGel (NovaSyn Tg amino resin, Novabiochem) and Rapp TentaGel were used. Reactions were monitored by thin-layer chromatography with pre-coated silica gel 60 $F_{254}$ aluminium plates (Merck KGaA, Darmstadt). Flash column chromatography was performed with silica gel (40–63 μm) purchased from Merck KGaA, Darmstadt. Optical rotations $[\alpha]_D$ were measured with a Perkin-Elmer 241 polarimeter. RP-HPLC analyses were carried out on a Knauer HPLC system with Phenomenex Luna C18(2) (250×4.6 mm, 5 μ) and Phenomenex Jupiter C18 columns (250×4.6 mm, 5 μ) at a pump rate of 1 mL min$^{-1}$. Preparative HPLC separations were performed on a Knauer HPLC system with a Phenomenex Luna C18(2) column (250×50 mm, 10 μm) and a pump rate of 20 mL min$^{-1}$. Semi-preparative HPLC separations were carried out on a Knauer HPLC system with Phenomenex Luna C18(2) (250×21.20 mm, 10 μ) and Phenomenex Jupiter (250×21.20 mm, 5 μ) columns at a flow rate of 10 mL min$^{-1}$. Water and $CH_3CN$ were used as solvents. $^1$H, $^{13}$C, and 2D NMR spectra were recorded on Bruker AC 200, AM 400, ARX 400, or DRX 600 spectrometers. Proton chemical shifts are reported in ppm relative to residual $CHCl_3$ ($\delta$ = 7.24), DMSO ($\delta$ = 2.49), methanol ($\delta$ = 3.31), or water ($\delta$ = 4.76). Multiplicities are given as: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). $^{13}$C chemical shifts are reported relative to $CDCl_3$ ($\delta$ = 77.0), DMSO ($\delta$ = 39.5), or methanol ($\delta$ = 49.00). Assignment of proton and carbon signals was achieved by COSY, TOCSY, HMQC, and HMBC experiments when noted. For $^1$H and $^{13}$C signals of the saccharide portions the following denominations were used: *N*-acetyl-D-galactosamine (no prime), D-galactose (′), and *N*-acetyl-neuraminic acid (″). MALDI-TOF mass spectra were acquired on a Micromass Tofspec E spectrometer while ESI-mass spectra were obtained on a ThemoQuest-Navigator spectrometer. HR-ESI mass spectra were recorded on a Micromass Q-TOF Ultima spectrometer.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu) (4): Procedure A**: A solution of sodium methoxide in methanol (5%) was added dropwise to Fmoc-Thr(α-$Ac_3$-GalNAc)-O*t*Bu **3**[31,32] (186 mg, 0.256 mmol) dissolved in dry methanol (6 mL) until pH 8.5 was reached. The reaction mixture was stirred for 3 h and was subsequently neutralized by addition of acidic ion-exchange resin (Amberlyst 15). After filtration, the solvent was removed in vacuo. The resulting solid was dissolved in *N*,*N*-dimethylformamide (10 mL), and α,α-dimethoxytoluene (77 μL) was added. The pH was adjusted to 3.5 with a catalytic amount of *p*-toluenesulfonic acid (10 mg). The reaction was carried out under reduced pressure (15 mbar) at 50 °C in a rotary evaporator. After 2 h the mixture was neutralized with ethyldiisopropylamine (4 drops), and the solvent was removed in vacuo. The resulting residue was co-evaporated with toluene (3×20 mL) and purified by flash chromatography (silica gel, petroleum ether/ethyl acetate 1:2) to afford compound **4** (78 mg, 44%) as a colorless, amorphous solid.

**Procedure B**: A solution of sodium methoxide in methanol (5%) was added dropwise to a solution of Fmoc-Thr(α-$Ac_3$GalNAc)-O*t*Bu **3** (1.65 g, 2.27 mmol) in dry methanol (50 mL) until pH 8.5 was reached. The reaction mixture was stirred for 3 h and was subsequently neutralized by addition of acidic ion-exchange resin (Amberlyst 15). After filtration, the solvent was removed in vacuo. The resulting solid was dissolved in dry acetonitrile (300 mL), and α,α-dimethoxytoluene (684 μL) was added. The pH was adjusted to 4 with a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was stirred for 15 h at room temperature and was neutralized with triethylamine (4–5 drops). After removal of the solvent in vacuo, purification of the resulting residue by flash chromatography (silica gel, petroleum ether/ethyl acetate 1:2) gave compound **4** (1.18 g, 75%) as a colorless amorphous solid. $R_f$ = 0.60 (toluene/ethanol 4:1); $[\alpha]_D^{23}$ = 64.4 ($c$ = 1, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.76 (d, $J_{H3,H4} = J_{H5,H6}$ = 7.4 Hz, 2H; H4-, H5-Fmoc), 7.61 (d, $J_{H1,H2} = J_{H7,H8}$ = 7.4 Hz, 2H; H1-, H8-Fmoc), 7.51–7.49 (m, 2H; H3-, H6-Fmoc), 7.42–7.30 (m, 7H; H2-, H7-Fmoc, $H_{arom}$-Bzn), 6.49 (d, $J_{NH,H2}$ = 8.6 Hz, 1H; NH (GalNAc$^{NH}$)), 5.56 (s, 1H; CH-Bzn), 5.49 (d, $J_{NH,T\alpha}$ = 9.4 Hz, 1H; NH-T), 4.93 (d, $J_{H1,H2}$ = 3.1 Hz, 1H; H1), 4.50–4.44 (m, 3H; H2, $CH_2$-Fmoc), 4.25–4.04 (m, 6H; H5, H6a, H6b, H9- Fmoc, $T^\alpha$, $T^\beta$), 3.86–3.82 (m, 1H; H3), 3.70 (s, 1H; H4), 2.44 (d, 1H; OH, $J_{OH,H3}$ = 9.4 Hz), 2.07 (s, 3H; $CH_3$-Ac), 1.45 (s, 9H; $CH_3$–*t*Bu), 1.27 (d, 3H; $T^\gamma$, $J_{T\beta,T\gamma}$ = 6.3 Hz) ppm; $^{13}$C NMR (100.6 MHz, BB, DEPT, $CDCl_3$): $\delta$ = 172.5, 170.7 (C=O), 156.5 (C=O-urethane), 143.7 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 137.5 ($C_q$-Bzn), 129.1, 128.2, 126.3 ($C_{arom}$-Bzn), 127.8 (C3-, C6-Fmoc), 127.1 (C-2, C7-Fmoc), 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C-5-Fmoc), 101.2 (CH-Bzn), 100.0 (C1), 83.4 ($C_q$-*t*Bu), 76.7 ($T^\beta$), 75.5 (C3), 69.6 (C4), 69.2 ($CH_2$-Fmoc), 67.2 (C6), 63.7 (C5), 58.9 ($T^\alpha$), 50.4 (C2), 47.2 (C9-Fmoc), 28.1 ($CH_3$-*t*Bu), 23.1 ($CH_3$-Ac), 19.1 ($T^\gamma$) ppm; ESI-MS: *m/z*: found: 711.3 [*M*+Na]$^+$; $C_{38}H_{44}N_2O_{10}Na$ calcd 711.3; elemental analysis calcd (%): C 66.26, H 6.44, N 4.07; found: C 66.32, H 6.35, N 3.90.

**Ethyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-α,β-D-thiogalactopyranoside (6-*O*-Bn-$Ac_3$Gal-Set) (9)**: Ethanethiol (169 μL, 2.29 mmol) was added under argon to a solution of 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl-α,β-D-galactopyranose **5**[35] (911 mg, 2.08 mmol) in dry dichloromethane (40 mL). The mixture was treated with boron trifluoride etherate (48%, 650 μL, 5.20 mmol) at 0 °C and stirred for 2 days at ambient temperature. The reaction mixture was then diluted with dichloromethane (100 mL), washed with saturated aqueous $NaHCO_3$ (3×70 mL), dried over $MgSO_4$, and filtered, and the solvent was removed at reduced pressure. The residue was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate 6:1) to give the anomeric products α-**9** and β-**9** as colorless oils in a ratio of $\alpha/\beta$ = 1.2:1 (overall yield: 573 mg, 63%).

***α Anomer***: $R_f$ = 0.63 (petroleum ether/ethyl acetate 3:1); $[\alpha]_D^{23}$ = 148.2 ($c$ = 1, $CH_2Cl_2$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.31–7.23 (m, 5H; $H_{ar}$-Bn), 5.71 (d, $J_{H1,H2}$ = 5.5 Hz, 1H; H1), 5.47 (d, $J_{H3,H4}$ = 2.0 Hz, 1H; H4), 5.26–5.17 (m, 2H; H2, H3), 4.56–4.50 (m, 2H; H5, $CH_{2a}$-Bn), 4.39 (d, 1H; $CH_{2b}$-Bn), $J_{a,b}$ = 12.1 Hz), 3.48–3.45 (m, 2H; H6a,b), 2.58–2.46 (m, 2H; $CH_2$-Et), 2.03 (s, 3H; $CH_3$-Ac), 2.02 (s, 3H; $CH_3$-Ac), 1.95 (s, 3H; $CH_3$-Ac), 1.21 (t, $J_{CH2,CH3}$ = 7.4 Hz, 3H; $CH_3$-Et) ppm; $^{13}$C NMR (100.6 MHz, BB, $CDCl_3$): $\delta$ = 170.1, 169.7 (C=O), 137.6 ($C_q$-Bn), 128.3, 127.7, 127.6 ($C_{ar}$-Bn), 81.6 (C1), 73.3 ($CH_2$-Bn), 68.4, 68.3, 68.1, 67.4 (C2, C3, C4, C5), 67.8 (C6), 23.8 ($CH_2$-Et), 20.8, 20.6, 20.5 ($CH_3$-Ac), 14.5 ($CH_3$-Et) ppm; MS (ESI): *m/z*: found: 463.3 [*M*+Na]$^+$; $C_{21}H_{28}NaO_8S$ calcd 463.1.

***β Anomer***: $R_f$ = 0.53 (petroleum ether/ethyl acetate 3/1); $[\alpha]_D^{23}$ = −20.3 (c = 1, $CH_2Cl_2$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.31–7.22 (m, 5H; $H_{ar}$-Bn), 5.46 (d, $J_{H3,H4}$ = 3.1 Hz, 1H; H4), 5.17 (t, $J_{H1,H2}$ = 9.8 Hz, $J_{H2,H3}$ = 10.1 Hz, 1H; H2), 5.00 (dd, $J_{H2,H3}$ = 10.1 Hz, $J_{H3,H4}$ = 3.1 Hz, 1H; H3), 4.50 (d, $J_{a,b}$ = 12.1 Hz, 1H; $CH_{2a}$-Bn), 4.45 (d, $J_{H1,H2}$ = 9.8 Hz, 1H; H1), 4.36 (d, $J_{a,b}$ = 11.7 Hz, 1H; $CH_{2b}$-Bn), 3.83 (t, $J_{H5,H6a}$ = 6.3 Hz, $J_{H5,H6b}$ = 6.7 Hz, 1H; H5), 3.52 (dd, $J_{H5,H6a}$ = 6.3 Hz, $J_{H6a,b}$ = 9.4 Hz, 1H; H6a), 3.52 (dd, $J_{H5,H6b}$ = 7.1 Hz, $J_{H6a,b}$ = 9.4 Hz, 1H; H6b), 2.73–2.62 (m, 2H; $CH_2$-Et), 2.01 (s, 3H; $CH_3$-Ac), 2.00 (s, 3H; $CH_3$-Ac), 1.93 (s, 3H; $CH_3$-Ac), 1.23 (t, $J_{CH2,CH3}$ = 7.4 Hz, 3H; $CH_3$-Et) ppm; $^{13}$C NMR (100.6 MHz, BB, $CDCl_3$): $\delta$ = 170.1, 170.0, 169.6 (C=O), 137.5 ($C_q$-Bn), 128.4, 127.9, 127.8 ($C_{ar}$-Bn), 83.9 (C1), 75.8 (C2), 73.5 ($CH_2$-Bn), 72.1 (C3), 67.7 (C4), 67.4 (C5, C6), 24.3 ($CH_2$-Et), 20.8, 20.6 ($CH_3$-Ac (3×)), 14.8 ($CH_3$-Et) ppm; MS (ESI): *m/z*: found: 463.1 [*M*+Na]$^+$; $C_{21}H_{28}NaO_8S$ calcd 463.1.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-4,6-*O*-benzylidene-2-desoxy-3-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (Fmoc-Thr(β-6-*O*-Bn-$Ac_3$Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu) (7)**: ***Procedure A***: Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **4** (1.00 g, 1.45 mmol) and 6-*O*-Bn-$Ac_3$Gal-TCI α-**6**[36–38] (979 mg, 1.81 mmol) were each separately co-evaporated with toluene (3×50 mL) and dried under high vacuum. The glycosyl acceptor **4** and the glycosyl donor α-**6** were dissolved in dry dichloromethane (25 mL), and activated molecular sieves (1 g, 4 Å) were added. The suspension was stirred for 1 h at ambient temperature under argon. After the system had been cooled to −15 °C, a solution of trimethylsilyl triflate (29 μL) in dry dichloromethane (2.5 mL) was added. The reaction mixture was allowed to warm up to room temperature over 1 h, diluted with dichloromethane (50 mL), and neutralized with triethylamine. The mixture was filtered through Hyflo Super Cel®, and the solvent was removed from the filtrate in vacuo. Purification of the residue by flash chromatography (silica gel, ethyl acetate/petroleum ether 2:1) resulted in a mixture of the desired β-glycosylation product **7** and the orthoester **8**. The two components were separated by preparative RP-HPLC (column: Phenomenex Luna, isocratic: $CH_3CN/H_2O$ 70:30) to give the product **7** ($R_t$ =


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96 min) and the orthoester **8** ($R_t$ = 108 min) as colorless, amorphous solids in a ratio of **7**/**8** = 1:2.7 (overall yield: 1.22 g, 79%).

***Procedure B***: Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **4** (100 mg, 0.145 mmol) and 6-*O*-Bn-$Ac_3$Gal-SEt β-**9** (80 mg, 0.182 mmol) were each co-evaporated with toluene (3×10 mL), and dried under high vacuum. The glycosyl acceptor **4** and the glycosyl donor β-**9** were dissolved in dry dichloromethane (3 mL). Flame-dried molecular sieves (300 mg, 4 Å) were added to this solution, and the mixture was stirred for 20 min at ambient temperature under argon. The suspension was cooled to 0°C, *N*-iodosuccinimide (82 mg, 0.363 mmol) was added, and the mixture was stirred for an additional 20 min. Subsequently, trifluoromethanesulfonic acid (6.3 μL) was added dropwise, and the reaction mixture was stirred for 20 h at ambient temperature. It was diluted with dichloromethane (25 mL) and filtered through Hyflo Super Cel. The filtrate was washed with saturated aqueous $NaHCO_3$ (3×15 mL) and saturated aqueous $Na_2S_2O_3$ (2×15 mL), dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. The crude product was purified by flash chromatography (silica gel, petroleum ether/ethyl acetate 2:3) to give **7** as a colorless, amorphous solid (81 mg, 52%).

***Procedure C***: Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **4** (100 mg, 0.145 mmol) and 6-*O*-Bn-$Ac_3$Gal-Br α-**10**[41] (100 mg, 0.218 mmol) were separately co-evaporated with toluene (3×10 mL) and dried under high vacuum. Molecular sieves (200 mg, 4 Å) and silver triflate (75 mg, 0.290 mmol) were added to a solution of glycosyl acceptor **4** in dry dichloromethane (2 mL). After the suspension had been stirred for 30 min at ambient temperature under an argon atmosphere with exclusion of light, the glycosyl donor α-**10**, dissolved in dry dichloromethane (2 mL), was added at −40°C. The reaction mixture was allowed to warm up to room temperature over 20 h. Since the reaction was not complete at this stage (monitoring by TLC), 6-*O*-Bn-$Ac_3$Gal-Br α-**10** (100 mg, 0.218 mmol) was again added to the reaction mixture, which was stirred for an additional two days at ambient temperature with protection from light. The mixture was diluted with dichloromethane (100 mL) and filtered through Hyflo Super Cel. The filtrate was washed with saturated aqueous $NaHCO_3$ (3×50 mL), dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. Purification of the residue by flash chromatography (silica gel, petroleum ether/ethyl acetate 2:3) afforded the β-glycosylation product **7** as a colorless, amorphous solid (102 mg, 66%).

***Procedure D***: Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **4** (890 mg, 1.29 mmol) and 6-*O*-Bn-$Ac_3$Gal-Br α-**10** (1.187 g, 2.58 mmol) were each separately co-evaporated with toluene (3×50 mL) and dried under high vacuum. The glycosyl acceptor **4** was dissolved in a mixture of dry nitromethane (25 mL) and dry dichloromethane (5 mL), was treated with molecular sieves (3 g, 3 Å) and $Hg(CN)_2$ (652 mg, 2.58 mmol), and was stirred for 30 min at ambient temperature under argon. The glycosyl donor α-**10**, dissolved in dry dichloromethane (10 mL), was subsequently added at 0°C. The reaction mixture was stirred for four days at room temperature, was diluted with dichloromethane (150 mL), and was filtered through Hyflo Super Cel. The filtrate was washed with saturated aqueous $NaHCO_3$ (3×100 mL) and saturated aqueous NaI (2×100 mL), dried over $MgSO_4$, and filtered, and the solvents were removed under reduced pressure. After purification of the crude product by flash chromatography (silica gel, petroleum ether/ethyl acetate 2:3), the β-glycosylation product **7** was isolated as a colorless, amorphous solid (1.288 g, 93%).

$R_f$ = 0.63 (ethyl acetate/petroleum ether 3:1); $[\alpha]_D^{23}$ = 74.4 ($c$ = 1, $CH_2Cl_2$); $^1$H NMR (400 MHz, COSY, HMQC, $CDCl_3$): $\delta$ = 7.76 (d, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.4 Hz, 2H; H4-, H5-Fmoc), 7.61 (d, $J_{H1,H2}$ = $J_{H7,H8}$ = 7.8 Hz, 2H; H1-, H8-Fmoc), 7.52 (d, $J_{a,b}$ = 6.3 Hz, 2H; $H_{ar}$-Bzn), 7.43–7.20 (m, 12H; H2-, H3-, H6-, H7-Fmoc, $H_{ar}$-Bzn (3H), $H_{ar}$-Bn (5H)), 5.81 (d, $J_{NH,H2}$ = 9.0 Hz, 1H; NH-GalNAc), 5.50 (s, 1H; CH-Bzn), 5.46 (d, $J_{NH,T\alpha}$ = 9.4 Hz, 1H; NH-T), 5.41 (d, $J_{H3',H4'}$ = 3.1 Hz, 1H; H4′), 5.19 (dd, $J_{H1',H2'}$ = 7.8 Hz, $J_{H2',H3'}$ = 10.2 Hz, 1H; H2′), 5.00–4.90 (m, 2H; H3′ {4.97, dd, $J_{H2',H3'}$ = 10.2 Hz, $J_{H3',H4'}$ = 3.5 Hz}, H1 {4.93}), 4.68 (d, $J_{H1',H2'}$ = 8.2 Hz, 1H; H1′), 4.66–4.59 (m, 1H; H2), 4.52–4.37 (m, 4H; $CH_2$-Fmoc {4.47}, $CH_2$-Bn {4.39}), 4.30–4.10 (m, 5H; H4 {4.26}, H9-Fmoc {4.22}, $T^\alpha$ {4.18}, $T^\beta$ {4.16}, H6a {4.12}), 3.93–3.79 (m, 3H; H6b {3.88}, H5′ {3.85}, H3 {3.81}), 3.60 (s, 1H; H5), 3.55 (t, $J_{H5',H6'a}$ = 6.7 Hz, 1H; H6a′), 3.51–3.45 (m, 1H; H6b′), 2.05 (s, 3H; $CH_3$-Ac), 2.04 (s, 3H; $CH_3$-Ac), 1.98 (s, 3H; $CH_3$-Ac), 1.95 (s, 3H; $CH_3$-Ac), 1.43 (s, 9H; $CH_3$-*t*Bu), 1.24 ($s_b$, 3H; $T^\gamma$) ppm; $^{13}$C NMR (100.6 MHz, BB, HMQC, $CDCl_3$): $\delta$ = 170.3, 170.2, 169.8, 169.7, 169.4 (C=O), 156.4 (C=O-urethane), 143.6 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 137.6, 137.4 ($C_q$-Bn, $C_q$-Bzn), 128.7, 128.1, 126.2 ($C_{ar}$-Bzn), 128.4, 127.9 ($C_{ar}$-Bn), 127.8 (C3-, C6-Fmoc), 127.1 (C2-, C7-Fmoc), 124.9 (C1-, C8-Fmoc), 120.1 (C4-, C5-Fmoc), 101.7 (C1′), 100.5 (CH-Bzn), 100.3 (C1), 83.1 ($C_q$-*t*Bu), 76.1 ($T^\beta$), 75.5 (C4), 74.9 (C3), 73.5 ($CH_2$-Bn), 72.2 (C5′), 71.2 (C3′), 67.0 (C6), 68.9 (C2′), 67.9 (C6′), 67.6 (C4′), 66.9 ($CH_2$-Fmoc), 63.6 (C-5), 59.0 ($T^\alpha$), 47.9 (C2), 47.2 (C9-Fmoc), 28.0 ($CH_3$–*t*Bu), 23.4 ($CH_3$-NHAc), 20.7, 20.6 ($CH_3$-OAc (3×)), 18.9 ($T^\gamma$) ppm; HR-MS (ESI-TOF): *m/z*: found: 1089.4196 $[M+Na]^+$; $C_{57}H_{66}N_2O_{18}Na$ calcd 1089.4208.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-2-deoxy-4,6-*O*-benzylidene-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (11)**: A solution of sodium methoxide in methanol (0.1 M) was added dropwise to a solution of Fmoc-Thr(β-6-*O*-Bn-$Ac_3$Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **7** (1.03 g, 0.965 mmol) in methanol (25 mL) until pH 8.5 was reached. The reaction mixture was stirred for 12 h, during which the pH was carefully monitored and readjusted when necessary. Neutralization with acetic acid (0.05 mL), removal of the solvent in vacuo, and purification by flash chromatography (silica gel, ethyl acetate/ethanol 50:1→25:1) afforded compound **11** (560 mg, 62%) as a colorless, amorphous solid. $R_f$ = 0.35 (toluene/ethanol 4:1); $[\alpha]_D^{24}$ = 62.4 ($c$ = 1, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.76 (d, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.8 Hz, 2H; H4-, H5-Fmoc), 7.60 (dd, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.0 Hz, 2H; H1-, H8-Fmoc), 7.49 (d, $J_{Ha,Hb}$ = 7.4 Hz, 2H; $H_{ar}$-Bzn), 7.41–7.35 (m, 2H; H3-, H6-Fmoc), 7.35–7.25 (m, 10H; H2-, H7-Fmoc, $H_{ar}$-Bzn (3H), $H_{ar}$-Bn (5H), 6.31 (d, $J_{NH,H2}$ = 9.1 Hz, 1H; NH-GalNAc), 5.58 (d, $J_{NH,T\alpha}$ = 9.4 Hz, 1H; NH-T), 5.45 (s, 1H; CH-Bzn), 4.92 (d, $J_{H1,H2}$ = 3.1 Hz, 1H; H1), 4.65–4.43 (m, 5H; H2, $CH_2$-Bn, $CH_2$-Fmoc), 4.31–4.08 (m, 6H; H4, $T^\alpha$, $T^\beta$, H9-Fmoc, H1′, H6a), 3.90–3.86 (m, 2H; H3, H6b), 3.73–3.68 (m, 3H; H3′, H4′, H5′), 3.62–3.55 (m, 3H; H6a′, H6b′, H5), 3.42–3.37 (m, 1H; H2′), 2,99 ($s_b$, 1H; OH), 2.02 (s, 3H; $CH_3$-NHAc), 1.42 (s, 9H; $CH_3$-*t*Bu), 1.26 (d, 3H; $T^\gamma$, $J_{T\gamma,T\beta}$ = 5.9 Hz) ppm; $^{13}$C NMR (100.6 MHz, $CDCl_3$, BB, HMQC): $\delta$ = 172.2 (2×C=O), 156.4 (C=O-urethane), 143.7 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 138.1, 137.5 ($C_q$-Bn, $C_q$-Bzn), 128.9, 128.1, 126.1 ($C_{ar}$-Bzn), 128.4, 127.8 ($C_{ar}$-Bzn), 127.6 (C3-, C6-Fmoc), 127.1 (C2-, C7-Fmoc), 125.0 (C1-, C8-Fmoc), 120.1 (C4-, C5-Fmoc), 105.5 (CH-Bzn), 101.1 (C1′), 100.6 (C1), 83.4 ($C_q$-*t*Bu), 77.2 ($T^\beta$) 76.7 (C4), 75.8 (C3), 73.7 (C5′), 73.6 ($CH_2$-Bn), 73.4 (C3′), 70.9 (C2′), 69.8 (C6), 68.9 (C6′), 68.7 (C4), 67.1 ($CH_2$-Fmoc), 63.7 (C5), 59.0 ($T^\alpha$), 48.3 (C2), 47.2 (C9-Fmoc), 28.1 ($CH_3$–*t*Bu), 23.5 ($CH_3$-NHAc), 18.9 ($T^\gamma$) ppm; HR-MS (ESI-TOF): *m/z*: found: 963.3879 $[M+Na]^+$; $C_{51}H_{60}N_2O_{15}Na$ calcd 963.3891.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-2-deoxy-4,6-*O*-benzylidene-3-*O*-[6-*O*-benzyl-3-*O*-{benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-galacto-2-nonulopyranosyl)onat}-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (Fmoc-Thr(α-$Ac_4$Neu-NAcCOOBn-(2–3)-β-6-*O*-Bn-Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu) (14)** ***Procedure A***: Fmoc-Thr(β-6-*O*-Bn-Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **11** (110 mg, 0.177 mmol) and $Ac_4$NeuNAcCOOBn-SEt **13** (147 mg, 0.240 mmol) were separately co-evaporated with toluene (3×20 mL) and dried under high vacuum. The glycosyl acceptor **11** and the glycosyl donor **13** were dissolved in anhydrous acetonitrile (10 mL), treated with molecular sieves (350 mg, 3 Å), and stirred for 30 min at ambient temperature under argon. The suspension was cooled to −37°C, and *N*-iodosuccinimide (108 mg, 0.480 mmol), dissolved in dry acetonitrile (1 mL), and trifluoromethanesulfonic acid (125 μL of a 0.4 M solution in dry acetonitrile) were added slowly. The reaction mixture was stirred for an additional 30 min at −37°C and then for 20 h at −23°C. It was neutralized with pulverized $NaHCO_3$ and allowed to warm to ambient temperature. The suspension was diluted with dichloromethane (100 mL) and filtered through Hyflo Super Cel. The filtrate was washed with saturated aqueous $NaHCO_3$ (2×50 mL) and saturated aqueous $Na_2S_2O_3$ (1×50 mL), and the combined aqueous layers were re-extracted with dichloromethane. The combined organic phases were dried over $MgSO_4$, and filtered, and the solvents were removed at reduced pressure. Flash chromatography of the residue (silica gel, ethyl acetate/ethanol 10:1) gave one fraction of the pure α-glycosylation product α-**14** as a colorless, amorphous solid (66 mg, 38%), together with a second fraction containing the β-glycosylation product β-**14** and the sialic acid glycal. Separation of the β-product and the glycal was achieved by further flash chromatography on silica gel (ethyl acetate/cyclohexane 10:1) and afforded β-**14** (42 mg, 24%) as a colorless, amorphous solid. The sialic acid glycal (62 mg, 47% yield

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based on the sialic acid donor **13**) was formed as a by-product, and a small amount of the glycosyl acceptor **11** (17 mg, 15%) was re-isolated.

***Procedure B***: Fmoc-Thr(β-6-*O*-Bn-Gal(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **11** (338 mg, 0.359 mmol) and α-$Ac_4$NeuNAcCOOBnXan **15** (482 mg, 0.718 mmol, 2 equiv) were dissolved in a mixture of acetonitrile and dichloromethane (55 mL, 2:1). The solution was stirred for 1 h in a Schlenk flask (brown glass) in the presence of flame-dried molecular sieves (2.1 g, powder, 3 Å) under an argon atmosphere and with exclusion of moisture. After the reaction mixture had been cooled to −68°C, silver triflate (200 mg, 0.78 mmol) and di-*tert*-butylpyridine (186 μL, 213.8 mg, 1.12 mmol) were added. A pre-cooled (−15°C) solution of phenylsulfenyl chloride[47] (90 μL, 0.78 mmol) in dry dichloromethane (0.5 mL) was added dropwise, and stirring was continued at −68°C for 3.5 h. The reaction mixture was diluted with a suspension of silica gel (0.7 g) in ethyl acetate (10 mL), filtered through Hyflo Super Cel, and washed with saturated aqueous $NaHCO_3$ (2×75 mL) and brine (75 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification of the crude product by flash chromatography (silica gel, ethyl acetate→ethyl acetate/ethanol 65:1) yielded **14** (262 mg, 50%) as a colorless, amorphous solid (α/β, 97:3).

***Procedure C***: Fmoc-Thr(β-6-*O*-Bn-Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu **11** (210 mg, 0.223 mmol) and $Ac_4$NeuNAcCOOBn-Xan **15** (375 mg, 0.558 mmol) were each separately co-evaporated with toluene (3× 10 mL) and dried under high vacuum. The glycosyl acceptor **11** and the glycosyl donor **15** were dissolved in a mixture of dry dichloromethane (3 mL) and dry acetonitrile (7 mL), powdered molecular sieves (600 mg, 3 Å) were added, and the reaction mixture was stirred for 1 h at ambient temperature under argon. The suspension was cooled to −68°C, and silver triflate (143 mg, 0.558 mmol) and a solution of methylsulfenyl bromide in dry 1,2-dichloroethane (0.35 mL of a 1.6 M solution; the methylsulfenyl bromide solution in 1,2-dichloroethane was prepared by addition of bromine (410 μL, 7.99 mmol) to a solution of dimethyl disulfide (709 μL, 7.99 mmol) in dry 1,2-dichloroethane (10 mL) and stirring at ambient temperature with exclusion of light for 15 h) were added slowly. The reaction mixture was stirred for 4 h at −68°C under an argon atmosphere with exclusion of light. It was then neutralized with Huenig's base (115 μL, 3 equiv) and allowed to warmed to room temperature. The suspension was diluted with dichloromethane (100 mL) and filtered through Hyflo Super Cel, and the solvents were removed from the filtrate in vacuo. The residue was purified by flash chromatography (silica gel; 100% ethyl acetate) to afford the desired α-glycosylation product α-**14** (194 mg, 58%) as a colorless, amorphous solid. In addition, a small amount of the unreacted glycosyl acceptor **11** (17 mg, 8%) and the sialic acid glycal (235 mg, 77% yield based on the sialic acid donor **15**) formed as a by-product were isolated.

**α Anomer**: $R_f$ = 0.28 (ethyl acetate/ethanol 10:1); $R_t$ = 30.1 min (Phenomenex Luna C18(2), grad.: $CH_3CN/H_2O$ (60:40)→(90:10), 40 min); $[\alpha]_D^{27}$ = 32.0 (*c* = 1, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HMQC): δ = 7.74 (d, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.8 Hz, 2H; H4-, H5-Fmoc), 7.58 (dd, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.8 Hz, 2H; H1-, H8-Fmoc), 7.49 (d, $J_{Ha,Hb}$ = 5.8 Hz, 2H; $H_{ar}$-Bzn), 7.42–7.35 (m, 2H; H3-, H6-Fmoc), 7.35–7.24 (m, 15H; H2-, H7-Fmoc, $H_{ar}$-Bzn (3H), $H_{ar}$-Bn (10H)), 6.59 (d, $J_{NH,H2}$ = 9.4 Hz, 1H; NH-GalNAc), 6.12 (d, $J_{NH,T\alpha}$ = 9.7 Hz, 1H; NH-T), 5.45 (s, 1H; CH-Bzn), 5.40 (t, $J_{H8'',H9''}$ = 6.6 Hz, 1H; H8''), 5.28–5.13 (m, 2H; H7'', NH-NeuNAc), 5.16 (s, 2H; $CH_2$-Bn), 4.98 (d, $J_{H1,H2}$ = 3.5 Hz, 1H; H1), 4.92–4.81 (m, 1H; H4''), 4.74–4.63 (m, 1H; H2), 4.55 (s, 2H; $CH_2$-Bn), 4.52–4.03 (m, 10H; H9a''{4.46}, $CH_2$-Fmoc {4.39, 4.34}, $T^\beta$ {4.33}, H4 {4.26}, $T^\alpha$ {4.25}, H9-Fmoc {4.19}, H1' {4.15}, H6a {4.13}, H5'' {4.06}), 3.99–3.84 (m, 4H; H6'' {3.93}, H9b'' {3.92}, H3' {3.90}, H6b {3.87}), 3.72–3.55 (m, 4H; H3 {3.67}, H2' {3.61}, H6a' {3.59}, H5 {3.57}), 3.54–3.39 (m, 2H; H6b'{3.87}, H5'{3.41}), 3.24 (s, 1H; H4'), 2,78 ($s_b$, 1H; OH), 2.71 (dd, $J_{H3eq'',H3ax''}$ = 12.7 Hz, $J_{H3eq'',H2''}$ = 4.3 Hz, 1H; $H3_{eq}''$), 2.36 (s, 1H; OH), 2.03–2.00 (m, 1H; $H3_{ax}''$), 2.09, 2.07, 2.02, 2.00, 1.92, 1.82 (6×s, 18H; 6×$CH_3$-Ac), 1.42 (s, 9H; $CH_3$-*t*Bu), 1.26 (d, $J_{T\gamma,T\beta}$ = 5.9 Hz, 3H; $T^\gamma$) ppm; $^{13}C$ NMR (100.6 MHz, $CDCl_3$, BB, HMQC): δ = 171.53, 170.91, 170.82, 170.24, 170.09, 169.76, 167.77 (C=O), 156.83 (C=O-urethane), 143.71 (C1a-, C8a-Fmoc), 141.29, 141.15 (C4a-, C5a-Fmoc), 138.07, 137.51 ($C_q$-Bn), 134.21 ($C_q$-Bn), 128.97, 128.84, 128.78, 128.43, 127.51 ($C_{ar}$-Bn), 128.78, 128.04, 126.44 ($C_{ar}$-Bzn), 127.79 (C3-, C6-Fmoc), 127.09, 126.97 (C2-, C7-Fmoc), 125.17, 124.94 (C1-, C8-Fmoc), 120.03 (C4-, C5-Fmoc), 106.55 (C1'), 100.96 (CH-Bzn), 100.45 (C1), 97.47 (C2''), 83.13 ($C_q$-*t*Bu), 78.15 (C3), 75.94, 75.15 ($T^\beta$, C4, C3'), 73.60 ($CH_2$-Bn), 73.43, 72.92 (C6'',C5'), 69.72 (C8'', C6'), 69.07 (C6), 68.61 (C4''), 68.22, 68.11 ($CH_2$-Bn, C2',C4',C7''), 67.25 ($CH_2$-Fmoc), 63.64 (C9''), 63.52 (C5), 59.24 ($T^\alpha$), 49.08 (C5''), 47.79 (C2), 47.09 (C9-Fmoc), 37.42 (C3''), 28.06 ($CH_3$-*t*Bu), 23.16, 23.07 ($CH_3$-NHAc), 21.33, 21.21, 21.01, 20.78, 20.64 ($CH_3$-OAc), 19.47 ($T^\gamma$) ppm; MS (HR-ESI-TOF): *m/z*: found: 1512.5729 $[M+Na]^+$; $C_{77}H_{91}N_3O_{27}Na$ calcd 1512.5737.

**β Anomer**: $R_f$ = 0.43 (ethyl acetate/ethanol 10/1); $[\alpha]_D^{23}$ = 30.9 (*c* = 1, $CH_2Cl_2$); $^1H$ NMR (400 MHz, COSY, HMQC, $CDCl_3$): δ = 7.75 (d, $J_{H3,H4}$ = $J_{H5,H6}$ = 6.3 Hz, 2H; H4-, H5-Fmoc), 7.61 (dd, $J_{H1,H2}$ = $J_{H7,H8}$ = 7.1 Hz, 2H; H1-, H8-Fmoc), 7.52–7.48 (m, 2H; $H_{ar}$-Bzn), 7.41–7.24 (m, 17H; H2-, H3-, H6-, H7-Fmoc, $H_{ar}$-Bzn (3H), $H_{ar}$-Bn (2×, 10H)), 6.40 (d, $J_{NH,2H}$ = 9.0 Hz, 1H; NH-GalNAc), 5.86 (d, $J_{NH,H5''}$ = 9.8 Hz, 1H; NH-NeuNAc), 5.63 (d, $J_{NH,T\alpha}$ = 9.4 Hz, 1H; NH-T), 5.49 (s, 1H; CH-Bzn), 5.43 (dt, $J_{H3''ax,H4''}$ = $J_{H4'',H5''}$ = 10.6 Hz, $J_{H3''eq,H4''}$ = 4.7 Hz, 1H; H4''), 5.34 (dd, $J_{H6'',H7''}$ = 2.0 Hz, $J_{H7'',H8''}$ = 6.3 Hz, 1H; H7''), 5.26–5.22 (m, 1H; H8''), 5.21 (d, $J_{a,b}$ = 12.1 Hz, 1H; $CH_{2a}$-Bn), 5.12 (d, $J_{a,b}$ = 11.7 Hz, 1H; $CH_{2a'}$-Bn), 4.94 (d, $J_{H1,H2}$ = 3.1 Hz, 1H; H1), 4.66–3.86 (m, 16H; H2 {4.62}, H9a,b'' {4.59, 3.95}, $CH_2$-Bn {4.55}, $CH_2$-Fmoc {4.45}, H6'' {4.37, dd, $J_{H5'',H6''}$ = 10.6 Hz, $J_{H6'',H7''}$ = 2.0 Hz}, H1' {4.31, d, $J_{H1',H2'}$ = 7.4 Hz}, $T^\alpha$ {4.27}, H4 {4.26}, H9-Fmoc {4.26}, $T^\beta$ {4.18}, H6a,b {4.15, 3.94}, H5'' {4.14}), 3.82–3.58 (m, 8H; H3 {3.78}, H3' {3.78}, H2' {3.70}, H4' {3.66}, $H6'_{a,b}$ {3.66}, H5' {3.65}, H5 {3.61}), 2.55 (dd, $J_{H3''ax,eq''}$ = 13.7 Hz, $J_{H3''eq,H4''}$ = 4.3 Hz, 1H; $H3''_{eq}$), 2.10 (s, 3H; $CH_3$-Ac), 2.01 (s, 3H; $CH_3$-Ac), 2.06–2.03 (m, 1H; $H3''_{ax}$), 1.99 (s, 3H; $CH_3$-Ac), 1.96 (s, 3H; $CH_3$-Ac), 1.95 (s, 3H; $CH_3$-Ac)), 1.86 (s, 3H; $CH_3$-Ac), 1.44 (s, 9H; $CH_3$–*t*Bu), 1.28 (d, $J_{T\beta,\gamma}$ = 6.3 Hz, 3H; $T^\gamma$) ppm; $^{13}C$ NMR (100.6 MHz, BB, HMQC, $CDCl_3$): δ = 172.6, 170.9, 170.7, 170.4, 170.2, 170.1, 167.7 (C=O), 156.5 (C=O-urethane), 143.7 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 138.1, 137.6 ($C_q$-Bn, $C_q$-Bzn), 134.7 ($C_q$-Bn), 128.9, 128.5, 128.4, 127.6 ($C_{ar}$-Bn (2×)), 128.6, 128.1, 126.4 ($C_{ar}$-Bzn), 127.8 (C3-, C6-Fmoc), 127.1 (C2-, C7-Fmoc), 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 105.3 (C1'), 100.9 (CH-Bzn), 100.5 (C1), 98.9 (C2''), 83.4 ($C_q$-*t*Bu), 76.8 (C3, C3'), 75.7 (C4, $T^\beta$), 73.5 ($CH_2$-Bn), 73.4 (C5'), 71.2 (C6''), 70.0 (C8''), 69.5 (C6'), 69.1 (C4''), 68.9 (C6), 68.0 ($CH_2$-Bn), 67.8 (C2', C4', C7''), 67.3 ($CH_2$-Fmoc), 63.7 (C5), 62.4 (C9''), 59.0 ($T^\alpha$), 48.5 (C2, C5''), 47.2 (C9-Fmoc), 34.2 (C3''), 28.1 ($CH_3$–*t*Bu), 23.3, 23.1 ($CH_3$-NHAc), 21.0, 20.9, 20.8 ($CH_3$-OAc (4×)), 19.4 ($T^\gamma$) ppm; MS (MALDI-TOF, dhb, positive ion mode): m/z: found: 1513.3 $[M+Na]^+$; $C_{77}H_{91}N_3NaO_{27}$ calcd 1513.5; found: 1529.4 $[M+K]^+$; $C_{77}H_{91}KN_3O_{27}$ calcd: 1529.5; MS (HR-ESI-TOF): *m/z*: found: 1512.5740 $[M+Na]^+$; $C_{77}H_{91}N_3O_{27}Na$ calcd 1512.5737.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[6-*O*-benzyl-3-*O*-{benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-galacto-2-nonulopyranosyl)onat}-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (16)**: A solution of trisaccharide **14** (359 mg, 0.241 mmol) in aqueous acetic acid (80%, 15 mL) was stirred at 80°C for 1 h. Toluene (15 mL) was added, and the reaction mixture was concentrated in vacuo and subsequently co-evaporated with toluene (3×15 mL). The resulting crude product was purified by flash chromatography (silica gel, ethyl acetate/ethanol 15:1) to give the title compound as a colorless, amorphous solid (308 mg, 82%). $R_f$ = 0.12 (ethyl acetate/ethanol 10:1); $[\alpha]_D^{28}$ = 12.0 (*c* = 1, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$, COSY, HMQC): δ = 7.73 (d, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.4 Hz, 2H; H4-, H5-Fmoc), 7.56 (d, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.8 Hz, 2H; H1-, H8-Fmoc), 7.42–7.23 (m, 14H; H2-, H3-, H6-, H7-Fmoc, $H_{ar}$-Bn (10H)), 6.61 (d, $J_{NH,H2}$ = 9.4 Hz, 1H; NH−GalNAc), 5.99 (d, $J_{NH,T\alpha}$ = 9.8 Hz, 1H; NH-T), 5.49–5.41 (m, 1H; H8), 5.23–5.07 (m, 4H; $CH_2$-$Bn^1$ {5.16, 5.14} H7'' {5.10}, NH-NeuNAc), 4.94–4.88 (d, $J_{H1,H2}$ = 1.6 Hz, 1H; H1), 4.87–4.77 (m, 1H; H4''), 4.59–4.44 (m, 4H; H9a'' {4.52}, H2 {4.52}, $CH_2$-Bn {4.50}), 4.43–4.35 (m, 2H; $CH_2$-Fmoc), 4.34–4.26 (m, 1H; $T^\beta$), 4.26–4.12 (m, 4H; $T^\alpha$ {4.23}, H1' {4.19}, H9-Fmoc {4.18}, H4 {4.16}), 4.11–4.01 (m, 1H; H5''), 3.95 (dd, $J_{H6'',H5''}$ = 10.8 Hz, $J_{H6'',H7''}$ = 1.6 Hz, 1H; H6''), 3.91–3.83 (m, 2H; H6a {3.87}, H3' {3.86}), 3.82–3.63 (m, 4H; H5 {3.78}, H9b'' {3.74}, H6b {3.73}, H2' {3.67}), 3.61–3.51 (m, 2H; H3 {3.57}, H6a' {3.54}), 3.49–3.35 (m, 2H; H6b' {3.44}, H5' {3.39}), 3.26–3.20 (m, 1H; H4'), 2.72 (dd, $J_{H3eq'',H3ax''}$ = 13.1 Hz, $J_{H3eq'',H4''}$ = 4.7 Hz, 1H; $H3_{eq}''$), 2.15–1.90 (m, 16H; 4×$CH_3$OAc, 1× $CH_3$NAc, $H3_{ax}''$ {2.02}), 1.83 (s, 3H; $CH_3$NAc), 1.42 (s, 9H; $CH_3$-*t*Bu), 1.26 (d, $J_{T\gamma,T\beta}$ = 6.26 Hz, 3H; $T^\gamma$) ppm; $^{13}C$ NMR (100.6 MHz, $CDCl_3$, BB, HMQC): δ = 171.77, 171.38, 170.86, 170.57, 170.06, 169.62 (C=O), 167.59 (C1''), 156.73 (C=O-urethane), 144.64, 143.66 (C1a-, C8a-Fmoc), 141.24 (C4a-, C5a-Fmoc), 137.89, 134.16 ($C_q$ (2×Bn)), 128.94, 128.82,

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128.73, 128.73, 128.39, 128.12, 127.76, 127.69, 127.54 ($C_{arom}$ (2×Bn), C3-, C6-Fmoc), 127.03, 126.92 (C2-, C7-Fmoc), 125.20, 125.09 (C1-, C8-Fmoc), 124.86 (C4-, C5-Fmoc), 105.90 (C1′), 100.07 (C1), 97.44 (C2″), 80.68 (C3), 76.13 (C3′), 75.87 ($T^{\beta}$), 73.48 ($CH_2$-Bn), 73.40 (C5′), 73.10 (C6″), 69.69 (C6′), 69.60 (C4), 69.51 (C5), 68.79 (C8″), 68.49 (C4″), 68.40 (C4′), 68.27 (C2′), 68.17 ($CH_2$-Bn), 67.89 (C7), 67.11 ($CH_2$-Fmoc), 63.52 (C9″), 62.94 (C6), 59.15 ($T^{\alpha}$), 49.01 (C5″), 47.43 (C2), 47.06 (C9-Fmoc), 37.41 (C3″), 23.16, 23.01 ($CH_3$-NHAc), 21.08, 21.02, 20.72, 20.57, ($CH_3$ (4×OAc)), 19.22 ($T^{\gamma}$) ppm; MS (HR-ESI-TOF): *m/z*: found: 1402.5597; $C_{70}H_{87}N_3O_{27}$ calcd 1402.5606.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-{benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-galacto-2-nonulopyranosyl)onat}-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (17)**: Conjugate **16** (286 mg, 0.204 mmol) was dissolved in pyridine (5 mL) and cooled to 0°C. After the addition of catalytic *N*,*N*-dimethylaminopyridine, acetic anhydride (2.2 mL) was added dropwise. The solution was stirred at ambient temperature for 6 h, diluted with toluene (10 mL), and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (ethyl acetate/ethanol 20:1) afforded the protected compound **17** as a colorless, amorphous solid (279 mg, 87%). $R_f$ = 0.13 (ethyl acetate); $[\alpha]_D^{28}$ = 36.0 (*c* = 1, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, COSY, HMQC): $\delta$ = 7.74 (d, $J_{H4,H3} = J_{H5,H6}$ = 7.4 Hz, 2H; H4-, H5-Fmoc), 7.57 (t, $J_{H1,H2} = J_{H8,H7}$ = 7.8 Hz, 2H; H1-, H8-Fmoc), 7.43–7.19 (m, 14H; H2-, H3-, H6-, H7-Fmoc, $H_{ar}$-Bn (10H)), 6.36 (d, $J_{NH,H2}$ = 9.4 Hz, 1H; NH−GalNAc), 5.85 (d, $J_{NH,T\alpha}$ = 9.8 Hz, 1H; NH-T), 5.50–5.43 (m, 1H; H8″), 5.41 (s, 1H; $CH_{2a}$-Bn), 5.40–5.33 (m, 1H; H4); 5.22 (dd, $J_{H6'',H7''}$ = 2.3 Hz, $J_{H7'',H8''}$ = 7.6 Hz, 1H; H7″), 5.08 (d, $J_{H4',H3'}$ = 3.12 Hz, 1H; H4′), 5.04 (s, 1H; $CH_{2b}$-Bn), 4.99–4.90 (m, 2H; H2′ {4.94}, NH−NeuNAc), 4.89–4.74 (m, 2H; H1 {4.86}, H4″ {4.81}), 4.58–4.30 (m, 8H; H2 {4.52}, $CH_{2a}$-Bn {4.51}, H1 {4.50}, $CH_{2b}$-Bn {4.44}, H3′ {4.41}, $CH_2$-Fmoc {4.36}, H9a″ {4.35}), 4.28–4.06 (m, 5H; $T^{\beta}$ {4.25}, $T^{\alpha}$ {4.22}, H9-Fmoc {4.20}, H6a {4.11}, H5 {4.08}), 4.01 (d, $J_{H4'',H5''} = J_{H5'',H6''}$ = 10.6 Hz, 1H; H5″), 3.94 (dd, $J_{H5,H6b}$ = 7.4 Hz, $J_{H6a,H6b}$ = 11.3 Hz, 1H; H6b), 3.90–3.76 (m, 2H; H9b″ {3.89}, H3 {3.81}) 3.75–3.68 (m, 1H; H5′), 3.53 (dd, $J_{H5',H6a'}$ = 6.24 Hz, $J_{H6a',H6b'}$ = 9.40 Hz, 1H; H6a′), 3.47 (dd, $J_{H5'',H6''}$ = 10.9 Hz, $J_{H6'',H7''}$ = 2.4 Hz, 1H; H6″), 3.40 (dd, $J_{H5',H6b'}$ = 6.7 Hz, $J_{H6a',H6b'}$ = 9.6 Hz, 1H; H6b′), 2.59 (dd, $J_{H3eq'',H3ax''}$ = 12.7 Hz, $J_{H3eq'',H4''}$ = 4.3 Hz, 1H; $H3_{eq}$″), 2.24 (s, 3H; $CH_3$-Ac), 2.03–2.00 (m, 1H; $H3_{ax}$″), 2.09, 2.07, 2.03, 2.02, 2.00, 1.92, 1.82 (7×s, 27H; 9×$CH_3$-Ac), 1.42 (s, 9H; $CH_3$-*t*Bu), 1.26 (d, $J_{T\gamma,T\beta}$ = 5.92 Hz, 3H; $T^{\gamma}$) ppm; $^{13}$C NMR (100.6 MHz, $CDCl_3$, BB, HMQC): $\delta$ = 171.6, 171.0, 170.6, 170.3, 170.2, 169.9, 169.7, 167.2 (C=O), 156.7 (C=O (urethane)), 143.7, 143.6 (C1a-, C8a-Fmoc), 141.2, 141.3 (C4a-, C5a-Fmoc), 137.9, 134.7 ($C_q$-Bn), 128.9, 128.6, 128.3, 127.6, 127.5 ($C_{arom}$ (2×Bn), 127.8 (C3-, C6-Fmoc), 127.0 (C2-, C7-Fmoc), 125.1, 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 101.6 (C1′), 99.8 (C1), 96.8 (C2″), 83.0 ($C_q$-*t*Bu), 76.2 ($T^{\beta}$), 74.1 (C3), 73.4 ($CH_2$-Bn), 72.5 (C6″), 72.2 (C5′), 71.6 (C3′), 69.5 (C4), 69.2 (C2′), 69.1 (C4″), 68.5 (C8″, $CH_2$-Bn), 68.1 (C5), 68.0 (C6′), 67.8 (C4′), 67.7 (C7″), 67.4 ($CH_2$-Fmoc), 63.3 (C6), 63.1 (C9″), 59.2 ($T^{\alpha}$), 48.9 (C5″), 48.5 (C2), 47.0 (C9-Fmoc), 37.4 (C3″), 28.1 ($CH_3$-*t*Bu)), 23.3, 23.1 ($CH_3$-NHAc), 21.2, 21.1, 21.0, 20.8, 20.7, 20.6 ($CH_3$ (8×OAc), 19.0 ($T^{\gamma}$) ppm; HR-MS (ESI-TOF): *m/z*: found: 1592.5853 $[M+Na]^+$; $C_{78}H_{95}N_3O_{31}Na$ calcd 1592.5847.

***N*-Fluorenylmethoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[2,4-di-*O*-acetyl-6-*O*-benzyl-3-*O*-{benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-galacto-2-nonulopyranosyl)onat}-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine (18)**: A solution of protected trisaccharide **17** (250 mg, 0.159 mmol) in a mixture of TFA (5 mL) and anisole (0.5 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with toluene (25 mL) and the solvent was removed in vacuo. The resulting residue was co-evaporated with toluene (4×25 mL) and purified by flash chromatography (silica gel, ethyl acetate/methanol 2:1) to yield compound **18** (242 mg, quant.) as a colorless, amorphous solid. $R_f$ = 0.27 (ethyl acetate/ethanol 1:1); $[\alpha]_D^{28}$ = 24.8 (*c* = 1, $CHCl_3$); $^1$H NMR (400 MHz, $CD_3OD$, COSY, HMQC): $\delta$ = 7.82 (d, $J_{H3,H4} = J_{H5,H6}$ = 7.4 Hz, 2H; H4-, H5-Fmoc), 7.72–7.68 (m, 2H; H1-, H8-Fmoc), [7.52–7.48 (m, 2H), 7.44–7.27 (m, 12H)] (H2-, H3-, H6-, H7-Fmoc, $H_{arom}$ (2×Bn, 10H)), 5.60–5.54 (m, 1H; H8″), 5.46–5.39 (m, 3H; H4 {5.44}, H7″ {5.41}, $CH_{2a}$-Bn {d, $J_{a,b}$ = 12.1 Hz}), 5.20 (d, $J_{H3',H4'}$ = 3.1 Hz, 1H; H4′), 5.15 (d, $J_{a,b}$ = 12.1 Hz, 1H; $CH_{2b}$-Bn), 5.02–4.95 (m, 2H; H1 {5.00}, H4″ {4.96}), 4.87 (d, $J_{H2',H3'}$ = 10.2 Hz, 2H; H2′), 4.76 (d, $J_{H1',H2'}$ = 7.8 Hz, 1H; H1′), 4.66–4.40 (m, 6H; H3′ {4.64}, $CH_2$-Bn {4.61, 4.54}, $CH_2$-Fmoc {4.60, 4.48}, $T^{\beta}$ {4.44}), 4.38–4.29 (m, 2H; H2 {4.33}, H9a″ {4.31}), 4.28–4.21 (m, 2H; H9-Fmoc {4.26}, H5 {4.23}), 4.16–4.11 (m, 2H; $T^{\alpha}$ {4.14}, H6a {4.12}), 4.08–3.94 (m, 4H; H3 {4.05}, H9b″ {4.01}, H6b {4.00}, H5″ {3.96}), 3.92–3.86 (m, 1H; H5′), 3.82–3.77 (m, 1H; H6″), 3.63 (dd, $J_{H5',H6'a}$ = 6.3 Hz, $J_{H6'a,b}$ = 9.4 Hz, 1H; H6a′), 3.49 (dd, $J_{H5',H6'b}$ = 6.3 Hz, $J_{6'a,6'b}$ = 9.4 Hz, 1H; H6b′), 2.65 (dd, $J_{H3''ax,eq}$ = 12.5 Hz, $J_{H3''eq,H4''}$ = 4.7 Hz, 1H; $H3_{eq}$″), 2.29 (s, 3H; $CH_3$-Ac), 2.19 (s, 3H; $CH_3$-Ac), 2.13 (s, 3H; $CH_3$-Ac), 2.10 (s, 3H; $CH_3$-Ac), 2.08 (s, 3H; $CH_3$-Ac), 2.05 (s, 6H; $CH_3$ (2×Ac)), 2.03 (s, 3H; $CH_3$-Ac), 1.99 (s, 3H; $CH_3$-Ac), 1.84 (s, 3H; $CH_3$-Ac), 1.55 (dd, $J_{H3''ax,H3''eq}$ = 12.5 Hz, $J_{H3''ax,H4''}$ = 12.1 Hz, 1H; $H3_{ax}$″), 1.25 (d, $J_{T\beta,\gamma}$ = 5.5 Hz, 3H; $T^{\gamma}$) ppm; $^{13}$C NMR (100.6 MHz, $CD_3OD$, BB, HMQC): $\delta$ = 173.5, 172.7, 172.5, 172.2, 172.1, 171.8, 171.5, 171.0, 168.7 (C=O), 159.0 (C=O-urethane), 145.4, 145.2 (C1a-, C8a-Fmoc), 142.7 (C4a-, C5a-Fmoc), 139.4, 136.1 ($C_q$ (2×Bn)), 129.8, 129.7, 129.6, 129.5, 128.9, 128.8, 128.2 ($C_{arom}$ (2×Bn)), C2-, C3-, C6-, C7-Fmoc), 126.1, 126.0 (C1-, C8-Fmoc), 121.0 (C4-, C5-Fmoc), 102.7 (C1′), 100.3 (C1), 98.1 (C2″), 77.5 ($T^{\beta}$), 74.9 (C3), 74.5 ($CH_2$-Bn), 73.2 (C5′), 73.0 (C6″), 72.8 (C3′), 71.4 (C4, C2′), 70.9 (C4″), 69.8 ($CH_2$-Bn), 69.4 (C4′), 69.3 (C6′), 69.0 (C8″), 68.8 (C5), 68.5 (C7″), 67.5 ($CH_2$-Fmoc), 64.3 (C6), 63.5 (C9″), 61.2 ($T^{\alpha}$), 50.5 (C2), 50.0 (C5″), 48.7 (C9-Fmoc), 38.7 (C3″), 23.6, 22.7 ($CH_3$-NHAc), 21.7, 21.0, 20.8, 20.7, 20.6, 20.5 ($CH_3$ (8×OAc)), 19.5 ($T^{\gamma}$) ppm; MS (HR-ESI-TOF): *m/z*: found: 1558.5040 $[M+2Na-H]^+$; $C_{74}H_{86}N_3NaO_{31}$ calcd 1558.5041.

**General procedure for the automated solid-phase glycopeptide synthesis**: Peptide syntheses were carried out in a Perkin–Elmer ABI 433A peptide synthesizer by use of Fmoc-Pro-PTMSEL[52] and Fmoc-Asp(O*t*Bu)-PHB preloaded Tentagel resins.[56] In iterative coupling cycles, the subsequent amino acids were attached sequentially. In every coupling step, the *N*-terminal Fmoc group was removed by three 2.5 min treatments with 20% piperidine in NMP. Amino acid couplings were performed with Fmoc-protected amino acids (1 mmol) activated by HBTU/HOBt[53] (1 mmol each) and DIPEA (2 mmol) in DMF (20–30 min vortex). After every coupling step, unreacted amino groups were capped by treatment with a mixture of $Ac_2O$ (0.5 M), DIPEA (0.125 M), and HOBt (0.015 M) in NMP (10 min vortex). Attachment of the glycosylated amino acids was performed manually as described in the procedures for the corresponding glycopeptides.

**Ac-Gly-Val-Thr(α-Ac₄NeuNAcCOOBn-(2–3)-β-6-*O*-Bn-Ac₂Gal-(1–3)-α-Ac₂GalNAc)-Ser(*t*Bu)-Ala-Pro-Asp(O*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Pro-Ala-Pro-OH (20)**: Starting from Fmoc-Pro-PTMSEL-preloaded NovaSyn Tg resin **19**[52] (182 mg, 0.05 mmol, loading: 0.275 mmol $g^{-1}$), the coupling of the first eight amino acids was performed by the automated standard procedure. The glycosylated amino acid **18** (110 mg, 0.073 mmol, 1.45 equiv) was coupled manually with the aid of HATU (31.5 mg, 0.082 mmol, 1.64 equiv), HOAt (11.2 mg, 0.082 mmol, 1.64 equiv), and NMM (18.2 µL, 16.7 mg, 0.165 mmol, 3.32 equiv) for activation (coupling time 6 h). Then, in a resumption of the standard procedure, the final two amino acids were attached to the polymer-bound glycopeptide, and the *N*-terminal Fmoc-group was exchanged for an acetyl group. For the cleavage procedure the resin was placed in a Merrifield glass reactor, washed with dichloromethane (3×20 mL), treated with a solution of tetrabutylammonium fluoride trihydrate (38 mg, 0.12 mmol, 2.4 equiv) in dry dichloromethane (8 mL), and shaken for 35 min at room temperature. The mixture was filtered, and the resin was washed with $CH_2Cl_2$ (4×10 mL). The cleavage process was repeated with a solution of TBAF·3$H_2O$ (35 mg, 0.11 mmol) in dichloromethane (8 mL). Filtrates and washing solutions of both cleavage procedures were combined, washed with water (3×20 mL), dried over anhydrous $MgSO_4$, and liberated from the solvents in vacuo to yield 100 mg of a colorless solid. After purification by preparative RP-HPLC ($R_t$ = 55.7 min, column: Phenomenex Jupiter, grad: $CH_3CN/H_2O$+0.1% TFA (10:90)→(100:0), 80 min, $R_t$ = 56.3 min) and lyophilization, the protected glycopeptide **20** was obtained as a colorless solid (55.0 mg, 39%). $R_t$ = 29.0 min (Phenomenex Luna C18(2), grad.: $CH_3CN/H_2O$ + 0.1% TFA (10:90)→(0:90), 40 min); $[\alpha]_D^{22}$ = 4.58 (*c* = 1, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$, COSY, HMQC): $\delta$ = 7.66–7.44 (m, 2H; $D^{NH}$ {7.65}, $V^{NH}$ {7.45}) 7.41–7.25 (m, 12H; $A^{NH}_{1/2}$ {7.37}, 2×5 $H_{ar}$-Bn), 6.96–6.88 (m, 1H; $G^{NH}$), 6.76–6.68 (m, 1H; NH-GalNAc), 5.49–5.41 (m, 1H; H8″), 5.40–5.30 (m, 3H; $CH_{2a}$-$Bn_1$ {5.37}, H4 {5.33}, H7″ {5.28}), 5.29–5.26 (m, 1H; H7″), 5.12–4.94 (m, 4H; NH−NeuAc {5.08}, H4′ (5.06), $CH_{2b}$-$Bn_1$ {5.02}, H1 {4.99}), 4.93–4.78 (m,

2H; H4′′ {4.84}, H2′ {4.90}), 4.72–4.19 (m, 18H; $D^{\alpha}$ {4.62}, H1′ {4.61}, $A^{\alpha}_{1/2}$ {4.60}, $CH_{2a}$-$Bn_2$ {4.52}, $R^{\alpha}$ {4.49}, H3′ {4.44}, $CH_{2b}$-$Bn_2$ {4.43}, 3×$P^{\alpha}$ {4.43}, H9a′′ {4.40}, $S^{\alpha}$ {4.40}, $V^{\alpha}$ {4.32}, $T^{\alpha}_{1}$ {4.31}, $T^{\beta}_{ST}$ {4.29}, H2 {4.29}, $T^{\beta}_{ST}$), 4.15–3.88 (m, 9H; H5 {4.10}, $T^{\beta}_{1}$ {4.05}, $S^{\beta}$ {4.08}, H3 {3.99}, H5′′ {3.99}, $G^{\alpha}$ {3.97}, H9b {3.94}), 3.76–3.68 (2H; H5′ {3.72}, H6a {3.72}), 3.66–3.42 (m, 11H; 3×$P^{\delta}$ {3.59, 3.50}, H6a′ {3.53}, H6′′ {3.47}, H6 {3.47}, $R^{\delta}$), 3.37 (dd, $J_{H6a',H6b'}$ = 6.8 Hz, $J_{H6b',H5'}$ = 9.2 Hz, 1H; H6b′), 3.17 ($s_b$, 1H; OH), 2.88–2.71 (m, 1H; $D^{\beta}$ {2.84, 2.75}), 2.64–2.45 (m, 9H; $CH^4_2$-Pmc {2.60}, $CH^2_3$–Pmc, $CH^8_3$-Pmc {2.52, 2.51}}, $H3_{eq}$′′ {2.56}), 2.21 (s, 3H; $CH_3$-Ac), 2.13–1.86 (m, 18H; 3×$P^{\beta}$ {2.12, 2.02}, $V^{\beta}$ {2.08}, 3×$P^{\gamma}$ {2.04, 1.93}, $CH^7_3$-Pmc {2.08}, $R^{\beta}$), 2.12, 2.08, 2.05, 2.04, 2.00, 1.95 (6 s, 27H; 7×$CH_3$-Ac, 2×$CH_3$–NAc {2.00, 1.94}), 1.82–1.75 (m, 6H; $CH_3$–NAc {s, 1.79}, $CH^3_2$–Pmc {1.78}), 1.68–1.56 (m, 3H; $R^{\gamma}$ {1.67}, $H3_{ax}$′′ {t, 1.65, $J_{H3''ax,eq}$ = 12.5 Hz, $J_{H3''ax,H4''}$ = 12.2 Hz}), 1.39 (s, 9H; $tBu^D$), 1.32–1.25 (m, 12H; 2×$A^{\beta}$, 2×$CH^5_3$-Pmc {s, 1.28}), 1.24–1.15 (m, 12H; $T^{\gamma}_{ST}$ {1.19}, $tBu^S$ {s, 1.18}), 1.15–0.99 (m, 12H; $tBu^T$ {s, 1.07}, $T^{\gamma}_{1}$ {1.03}), 0.88 (dd, $J_{Va\gamma,Vb\gamma}$ = 17.2 Hz, $J_{V\gamma,V\beta}$ = 6.2 Hz, 6H; $V^{\gamma}$) ppm; $^{13}$C NMR ($CDCl_3$, $\delta$ obtained from HMQC): $\delta$ = 129.0, 128.7, 128.4, 127.9, 126.0 ($C_{ar}$-Bn), 100.9 (C1′), 100.3 (C1), 78.4 ($T^{\beta}_{ST}$), 73.7 (C3), 73.6 ($CH_2$-$Bn^2$), 72.1 (C6′′), 71.8 (C5′), 71.6 (C3′), 69.8 (C2′), 69.4 (C4, C4′′), 68.6 ($CH_2$-$Bn^1$), 68.4 (C8′′), 68.0 (C5), 67.9 (C6′), 67.7 (C4′), 67.3 (C7′′), 66.2 ($T^{\beta}_{1}$), 63.1 ($S^{\beta}$), 62.6 (C9′′), 61.6 (C6), 60.9 ($P^{\alpha}$), 59.4 ($S^{\alpha}$), 58.4 ($V^{\alpha}$), 58.2 ($T^{\alpha}_{1}$), 50.3 ($D^{\alpha}$), 50.0 ($A^{\alpha}$), 48.8 (C2), 48.7 (C5′′), 47.4 ($P^{\delta}$), 43.1 ($G^{\alpha}$), 37.4 ($D^{\beta}$), 37.2 (C3′′), 32.8 ($CH^3_2$–Pmc), 32.7 ($R^{\beta}$), 28.9, 28.6 ($P^{\beta}$), 28.4 ($tBu^S$) 28.2 ($R^{\gamma}$), 28.0 ($tBu^D$), 27.4 ($tBu^T$), 26.8 (2×$CH^5_3$-Pmc), 25.1 ($P^{\gamma}$), 23.2, 22.9, 22.8 (3×NHAc), 21.4 ($CH^4_2$-Pmc), 21.6, 21.0, 20.9 (8×OAc), 20.9 ($V^{\beta}$), 19.5, 18.2 ($V^{\gamma}$), 18.4 ($T^{\gamma}_{1}$), 18.8 ($T^{\gamma}_{ST}$), 18.5 ($CH^8_3$-Pmc), 17.7 ($A^{\beta}$), 17.5 ($CH^2_3$-Pmc), 12.2 ($CH^7_3$-Pmc); MALDI-TOF-MS (dhb, positive-ion mode): found: 2818.4 $[M+H]^+$; $C_{132}H_{194}N_{17}O_{48}S$ calcd 2817.3; found: 2840.0 $[M+Na]^+$; calcd 2839.3; found: 2856.1 $[M+K]^+$; calcd 2855.4; found: 2879.0 $[M-H+Na+K]^+$ calcd 2877.4.

**Ac-Gly-Val-Thr(α-Ac₄NeuNAcCOOBn-(2–3)-β-6-*O*-Bn-Ac₂Gal-(1–3)-α-Ac₂GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-OH (21)**: A solution of **20** (48.5 mg, 0.017 mmol) in a mixture of TFA (15 mL), triisopropylsilane (0.9 mL), and water (0.9 mL) was stirred at room temperature for 2.5 h. The reaction mixture was then concentrated in vacuo and co-evaporated with toluene (4×20 mL). The product **21** was precipitated into cold (0°C) diethyl ether (20 mL) to furnish a colorless solid, which was washed with diethyl ether (3×20 mL). The obtained glycopeptide **21** (41 mg) was sufficiently pure to be characterized and subsequently deprotected without further purification. $R_t$ = 20.2 min (Phenomenex Jupiter C18, grad.: $CH_3CN/H_2O$ + 0.1% TFA (10:90)→(60:40), 40 min); $^1$H NMR (400 MHz, $CD_3OD$, COSY, HMQC): $\delta$ = 7.53–7.45 (m, 10H; $H_{ar}$-Bn), 5.61–5.52 (m, 1H; H8′′), 5.50–5.35 (m, 3H; H7′′ {5.44}, H4 {5.45}, $CH_{2a}$-$Bn^1$ {5.39}), 5.23 (d, $J_{H3,H4}$ = $J_{H4,H5}$ = 3.5 Hz, 1H; H4′), 5.19–5.11 (m, 1H; $CH_{2b}$-$Bn^1$), 5.07–4.97 (m, 2H; H1 {5.02}, H4′′ {4.98}), 4.83–4.56 (m, 9H; H1′ {4.80}, H2′ {4.79}, $R^{\alpha}$ {4.72}, $T^{\alpha}_{ST}$ {4.68}, $D^{\alpha}$ {4.66}, H3′ {4.65}, $CH_{2a}$-$Bn^2$ {4.62}, 2×$A^{\alpha}$ {4.60}), 4.55–4.44 (m, 5H; $CH_{2b}$-$Bn^2$ {4.52}, $S^{\alpha}$ {4.48}, 3×$P^{\alpha}$ {4.45}), 4.43–4.24 (m, 7H; $T^{\alpha}_{1}$ {4.37}, H2 {4.34}, $T^{\beta}_{ST}$ {4.33}, H9a′′ {4.32}, $V^{\alpha}$ {4.31}, H5 {4.29}, $T^{\beta}_{1}$ {4.29}), 4.19–3.59 (m, 18H; H6a {4.13}, H3 {4.12}, H9b′′ {4.08}, H6b {4.04}, H5′′ {3.97}, $G^{\alpha}_{a}$ {3.95}, $G^{\alpha}_{b}$ {3.91}, H5′ {3.88}, $S^{\beta}$ {3.82}, 3×$Pa^{\delta}$ {3.81}, H6′′ {3.77}, 3×$P^{\delta}_{b}$ {3.68}, H6a′ {3.62}), 3.45 (dd, $J_{H6b',H6a'}$ = 7.44 Hz, $J_{H6b',H5'}$ = 9.6 Hz, 1H; H6b′), 3.30–3.16 (m, 2H; $R^{\delta}$), 3.00–3.16 (d, $J_{D\beta,D\alpha}$ = 5.9 Hz, 2H; $D^{\beta}$), 2.64 (dd, $J_{H3eq'',H3ax''}$ = 12.3 Hz, $J_{H3eq'',H4''}$ = 4.7 Hz, 1H; $H3_{eq}$′′), 2.39–1.97 (m, 13H; 3×$Pa^{\beta}$ {2.29}, 3×$P^{\gamma}$ {2.07}, $V^{\beta}$ {2.12}, 3×$P^{\beta}_{b}$ {2.04}), 2.31 (s, 3H; $CH_3$-NHAc), 2.18, 2.14, 2.09 (3×s, 9H; 3×$CH_3$-OAc), 2.08 (s, 3H; $CH_3$-NHAc), 2.07, 2.05, 1.99 (3×s, 15H; 5×$CH_3$-OAc), 1.95–1.86 (m, 1H; $R^{\beta}_{a}$), 1,84 (s, 3H; $CH_3$-NHAc), 1,82–1,69 (m, 2H; $R^{\beta}_{b}$ {1.78}, $R^{\gamma}$ {1.73}), 1.55 (t, $J_{H3eq'',H3ax''}$ = $J_{H3ax'',H4''}$ = 12.5 Hz, 1H; $H3_{ax}$′′), 1.49–1.39 (m, 6H; 2×$A^{\beta}$), 1.33–1.27 (d, $J_{T\gamma,T\beta}$ = 6.3 Hz, 3H; $T^{\gamma}_{ST}$), 1.26–1.18 (m, 3H; $T^{\gamma}_{1}$), 1.02 (t, $J_{Va\gamma,Vb\gamma}$ = $J_{V\gamma,V\beta}$ = 7.4 Hz, 6H; $V^{\gamma}$) ppm; $^{13}$C NMR (100.6 MHz, $CDCl_3$, BB, HMQC): $\delta$ = 175.30, 174.47, 174.30, 174.03, 173.87, 173.80, 173.57, 173.18, 173.06, 172.83, 172.52, 172.34, 172.08, 171.93, 171.72, 171.51, 170.95, 168.72 (C=O), 139.40, 136.14 ($C_q$-Bn), 129.80, 129.64, 129.41, 128.94, 128.80 ($C_{ar}$-Bn), 102.54 (C1′), 101.11 (C1), 98.15 (C2′′), 79.03 ($T^{\beta}_{ST}$), 75.50 (C3), 74.40 ($CH_2$-$Bn^2$), 72.91 (C6′′, C3′, C5′), 71.64 (C2′, C4), 70.90 (C4′′), 69.76 ($CH_2$-$Bn^1$), 69.19 (C4′), 69.03 (C8′′, $T^{\beta}_{1}$, 68.79 (C6′, C5), 68.55 (C7′′), 64.35 (C6), 63.23 (C9′′, $S^{\beta}$), 60.28 ($T^{\alpha}_{1}$, $P^{\alpha}$, $V^{\alpha}$), 57.95 ($T^{\alpha}_{ST}$), 56.54 ($S^{\alpha}$), 51.99 ($R^{\alpha}$), 51.82 ($D^{\alpha}$), 50.09 (C5′′), 49.99 (C2), 48.90 ($A^{\alpha}$), 48.11 ($P^{\delta}$), 43.60 ($G^{\alpha}$), 42.13 ($R^{\delta}$), 38.76 (C3′′), 35.80 ($D^{\beta}$), 31.82 ($V^{\beta}$), 30.41 ($P^{\beta}$), 29.40 ($R^{\beta}$),26.16 ($P^{\gamma}$), 25.90 ($R^{\gamma}$), 23.76, 22.69, 22.55 (3×NHAc), 22.06 21.79, 21.04, 20.96, 20.88, 20.74, 20.62 (8×OAc), 20.54 ($T^{\gamma}_{1}$), 19.94 ($V^{\gamma a}$), 19.41 ($T^{\gamma}_{ST}$), 19.23 ($V^{\gamma b}$), 16.67 ($A^{\beta}$); MALDI-TOF-MS (dhb, positive-ion mode): found: 2383.7 $[M+H]^+$; $C_{106}H_{152}N_{17}O_{45}$ calcd 2383.0; found: 2405.7 $[M+Na]^+$; calcd 2405.0; found: 2421.8 $[M+K]^+$; calcd 2421.1; found: 2443.8 $[M-H+Na+K]^+$; calcd 2443.1.

**Ac-Gly-Val-Thr(α-NeuNAcCOOH-(2–3)-β-6-*O*-Bn-Gal-(1–3)-α-GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-OH (22)**: Catalytic palladium on activated charcoal (10%; 0.1 mg) was added under argon to a solution of **21** (41.0 mg, max. 0.017 mmol) in methanol (35 mL). The reaction flask was purged with hydrogen, and the solution was stirred under a hydrogen atmosphere for two days. The mixture was filtered through Hyflo Super Cel, which was afterwards washed with methanol (3×25 mL). The filtrate and washing solutions were combined. After removal of the solvent in vacuo, the resulting crude product (33.6 mg) was of sufficient purity to be employed for the final deprotection without additional purification. $R_t$ = 16.0 min (Phenomenex Jupiter C18, grad.: $CH_3CN/H_2O$ + 0.1% TFA (10:90)→(60:40), 40 min); *MALDI-TOF-MS* (dhb, positive-ion mode): *m/z*: found: 2204.0 $[M+H]^+$; $C_{92}H_{140}N_{17}O_{45}$ calcd. 2204.2; found: 2226.0 $[M+Na]^+$; calcd 2226.2.

For the concluding deacetylation, one part of the crude glycopeptide (16.0 mg, max. 0.0072 mmol) was dissolved in a 5 mM aqueous solution of sodium hydroxide (5 mL, pH 11.5). The mixture was stirred for 59 h, during which the course of the reaction was continuously monitored by analytical RP-HPLC. After 89% conversion, the solution was neutralized by the addition of acetic acid and lyophilized, and the resulting crude product was purified by semi-preparative RP-HPLC (Phenomenex Jupiter, grad: $CH_3CN/H_2O$ + 0.1% TFA (5:95)→(30:70), 80 min, $R_t$ = 27.3 min) to yield the title compound as a colorless, amorphous solid (8.6 mg, 56% over three steps). $[\alpha]^{26}_{D}$ = −40.7 (*c* = 0.86, $H_2O$), $R_t$ = 14.5 min (Phenomenex Jupiter C18, grad.: $CH_3CN/H_2O$ + 0.1% TFA (5:95)→(30:70), 40 min); $^1$H NMR (400 MHz, $D_2O$, COSY, HMQC): $\delta$ = 4.92 (d, $J_{H1,H2}$ = 3.7 Hz, 1H; H1), 4.69 (t, $J_{D\alpha,D\beta}$ = 6.4 Hz, 1H; $D^{\alpha}$), 4.65–4.58 (m, 2H; $T^{\alpha}_{ST}$ {4.63}, $R^{\alpha}$ {4.62}), 4.57–4.49 (m, 1H; $A_1^{\alpha}$), 4.49–4.41 (m, 3H; H1′ {4.47}, $S^{\alpha}$ {4.45}, $A^{\alpha}_{2}$ {4.44}), 4.40–4.34 (m, 3H; 3×$P^{\alpha}$), 4.33–4.26 (m, 3H; $T^{\beta}_{ST}$ {4.31}, $V^{\alpha}$ {4.29}, $T^{\alpha}_{1}$ {4.28}), 4.22–4.14 (m, 3H; H4 {4.21}, H2 {4.19}, $T^{\beta}_{1}$ {4.18}), 4.08–3.95 (m, 3H; H3′ {4.04}, H7′′ {4.03}, H3 {4.00}), 3.94–3.87 (m, 3H; $G^{\alpha}$ {3.91}, H4′ {3.90}), 3.86–3.52 (m, 20H; H5′, H8′′ {3.83}, H6a {3.80}, $S^{\beta}$ {3.76}, 3×$Pa^{\delta}$ {3.73}, H5′′ {3.71}, H9a′′, H9b′′, H4′′ {3.67}, 3×$Pa^{\alpha}$ {3.63}, H6b {3.62}, H6′′, H5, H6a′, H6b′), 3.48 (dd, $J_{H1',H2'}$ = 7.9 Hz, $J_{H2',H3'}$ = 1.6 Hz, 1H; H2′), 3.21–3.14 (m, 2H; $R^{\delta}$), 2.99–2.83 (m, 2H; $D^{\beta}$), 2.71 (dd, $J_{H3eq'',H3ax''}$ = 12.2 Hz, $J_{H3eq'',H4''}$ = 4.4 Hz, 1H; $H3_{eq}$′′), 2.35–2.19 (m, 3H; 3×$Pa^{\beta}$), 2.10–1.94 (m, 8H; $V^{\beta}$ {2.03}, 3×$P^{\gamma}$ {2.01}, $R^{\beta}_{a}$), 2.02, 1.99, 1.97 (3×s, 9H; 3×$CH_3$-NHAc), 1.93–1.77 (m, 4H; 3×$P^{\beta}_{b}$ {1.88}, $H3_{ax}$′′ {1.80}), 1.77–1.59 (m, 3H; $R^{\beta}_{b}${1.70}, $R^{\gamma}$ {1.63}), 1.38–1.31 (m, 6H; 2×$A^{\beta}$), 1.24 (d, $J_{T\gamma,T\beta}$ = 6.1 Hz, 3H; $T^{\gamma}_{ST}$), 1.15 (d, $J_{T\gamma,T\beta}$ = 6.4 Hz, 3H; $T^{\gamma}_{1}$), 0.94 (dd, *J* = 6.6 Hz, *J* = 2.0 Hz, 6H; $V^{\gamma}$); MALDI-TOF-MS *(dhb, positive ion mode)*: found: 1868.8 $[M+H]^+$; $C_{76}H_{124}N_{17}O_{37}$ calcd 1867.9; found: 1890.9 $[M+Na]^+$; calcd 1889.9; found: 1907.1 $[M+K]^+$; calcd 1905.9; found: 1912.8 $[M-H+2\times Na]^+$; calcd 1911.9; ESI-MS (positive-ion mode): found: 977.87 $[M-2H+4Na]^{2+}$; $[C_{76}H_{121}N_{17}O_{37}Na_4]^{2+}$ calcd 977.88,

**Ac-Thr-Ser-Ser-Ala-Ser-Thr-Gly-His-Ala-Thr(α-Ac₄NeuNAcCOOBn-(2–3)-β-Ac₂BnGal-(1–3)-α-Ac₂GalNAc)-Pro-Leu-Pro-Val-Thr-Asp-OH (24)**: The automated solid-phase synthesis of the MUC4 glycopeptide **24** was performed starting from the preloaded Fmoc-Asp(O*t*Bu)-PHB Tentagel S resin[56] **23** (238 mg, 0.05 mmol, loading: 0.21 mmol g$^{-1}$). The first five Fmoc-amino acids were coupled with excesses of 20 equivalents (1.00 mmol), by the standard procedure. For the coupling of the glycosylated threonine **18**, which was incorporated in the MUC4 sequence at the threonine-10 position, only 1.3 equivalents (100 mg, 0.066 mmol) were used. The coupling of the α-(2,3)-sialyl-T-threonine building block **18** was activated with a mixture of HATU (35 mg, 0.092 mmol), HOAt (13 mg, 0.092 mmol), and *N*-methylmorpholine (20 µL, 0.185 mmol) in *N*-methylpyrrolidone (2 mL) and carried out over 3 h. The nine subsequent Fmoc-amino acids were coupled to the glycopeptide by the standard procedure. After completion of the 16-amino acid MUC4 sequence, the terminal Fmoc group was removed with piperidine (20%) in *N*-methylpyrrolidone, and the *N*-terminus of the glycopeptide was acetylated with capping reagent on the resin. In order to detach the glycopeptide from the polymer and simultaneously to cleave the amino acid side chain protection

groups, the resin was transferred into a Merrifield glass reactor and treated with a mixture of trifluoroacetic acid (7.50 mL), dist. water (0.45 mL), and triisopropylsilane (0.45 mL) for 2 h. After filtration, the resin was washed with trifluoroacetic acid (4×5 mL), and the combined filtrates were concentrated in vacuo to a volume of 5 mL and added dropwise to dry diethyl ether (20 mL) at 0 °C. The formed precipitate was separated from the mother liquor by centrifugation and washed with diethyl ether (10 mL). The residue was dissolved in dist. water and lyophilized. The crude product was purified by semipreparative RP-HPLC (column: Phenomenex Luna, gradient: $CH_3CN/H_2O$ (+ 0.1% TFA) 5:95→$CH_3CN/H_2O$ (+ 0.1% TFA) 50:50 in 60 min, $R_t$ = 55.2 min), and the MUC4 glycopeptide **24** (64 mg, 46% yield over 33 steps, 98% yield per coupled amino acid) was obtained as a colorless lyophilizate.

$R_t$ = 27.1 min (analytical RP-HPLC, column: Phenomenex Luna, gradient: $CH_3CN/H_2O$ (+ 0.1% TFA) 5:95→$CH_3CN/H_2O$ (+ 0.1% TFA) 100:0 in 60 min); $[\alpha]_D^{23}$ = −47.31 (*c* = 1, $H_2O$); $^1H$ NMR (400 MHz, COSY, HMQC, $D_2O$): $\delta$ = 8.61 (d, $J_{H\varepsilon,NH}$ = 1.6 Hz, 1H; $H^\varepsilon$), 7.46–7.30 (m, 10H; $H_{ar}$-Bn (2×)), 7.26 (s, 1H; $H^\delta$), 5.44–5.38 (m, 4H; H4 {5.41}, H7″ {5.41}, H8″ {5.40}, $CH_{2a}$-Bn {5.40}), 5.12–5.05 (m, 2H; H1 {5.09}, $CH_{2b}$-Bn {5.09}), 5.00–4.47 (m, 1H; H4′), 4.92–4.73 (m, 5H; H4″ {4.87}, H1′ {4.79}, H2′ {4.79}, $D^\alpha$ {4.78}, $T^{\alpha*}$ {4.74}), 4.70 (dd, $J_{H\alpha,H\beta}$ = 5.5 Hz, $J_{H\alpha,H\beta'}$ = 8.6 Hz, 1H; $H^\alpha$), 4.60 (d, $J_{a,b}$ = 11.7 Hz, 1H; $CH_{2a}$-Bn), 4.54–4.40 (m, 9H; $S^\alpha$ {4.52, 4.45 (3×)}, $P^\alpha$ {4.50, 4.42}, $CH_{2b}$-Bn {4.49}, H3′ {4.49}, $A^\alpha$ {4.48}, $L^\alpha$ {4.45}), 4.38–4.28 (m, 9H; $T^\alpha$ {4.36, 4.33 (3×)}, H5′ {4.35}, $A^\alpha$ {4.34}, H9a″ {4.33}, 2-H {4.31}, H3 {4.31}, $T^{\beta*}$ {4.29}), 4.26–4.14 (m, 5H; $T^\beta$ {4.22 (3×)}, H6a′ {4.16}, $V^\alpha$ {4.16}), 4.12–4.02 (m, 2H; H9b″ {4.09}, H6b′ {4.05}), 3.97–3.81 (m, 10H; $P^\delta$ {3.95}, $S^\beta$ {3.87 (3×)}, H5″ {3.86}, $G^\alpha$ {3.85}), 3.79–3.54 (m, 6H; H5 {3.75}, H6″ {3.75}, $P^\delta$ {3.73, 3.67, 3.61}, H6a {3.56}), 3.46–3.39 (m, 1H; H6b), 3.26 (dd, $J_{H\alpha,H\beta}$ = 5.1 Hz, $J_{H\beta,H\beta'}$ = 15.7 Hz, 1H; $H^\beta$), 3.10 (dd, $J_{H\alpha,H\beta'}$ = 8.6 Hz, $J_{H\beta,H\beta'}$ = 15.7 Hz, 1H; $H^{\beta'}$), 2.96 (d, $J_{D\alpha,\beta}$ = 5.9 Hz, 2H; $D^\beta$), 2.64–2.58 (m, 1H; $H3_{eq}$″), 2.30–2.23 (m, 2H; $P^\beta$), 2.29 (s, 3H; $CH_3$-Ac), 2.19 (s, 3H; $CH_3$-Ac), 2.15 (s, 3H; $CH_3$-Ac), 2.10–2.08 (m, 1H; $V^\beta$), 2.09 (s, 3H; $CH_3$-Ac), 2.08 (s, 3H; $CH_3$-Ac), 2.07 (s, 3H; $CH_3$-Ac), 2.06 (s, 3H; $CH_3$-Ac), 2.05–1.96 (m, 4H; $P^\gamma$ {2.03, 1.98}), 2.02 (s, 3H; $CH_3$-Ac), 1.98 (s, 3H; $CH_3$-Ac), 1.92–1.86 (m, 2H; $P^\beta$), 1.85 (s, 3H; $CH_3$-Ac), 1.72–1.48 (m, 4H; $L^\gamma$ {1.68}, $L^\beta$ {1.58, 1.53}, $H3_{ax}$″ {1.56}), 1.39 (d, $J_{A\alpha,\beta}$ = 7.1 Hz, 6H; $A^\beta$ (2×)), 1.33 (d, $J_{T\beta*,\gamma}$ = 6.3 Hz, 3H; $T^{\gamma*}$), 1.23–1.17 (m, 9H; $T^\gamma$ (3×)), 1.00–0.93 (m, 12H; $L^\delta$ {0.99, 0.96}, $V^\gamma$ {0.96, 0.94}) ppm; $^{13}C$ NMR (100.6 MHz, BB, HMQC, $D_2O$): $\delta$ 175.0, 174.9, 174.6, 174.0, 173.9, 173.5, 173.4, 173.3, 173.1, 172.7, 172.6, 172.3, 172.2, 172.1, 171.9, 171.7, 171.5, 171.1, 170.6, 169.5, 167.1 (C=O), 137.0, 134.1 ($C_q$-Bn (2×)), 133.3 ($H^\varepsilon$), 129.0, 128.7, 128.6, 127.9 ($C_{ar}$-Bn (2×)), 128.2 ($H^\gamma$), 117.2 ($H^\delta$), 100.5 (C1′), 99.1 (C1), 97.0 (C2″), 75.5 ($T^{\beta*}$), 72.9 (C3, $CH_2$-Bn), 71.6 (C5, C3′, C6″), 70.5 (C8″), 70.1 (C2′), 69.4 (C4″), 68.8 ($CH_2$-Bn), 68.3 (C4′), 67.5 (C4, C6, C5′, C7″), 66.9, 66.7 ($T^\beta$ (3×)), 63.1 (C6′), 61.9 (C9″), 60.9, 60.8 ($S^\beta$ (3×)), 60.2, 59.6 ($P^\alpha$), 59.4 ($V^\alpha$), 59.1, 58.6 ($T^\alpha$ (3×)), 55.6, 55.3 ($S^\alpha$ (3×)), 55.3 ($T^{\alpha*}$), 51.8 ($H^\alpha$), 50.3 ($L^\alpha$), 49.9, 49.3 ($A^\alpha$), 48.9 ($D^\alpha$), 48.5 (C5″, C2), 48.1, 47.7 ($P^\delta$), 42.3 ($G^\alpha$), 39.7 ($L^\beta$), 36.7 (C3″), 35.3 ($D^\beta$), 30.0 ($V^\beta$), 29.3, 29.2 ($P^\beta$), 26.5 ($H^\beta$), 24.6, 24.3 ($P^\gamma$), 24.2 ($L^\gamma$), 22.4 ($L^\delta$), 22.3, 21.7 ($CH_3$-NHAc (3×)), 21.6 ($L^\delta$), 20.8, 20.7, 20.2, 20.0, 19.9, 19.7 ($CH_3$-OAc (8×)), 18.6 ($T^\gamma$ (3×), $T^{\gamma*}$), 18.3, 17.5 ($V^\gamma$), 16.6, 16.2 ($A^\beta$) ppm; MS (MALDI-TOF, dhb, positive-ion mode): m/z: found: 2755.7 $[M+H]^+$; $C_{121}H_{175}N_{20}O_{53}$ calcd 2756.2; found: 2777.6 $[M+Na]^+$; $C_{121}H_{174}N_{20}NaO_{53}$ calcd 2778.1; found: 2793.1 $[M+K]^+$; $C_{121}H_{174}KN_{20}O_{53}$ calcd 2794.1; found: 2799.6 $[M+2Na-H]^+$; $C_{121}H_{173}N_{20}Na_2O_{53}$ calcd 2800.1.

**Ac-Thr-Ser-Ser-Ala-Ser-Thr-Gly-His-Ala-Thr(α-NeuNAc-(2–3)-β-Gal-(1–3)-α-GalNAc)-Pro-Leu-Pro-Val-Thr-Asp-OH (25)**: A catalytic amount of palladium (10%) on activated charcoal was added under argon atmosphere to a solution of Ac-Thr-Ser-Ser-Ala-Ser-Thr-Gly-His-Ala-Thr(α-$Ac_4$NeuNAcCOOBn-(2–3)-β-$Ac_2$BnGal-(1–3)-α-$Ac_2$GalNAc)-Pro-Leu-Pro-Val-Thr-Asp-OH **24** (36 mg, 0.013 mmol) in dry methanol (15 mL). The reaction flask was then flooded with hydrogen, and the suspension was stirred for 48 h under hydrogen atmosphere. After completion of the reaction (monitoring by analytical RP-HPLC), the mixture was filtered through Hyflo Super Cel and washed with methanol, and the solvent was removed from the filtrate at reduced pressure. The residue was dissolved in dist. water (20 mL) and lyophilized. The crude product of the debenzylation reaction was obtained as a colorless lyophilizate (31 mg, 92%) and subjected to the subsequent deacetylation procedure without further purification.

$R_t$ = 16.6 min (analytical RP-HPLC, column: Phenomenex Luna, gradient: $CH_3CN/H_2O$ (+ 0.1% TFA) 5:95→$CH_3CN/H_2O$ (+ 0.1% TFA) 100:0 in 60 min); MS (MALDI-TOF, dhb, positive-ion mode): *m/z*: found: 2576.1 $[M+H]^+$; $C_{107}H_{163}N_{20}O_{53}$ calcd 2576.1; found: 2597.8 $[M+Na]^+$; $C_{107}H_{162}N_{20}NaO_{53}$ calcd 2598.1; found: 2613.5 $[M+K]^+$; $C_{107}H_{162}KN_{20}O_{53}$ calcd 2614.0; found: 2619.7 $[M+2Na-H]^+$; $C_{107}H_{161}N_{20}Na_2O_{53}$ calcd 2620.0.

The debenzylated MUC4 glycopeptide (29 mg, 0.011 mmol) was dissolved in anhydrous methanol (15 mL) and treated with a solution of NaOMe (5%) in methanol. The reaction mixture was adjusted to pH 10 and stirred for 48 h at ambient temperature. The solution was neutralized by addition of acetic acid, and the solvent was evaporated in vacuo. Analytical RP-HPLC analyses and mass spectra confirmed that the residue contained the monoacetylated MUC4 glycopeptide as the main product. The residue was therefore again dissolved in an aqueous solution of NaOH (5 mM, 10 mL) and stirred at pH 11.5. The final deacetylation was monitored carefully by analytical RP-HPLC. The reaction mixture was neutralized with acetic acid after 36 h, and the water was removed by lyophilization. The residue was purified by semipreparative RP-HPLC (column: Phenomenex Luna, gradient: $CH_3CN/H_2O$ (+ 0.1% TFA) 5:95→$CH_3CN/H_2O$ (+ 0.1% TFA) 25:75 in 60 min, $R_t$ = 40.0 min) to give the (2,3)-sialyl-T-MUC4-glycopeptide **25** (15 mg, 64%) as a colorless, amorphous solid. In addition, the corresponding monoacetylated MUC4-glycopeptide was isolated as a by-product (3 mg, 12%).

$R_t$ = 15.6 min (analytical RP-HPLC, column: Phenomenex Luna, gradient: $CH_3CN/H_2O$ (+ 0.1% TFA) 5:95→$CH_3CN/H_2O$ (+ 0.1% TFA) 50:50 in 60 min); $[\alpha]_D^{23}$ = −28.73 (c = 1, $H_2O$); $^1H$ NMR (600 MHz, DQF-COSY, HMQC, $D_2O$): $\delta$ = 8.62 (s, 1H; $H^\varepsilon$), 7.28 (d, $J_{H\delta,NH}$ = 3.1 Hz, 1H; $H^\delta$), 5.06 (d, $J_{H1,H2}$ = 3.5 Hz, 1H; H1), 4.78–4.65 (m, 3H; $D^\alpha$ {4.73}, $H^\alpha$ {4.73}, $T^{\alpha*}$ {4.69}), 4.55–4.14 (m, 19H; $A^\alpha$ {4.50, 4.35}, H1′ {4.49}, $S^\alpha$ {4.49, 4.43 (3×)}, $P^\alpha$ {4.46, 4.42}, $L^\alpha$ {4.41}, $T^\alpha$ {4.35, 4.34 (3×)}, $T^{\beta*}$ {4.29}, $T^\beta$ {4.26, 4.20 (3×)}, H2 {4.23}, H8″ {4.19}, $V^\alpha$ {4.15}), 4.10–4.01 (m, 3H; H7″ {4.07}, H3′ {4.04}, H3 {4.03}), 3.94–3.55 (m, 26H; $G^\alpha$ {3.91}, H4 {3.90}, $P^\delta$ {3.89, 3.74, 3.68, 3.60}, $S^\beta$ {3.88 (3×)}, H4′ {3.86}, H6a, H6b {3.82, 3.62}, H5″ {3.82}, H9a″, H9b″{3.81, 3.60}, $6H_a$′, $6H_b$′ {3.73}, H6″ {4.73}, H4″ {3.67}, H5′ {3.61}, H5 {3.57}), 3.51 (dd, $J_{H1',H2'}$ = 7.8 Hz $J_{H2',H3'}$ = 9.4 Hz, 1H; H2′), 3.29 (dd, $J_{H\alpha,\beta}$ = 5.1 Hz, $J_{H\beta,H\beta'}$ = 15.3 Hz, 1H; $H^\beta$), 3.17–3.10 (m, 1H; $H^{\beta'}$), 2.96 (d, $J_{D\alpha,\beta}$ = 5.9 Hz, 2H; $D^\beta$), 2.74 (dd, $J_{3''ax,eq}$ = 12.5 Hz, $J_{H3''eq,H4''}$ = 4.3 Hz, 1H; $H3_{eq}$″), 2.32–2.22 (m, 2H; $P^\beta$), 2.10–1.96 (m, 14H; $CH_3$-Ac {2.09, 2.02, 1.97}, $V^\beta$ {2.08}, $P^\gamma$ {2.02, 1.99}), 1.93–1.84 (m, 2H; $P^\beta$), 1.80 (t, $J_{H3''ax,H3''eq}$ = $J_{H3''ax,H4}$ = 12.1 Hz, 1H; $H3_{ax}$″), 1.74–1.66 (m, 1H; $L^\gamma$), 1.63–1.51 (m, 2H; $L^\beta$), 1.40 (d, $J_{A\alpha,\beta}$ = 7.4 Hz, 6H; $A^\beta$ (2×)), 1.33 (d, $J_{T\beta*,\gamma*}$ = 6.3 Hz, 3H; $T^{\gamma*}$), 1.26–1.17 (m, 9H; $T^\gamma$ (3×)), 1.00–0.92 (m, 12H; $L^\delta$ {0.96, 0.94}, $V^\gamma$ {0.95, 0.94}) ppm; $^{13}C$ NMR (150.9 MHz, BB, HMQC, $D_2O$): $\delta$ = 175.0, 174.8, 174.6, 174.3, 173.9, 173.6, 173.5, 173.4, 173.3, 172.8, 172.3, 171.5, 171.0, 170.9, 169.5 (C=O), 133.3 ($H^\varepsilon$), 128.2 ($H^\gamma$), 117.2 ($H^\delta$), 104.4 (C-1′), 99.4 (C2″), 98.6 (C1), 77.9 (C3), 75.5 (C3′), 74.6 ($T^{\beta*}$), 72.6 (C5′), 71.6 (C4′), 70.9 (C7″), 70.2 (C6″), 70.0 (C2′), 68.9 (C8″), 68.2 (C4″), 67.9 (C5), 67.2 (C4), 66.9, 66.8 ($T^\beta$ (3× )), 63.1 (C9″), 62.4 (C6), 61.2 (C6′), 60.9, 60.7 ($S^\beta$ (3×)), 60.1, 59.6 ($P^\alpha$), 59.3 ($V^\alpha$), 59.1, 59.0, 58.6 ($T^\alpha$), 55.6 ($T^{\alpha*}$), 55.5, 55.4 ($S^\alpha$ (3×)), 51.9 ($H^\alpha$), 51.5 (C5″), 50.3 ($L^\alpha$), 49.9, 49.4 ($A^\alpha$), 49.2 ($D^\alpha$), 48.2 (C-2), 48.0, 47.8 ($P^\delta$), 42.4 ($G^\alpha$), 39.3 (C3″), 38.9 ($L^\beta$), 35.5 ($D^\beta$), 30.0 ($V^\beta$), 29.3, 29.2 ($P^\beta$), 26.4 ($H^\beta$), 24.6, 24.2 ($P^\gamma$), 24.3 ($L^\gamma$), 22.3 ($L^\delta$), 22.2, 21.9, 21.6 ($CH_3$-Ac), 21.4 ($L^\delta$), 18.6 ($T^\gamma$ (3×), $T^{\gamma*}$), 18.3, 17.6 ($V^\gamma$), 16.6, 16.3 ($A^\beta$) ppm; MS (MALDI-TOF, dhb, positive-ion mode): *m/z*: found: 2242.1 $[M+H]^+$; $C_{91}H_{147}N_{20}O_{45}$ calcd 2241.3; found: 2263.9 $[M+Na]^+$; $C_{91}H_{146}N_{20}NaO_{45}$ calcd 2263.2; found: 2280.1 $[M+K]^+$; $C_{91}H_{146}KN_{20}O_{45}$ calcd 2279.2.

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