CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.201302921

A Unified Strategy for the Synthesis of Mucin Cores 1–4 Saccharides and the Assembled Multivalent Glycopeptides

Christian Pett, Manuel Schorlemer, and Ulrika Westerlind*[a]

Dedicated to Professor Thomas Norberg on the occasion of his 65th birthday

Abstract: By displaying different *O*-glycans in a multivalent mode, mucin and mucin-like glycoproteins are involved in a plethora of protein binding events. The understanding of the roles of the glycans and the identification of potential glycan binding proteins are major challenges. To enable future binding studies of mucin glycan and glycopeptide probes, a method that gives flexible and efficient access to all common mucin core-glycosylated amino acids was developed. Based on a convergent synthesis strategy starting from a shared early stage intermediate by differentiation in the glycoside acceptor reactivity, a common disaccharide building block allows for the creation of extended glycosylated amino acids carrying the mucin type-2 cores 1–4 saccharides. Formation of a phenyl-sulfenyl-*N*-Troc (Troc = trichloroethoxycarbonyl) byproduct during *N*-iodosuccinimide-promoted thioglycoside couplings was further characterized and a new methodology for the removal of the Troc group is described. The obtained glycosylated 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acid building blocks are incorporated into peptides for multivalent glycan display.

Keywords: amino acids · carbohydrates · glycopeptides · glycosylation · reactivity

Introduction

Mucins are highly glycosylated proteins that populate the cell surface of epithelial tissues in a membrane-bound or secreted form.[1] By displaying different *O*-glycans in a multivalent mode, mucins and mucin-like glycoproteins are involved in a plethora of protein binding events.[2] With the diversity of glycan structures found in biological systems in mind, the understanding of the roles of the glycan and the identification of potential glycan binding proteins is a major challenge. Here, chemical synthesis can enable glycobiology by supplying well-defined glycan and glycopeptide probes, thus making systematic glycan binding studies feasible. In spite of the structural diversity of mucin-type *O*-glycans, they all have in common that a GalNAc (Ac = acetyl) residue is connected to the protein backbone through an *O*-glycosidic linkage to serine or threonine.[3] The branching of the GalNAc residue in the 3- or 6-position with Gal, GlcNAc, or GalNAc gives rise to different core structures (1–8), and the core structures 1–4 are the most common structures identified in humans (Figure 1 A).[4] These structures are often extended with Gal and GlcNAc residues and terminated with fucose, sialic acid, sulfation, Gal, and/or GalNAc. Various bioactive glycoconjugates contain the type-2 disaccharide unit *N*-acetyllactosamine [β-D-Gal-(1→4)-β-D-GlcNAc], which serves as a common scaffold for the Lewis antigens for example, SLe^x, Le^x, and Le^y. These epitopes are responsible for generating diversity by extending or terminating the mucin-type core glycan structures.[5]

Several syntheses of mucin-type core-glycosylated amino acids and glycopeptides have been described,[6] which have mainly focused on specific protein targets, in particular tumor-associated mucin-1 epitopes and E-, P-, and L-selectin ligands.[7] In contrast to previously prepared P-selectin glycopeptide ligands involving a single glycan binding site, the multivalent mucin peptide tandem repeats are densely glycosylated, thereby posing a synthesis problem that differs fundamentally from the challenge posed by monovalent glycopeptide structures. In the diverse mucin glycopeptide structures oligosaccharide diversity is generated by differing the extensions of structurally common core-glycosylated amino acids. Therefore, a synthesis strategy could be envisaged that gives flexible and efficient access to all mucin core-glycosylated amino acids, and in extension also glycopeptides, based on a small set of common building blocks.

We here report a unified synthesis of *N*-acetyllactosamine-extended core 1-, core 2-, core 3-, and core 4-glycosylated amino acids and of the multivalent mucin 1 (MUC1) and mucin 5B (MUC5B) glycopeptides. The method employs a disaccharide key building block responsible for selective extension of GalNAc- (Tn) or Gal-GalNAc-Thr (T-

[a] Dipl.-Chem. C. Pett, M. Sc. M. Schorlemer, Dr. U. Westerlind
Gesellschaft zur Förderung der Analytischen Wissenschaften e.V. ISAS–Leibniz
Institute for Analytical Sciences
Otto-Hahn-Strasse 6b, 44227 Dortmund (Germany)
Fax: (+49) 231-1392-4850
E-mail: ulrika.westerlind@isas.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201302921.

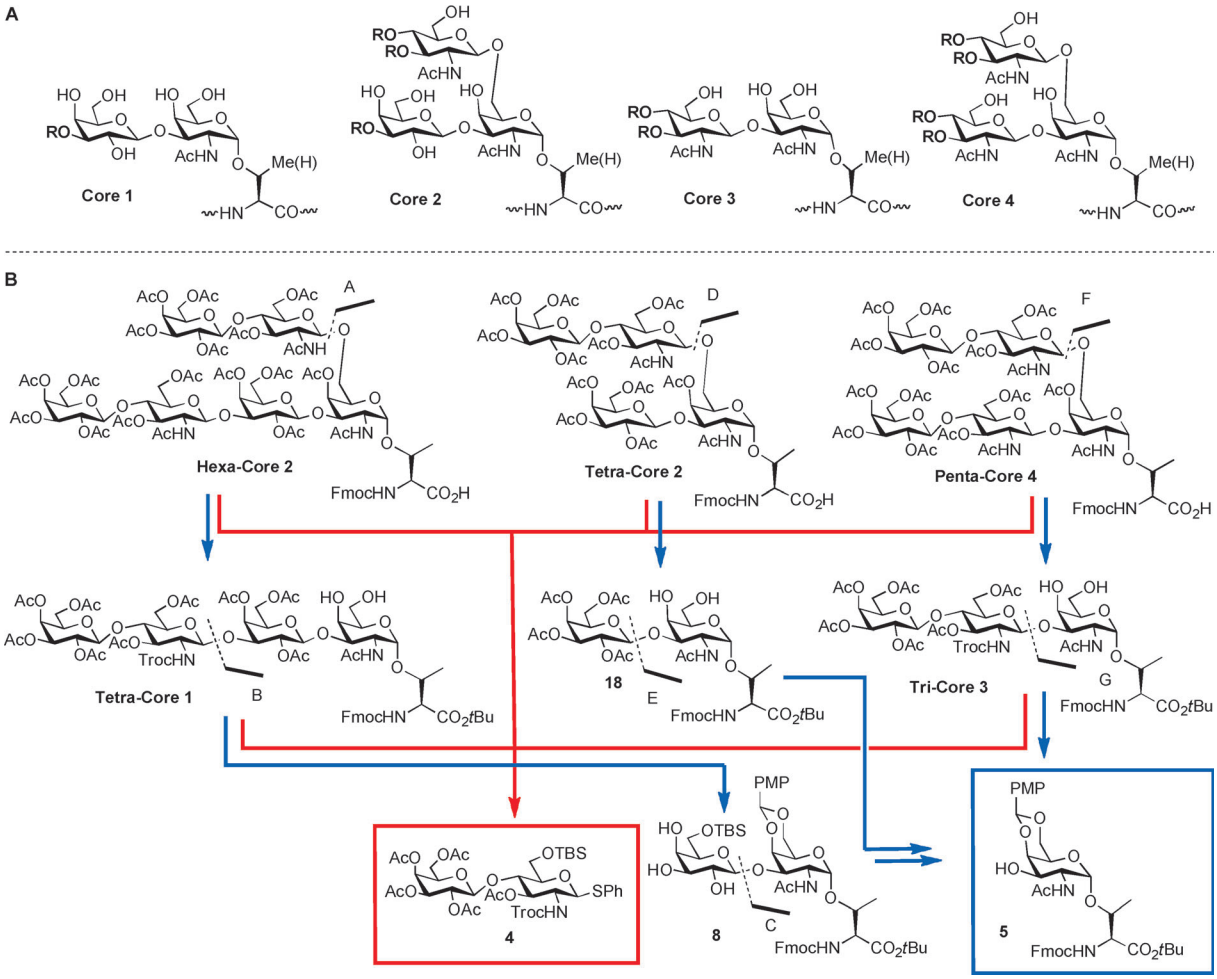


Figure 1. A) Core 1–4 structures of mucin-type glycosylation. B) A unified synthesis strategy for building up extended core 1–4 structures by using common precursor building blocks. *t*Bu = *tert*-butyl, Troc = trichloroethoxycarbonyl, TBS = *tert*-butyl dimethylsilyl, PMP = *p*-methoxyphenyl.

antigen) amino acids. By using orthogonal protecting groups that direct the reactivity, the stereoselectivity, and are further removable under mild conditions, the obtained core-glycosylated 9-fluorenylmethoxycarbonyl solid-phase peptide synthesis (Fmoc-SPPS) building blocks are readily available for glycopeptide synthesis after just a few steps from the common precursors.

Results and Discussion

Synthetic strategy: We outline a strategy to synthesize mucin glycopeptides by using glycosylated Fmoc-protected amino acid building blocks incorporated by a stepwise peptide assembly according to the standard Fmoc solid-phase peptide synthesis protocol. A triethylene glycol spacer was incorporated at the N terminus of the glycopeptides to have additional spacing for later immobilization on an *N*-hydroxysuccinimde (NHS)-activated microarray surface.[8] The choice of the protecting groups on the glycosylated amino acid is critical for a successful glycopeptide synthesis. Here, *O*-acetyl protection of the saccharide portion was selected, because these groups can be removed under mild conditions like diluted sodium methoxide in methanol.[9] The acetyl groups further stabilize the *O*-glycosidic bond during the acidic trifluoroacetic acid (TFA) treatment, required for resin cleavage of the synthesized peptides.[10] For the design of a straightforward route to mucin-type Fmoc-protected amino acid building blocks, a retrosynthetic analysis was carried out. We focused on a convergent coupling approach starting from common precursor building blocks, for example, an *N*-trichloroethoxycarbonyl (NHTroc)-protected thioglycoside disaccharide donor and Tn or T-antigen acceptors. By using this strategy and further relying on orthogonal protecting groups, as well as internal reactivity differences of the acceptors, the different type-2 *N*-acetyllactosamine-extended core 1, core 2, core 3, and core 4 Fmoc-SPPS amino acids were obtained in a few steps from the precursor building blocks. We used the thioglycoside donor **4**, containing a *tert*-butyldimethylsilyl group in the 6-position and *N*-Troc protection in the 2-position of the GlcN residue as a common building block for core extension. The choice of the glycan protecting groups was considered to be critical for the proceeding key glycosylation steps. The *O*-TBS

group and the *N*-Troc protection contributed positively by stereoelectronic effects to the increased reactivity of the common donor **4**, which in the later deprotection steps were easily removed under mild conditions.[11] Furthermore, the Troc group directs the stereochemistry of the glycosylation by neighboring group participation, resulting in the formation of the desired β-glycosidic bond.[11a,b] To obtain good yields and avoid negative steric effects during the key glycosylation steps, a strategy was selected making use of the increased reactivity of the 3- and 6-position on the Gal and GalNAc residues. By disconnections A-, D-, and F-selective glycosylation would take place on the more reactive primary hydroxyl group in the 6-position of GalNAc, without protection of the neighboring 4-position, yielding the core 2 hexasaccharide **17**, the core 2 tetrasaccharide **22**, and the core 4 pentasaccharide **37** starting from the acceptors core 1 tetrasaccharide **11**, the T antigen disaccharide **18**, and the core 3 trisaccharide **30**, respectively. Accordingly, the core 1 tetrasaccharide **11** (disconnection B), is prepared by selective glycosylation in the 3-position of acceptor **8** without protection of the less reactive 2- and 4-hydroxyl groups. The disaccharide acceptors **8** and **18** as well as the core 3 trisaccharide **30** are all obtained from the common precursor GalNAc-Thr amino acid **5** by disconnections C, E and G (Figure 1B).

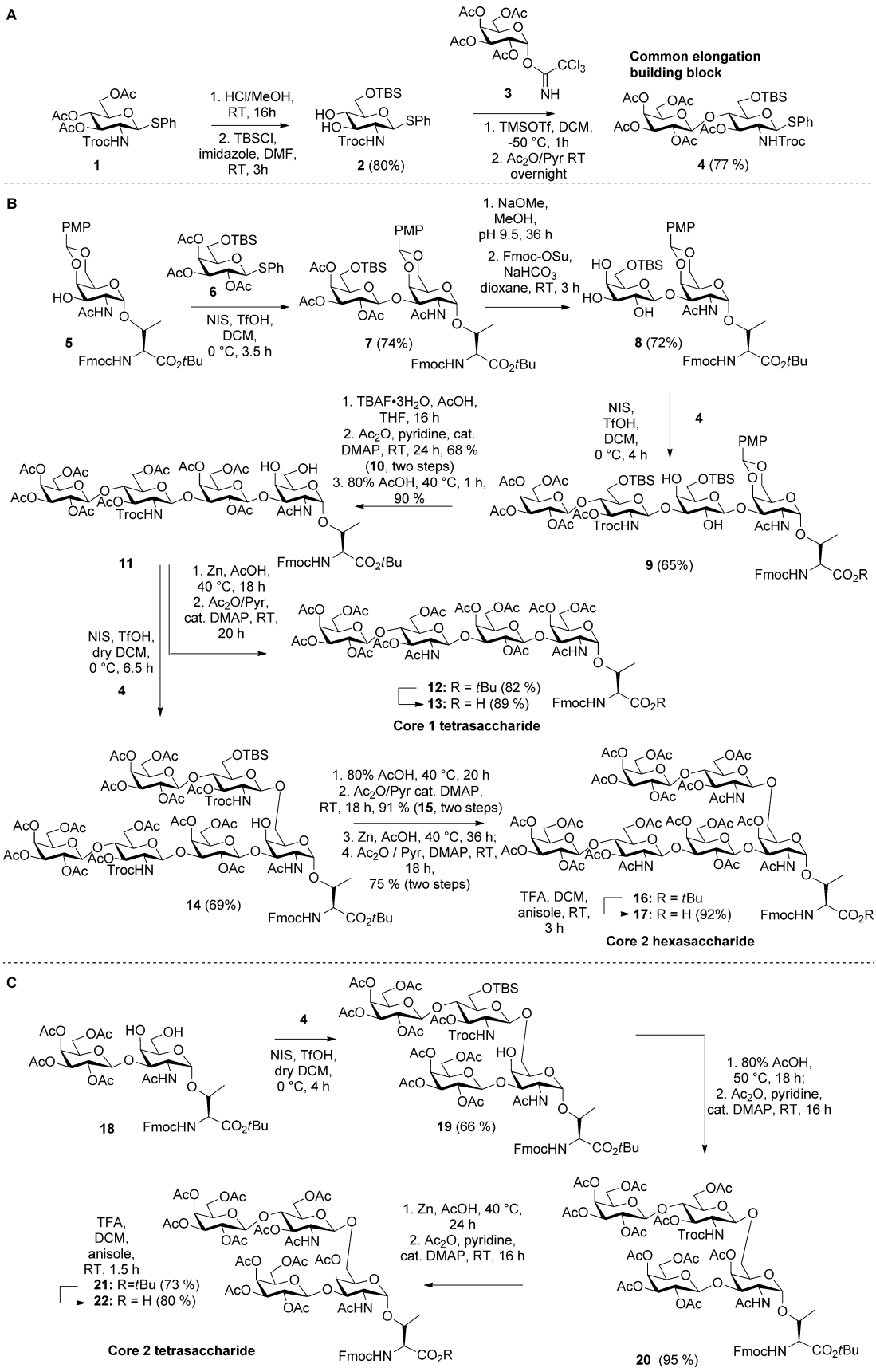
The chemical synthesis commenced with the preparation of the common disaccharide donor **4**, which in later steps is responsible for the *N*-acetyllactosamine extension of the different glycan core structures. Starting from the known GlcN-Troc-protected thioglycoside **1**,[12] removal of the acetyl protecting groups by using HCl/MeOH was followed by *tert*-butyldimethylsilyl protection of the 6-position to form compound **2**. Regioselective glycosylation at the more reactive 4-position of the GlcN-Troc acceptor **2** with trichloroacetimidate (**3**)[13] activated by trimethylsilyltriflate (TMSOTf)[14] at −40 °C gave disaccharide **4** (Scheme 1A).

For preparation of the core 1 tetrasaccharide **13** and core 2 hexasaccharide **17** Fmoc-protected amino acids, two regioselective glycosylations were carried out starting from the thioglycoside donor **4** and the T-antigen Gal-GalNAc-Thr disaccharide acceptor **8**, respectively. Initially, the T-antigen Thr conjugate **8** was formed by reaction of the GalNAc-Thr compound **5**[15] with the donor thioglycoside **6**,[16] activated by NIS/TfOH[17] to give the disaccharide **7**. Removal of the galactose acetyl protecting groups by using NaOMe in methanol at pH 9.5 was followed by reintroduction of the partially cleaved Fmoc group yielding the disaccharide acceptor **8**. By NIS/TfOH activation at 0 °C, glycosylation took place providing the core 1 tetrasaccharide **9** in good yield and with complete regio- and stereoselectivity resulting from the higher reactivity of the 3-position of the galactose in the T-antigen acceptor, compared with the hydroxyl groups in 2- and 4-position. Avoiding steric hindrance from neighboring protecting groups of the acceptor during disaccharide extension further contribute to the good yield of the coupling product **9** (Scheme 1B). The conversion of tetrasaccharide **9** to a stable building block compatible with solid-phase peptide synthesis, was achieved by a few protecting group manipulations. Initially, the TBS groups of the tetrasaccharide **9** were removed by using tetrabutylammonium-fluoride buffered with acetic acid to avoid cleavage of the base-sensitive Fmoc group. The TBS removal was followed by acetylation yielding compound **10**. Hydrolysis of the benzylidene acetal by 80% aqueous acetic acid gave compound **11**. Reductive elimination of the *N*-Troc group and subsequent acetylation followed. In a final step, the *t*Bu ester was cleaved by treatment with TFA in dichloromethane to give the core 1 tetrasaccharide-containing Fmoc-protected building block **13** in a total yield of 29% over seven steps starting from the common T-antigen precursor **8** (Scheme 1B).

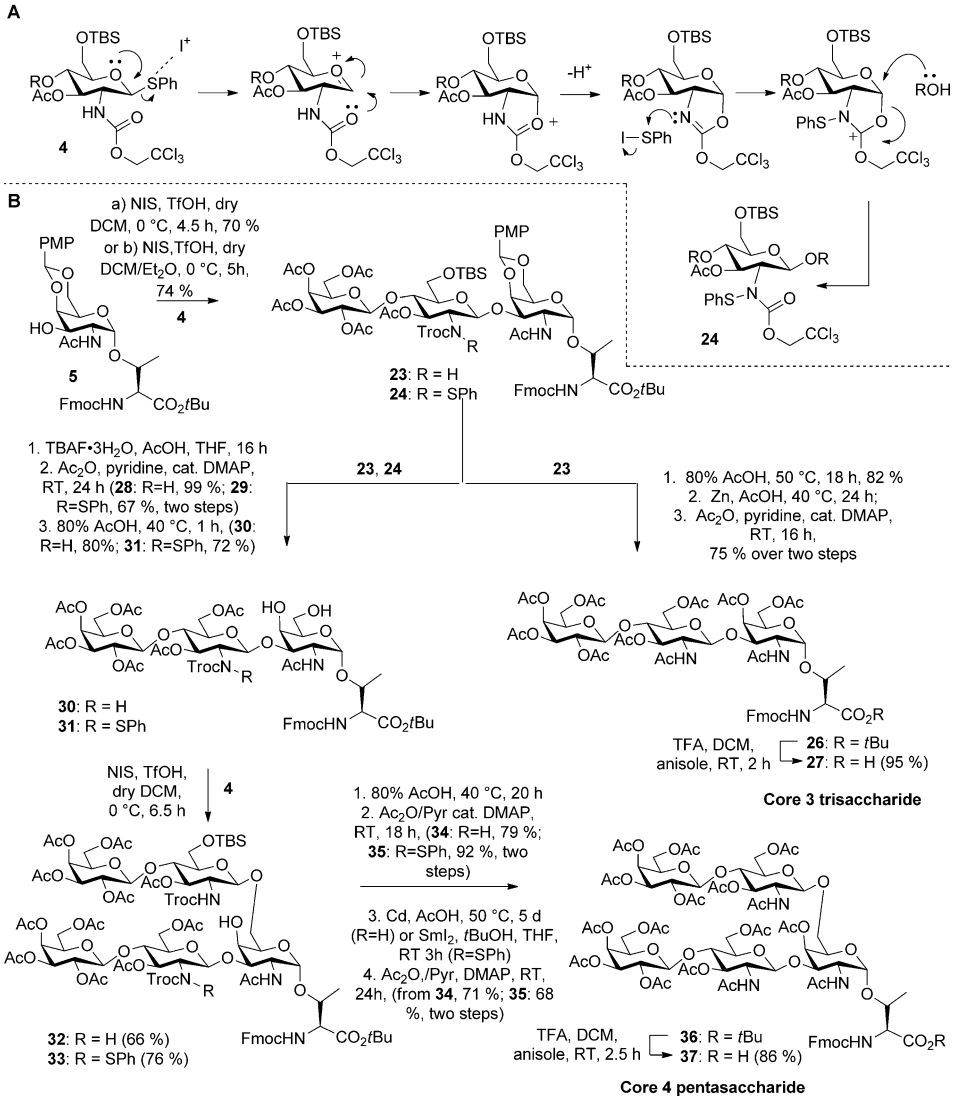
For the preparation of the core 2 hexasaccharide **17**, a second glycosylation of the core 1 tetrasaccharide intermediate **11** was performed by using the thioglycoside donor **4** activated with NIS/TfOH. The hexasaccharide coupling product **14** was formed with the desired stereo- and regioselectivity. A few protecting group manipulations followed to generate the Fmoc-protected core 2 hexasaccharide building block **17** in a total yield of 17% over ten steps starting from the common T-antigen precursor **8** (Scheme 1C).

Starting from the known T-antigen amino acid intermediate **18**[6d] and the thioglycoside donor **4** activated by NIS/TfOH at 0 °C, the core 2 tetrasaccharide **19** was obtained. In accordance with the procedures described above, the formed coupling product **19** was converted to the Fmoc-protected core 2 tetrasaccharide building block **22**, which was obtained in a total yield of 37% over six steps starting from the common T-antigen precursor **18** (Scheme 1C).

In the initial attempts to generate the core 3 Fmoc-SPPS building block starting from the Tn amino acid acceptor **5** and thioglycoside donor **4** promoted by NIS/TfOH at 0 °C, the coupling reaction proceeded very slowly and gave a mixture of two compounds; the desired trisaccharide coupling product **23** and byproduct **24**. Supported by NMR spectroscopic and mass spectrometry data, the formed byproduct was found to contain an extra phenyl-sulfenyl group at the *N*-Troc amine. A plausible mechanism for the formation of compound **24** suggests that the nitrogen atom in the oxazoline intermediate obtained from thioglycoside donor **4** acted as a nucleophile on the phenyl-sulfenyl iodide intermediate to form the PhS-N-Troc product **24** (Scheme 2A). A number of conditions were investigated to suppress the formation of compound **24**, however the generation of **24** could not be completely avoided. Because the Troc group and the labile phenyl-sulfenyl N–S bond could be cleaved during reductive zinc treatment used in later steps, conditions with increased amounts of NIS were selected instead, thus promoting the formation of product **24** exclusively. In a few steps, the coupling product **24** was converted to the desired Fmoc-SPPS building block **27** in a total yield of 41% over four steps starting from the Tn precursor **5** (Scheme 2B). In accordance with the synthesis of the core 2 hexasaccharide, the two core 3 products **23** and **24** were converted to acceptors **30** and **31**, respectively, suitable for further disaccharide extension resulting in the pentasaccharide core 4 coupling products **32** and **33**, respectively. After ex-



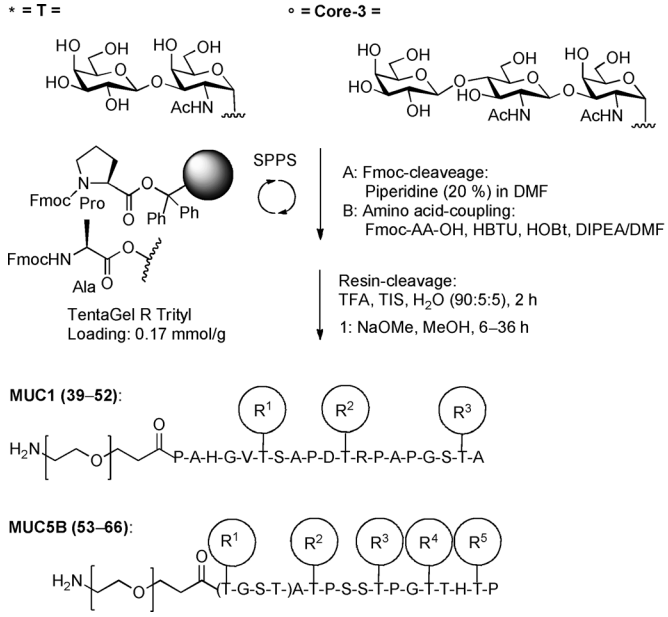
Scheme 1. A) Synthesis of the disaccharide donor elongation building block. B) Synthesis of extended core 1 and core 2, tetra- and hexasaccharide Fmoc-protected amino acids. C) Synthesis of an extended core 2 tetrasaccharide Fmoc-protected amino acid. Pyr = pyridine, Tf = triflate, Su = succinimidyl, TBAF = tetrabutylammonium fluoride, DMAP = 4-dimethylaminopyridine.



Scheme 2. A) A plausible mechanism for the formation of the PhS-N-Troc product **24**. B) Synthesis of extended core 3 and core 4 tri- and pentasaccharide Fmoc-protected amino acids.

change of the TBS group for an acetyl group the removal of the Troc groups to give the common intermediate **36** followed. The reduction of the Troc groups by treatment with zinc or cadmium in acetic acid[18] were very slow for both compounds and did not affect the PhS-N-Troc product **35**. Instead, samarium iodide (SmI_2) was investigated as a reducing agent; it has previously been employed to break labile heteroatom linkages like N–S bonds.[19] An earlier study showed that SmI_2 in THF cleaves 2-chloroethoxy carbamate groups.[20] In a modified procedure that might have broader applications for Troc deprotection of complex carbohydrates or natural products, treatment of compound **35** with SmI_2 in THF/*t*BuOH removed the Troc groups and PhS adducts within a few hours at room temperature. After subsequent acetylation compound **36** was obtained in a good yield. Final *t*Bu removal by using TFA in dichloromethane gave the Fmoc-protected core 4 pentasaccharide **37** in a total yield of 19 (via **23**) and 15 % (via **24**) over ten steps starting from the Tn precursor **5**. (Scheme 2 B).

To optimize the synthesis of multivalent mucin glycopeptides, the shorter T-antigen disaccharide **38** and the core 3 trisaccharide **27** Fmoc-SPPS building blocks were used. Coupling of the standard Fmoc-protected amino acids (8 equiv) was carried out by using *O*-(benzotrialzol-1-yl)-tetramethyluronium hexafluorophosphate/1-hydroxybenzotriazole (HBTU/HOBt),[21] the glycosylated Fmoc-protected threonine building blocks (1.5 equiv) were coupled with *O*-(7-azabenotriazol-1-yl)-tetramethyluronium hexafluorophosphate/1-Hydroxy-7-azabenzotriazole (HATU/HOAt).[22] The glycosylated amino acids were pre-activated in a smaller volume of solvent and added manually to the resin. Furthermore, the reaction times of the glycosylated amino acids were extended and the two amino acids followed were double coupled. After assembly of the full mucin tandem repeat pep-



39	PAHGVT*SAPDTRPAPGSTA (85 %)	**53**	AT*PSSTPGTTHTP (72 %)
40	PAHGVTSAPDT*RPAPGSTA (85 %)	**54**	ATPSSTPGT*THTP (78 %)
41	PAHGVTSAPDTRPAPGST*A (76 %)	**55**	AT*PSSTPGT*THTP (79 %)
42	PAHGVT*SAPDT*RPAPGSTA (67 %)	**56**	AT*PSST*PGTTHTP (62 %)
43	PAHGVT*SAPDTRPAPGST*A (84 %)	**57**	AT*PSST*PGT*THTP (61 %)
44	PAHGVTSAPDT*RPAPGST*A (62)	**58**	AT*PSST*PGT*THT*P (64 %)
45	PAHGVT*SAPDT*RPAPGST*A (59 %)	**59**	T*GSTAT*PSST*PGT*THTP (33 %)
46	PAHGVT°SAPDTRPAPGSTA (70 %)	**60**	AT°PSSTPGTTHTP (75 %)
47	PAHGVTSAPDT°RPAPGSTA (65 %)	**61**	ATPSSTPGT°THTP (67 %)
48	PAHGVTSAPDTRPAPGST°A (72 %)	**62**	AT°PSSTPGT°THTP (62 %)
49	PAHGVT°SAPDT°RPAPGSTA (74 %)	**63**	AT°PSST°PGTTHTP (67 %)
50	PAHGVT°SAPDTRPAPGST°A (62 %)	**64**	AT°PSST°PGT°THTP (65 %)
51	PAHGVTSAPDT°RPAPGST°A (62 %)	**65**	AT°PSST°PGT°THT°P (8 %)
52	PAHGVT°SAPDT°RPAPGST°A (59 %)	**66**	T°GSTAT°PSST°PGT°THTP (49 %)

Scheme 3. Synthesis of multivalent MUC1 and MUC5B glycopeptides containing core 1 and core 3 glycans. Overall yields in parenthesis starting from preloaded Fmoc-Ala or Fmoc-Pro trityl resin. DIPEA = diisopropylethylamine

tides on the resin, the peptides were terminated with coupling of a triethylene glycol spacer and subsequent detachment from the resin by applying a mixture of trifluoracetic acid/triisopropylsilane (TIPS)/H_2O (90:5:5). After a desalting step on a C-18 cartridge, the *O*-acetyl groups were removed by treatment with catalytic amounts of NaOMe in methanol at pH 9.5. Finally, the deprotected T-antigen and core 3 glycopeptides were purified by preparative HPLC to obtain compounds **39–66** (Scheme 3).

Conclusion

In conclusion, we have developed an efficient methodology for the synthesis of extended mucin core-glycosylated amino acids. Based on a convergent approach with common precursor building blocks, the core 1–4 Fmoc-protected building blocks were obtained in excellent yields. Key glycosylation reactions proceeded with complete regio- and stereoselectivity without protection of neighboring hydroxyl groups taking advantage of the increased reactivity of the galactose 3- and 6-position. The described methodology opens up the way for the syntheses of other extended core amino acid building blocks by using similar strategies. With the first *N*-acetyllactosamine-extended core amino acids in hand, different multivalent mucin glycopeptides can be prepared, which will enable future biological studies. The first syntheses of mucin multivalent peptides were optimized by using the di- and trisaccharide core 1- and core 3-glycosylated amino acids.

Experimental Section

General methods and the synthesis of compounds **2–4**, **7**, **8**, **19**—**37**, and **39–66** are described in the Supporting Information.

Synthesis of the type-2 core 1 tetrasaccharide building block

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-2-deoxy-4,6-O-para-methoxybenzylidenacetal-3-O-[6-tert-butyldimethylsilyl-3-O-[3-O-acetyl-2-deoxy-6-tert-butyldimethyldimethylsilyl-2-N-(2,2,2-trichloroethoxycarbonyl-amino)-4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-galactopyranosyl]-α-*D*-galactopyranosyl)-*L*-threonine-tert-butyl-ester (Fmoc-Thr(βAc4Gal-(1→4)-βAc-TBDMS-GlcNHTroc-(1→3)-βTBDMS-Gal-(1→3)-αPMP-GalNAc)-OtBu) (**9**)*: Compounds **8** (3.98 g, 4.00 mmol) and **4** (4.88 g, 5.23 mmol) were dissolved in anhydrous dichloromethane (60 mL) followed by addition of activated molecular sieves 4 Å (5 g). The mixture was stirred for 1 h under an argon atmosphere, before it was cooled down to 0 °C. Then slow addition of *N*-iodosuccinimide (1.18 g, 5.23 mmol) and a suspension of trifluoromethanesulfonic acid (53 μL, 91 mg, 0.61 mmol) in dry dichloromethane (1 mL) followed. After 2.5 h additional portions of the thioglycoside donor **4** (1.12 g, 1.20 mmol) and *N*-iodosuccinimide (270 mg, 1.2 mmol) were added and the reaction was stirred for 1.5 h at 0 °C. Then it was diluted with dichloromethane (40 mL) and the molecular sieve was filtered off and washed with dichloromethane. The mixture was diluted with additional dichloromethane to reach a total volume of 200 mL. The organic phase was washed with a 0.5 M aqueous solution of sodium thiosulfate, a saturated aqueous solution of sodium bicarbonate, water and brine (80 mL each). Then the solution was dried over sodium sulfate, filtered, and concentrated in vacuum. The residue was purified by silica column chromatography (cyclohexane/EtOAc gradient from 2:1 to 1:1) to give compound **9** (4.71 g, 65 % 2.59 mmol) as a colorless solid. $R_f = 0.45$ (cyclohexane/EtOAc 2:3); $[\alpha]_D^{20} = +42.22$ ($c = 0.99$ in $CHCl_3$); ^{1}H NMR (600 MHz, [D_6]DMSO, 30 °C, internal DMSO δ(H) = 2.50 ppm): $\delta = 7.90$–7.88 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.76–7.73 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.63 (d, ^3J(NH,H-2″) = 8.3 Hz, 1H; NH-Troc,), 7.56 (d, ^3J(NH,Tα) = 9.8 Hz, 1H; NH-Fmoc), 7.47 (d, ^3J(NH,H-2) = 9.7 Hz, 1H; NH-Ac), 7.42 (t, ^3J(H-2,H-1) = ^3J(H-2,H-3) = ^3J(H-7,H-6) = ^3J(H-7,H-8) = 7.4 Hz, 2H; H-2-Fmoc, H-7-Fmoc), 7.37 (d, ^3J(H-2,H-3) = ^3J(H-6,H-5) = 8.8 Hz, 2H; H-2-PMP, H-6-PMP), 7.31 (t, ^3J(H-3,H-4) = ^3J(H-3,H-2) = ^3J(H-6,H-7) = ^3J(H-6,H-5) = 7.4 Hz, 2H; H-3-Fmoc, H-6-Fmoc), 6.93 (d, ^3J(H-3,H-2) = ^3J(H-5,H-6) = 8.8 Hz, 2H; H-3-PMP, H-5-PMP), 5.52 (s, 1H;

CH-(PMP)), 5.24 (d, ^3J(H-4‴,H-3‴) = 3.6 Hz, 1H; H-4‴), 5.11 (dd, ^3J(H-3‴,H-4‴) = 3.5, ^3J(H-3‴,H-2‴) = 10.3 Hz, 1H; H-3‴), 4.98 (d, ^3J(H-1″,H-2″) = 8.5 Hz, 1H; H-1″), 4.93 (t, ^3J(H-3″,H-2″) = ^3J(H-3″,H-4″) = 6.6 Hz, 1H; H-3″), 4.89 (d, $^3J(CH_{2a},CH_{2b})$ = 12.4 Hz, 1H; CH_{2a}-(Troc)), 4.85–4.82 (m, 1H; H-2‴), 4.77 (d, ^3J(H-1,H-2) = 3.3 Hz, 1H; H-1), 4.72 (d, ^3J(H-1‴,H-2‴) = 7.9 Hz 1H; H-1‴), 4.68–4.65 (m, 2H; CH_{2b}-(Troc), OH-2′), 4.48–4.40 (m, 3H; CH_{2ab}-(Fmoc), OH-4′), 4.36–4.26 (m, 5H; H-2, H-5, H-1′, H-9-(Fmoc), T^{β}), 4.15–4.10 (m, 2H; H-5‴, T^{α}), 4.06–3.97 (m, 4H; H-6_{ab}, H-$6'''_{ab}$), 3.83–3.62 (m, 11H; H-3, H-4, H-4′, H-4″, H-$6'_{ab}$, H-$6''_{ab}$, CH_3-(PMP)), 3.45–3.41 (m, 3H; H-2′, H-2″, H-5″), 3.36–3.30 (m, 2H; H-5′, H-3′), 2.09–1.86 (m, 18H; CH_3-(Ac)), 1.39 (s, 9H; *t*Bu-(Thr)), 1.12 (d, ^3J-(Tγ,Tβ) = 6.2 Hz, 3H; T^{γ}), 0.87 (s, 9H; *t*Bu-(TBS)), 0.83 (s, 9H; *t*Bu-(TBS)), 0.05 (s, 6H; 2×Me(TBS)), 0.04 (s, 3H; Me(TBS)), 0.03 ppm (s, 3H; Me(TBS)); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO, internal DMSO δ(C) = 39.51 ppm): δ = 169.9, 169.5, 169.3, 169.0 (C=O-(Ac), C=O-(*t*Bu)), 159.5 (C-1-PMP), 156.7 (C=O-(Fmoc)), 154.4 (C=O-(Troc)), 143.8, 143.5 (C-1_a-Fmoc, C-8_a-Fmoc), 140.8 (C-4_a-Fmoc, C-5_a-Fmoc), 130.8 (C-4-PMP), 127.6 (C-2-Fmoc, C-7-Fmoc, C-2-PMP, C-6-PMP), 127.0 (C-3-Fmoc, C-6-Fmoc), 125.2 (C-1-Fmoc, C-8-Fmoc), 120.2, 120.1 (C-4-Fmoc, C-5-Fmoc), 113.3 (C-3-PMP, C-5-PMP), 105.4 (C-1′), 100.3 (C-1″), 2× 99.8, 99.7 (C-1, C-1‴, CH-(PMP)), 96.2 (C_{quart}-(Troc)), 81.4 (C_{quart}-(*t*Bu-(Thr))), 80.4 (C-5″), 75.8 (T^{β}), 75.6 (C-4″), 75.1, 75.0 (C-3, C-3′, C-5′), 74.2 (C-5), 73.5 (C-3″), 73.4 (CH_{2ab}-(Troc)), 70.3 (C-3‴), 69.9 (C-2′), 69.8 (C-5‴), 69.0 (C-2‴), 68.4 (C-6_{ab}), 67.1 (C-4′), 67.0 (C-4‴), 65.6 (CH_{2ab}-(Fmoc)), 63.1 (C-4), 62.1 (C-$6'_{ab}$), 61.6 (C-$6''_{ab}$), 60.9 (C-$6'''_{ab}$), 59.3 (T^{α}), 55.8 (C-2″), 55.1 (CH_3-(PMP)), 47.3, 46.8 (C-2, C-9-(Fmoc)), 27.6 (*t*Bu-(Thr)), 25.8, 25.6 (2×*t*Bu-(TBS)), 23.2, 20.6, 20.5, 20.4, 20.3 (6×CH_3-(Ac)), 19.3 (T^{γ}), 18.0, 17.9 (2 C_{quart}-(TBS)), −5.3, −5.4 ppm (4×Me(TBS)); HRMS (ESI) (pos): *m/z* calcd for $C_{82}H_{117}Cl_3N_3O_3Si_2^+$: 1816.6224 $[M+H]^+$; found: 1816.6224.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-2-deoxy-4,6-O-para-methoxybenzylidene-3-O-[2,4,6-tri-O-acetyl-3-O-[3,6-di-O-acetyl-2-deoxy-2-N-(2,2,2-trichloroethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactosylpyranosyl]-α-D-galactopyranosyl)-L-threonine-tert-butylester (Fmoc-Thr(βAc_4Gal-(1→4)-βAc_2GlcNHTroc-(1→3)-βAc_3Gal-(1→3)-αPMP-GalNAc)-OtBu) (**10**)*: A solution of compound **9** (3.80 g, 2.09 mmol) in tetrahydrofuran (60 mL) was cooled to 0 °C. Then a mixture of tetrabutylammoniumfluoride trihydrate (6.59 g, 20.9 mmol) and acetic acid (2.39 mL, 2.51 g, 41.8 mmol) in tetrahydrofuran (20 mL) was added. After 1 h the ice bath was removed and the reaction was stirred for 16 h at room temperature. The mixture was diluted with ethyl acetate (400 mL) and the organic phase was washed twice with an aqueous saturated solution of bicarbonate/brine (1:1, 450 mL) and once with brine (200 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in pyridine (60 mL) followed by addition of *N,N*-dimethylaminopyridine (25 mg, 0.20 mmol) and acetic anhydride (30 mL, 32.4 g, 317 mmol). The reaction was stirred for 24 h. The solvent was removed by co-evaporation with toluene and the crude material was purified by silica column chromatography (cyclohexane/EtOAc 1:2) to give compound **10** (2.50 g, 68 %, 1.44 mmol) as a colorless solid. R_f = 0.43 (toluene (Tol)/EtOAc 1:3); $[\alpha]_D^{20}$ = +56.94 (c = 0.49 in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30 °C, internal DMSO): δ = 7.92–7.90 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.81 (d, ^3J(NH,H-2) = 9.1 Hz, 1H; NH-Troc), 7.77–7.34 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.43–7.41 (m, 4H; H-2-Fmoc, H-7-Fmoc, NH-Ac, NH-Fmoc), 7.34–7.30 (m, 4H; H-2-PMP, H-6-PMP, H-3-Fmoc, H-6-Fmoc), 6.93 (d, ^3J(H-3,H-2) = ^3J(H-5,H-4) = 8.8 Hz, 2H; H-3-PMP, H-5-PMP), 5.44 (s, 1H; CH-(PMP)), 5.34–5.32 (m, 1H; H-4′), 5.22 (d, ^3J(H-4‴,H-3‴) = 3.2 Hz, 1H; H-4‴), 5.16 (dd, ^3J(H-3‴,H-2‴) = 10.3, ^3J(H-3‴,H-4‴) = 3.3 Hz, 1H; H-3‴), 4.98–4.97 (m, 2H; H-3″, CH_{2a}-(Troc)), 4.84–4.80 (m, 2H; H-2′, H-2‴), 4.71–4.69 (m, 2H; H-1, H-1‴), 4.63–4.61 (m, 2H; H-1′, H-1″), 4.53–4.45 (m, 3H; CH_{2ab}-(Fmoc), CH_{2b}-(Troc)), 4.32 (t, ^3J(H-9,CH_2) = 6.7 Hz, 1H; H-9-(Fmoc)), 4.28–4.26 (m, 3H; H-5, H-$6'_a$, T^{β}), 4.23–4.17 (m, 2H; H-2, H-5‴), 4.09–3.99 (m, 6H; H-6_{ab}, H-$6'_a$, H-$6'''_{ab}$, T^{α}), 3.93–3.89 (m, 3H; H-5′, H-$6'_b$, H-$6''_b$), 3.97 (dd, ^3J(H-3′,H-4′) = 4.0, ^3J(H-3′,H-2′) = 10.2 Hz, 1H; H3′), 3.75–3.72 (m, 4H; H-3, CH_3-(PMP)), 3.70–3.65 (m, 2H; H-4, H-4″), 3.54–3.51 (m, 1H; H-5″), 3.29 (q, ^3J(H-2″,H-1″) = ^3J(H-2″,NH″) = ^3J(H-2″,H-3″) = 9.1 Hz 1H; H-2″), 2.09–1.83 (m, 30H; CH_3-(Ac)), 1.36 (s, 9H; *t*Bu), 1.15 ppm (d, ^3J(Tγ,Tβ) = 6.2 Hz, 3H; T^{γ}); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): δ = 170.3, 170.0, 169.9, 169.5, 169.2, 169.1, 168.7 (C=O-(Ac), C=O-(*t*Bu)), 159.5 (C1-PMP), 156.8 (C=O-(Fmoc)), 154.0 (C=O-(Troc)), 143.7 (C-1_a-Fmoc, C-8_a-Fmoc), 140.8 (C-4_a-Fmoc, C-5_a-Fmoc), 130.8 (C-4-PMP), 127.7 (C-2-PMP, C-6-PMP), 127.0 (C-3-Fmoc, C-6-Fmoc, C-2-Fmoc, C-7-Fmoc), 125.3, 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 113.4 (C-3-PMP, C-5-PMP), 101.5 (C-1′), 100.6 (C-1″), 100.0 (C-1‴), 99.8 (CH-(PMP)), 99.1 (C-1), 96.1 (C_{quart}-(Troc)), 81.5 (C_{quart}-(*t*Bu)), 78.1 (C-3′), 76.0 (C-4″), 75.1, 75.0 (T^{β}, C-3), 73.4 (C-5, CH_{2ab}-(Troc)), 72.8 (C-3‴), 71.5 (C-5″), 70.7 (C-5), 70.3 (C-3‴), 69.6 (C-5″), 69.4 (C-4′), 69.1, 68.9 (C-2′, C-2″), 68.6 (C-6_{ab}), 67.0 (C-4‴), 65.6 (CH_{2ab}-(Fmoc)), 62.9 (C-4), 62.5 (C-$6'_{ab}$), 61.8 (C-$6''_{ab}$), 60.8 (C-$6'''_{ab}$), 59.3 (T^{α}), 55.8 (C-2″), 55.1 (CH_3-(PMP)), 46.9, 46.8 (C-2, C-9-(Fmoc)), 27.6 (*t*Bu), 22.9, 20.8, 20.7, 20.6, 20.5, 20.4, 20.3 (CH_3-(Ac)), 19.3 ppm (T^{γ}); HRMS (ESI) (pos): *m/z* calcd for $C_{78}H_{97}Cl_3N_3O_{36}^+$: 1756.4917 $[M+H]^+$; found: 756.4922; calcd for $C_{78}H_{97}Cl_3KN_3O_{36}^{2+}$: 897.7277 $[M+K+H]^{2+}$; found: 898.7227.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-2-deoxy-3-O-[2,4,6-tri-O-acetyl-3-O-[3,6-di-O-acetyl-2-deoxy-2-N-(2,2,2-trichloroethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine-tert-butylester(Fmoc-Thr(βAc_4Gal-(1→4)-βAc_2GlcNHTroc-(1→3)-βAc_3Gal-(1→3)-αGalNAc)-OtBu) (**11**)*: A solution of compound **10** (2.45 g, 1.39 mmol) in a 80 % aqueous solution of AcOH (50 mL) was stirred for 1 h at 40 °C. The solvent was removed by co-evaporation with toluene in vacuum. The crude product was purified by silica column chromatography (100 % EtOAc) to give compound **11** (2.05 g, 90 %, 1.25 mmol) as a colorless solid. R_f = 0.27 (EtOAc); $[\alpha]_D^{20}$ = +36.86 (c = 0.51 in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30 °C): δ = 7.92–7.90 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.81 (d, ^3J(NH,H2″) = 9.1 Hz, 1H; NH-Troc), 7.75 (t, ^3J-(H1,H2) = ^3J(H8,H7) = 7.7 Hz, 2H; H-1-Fmoc, H-8-Fmoc), 7.46–7.41 (m, 4H; H-2-Fmoc, H-7-Fmoc, NH-Fmoc, NH-Ac), 7.34–7.29 (m, 2H; H-3-Fmoc, H-6-Fmoc), 5.31 (d, ^3J(H-4′,H-3′) = 3.5 Hz, 1H; H-4′), 5.21 (d, ^3J(H-4‴,H-3‴) = 3.6 Hz, 1H; H-4‴), 5.16 (dd, ^3J(H-3‴,H-2‴) = 10.3 Hz, ^3J(H-3‴,H-4‴) = 3.5 Hz, 1H; H-3‴), 4.99–4.97 (m, 2H; H-3″, CH_{2a}-(Troc)), 4.86 (t, ^3J(H-2′,H-3′) = ^3J(H-2′,H-1′) = 8.2 Hz, 1H; H-2′), 4.82 (dd, ^3J(H-2‴,H-3‴) = 10.3 Hz, ^3J(H-2‴,H-1‴) = 7.9 Hz, 1H; H-2‴), 4.70–4.58 (m, 4H; H-1, H-1′, H-1″, H-1‴), 4.52–4.49 (m, 2H; CH_{2b}-(Troc), CH_{2a}-(Fmoc)), 4.46–4.30 (m, 1H; CH_{2b}-(Fmoc)), 4.39 (d, ^3J(OH-4,H-4) = 5.4 Hz, 1H; OH-4,), 4.32 (t, ^3J(H-9,CH_{2ab}) = 6.7 Hz, 1H; H-9-(Fmoc)), 4.26–4.20 (m, 4H; H-2, H-5‴, H-$6''_a$, T^{β}), 4.08–4.06 (m, 1H; T^{α}), 4.01–3.91 (m, 6H; H-5′, H-$6'_{ab}$, H-$6''_b$, H-$6'''_{ab}$), 3.89–3.87 (m, 1H; H-4), 3.80–3.78 (m, 1H; H-3′), 3.68 (t, ^3J(H-4″,H-3″) = ^3J(H-4″,H-5″) = 9.5 Hz 1H; H-4″), 3.63–3.61 (m, 1H; H-5), 3.55–3.82 (m, 2H; H-3, H-5″), 3.48–3.46 (m, 2H; H-6_{ab}), 3.33–3.29 (m, 2H; OH-6, H-2″), 2.09- 1.85 (m, 30H; CH_3-(Ac)), 1.35 (s, 9H; *t*Bu), 1.16–1.15 ppm (m, 3H; T^{γ}); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): δ = 170.3, 170.0, 169.9, 169.6, 169.5, 169.2, 169.1, 168.8, 168.6 (C=O-(Ac), C=O-(*t*Bu)), 156.8 (C=O-(Fmoc)), 154.0 (C=O-(Troc)), 143.7 (C-1_a-Fmoc, C-8_a-Fmoc), 140.8 (C-4_a-Fmoc, C-5_a-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.0 (C-3-Fmoc, C-6-Fmoc), 125.3, 125.2 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 101.4 (C-1′), 100.6 (C-1″), 100.0 (C-1‴), 98.9 (C-1), 96.1 (C_{quart}-(Troc)), 81.4 (C_{quart}-(*t*Bu)), 78.3 (C-3′), 77.4 (C-3), 75.9 (C-4″), 73.5 (T^{β}), 73.4 (CH_{2ab}-(Troc)), 72.8 (C-3″), 71.7 (C-5), 71.5 (C-5″), 70.7 (C-5′), 70.3 (C-3‴), 69.6 (C-5‴), 69.3 (C-4′), 69.1 (C-2′), 68.9 (C-2‴), 67.4 (C-4), 67.0 (C-4‴), 65.6 (CH_{2ab}-(Fmoc)), 62.2 (C-6_{ab}), 61.8 (C-$6''_{ab}$), 60.8 (C-$6'''_{ab}$), 60.5 (C-6_{ab}), 59.4 (T^{α}), 55.8 (C-2″), 47.0 (C-2), 46.8 (C-9-(Fmoc)), 27.6 (*t*Bu), 22.9, 20.8, 20.7, 20.6, 20.5, 20.4, 20.3 (10× CH_3-(Ac)), 19.1 ppm (T^{γ}); HRMS (ESI) (pos): *m/z* calcd for $C_{70}H_{91}Cl_3N_3O_{35}^+$: 1638.4499 $[M+H]^+$; found: 1638.4480.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-4,6-O-acetyl-2-deoxy-3-O-[2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactosylpyranosyl]-α-D-galactosylpyranosyl)-L-threonine-tert-butylester (Fmoc-Thr(βAc_4Gal-(1→4)-βAc_2GlcNAc-(1→3)-βAc_3Gal-(1→3)-αAc_2GalNAc)-OtBu) (**12**)*: Zinc dust was activated by treatment with 1 N hydrochloric acid followed by washing with water, methanol, and diethyl ether. The activated zinc (393 mg, 6.0 mmol) was added to a solution of compound **11** (492 mg, 0.29 mmol) in acetic acid (6 mL). The reaction was stirred at 40 °C for 18 h and then the mixture was filtered over celite and the filtrate was co-evaporated several times with toluene. The residue was dis-

solved in pyridine/acetic anhydride (2:1, 9 mL). *N,N*-dimethylaminopyridine (3.7 mg, 0.03 mmol) was added to the solution and the reaction mixture was stirred for 20 h. The solvent was removed by co-evaporation with toluene and the crude product purified by silica column chromatography (100% EtOAc) to give compound **12** (389 mg, 82%, 0.25 mmol) as a colorless solid. R_f=(EtOAc/MeOH 20:1); $[\alpha]_D^{20}=+41.40$ ($c=0.50$ in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30°C): $\delta=7.77$ (d, ^3J(H-4,H-3)=^3J(H-5,H-6)=9.1 Hz, 2H; H-4-Fmoc, H-5-Fmoc,), 7.62–7.59 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.49–7.46 (m, 2H; H-2-Fmoc, H-7-Fmoc), 7.39–7.35 (m, 2H; H-3-Fmoc, H-6-Fmoc), 6.22 (d, ^3J(NH,H-2)=9.1 Hz, 1H; NH-Ac), 5.92 (d, ^3J(NH,Tα)=9.1 Hz, 1H; NH-Fmoc), 5.60–5.54 (m, 1H; NH″-Ac), 5.32–5.29 (m, 3H; H-4, H-4′, H-4‴), 5.15 (t, ^3J(H-3″, H-2″)=^3J(H-3″,H-4″)=9.5 Hz, 1H; H-3″), 5.07 (dd, ^3J(H-2‴,H-1‴)=8.0, ^3J(H-2‴,H-3‴)=10.3 Hz, 1H; H-2‴), 4.98–4.95 (m, 2H; H-2′, H-3‴), 4.83 (sbr, 1H; H-1), 4.76–4.68 (m, 2H; H-1″, H-6$''_a$), 4.57 (d, ^3J(H-1′,H-2′)=7.8 Hz, 1H; H-1′), 4.51–4.49 (m, 2H; H-1‴, H-2), 4.43 (m, 1H; CH_{2a}-(Fmoc)), 4.35–4.31 (m, 1H; CH_{2b}-(Fmoc)), 4.25–4.17 (m, 3H; T$^\beta$, T$^\alpha$, H-9-(Fmoc)), 4.12–4.02 (m, 6H; H-5, H-6$_{ab}$, H-6′$_a$, H-6‴$_{ab}$), 3.99–3.90 (m, 3H; H-3, H-6′$_b$, H-6″$_b$), 3.86–3.50 (m, 3H; H-3′, H-5, H-5′), 3.77 (t, ^3J(H-4″,H-3″)=^3J(H-4″,H-5″)=9.3 Hz, 1H; H-4″), 3.58–3.52 (m, 2H; H-2″, H-5″), 2.11–1.87 (m, 39H; CH_3-(Ac)), 1.43 (s, 9H; *t*Bu), 1.32–1.28 ppm (m, 3H; T$^\gamma$); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): $\delta=170.9$, 170.6, 170.5, 170.3, 170.2, 170.1, 170.0, 169.7, 169.3 (C=O-(Ac), C=O-(*t*Bu)), 156.6 (C=O-(Fmoc)), 143.8 (C-1$_a$-Fmoc, C-8$_a$-Fmoc), 141.5 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 128.0 (C-2-Fmoc, C-7-Fmoc), 127.3 (C-3-Fmoc, C-6-Fmoc), 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 101.2 (C-1‴), 100.6 (C-1′), 100.5 (C-1‴), 100.1 (C-1), 83.6 (C_{quart}-(*t*Bu)), 76.6 (T$^\beta$), 76.1 (C-5), 75.9 (C-4″), 72.7 (C-5‴), 71.9 (C-3, C-3″), 71.3 (C-5′), 71.0 (C-3‴), 70.8 (C-3′), 70.7 (C-2′), 69.3 (C-4, C-4′), 69.2 (C-2‴), 68.2 (C-5‴), 67.2 (CH_{2ab}-(Fmoc)), 66.8 (C-4‴), 63.2 (C-6′$_{ab}$), 61.9 (C-6‴$_{ab}$), 60.9 (C-6$_{ab}$, C-6″$_{ab}$), 59.2 (T$^\alpha$), 55.3 (C-2″), 48.7 (C-2), 47.4 (C-9-(Fmoc)), 28.2 (*t*Bu), 23.3–20.7 (CH_3-(Ac)), 18.8 ppm (T$^\gamma$); HRMS (ESI) (pos): *m/z* calcd for $C_{73}H_{96}N_3O_{36}^+$: 1590.5774 $[M+H]^+$; found: 1590.5763; calcd for $C_{73}H_{96}KN_3O_{36}^{2+}$: 814.7706 $[M+K+H]^{2+}$; found: 814.7661.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-4,6-O-acetyl-2-deoxy-3-O-{2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactosyl-pyranosyl}-α-D-galactosylpyranosyl)-L-threonine (Fmoc-Thr(βAc$_4$Gal-(1→4)-βAc$_2$GlcNAc-(1→3)-βAc$_3$Gal-(1→3)-αAc$_2$GalNAc)-OH) (**13**)*: A solution of compound **12** (718 mg, 0.45 mmol) in dichloromethane (3 mL), anisole (0.5 mL), and trifluoroacetic acid (9 mL) was stirred for 2.5 h. The solvent was removed by co-evaporation with toluene and the residue was purified by silica column chromatography (EtOAc→EtOAc/MeOH 25:1) to give compound **13** (616 mg, 89%, 0.40 mmol) as a colorless solid. R_f=0.21 (EtOAc/MeOH/AcOH/H$_2$O 50:3:3:2); $[\alpha]_D^{20}=+48.57$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta=12.90$ (COOH), 7.91, 7.90 (2×d, ^3J(H-4,H-3)=^3J(H-5,H-6)=7.4 Hz, 2×1H; H-4-Fmoc, H-5-Fmoc), 7.83 (d, ^3J(NH″,H-2″)=9.0 Hz, 1H; NH″-Ac), 7.77–7.72 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.45–7.30 (m, 6H; H-2-Fmoc, H-3-Fmoc, H-6-Fmoc, H-7-Fmoc, NH-Fmoc, NH-Ac), 5.28 (d, ^3J(H-4′,H-3′)=3.7 Hz, 1H; H-4′), 5.27 (d, ^3J(H4,H3)=3.4 Hz, 1H; H-4), 5.22 (d, ^3J(H-4‴,H-3‴)=3.5 Hz, 1H; H-4‴), 5.17 (dd, ^3J(H-3‴,H-4‴)=3.5, ^3J(H-3‴,H-2‴)=10.3 Hz, 1H; H-3‴), 5.00 (t, ^3J(H-3″,H-4″)=^3J(H-3″,H-2″)=9.5 Hz, 1H; H-3″), 4.83 (dd, ^3J(H-2‴,H-3‴)=10.3, ^3J(H-2‴,H-1‴)=8.0 Hz, 1H; H-2‴), 4.74–4.69 (m, 4H; H-1, H-2, H-1″, H-1‴), 4.59 (d, J(H-1′,H-2′)=8.0 Hz, 1H; H-1′), 4.52–4.43 (m, 2H; CH_{2ab}-(Fmoc)), 4.32–4.30 (m, 2H; H-9-(Fmoc), H-6$''_a$), 4.27–4.25 (m, 1H; T$^\beta$), 4.21 (t, ^3J(H-5‴,H-6‴)=6.8 Hz, 1H; H5‴), 4.15–4.08 (m, 4H; H-2, H-5, H-6′$_a$, T$^\alpha$), 4.00–3.98 (m, 2H; H-6‴$_{ab}$), 3.94–3.79 (m, 6H; H-3, H-3′, H-5′, H-6$_{ab}$, H-6′$_b$, H-6‴$_b$), 3.65 (t, ^3J(H-4″,H-3″)=^3J(H-4″,H-5″)=9.5 Hz, 1H; H-4″), 3.57–3.54 (m, 1H; H-5″), 3.41–3.37 (m, 1H; H-2″), 2.09–1.70 ppm (m, 39H; CH_3-(Ac)); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): $\delta=170.3$, 170.1, 170.0, 169.9, 169.7, 169.5, 169.4, 169.1, 169.0, 168.8 (C=O-(Ac)), 156.8 (C=O-(Fmoc)), 143.8, 143.7 (C-1$_a$-Fmoc, C-8$_a$-Fmoc), 140.8 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.1 (C-3, C-6-Fmoc), 125.3, 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 100.6 (C-1′), 100.0, 98.7 (C-1, C-1″, C-1‴), 76.9 (C-3′), 76.1 (C-4″), 74.5 (T$^\beta$), 73.4 (C-3), 72.7 (C-3″), 71.4 (C-5″), 70.3 (C-5′, C-3‴), 69.7 (C-4), 69.6 (C-5‴), 69.5 (C-2′), 69.0 (C-4′), 68.9 (C-2‴), 67.3 (C-5), 67.0 (C-4″), 65.6 (CH_{2ab}-(Fmoc)), 63.1 (C-6′$_{ab}$), 61.8, 61.7 (C-6$_{ab}$, C-6″$_{ab}$), 60.8 (C-6‴$_{ab}$), 58.5 (T$^\alpha$), 54.0 (C-2″), 47.7 (C-2), 46.8 (C-9-(Fmoc)), 22.8, 22.6, 20.64–20.38 (CH_3-(Ac)), 18.4 pm (T$^\gamma$); HRMS (ESI) (pos): *m/z* calcd for $C_{69}H_{88}N_3O_{36}^+$: 1534.5148 $[M+H]^+$; found: 1534.5139.

Synthesis of the type-2 core 2 hexasaccharide building block

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-2-deoxy-3-O-{2,4,6-tri-O-acetyl-3-O-[3,6-O-acetyl-2-N-(2,2,2-trichloroethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl]-β-D-galactosylpyranosyl}-6-O-[3-O-acetyl-2-deoxy-6-O-tert-butyldimethylsilyl-2-N-(2,2,2-trichloroethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactosylpyranosyl)-L-threonine-tert-butylester(Fmoc-Thr(βAc$_4$Gal-(1→4)-βAc$_2$GlcNHTroc-(1→3)-βAc$_3$Gal-(1→3)-[βAc$_4$Gal-(1→4)-βAc-TBS-GlcNHTroc-(1→6)]-αGalNAc)-OtBu) (**14**)*: A suspension of compound **11** (694 mg, 0.42 mmol), compound **4** (515 mg, 0.55 mmol), and 4 Å molecular sieves (0.6 g) in dry dichloromethane (15 mL) was stirred under argon at room temperature for 1 h before it was cooled to 0°C. Then *N*-iodosuccinimide (124 mg, 0.55 mmol) was added followed by the dropwise addition of trifluoromethanesulfonic acid (7.5 μmol, 13 mg, 0.09 mmol) in dry dichloromethane (100 μL). After 3 h another portion of donor **4** (119 mg, 0.13 mmol) and *N*-iodosuccinimide (28 mg, 0.13 mmol) was added. The reaction was stirred for additional 3.5 h under argon and at 0°C before the molecular sieve was filtered off and washed with dichloromethane. More dichloromethane was added to give a total volume of 120 mL. The organic phase was washed with a 0.5 M aqueous solution of sodium thiosulfate, as saturated aqueous solution of sodium bicarbonate, water, and brine (30 mL each). The organic phase was dried over sodium sulfate, filtered, and concentrated and the residue was purified by silica column chromatography (cyclohexane/EtOAc 1:1→1:3) to give compound **14** (722 mg, 69%, 0.29 mmol) as a colorless solid. R_f=0.39 (Tol/EtOAc 1:3); $[\alpha]_D^{20}=+20.27$ ($c=0.49$ in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$ DMSO, 30°C): $\delta=7.92$–7.88 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.81–7.73 (m, 4H; H-1-Fmoc, H-8-Fmoc, 2×NH-Troc), 7.46–7.38 (m, 4H; H-2-Fmoc, H-7-Fmoc, NH-Ac, NH-Fmoc), 7.34–7.30 (m, 2H; H-3-Fmoc, H-6-Fmoc), 5.32 (sbr, 1H; H-4′), 5.25 (d, ^3J(H-4‴″,H-3‴″)=3.6 Hz, 1H; H-4‴″), 5.22 (d, ^3J(H-4‴,H-3‴)=3.6 Hz, 1H; H4‴), 5.16 (dd, ^3J(H-3‴,H-4‴)=3.6, ^3J(H-3‴,H-2‴)=10.2 Hz, 1H; H3‴), 5.08 (dd, ^3J(H-3‴″,H-4‴″)=3.6, ^3J(H3‴″,H2‴″)=10.3 Hz, 1H; H-3‴″), 4.98–4.88 (m, 4H; H-3″, H-3‴, CH$''_{2a}$-(Troc), CH$''''_{2a}$-(Troc)), 4.86–4.80 (m, 3H; H-2′, H-2‴, H-2‴″), 4.72–4.69 (m, 2H; H-1‴, H-1‴″), 4.63–4.59 (m, 2H; H-1″, CH$''''_{2b}$-(Troc)), 4.57–4.54 (m, 2H; H-1, H-1′), 4.52–4.44 (m, 4H; H-1‴″, CH$''_{2b}$-(Troc), CH$_{2ab}$-(Fmoc)), 4.32 (t, ^3J(H-9,CH$_{2ab}$)=6.6 Hz, 1H; H-9-(Fmoc)), 4.27–4.25 (m, 1H; H-6$''_a$), 4.23–4.19 (m, 2H; H-2, H-5‴), 4.09–3.91 (m, 10H; H-5‴″, H-6′$_{ab}$, H-6′$_b$, H-6‴$_{ab}$, H-6‴″$_{ab}$, T$^\alpha$, T$^\beta$), 3.88–3.85 (m, 1H; H-5″), 3.84–3.73 (m, 8H; H-3′, H-4, H-4‴″, H-5, H-5′, H-6$_a$, H-6‴″$_{ab}$), 3.68 (t, ^3J(H-4″,H-3″)=^3J(H-4″,H-2″)=9.3 Hz, 1H; H-4″), 3.56–3.48 (m, 3H; H-3, H-5″, H-6$_b$), 3.42–3.37 (m, 1H; H-2‴″), 3.33–3.28 (m, 2H; H-2″, H-5‴″), 2.10–1.84 (m, 45H; CH_3-(Ac)), 1.35 (s, 9H; *t*Bu-(Thr)), 1.09 (d, J(Tγ,Tβ)=6.1 Hz, 3H; T$^\gamma$), 0.88 (s, 9H; *t*Bu-(TBS)), 0.07 (s, 3H; 2×Me-(TBS)), 0.06 ppm (s, 3H; 2×Me-(TBS)); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): $\delta=170.3$, 169.9, 169.7, 169.5, 169.4, 169.3, 169.1, 169.0, 168.8, 168.7 (C=O-(Ac), C=O-(*t*Bu)), 156.8 (C=O-(Fmoc)), 154.1, 154.0 (2×C=O-(Troc)), 143.7 (C-1$_a$-Fmoc, C-8$_a$-Fmoc), 140.8 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.0 (C-3-Fmoc, C-6-Fmoc), 125.2, 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 101.5 (C-1′), 100.5 (C-1″), 100.0 (C-1‴, C-1‴″), 99.6 (C-1‴″), 99.1 (C-1), 96.2, 96.1 (C_{quart}-(Troc)), 81.4 (C_{quart}-(*t*Bu)), 78.2 (C-3′), 76.8 (C-3), 75.9 (C-4″), 74.8 (C-4‴″), 74.5 (C-5‴″), 74.2 (T$^\beta$), 73.4 (CH$''_{2ab}$-(Troc)), 73.3 (CH$''''_{2ab}$-(Troc)), 72.8 (C-3″, C-3‴″), 71.5 (C-5″), 70.7 (C-5′), 70.3 (C-3‴, C-3‴″, C-5), 70.0 (C-6$_{ab}$), 69.9 (C-5‴″), 69.6 (C-5‴), 69.2 (C-4′), 69.0, 68.9 (C-2′, C-2‴, C-2‴″), 68.6 (C-4), 67.1 (C-4‴″), 67.0 (C-4‴), 65.6 (CH$_{2ab}$-(Fmoc)), 61.9 (C-6′$_{ab}$), 61.8 (C-6″$_{ab}$), 61.1 (C-6‴″$_{ab}$, C-6‴″″$_{ab}$), 60.8 (C-6‴$_{ab}$), 59.6 (T$^\alpha$), 55.8 (C-2″), 55.6 (C-2‴″), 46.9 (C-2), 46.8 (C-9-(Fmoc)), 27.6 (*t*Bu-(Thr)), 25.8 (*t*Bu-(TBS)), 23.0, 20.7–20.3 (CH_3-(Ac)), 18.9 (T$^\gamma$), 18.0 (C_{quart}(TBS), −5.2, −5.3 ppm (2×Me(TBS)); HRMS (ESI) (pos): *m/z* calcd for $C_{101}H_{137}Cl_6KN_4O_{51}Si_2^+$: 1250.7896 $[M+K+H]^{2+}$; found: 1250.7862.

N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-4-O-acetyl-2-deoxy-3-O-{2,4,6-tri-O-acetyl-3-O-[3,6-O-acetyl-2-deoxy-2-N-(2,2,2-trichloro-

*ethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl]-6-O-[3,6-O-acetyl-2-deoxy-2-N-(2,2,2-trichloroethoxycarbonylamino)-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranosyl)-L-threonine-tert-butylester (Fmoc-Thr(βAc$_4$Gal-(1→4)-βAc$_2$GlcNHTroc-(1→3)-βAc$_3$Gal-(1→3)-[βAc$_4$Gal-(1→4)-βAc$_2$-GlcNHTroc-(1→6)]-αAcGal-NAc)-OtBu) (**15**)*: Compound **14** (1.66 g, 0.67 mmol) was dissolved in 80% aqueous AcOH (18 mL) and stirred for 20 h at 40°C. The solvent was removed by co-evaporation with toluene in vacuum. The residue was dissolved in pyridine (20 mL) and cooled to 0°C. Addition of *N,N*-dimethylaminopyridine (8 mg, 0.07 mmol) and acetic anhydride (10 mL) followed and the solution was stirred at room temperature for 18 h. The solvent was removed by co-evaporation with toluene and the residue was purified by silica column chromatography (cyclohexane/EtOAc 3:2→4:3) to give compound **15** (1.48 g, 91%, 0.61 mmol) as a colorless solid. R_f = 0.48 (Tol/EtOAc 1:2); $[\alpha]_D^{20}$ = +24.30 (c = 1.00 in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30°C): δ = 7.91–7.89 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.76–7.72 (m, 4H; H-1-Fmoc, H-8-Fmoc, 2×NH-Troc), 7.45–7.41 (m, 4H; H-2-Fmoc, H-7-Fmoc, NH-Ac, NH-Fmoc), 7.33–7.31 (m, 2H; H-3-Fmoc, H-6-Fmoc), 5.29 (sbr, 1H; H-4′), 5.23–5.21 (m, 3H; H-4, H-4‴, H-4⁗), 5.17–5.14 (m, 2H; H-3‴, H-3⁗), 4.97–4.89 (m, 3H; H-3″, H-3‴, CH$^{\prime\prime}_{2a}$-(Troc)), 4.91–4.89 (m, 1H; CH$^{\prime\prime\prime\prime}_{2a}$-(Troc)), 4.83–4.80 (m, 2H; H-2‴, H-2⁗), 4.73–4.70 (m, 3H; H-1‴, H-1⁗, H-2′), 4.63–4.55 (m, 4H; H-1, H-1′, H-1″, CH$^{\prime\prime\prime\prime}_{2b}$-(Troc)), 4.51–4.47 (m, 4H; H-1‴, CH$^{\prime\prime}_{2b}$-(Troc), CH$_{2ab}$-(Fmoc)), 4.32–4.27 (m, 3H; H-9-(Fmoc), H-6$^{\prime\prime}_a$, H-6$^{\prime\prime\prime\prime}_a$), 4.22–4.19 (m, 2H; H-5‴, H-5⁗), 4.14–4.09 (m, 3H; H-2, H-6$^{\prime\prime}_b$, T$^\beta$), 4.05–3.99 (m, 6H; H-5⁗, H-6$^{\prime\prime\prime}_{ab}$, H-6$^{\prime\prime\prime\prime}_{ab}$, T$^\alpha$), 3.94–3.88 (m, 3H; H-6′$_{ab}$, H-6$^{\prime\prime\prime\prime}_b$), 3.81–3.76 (m, 3H; H-3, H-3′, H-6$_a$), 3.71–3.65 (m, 2H; H-4″, H-4‴), 3.61–3.58 (m, 1H; H-5″), 3.53–3.50 (m, 1H; H-5⁗), 3.44 (q, ^3J(H-2‴,H-3‴) = ^3J(H-2‴,H-4‴) = ^3J(H-2‴,NH) = 11.5 Hz, 1H; H-2⁗), 3.35–3.27 (m, 2H; H-2″, H-6$_b$), 2.09–1.82 (m, 51H; CH$_3$-(Ac)), 1.35 (s, 9H; *t*Bu), 1.13 ppm (d, J(Tγ,Tβ) = 7.7 Hz, 3H; T$^\gamma$); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): δ = 170.3, 170.0, 169.9 169.6, 169.5, 169.3, 169.2, 169.1, 168.8 (C=O-(Ac), C=O-(*t*Bu)), 156.8 (C=O-(Fmoc)), 154.2, 154.0 (2×C=O-(Troc)), 143.8, 143.7 (C-1$_a$-Fmoc, C-8$_a$-Fmoc), 140.82 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.0 (C-3-Fmoc, C-6-Fmoc), 125.2, 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 100.6 (C-1″), 100.5 (C-1′), 100.1 (C-1‴), 100.0, 99.9 (C-1‴, C-1⁗), 99.8 (C-1), 96.1, 96.0 (2 C$_{quart}$-(Troc)), 81.5 (C$_{quart}$-(*t*Bu)), 77.6 (C-3′), 76.4 (C-4‴), 75.9 (C-4″), 74.3 (T$^\beta$), 73.4, 73.3 (C-3, CH$^{\prime\prime}_{2ab}$-(Troc), CH$^{\prime\prime\prime\prime}_{2ab}$-(Troc)), 72.7 (C-3″, C-3‴), 71.8 (C-5″), 71.5 (C-5‴), 70.2 (C-4, C-3‴, C-3⁗, C-5′), 69.7, 69.6 (C-5‴, C-5⁗), 69.3 (C-2′), 69.2 (C-4′, C-6′$_{ab}$), 68.9 (C-2‴, C-2⁗, C-5), 67.0 (C-4‴, C-4⁗), 65.6 (CH$_{2ab}$-(Fmoc)), 62.2 (C-6$^{\prime\prime}_{ab}$), 61.8, 61.7 (C-6′$_{ab}$, C-6$^{\prime\prime\prime\prime}_{ab}$), 60.9, 60.8 (C-6$^{\prime\prime}_{ab}$, C-6$^{\prime\prime\prime\prime}_{ab}$), 59.5 (T$^\alpha$), 55.8 (C-2″), 55.6 (C-2‴), 47.6 (C-2), 46.8 (C-9-(Fmoc)), 27.6 (*t*Bu), 22.8, 20.6–20.3 (CH$_3$-(Ac)), 18.9 ppm (T$^\gamma$); HRMS (ESI) (pos): *m/z* calcd for $C_{99}H_{128}Cl_6N_4O_{53}^{2+}$: 1216.7790 $[M+2H]^{2+}$; found: 1216.7809.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-4-O-acetyl-2-deoxy-3-O-[2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl]-6-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranosyl)-L-threonine-tert-butylester (Fmoc-Thr(βAc$_4$Gal-(1→4)-βAc$_2$GlcNAc-(1→3)-βAc$_3$Gal-(1→3)-[βAc$_4$Gal-(1→4)-βAc$_2$-GlcNAc-(1→6)]-αAcGalNAc)-OtBu) (**16**)*: Zinc dust was activated by treatment with 1N hydrochloric acid followed by washing with water, methanol, and diethyl ether. Compound **15** (2.20 g, 0.90 mmol) was dissolved in acetic acid (30 mL) and then the pre-activated zinc (1.18 g, 18.0 mmol) was added. The reaction was stirred at 40°C for 36 h and then the solid was filtered off and washed with acetic acid. The filtrate was co-evaporated several times with toluene. The obtained residue was dissolved in pyridine/acetic anhydride (2:1, 30 mL) and *N,N*-dimethylaminopyridine (11 mg, 0.09 mmol) was added. The mixture was stirred for 18 h at room temperature. The solvent was removed by co-evaporation with toluene and the crude product was purified by silica column chromatography (EtOAc→EtOAc/MeOH 20:1) to give compound **16** (1.46 g, 75%, 0.68 mmol) as a colorless solid. R_f = 0.21 (EtOAc/MeOH 15:1); $[\alpha]_D^{20}$ = +21.05 (c = 0.51 in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30°C): δ = 7.91–7.89 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.81–7.78 (m, 2H; NH″-Ac, NH⁗-Ac), 7.76–7.72 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.52 (d, J(NH,H-2) = 9.7 Hz, 1H; NH-Ac), 7.45–7.41 (m, 2H; H-2-Fmoc, H-7-Fmoc), 7.40–7.38 (m, 1H; NH-Fmoc), 7.34–7.29 (m, 2H; H-3-Fmoc, H-6-Fmoc), 5.27 (d, ^3J(H4′,H3′) = 3.7 Hz, 1H; H-4′), 5.23–5.21 (m, 2H; H-4‴, H-4⁗), 5.19 (d, ^3J(H-4,H-3) = 1.9 Hz, 1H; H-4), 5.18–5.15 (m, 2H; H-3‴, H-3⁗), 5.00 (t, ^3J(H-3″,H-4″) = ^3J(H-3″,H-2″) = 9.3 Hz, 1H; H-3″), 4.91 (t, ^3J(H-3⁗,H-4⁗) = ^3J(H-3⁗,H-2⁗) = 9.3 Hz, 1H; H-3‴), 4.85–4.81 (m, 2H; H-2‴, H-2⁗), 4.73–4.67 (m, 4H; H-1″, H-1‴, H-1⁗, H-2′), 4.60 (d, ^3J(H-1,H-2) = 4.1 Hz 1H; H-1), 4.56 (d, ^3J(H-1′,H-2′) = 8.0 Hz, 1H; H-1′), 4.52–4.45 (m, 3H; H-1⁗, CH$_{2ab}$-(Fmoc)), 4.33–4.26 (m, 3H; H-6$^{\prime\prime}_a$, H-6$^{\prime\prime\prime\prime}_a$, H-9-(Fmoc)), 4.22–4.19 (m, 2H; H-5‴, H-5⁗), 4.15–4.11 (m, 2H; H-2, T$^\beta$), 4.08–4.05 (m, 1H; H-6$^{\prime\prime}_b$), 4.02–3.98 (m, 6H; H-5⁗, H-6$^{\prime\prime\prime}_{ab}$, H-6$^{\prime\prime\prime\prime}_{ab}$, T$^\alpha$), 3.94–3.89 (m, 3H; H-6′$_{ab}$, H-6$^{\prime\prime\prime\prime}_b$), 3.84 (t, ^3J(H-5′,H-6′) = 5.9 Hz, 1H; H-5′), 3.80–3.74 (m, 3H; H-3′, H-3⁗, H-6$_a$), 3.70–3.63 (m, 4H; H-2‴, H-4″, H-4⁗, H-5″), 3.56–3.54 (m, 1H; H-5⁗), 3.39 (q, ^3J(H-2″,H-1″) = ^3J(H-2″,H-3″) = ^3J(H-2″,NH) = 9.3 Hz, 1H; H-2″), 3.31–3.27 (m, 1H; H-6$_b$), 2.09–1.69 (m, 57H; CH$_3$-(Ac)), 1.34 (s, 1H; *t*Bu), 1.11 ppm (d, J(Tγ,Tβ) = 6.3 Hz, 3H; T$^\gamma$); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): δ = 170.3, 170.2, 170.0, 169.9, 169.6, 169.5, 169.4, 169.3, 169.1, 169.0, 168.9, 168.8 (C=O-(Ac), C=O-(*t*Bu)), 156.8 (C=O-(Fmoc)), 143.7 (C-1$_a$-Fmoc, C-5$_a$-Fmoc), 140.8 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.0 (C-3-Fmoc, C-6-Fmoc), 125.3, 125.1 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 100.5 (C-1′), 100.4 (C-1‴), 100.0, 99.9 (C-1″, C-1‴, C-1⁗), 98.4 (C-1), 81.4 (C$_{quart}$-(*t*Bu)), 76.8 (C-3′), 76.5 (C-4‴), 76.1 (C-4″), 73.8 (T$^\beta$), 73.4 (C-3, C-3⁗), 72.7 (C-3″), 71.7 (C-5″), 71.4 (C-5⁗), 70.2, 70.0 (C-3‴, C-3⁗, C-4, C-5′), 69.6 (C-5‴, C-5⁗), 69.5 (C-2′), 69.1 (C-6$_{ab}$), 69.0 (C-4′), 68.9 (C-2‴, C-2⁗), 68.6 (C-5), 67.0 (C-4‴, C-4⁗), 65.6 (CH$_{2ab}$-(Fmoc)), 62.2 (C-6$^{\prime\prime}_{ab}$), 61.6 (C-6′$_{ab}$, C-6$^{\prime\prime\prime\prime}_{ab}$), 60.8 (C-6$^{\prime\prime\prime}_{ab}$, C-6$^{\prime\prime\prime\prime}_{ab}$), 59.5 (T$^\alpha$), 54.0 (C-2″), 53.1 (C-2⁗), 47.7 (C-2), 46.8 (C-9-(Fmoc)), 27.6 (*t*Bu), 22.8, 22.7, 22.6, 20.6–20.3 (CH$_3$-(Ac)), 19.0 ppm (T$^\gamma$); HRMS (ESI) (pos): *m/z* calcd for $C_{97}H_{130}N_4O_{51}^{2+}$: 1083.8868 $[M+2H]^{2+}$; found: 1083.8872.

*N-9-Fluorenylmethyloxycarbonyl-O-(2-acetamido-2-deoxy-4-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranosyl]-6-O-[2-acetamido-3,6-O-acetyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-α-D-galactopyranosyl)-L-threonine (Fmoc-Thr(βAc$_4$Gal-(1→4)-βAc$_2$GlcNAc-(1→3)-βAc$_3$Gal-(1→3)-[βAc$_4$Gal-(1→4)-βAc$_2$-GlcNAc-(1→6)]-αAcGalNAc)-OH) (**17**)*: A solution of compound **16** (1.82 g, 0.84 mmol) in dichloromethane (5 mL), anisole (2 mL) and trifluoroacetic acid (15 mL) was stirred at room temperature for 3 h. The solvent was removed by co-evaporation with toluene. The residue was purified by silica column chromatography (EtOAc→EtOAc/MeOH/AcOH/H$_2$O 50:3:3:2) to give compound **17** (1.63 g, 92%, 0.77 mmol) as a colorless solid. R_f = 0.23 (EtOAc/MeOH/AcOH/H$_2$O 50:3:3:2); $[\alpha]_D^{20}$ = +27.60 (c = 1.00 in $CHCl_3$); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 30°C): δ = 12.87 (sbr, 1H; COOH), 7.91–7.89 (m, 2H; H-4-Fmoc, H-5-Fmoc), 7.83–7.79 (m, 2H; NH″-Ac, NH⁗-Ac), 7.77–7.73 (m, 2H; H-1-Fmoc, H-8-Fmoc), 7.45–7.41 (m, 3H; NH-Ac, H-2-Fmoc, H-7-Fmoc), 7.34–7.31 (m, 3H; NH-Fmoc, H-3-Fmoc, H-6-Fmoc), 5.27 (d, ^3J(H-4′,H-3′) = 3.5 Hz, 1H; H-4′), 5.23–5.22 (m, 2H; H-4‴, H-4⁗), 5.19 (d, ^3J(H-4,H-3) = 2.6 Hz, 1H; H-4), 5.18–5.15 (m, 2H; H-3‴, H-3⁗), 4.99 (t, ^3J(H-3″,H-2″) = ^3J(H-3″,H-4″) = 9.5 Hz, 1H; H-3″), 4.90 (t, ^3J(H-3⁗,H-2⁗) = ^3J(H-3⁗,H-4⁗) = 9.5 Hz, 1H; H-3⁗), 4.84–4.81 (m, 2H; H-2‴, H-2⁗), 4.72–4.67 (m, 4H; H-1″, H-1‴, H-1⁗, H-2′), 4.63 (d, ^3J(H-1,H-2) = 4.1 Hz, 1H; H-1), 4.55 (d, ^3J(H-1′,H-2′) = 7.9 Hz, 1H; H-1′), 4.51–4.47 (m, 3H; H-1⁗, CH$_{2a}$-(Fmoc)), 4.44 (m, 1H; CH$_{2b}$-(Fmoc)), 4.32–4.30 (m, 2H; H-6$^{\prime\prime\prime\prime}_a$, H-9-(Fmoc)), 4.26 (m, 1H; H-6$^{\prime\prime}_a$), 4.23–4.18 (m, 3H; H-5‴, H-5⁗, T$^\beta$), 4.11–4.03 (m, 3H; H-2, H-6$^{\prime\prime}_b$, T$^\beta$), 4.01–3.97 (m, 4H; H-6$^{\prime\prime\prime}_{ab}$, H-6$^{\prime\prime\prime\prime}_{ab}$), 3.94–3.83 (m, 5H; H-6′$_{ab}$, H-6$^{\prime\prime\prime\prime}_b$, H-5′), 3.80–3.76 (m, 2H; H-3, H-3′), 3.75–3.71 (m, 1H; H-6$_a$), 3.70–3.61 (m, 4H; H-2⁗, H-4″, H-4⁗, H-5″), 3.56–3.53 (m,1H; H-5⁗), 3.40–3.35 (m, 1H; H-2″), 3.31–3.27 (m, 1H; H-6$_b$), 2.09–1.69 (m, 57H; CH$_3$-(Ac)), 1.09 ppm (d, ^3J(Tγ,Tβ) = 6.4 Hz, 3H; T$^\gamma$); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO): δ = 170.3, 170.0, 169.9, 169.6, 169.5, 169.4, 169.1, 169.0, 168.8 (C=O-(Ac)), 156.8 (C=O-(Fmoc)), 143.8, 143.7 (C-1$_a$-Fmoc, C-8$_a$-Fmoc), 140.8 (C-4$_a$-Fmoc, C-5$_a$-Fmoc), 127.7 (C-2-Fmoc, C-7-Fmoc), 127.1 (C-3-Fmoc, C-6-Fmoc), 125.3, 125.2 (C-1-Fmoc, C-8-Fmoc), 120.2 (C-4-Fmoc, C-5-Fmoc), 100.6 (C-1′), 100.4 (C-1‴), 100.0, 99.9 (C-1″, C-1‴, C-1⁗), 98.5 (C-1⁗), 76.8 (C-3′), 76.6 (C-4⁗), 76.2 (C-4″), 74.1 (T$^\beta$), 73.5 (C-3), 73.4 (C-3⁗), 72.7 (C-3″), 71.7 (C-5″),

71.5 (C-5‴′), 70.3, 70.2, 70.1 (C-4, C-3‴, C-3‴″, C-5′), 69.6 (C-5‴, C-5‴″), 69.3 (C-2′), 69.0, 68.9 (C-4′, C-2‴, C-2‴″, C-6), 68.6 (C-5), 67.0 (C-4‴, C-4‴″), 65.6 (CH_{2ab}-(Fmoc)), 62.3 (C-6″$_{ab}$), 61.7 (C-6′, C-6‴$_{ab}$), 60.8 (C-6‴, C-6‴″$_{ab}$), 58.6 (T$^{\alpha}$), 54.0 (C-2″), 53.1 (C-2‴″), 47.8 (C-2), 46.8 (C-9-(Fmoc)), 22.8, 22.7, 22.6, 21.0–20.3 (CH_3-(Ac)), 18.70 ppm (T$^{\gamma}$); HRMS (ESI) (pos): *m/z* calcd for $C_{93}H_{122}N_4O_{51}^{2+}$: 1055.8555 $[M+2H]^{2+}$; found: 1055.8560; calcd for $C_{93}H_{121}KN_4O_{51}^{2+}$: 1074.8334 $[M+K+H]^{2+}$; found: 1074.8311.

General methods for the glycopeptide synthesis: The mucin tandem repeat peptides were synthesized by stepwise solid-phase peptide synthesis by using the Fmoc strategy, and starting with a preloaded Fmoc-proline or Fmoc-alanine trityl resin (13 μmol scale per peptide). The synthesis employed protected T- and core 3-threonine amino acid building blocks, which were pre-activated manually by using 1.5 equivalents that were activated with HATU/HOAt, whereas the other Fmoc-protected amino acids were coupled automatically on a Multisyntech peptide synthesizer by using eight equivalents of the amino acid and HBTU/HOBt. Fmoc deprotection was performed according to standard conditions, 20% piperidine in DMF. After assembly of the peptide backbone, a triethylene glycol amino acid spacer was coupled to the N terminus followed by Fmoc deprotection. The peptides were then released from the resin, and all acid-sensitive side chain protecting groups were simultaneously removed by treatment with TFA/TIPS/H_2O (90:5:5) followed by solvent concentration, lyophilization, and purification by using a C-18 cartridge (1 g of C-18 material, Waters). For saccharide deprotection the *O*-acetyl groups were cleaved by transesterification in methanol by using catalytic amounts of NaOMe at pH 9–9.5 (defined by using wet pH paper) for 4–36 h (deprotection followed by analytical HPLC) to yield peptides **39–66**, which were purified by preparative HPLC (Phenomenex Luna C18 (2), 21.20×250 mm). Additional experimental and characterization data for the glycopeptides **39–66** are given in the Supporting Information.

Acknowledgements

This work was supported by ISAS—Leibniz institute for Analytical Sciences, Fonds der Chemischen Industriegrant number Li 184/01 (Liebig Fellowship to U.W.) and Deutsche Forschungsgemeinschaftgrant number WE 4751/2-1. We are grateful to Prof. Dr. Herbert Waldmann (Max-Planck Institute of Molecular Physiology, Dortmund, Germany) for his unconditional support.

[1] a) E. P. Lillehoj, K. C. Kim, *Arch. Pharmacal Res.* **2002**, *25*, 770–780; b) M. C. Rose, J. A. Voynow, *Phys. Rev.* **2006**, *86*, 245–278; c) C. M. Evans, J. S. Koo, *Pharm. Therap.* **2009**, *121*, 332–348.

[2] a) I. Carlstedt, J. R. Davies, *Biochem. Soc. Trans.* **1997**, *25*, 214–219; b) M. Fukuda, S. Tsuboi, *Biochim. Biophys. Acta Molecular Basis of Disease* **1999**, *1455*, 205–217; c) N. Sharon, *Biochim. Biophys. Acta Gen. Subj.* **2006**, *1760*, 527–537; d) R. Ramphal, S. K. Arora, *Glycoconjugate J.* **2001**, *18*, 709–713; e) A. Walz, S. Odenbreit, J. Mahdavi, T. Boren, S. Ruhl, *Glycobiology* **2005**, *15*, 700–708.

[3] a) K. L. Carraway, S. Hull, *Glycobiology* **1991**, *1*, 131–138; b) S. Muller, S. Goletz, N. Packer, A. Gooley, A. M. Lawson, F.-G. Hanisch, *J. Biol. Chem.* **1997**, *272*, 24780–24793; c) H. Clausen, E. P. Bennett, *Glycobiology* **1996**, *6*, 635–646.

[4] a) P. Van den Steen, P. M. Rudd, R. A. Dwek, G. Opendenakker, *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 151–208; b) E. F. Hounsell, M. J. Davies, D. V. Renouf, *Glycoconjugate J.* **1996**, *13*, 19–26.

[5] a) F.-G. Hanisch, *Biol. Chem.* **2001**, *382*, 143–149; b) G. Lamblin, S. Degroote, J. M. Perini, P. Delmotte, A. Scharfman, M. Davril, J. M. Lo-Guidice, N. Houdret, V. Dumur, A. Klein, P. Rousse, *Glycoconjugate J.* **2001**, *18*, 661–684.

[6] a) H. Paulsen, J. P. Hoelck, *Carbohydr. Res.* **1982**, *109*, 89–107; b) H. Kunz, S. Birnbach, *Angew. Chem.* **1986**, *98*, 354–355; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 360–362; c) B. Liebe, H. Kunz, *Angew. Chem.* **1997**, *109*, 629–631; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 618–621; d) C. Brocke, H. Kunz, *Synthesis* **2004**, 525–542; e) S. Dziadek, H. Kunz, *Synlett* **2003**, 1623–1626; f) M. Elofsson, L. A. Salvador, J. Kihlberg, *Tetrahedron* **1997**, *53*, 369–390; g) E. Meinjohanns, M. Meldal, A. Schleyer, H. Paulsen, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1996**, 985–993; h) B. Luning, T. Norberg, J. Tejbrant, *Glycoconjugate J.* **1989**, *6*, 5–19; i) J. Geiger, B. G. Reddy, G. A. Winterfeld, R. Weber, M. Przybylski, R. R. Schmidt, *J. Org. Chem.* **2007**, *72*, 4367–4377; j) P. W. Glunz, S. Hintermann, L. J. Williams, J. B. Schwarz, S. D. Kuduk, V. Kudryashov, K. O. Lloyd, S. J. Danishefsky, *J. Am. Chem. Soc.* **2000**, *122*, 7273–7279; k) Y. Nakahara, C. Ozawa, E. Tanaka, K. Ohtsuka, Y. Takano, H. Hojo, Y. Nakahara, *Tetrahedron* **2007**, *63*, 2161–2169; l) M. Pratt, C. R. Bertozzi, *Org. Lett.* **2004**, *6*, 2345–2348; m) K. Baumann, D. Kowalczyk, H. Kunz, *Angew. Chem.* **2008**, *120*, 3494–3498; *Angew. Chem. Int. Ed.* **2008**, *47*, 3445–3449; n) K. Baumann, D. Kowalczyk, T. Gutjahr, M. Pieczyk, C. Jones, M. Wild, D. Vestweber, H. Kunz, *Angew. Chem.* **2009**, *121*, 3220–3224; *Angew. Chem. Int. Ed.* **2009**, *48*, 3174–3178; o) O. Seitz, C.-H. Wong, *J. Am. Chem. Soc.* **1997**, *119*, 8766–8776; p) K. M. Koeller, M. E. B. Smith, C.-H. Wong, *J. Am. Chem. Soc.* **2000**, *122*, 742–743; q) Y. Vohra, T. Buskas, G. J. Boons, *J. Org. Chem.* **2009**, *74*, 6064–6071; r) T. Matsushita, H. Hinou, M. Fumoto, M. Kurogochi, N. Fujitani, H. Shimizu, S.-I. Nishimura, *J. Org. Chem.* **2006**, *71*, 3051–3063.

[7] a) M. Pudelko, J. Bull, H. Kunz, *ChemBioChem* **2010**, *11*, 904–930; b) N. Gaidzik, U. Westerlind, H. Kunz, *Chem. Rev. Soc.* **2013**, *42*, 4421–4442.

[8] U. Westerlind, H. Schröder, A. Hobel, N. Gaidzik, A. Kaiser, C. M. Niemeyer, E. Schmitt, H. Waldmann, H. Kunz, *Angew. Chem.* **2009**, *121*, 8413–8417; *Angew. Chem. Int. Ed.* **2009**, *48*, 8263–8267.

[9] a) P. Sjölin, M. Elofsson, *J. Org. Chem.* **1996**, *61*, 560–565; b) K. B. Reimer, M. Meldal, S. Kusumoto, K. Fukase, K. Bock, *J. Chem. Soc. Perkin Trans. 1* **1993**, 925–932; c) P. Sjölin, S. K. George, K. E. Bergquist, R. Sarbari, A. Svensson, J. Kihlberg, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1731–1742.

[10] a) H. Kunz, C. Unverzagt, *Angew. Chem.* **1988**, *100*, 1763–1765; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1697–1699; b) P. Urge, D. C. Jackson, L. Gorbics, K. Wroblewski, G. Gracyk, L. Otvos, *Tetrahedron* **1994**, *50*, 2373–2390.

[11] a) S. Kusumoto, H. Yoshimura, M. Imoto, T. Shirnamoto, T. Shiba, *Tetrahedron Lett.* **1985**, *26*, 909–912; b) U. Ellervik, G. Magnusson, *Carbohydr. Res.* **1996**, *280*, 251–260; c) Z. Zhang, I. R. Ollman, X. S. Ye, R. Wischnat, T. Basov, C. H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734–753; d) M. Schultz, H. Kunz, *Tetrahedron: Asymmetry* **1993**, *4*, 1205–1220.

[12] F. Yan, S. Mehta, E. Eichler, W. W. Wakarchuck, M. Gilbert, M. J. Schur, D. M. Whitfield, *J. Org. Chem.* **2003**, *68*, 2426–2431.

[13] R. R. Schmidt, M. Stumpp, *Liebigs Ann. Chem.* **1983**, 1249.

[14] R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213—236; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212–235.

[15] S. Dziadek, C. Brocke, H. Kunz, *Chem. Eur. J.* **2004**, *10*, 4150–4162.

[16] S. Nambiar, J. F. Daeuble, R. J. Doyle, K. G. Taylor, *Tetrahedron Lett.* **1989**, *30*, 2179–2182.

[17] a) P. Konradsson, U. E. Udodong, B. Fraser-Reid, *Tetrahedron Lett.* **1990**, *31*, 4313–4316; b) G. H. Veeneman, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 275–278.

[18] G. Hancock, I. J. Galpin, *Tetrahedron Lett.* **1982**, *23*, 249–252.

[19] X. Fan, X. Zhang, Y. Zhang, *J. Chem. Res.* **2005**, 21–22.

[20] T. P. Ananthanarayan, T. Gallagher, P. Magnus, *J. Chem. Soc. Chem. Commun.* **1982**, 709–710.

[21] V. Dourtoglou, B. Gross, V. Lambropoulou, C. Zioudrou, *Synthesis* **1984**, 572–574.

[22] a) L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398; b) L. Carpino, A. El-Faham, C. A. Minor, F. Albericio, *J. Chem. Soc. Chem. Commun.* **1994**, 201–203.

Received: July 24, 2013
Published online: November 4, 2013

