The (2-Phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-Linker in the Synthesis of Glycopeptide Partial Structures of Complex Cell Surface Glycoproteins

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Dedicated to Professor Reinhard Hoffmann on the occasion of his 70th birthday

Abstract: The (2-phenyl-2-trimethylsilyl)ethyl-(PTMSEL) linker represents a novel fluoride-sensitive anchor for the solid-phase synthesis of protected peptides and glycopeptides. Its cleavage is achieved under almost neutral conditions using tetrabutylammonium fluoride trihydrate in dichloromethane thus allowing the construction of complex molecules sensitive to basic and acidic media commonly required for the cleavage of standard linker systems. The advantages of the PTMSEL linker are demonstrated in the synthesis of glycopeptides from the liver intestine (LI)-cadherin and the mucin MUC1, bearing carbohydrate moieties such as N-linked chitobiose or O-linked sialyl-T_N-residues. The synthesis of these types of glycopeptides is difficult because they are prone to secondary structure formation during the synthesis on the solid phase as well as in the completely deprotected form. Using the PTMSEL linker these molecules are accessible by automated synthesis according to the Fmoc strategy without frequently observed side reactions such as aspartimide or diketopiperazine formation.

Keywords: cadherins · glycopeptides · mucins · silicon-based linkers · solid-phase synthesis

Introduction

Due to posttranslational modifications, proteins and peptides exist in a variety of conjugated forms, most importantly as glycoconjugates. The N- and O-glycosidically bound carbohydrates affect the conformation of proteins and regulate their activity and biological half-lives. Glycoproteins and glycopeptides, for example cadherins or mucins, play fundamental roles in many biological processes, such as cell adhesion, regulation of cell growth and cell differentiation.[1] The synthesis of exactly specified partial structures of these glycoproteins provides a feasible approach to gain more information on structural and biological aspects of those regulatory processes and to detect or possibly influence pathological deficiencies.

Such peptides and glycopeptides can efficiently be synthesized applying solid-phase methodologies.[2] In all variations of solid-phase and combinatorial syntheses the linker is of importance. It must be stable throughout the multistep synthesis, but finally cleavable under mild conditions without affecting the produced compounds.[3] This especially holds true for the synthesis of protected glycopeptides, which shall be used in fragment condensations. Most of the common anchors are either acid- or base-labile. Many acid-labile anchors are based on substituted benzyl- or triphenylmethyl esters.[3,4] Their cleavage forms stable cations, which may cause undesired alkylation of nucleophilic moieties.[4] Furthermore, acid-labile protecting groups such as Boc, *tert*-butyl or trityl residues, for example in the amino acid side chains, are removed simultaneously. Base-labile linkages often are not compatible with the fluorenylmethoxycarbonyl (Fmoc)-strategy.[5] Under acidic and basic cleavage conditions undesired side reactions can take place on the produced peptides. Among those, aspartimide formation and rearrangements of aspartyl peptides are difficult to prevent.[6] Most of these side reactions can be avoided by using linker systems, which are cleavable under neutral conditions such as allylic anchors.[7] However, because of their insufficient steric hindrance, the allyl ester linkers are susceptible to aminolysis. Accordingly, they are prone to diketopiperazine formation[3f,4] at the stage of the polymer-bound dipeptides.

Recently, the (2-phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-linker **1** was introduced as a fluoride-labile linker for the solid-phase synthesis of protected peptides and glycopeptides according to Fmoc chemistry.[8] Cleavage of the PTMSEL-linker is achieved under almost neutral conditions by treatment with tetrabutylammonium fluoride trihydrate (TBAF·3 H_2O) in dichloromethane. Under these

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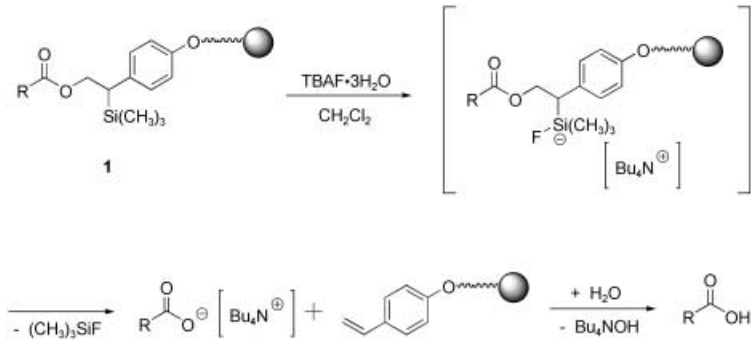
conditions, the hydrate shells of fluoride ions are unaffected, keeping the basicity at a low level. The increased sensitivity towards fluoridolysis is due to the benzylic position of the C–Si bond (Scheme 1).



Scheme 1. Mechanism of fluoride induced cleavage of the PTMSEL-linker.

Other fluoride-labile anchors, such as *p*-silylmethylbenzyl, *p*-silyloxy-benzyl and α-trimethylsilyl-benzyl (SAC) linkers,[9] require much more basic cleavage conditions, for example TBAF·3H_2O in dimethylformamide or tetrahydrofuran; these conditions are not compatible for retaining the Fmoc group. Therefore, the PTMSEL linker shows several advantageous properties compared with linkers described earlier: Common protecting groups (e.g. Fmoc, Boc, Z, Aloc, *tert*-butyl, benzyl, allyl, trityl)[10] are stable under the mild cleavage conditions of the PTMSEL linker and provide the possibility of orthogonal, three dimensional protecting group strategies. Early results showed that serious side reactions, such as aspartimide formations, are decisively suppressed. Furthermore, the PTMSEL linker is sterically so demanding, that even in sequences prone to diketopiperazine formation (Pro-Gly) this side reaction did not occur.[8]

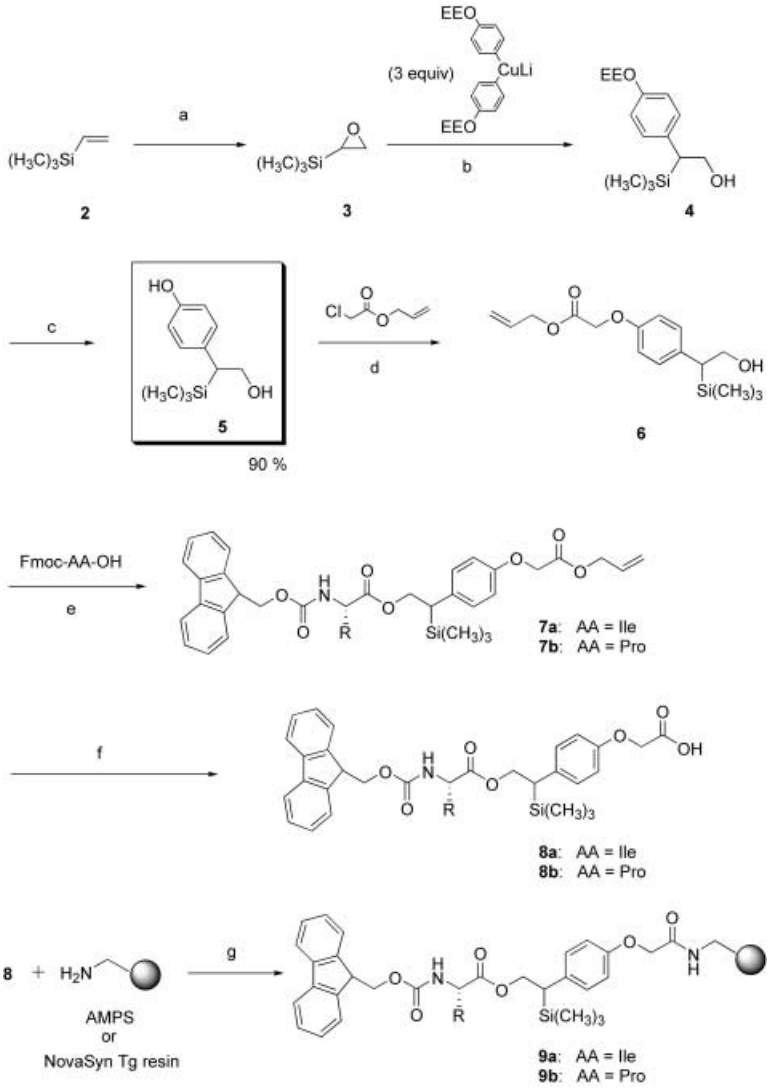
Abstract in German: *Der (2-Phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-Linker ist ein neuer fluorid-sensitiver Anker für die Festphasen-Synthese geschützter Peptide und Glycopeptide. Die Spaltung unter nahezu neutralen Bedingungen bei Verwendung von Tetrabutylammoniumfluoridtrihydrat in Dichlormethan ermöglicht den Aufbau komplexer Moleküle, die empfindlich gegenüber bei den Spaltungen gängiger Ankersysteme benötigten Basen bzw. Säuren sind. Die Vorteile des PTMSEL-Linkers werden gezeigt an Synthesen von Glycopeptiden aus dem leber-intestinalen (LI) Cadherin und aus dem Mucin MUC1, die Kohlenhydratstrukturen wie N-gebundene Chitobiose oder O-verknüpftes Sialyl-T_N-Antigen tragen. Aufgrund der Neigung solcher zur Ausbildung sekundärer Strukturen sowohl während der Synthese an fester Phase als auch in ungeschützter Form in Lösung, ist die Synthese solcher Glycopeptidstrukturen schwierig. Bei Verwendung des PTMSEL-Ankers können derartige Moleküle in automatisierten Synthesen nach der Fmoc-Strategie hergestellt werden, ohne dass es zu Nebenreaktion wie der häufig zu beobachtenden Aspartimid- oder Diketopiperazinbildung kommt.*

Results and Discussion

Taking advantage of its useful properties the (2-phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-linker was employed in syntheses of complex glycopeptides. On the one hand, glycopeptides from the LI-cadherin[11] prone to formation of secondary structures were synthesized. On the other hand, glycopeptide sequences of the tandem repeat region of the mucin[12] MUC1 bearing demanding carbohydrates were constructed.

Synthesis of the PTMSEL-linker-system: Trimethylvinylsilane **2** was treated with *m*-chloroperbenzoic acid to give trimethylsilyloxirane **3**.[13] Reaction of **3** with lithium di[4-(1-ethoxyethyloxy)phenyl]cuprate, generated in situ by lithiiation of 1-(4-bromophenoxy)-1-ethoxyethane with *n*-butyllithium and subsequent reaction with copper(I)iodide, efficiently yielded 2-[4-(1-ethoxyethyloxy)phenyl]-2-trimethylsilyl ethanol (**4**) in 85% yield.[8,14] The 1-ethoxy-ethyl-(EE)-protecting group was removed using catalytic amounts of pyridinium-*p*-toluenesulfonate (PPTS).[15] The PTMSEL linker unit, 2-(4-hydroxyphenyl)-2-trimethylsilyl-ethanol (**5**), was obtained in a yield of 90% (76% from **3**, Scheme 2).

Synthesis of anchor conjugates and resin loading: 2-(4-Hydroxyphenyl)-2-trimethylsilyl-ethanol (**5**) was linked to the polymeric support via its phenolic hydroxyl group (Scheme 2). Prior to this, the N-acyl amino acid was coupled to the linker molecule in solution since esterification on solid-phase is often accompanied by racemization. According to this strategy, 2-(4-hydroxyphenyl)-2-trimethylsilyl-ethanol (**5**) was treated with allyl chloroacetate to give allyl 4-[2-hydroxy-1-(trimethylsilyl)ethyl]phenoxy acetate (**6**). Acylation with the corresponding Fmoc-protected amino acid using dicyclohexylcarbodiimide (DCC) and catalytic amounts of 4-dimethylaminopyridine (DMAP)[16] gave compounds **7a** and **7b** (yields: >90%) protected as allyl esters. The allyl residue of **7a**/**b** was removed quantitatively using catalytic [$Pd(PPh_3)_4$] and sodium *p*-toluenesulfinate or other allyl trapping reagents furnishing acids **8a** and **8b**, respectively (yield ≈54% from **3**).[17] Acids **8a** and **8b** can be condensed with an amino-functionalized resin using *O*-(benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)/*N*-hydroxybenzotriazole (HOBt)/diisopropylethylamine (DIPEA) in DMF/CH_2Cl_2.[18] Aminomethyl polystyrene, AMPS (ACT; 200–400 mesh; loading: 1.00 mmol g^{-1}), or amino-functionalized TentaGel, NovaSyn Tg amino resin (Novabiochem, 110 μm beads, loading:



Scheme 2. Synthesis of the PTMSEL-linker and loading of the resin. a) *m*CPBA (1.0 equiv), $CHCl_3$, RT, 60 h, 74%; b) lithium [di(*p*-1-ethoxyethyloxy)-phenyl]cuprate (3.0 equiv), diethyl ether, −50°C, 5 h → −20°C, 14 h, 85%; c) PPTS (0.03 equiv), MeOH, RT, 2 h, 90%; d) allyl chloroacetate (1.9 equiv), K_2CO_3, KI, acetone, RT, 38 h, 78%; e) Fmoc-AA-OH (1.1 equiv), DCC (1.2 equiv), DMAP (0.06 equiv), CH_2Cl_2, 0°C, 4 h, > 90%; f) $[Pd(PPh_3)_4]$ (0.06 equiv), *p*-toluenesulfinate (1.3 equiv), THF/MeOH, RT, 90 min, quant.; g) TBTU (1.0 equiv), HOBt (1.0 equiv), NMM (2.0 equiv), CH_2Cl_2/DMF, RT, 18 h.

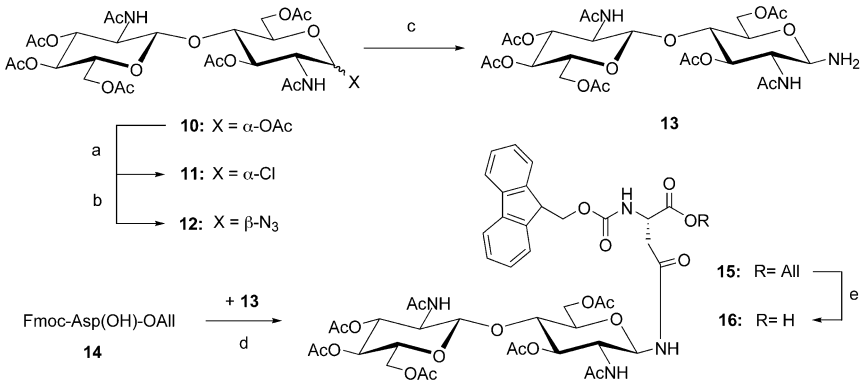
0.43 mmol g^{-1}) were used as polymeric supports. Unreacted amino groups were capped with acetic anhydride/pyridine 1:3 (Scheme 2).

Synthesis of an N-glycododecapeptide from human LI-cadherin: Cadherins[19] represent a class of important cell-adhesion proteins. LI-cadherin is a structurally distinct, intestine specific member of the cadherine gene superfamily. Recent data suggest, that down-regulation of cadherin expression parallels cellular dysregulations in human diseases.[19] Therefore, LI-cadherin mediated cell–cell adhesion could play an important role in maintaining physiological cellular functions in the intestinal mucosa.[20] It is assumed that LI-cadherin mediated homophilic binding proceeds exclusively via a specific binding region in the extracellular domain EC1. Therefore, synthetic partial structures of this homophilic binding region are considered useful tools for the elucidation of the mechanisms of LI-cadherin mediated cell-cell adhesion.

Synthesis of the glycosylated building block: Chitobiosyloctaacetate **10**[21] treated with a saturated solution of hydrogen chloride in acetyl chloride gave glycosyl chloride **11**. Subsequent reaction with sodium azide in the presence of a phase transfer catalyst rendered chitobiosyl azide **12**.[22] Hydrogenation over Raney nickel in dioxane/ethanol (4:1) efficiently yielded the chitobiosyl amine **13**, which was condensed via its amino group with Fmoc-Asp(OH)-OAll (**14**) using TBTU,[18] HOBt and DIPEA as coupling reagents to give the completely protected, N-glycosyl asparagine derivative **15** in 76% yield. The allyl ester of **15** was cleaved by palladium(0) catalyzed allyl transfer to sodium *p*-toluenesulfinate[17] to form the Fmoc-protected *N*-asparagine building block **16** ready for use in the solid-phase synthesis (Scheme 3).

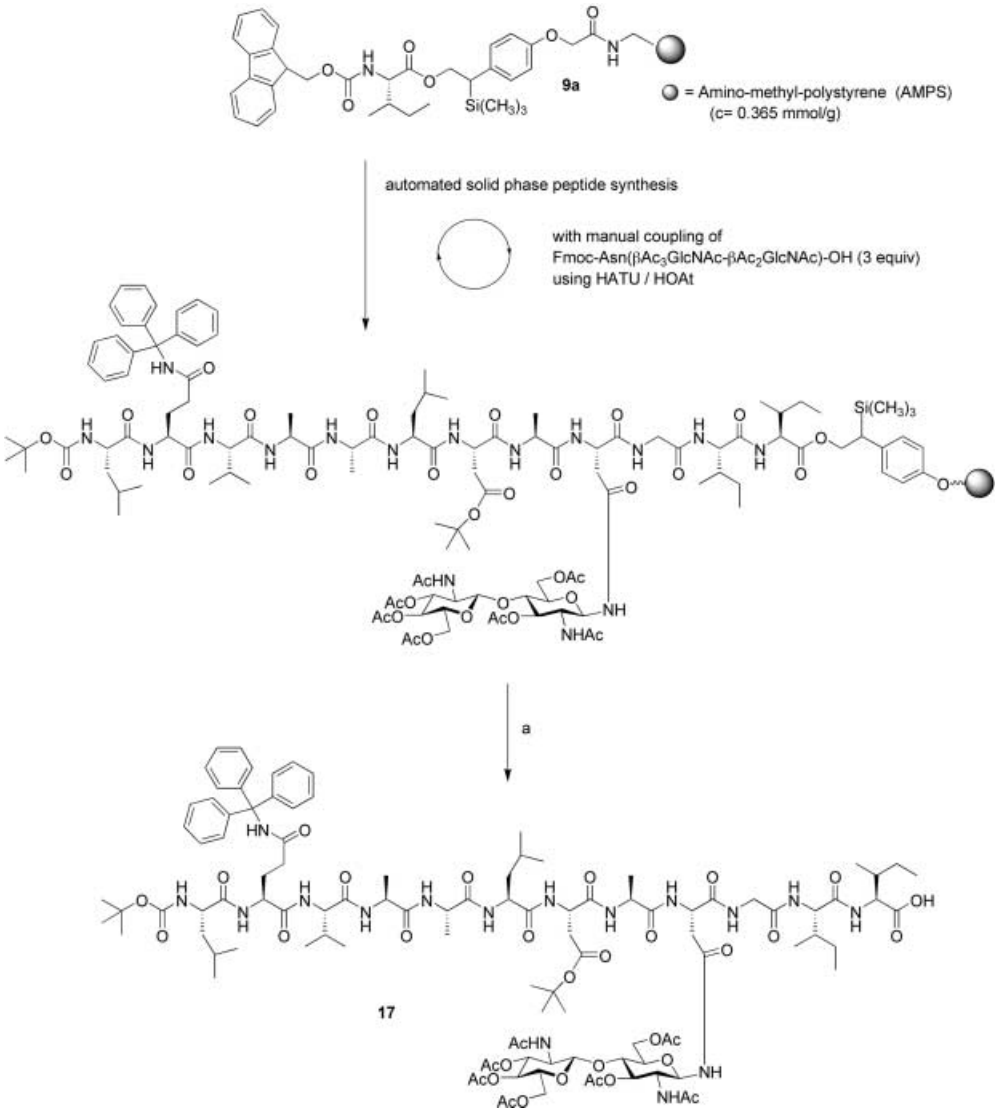
Solid-phase synthesis: Starting from polymer **9a** loaded with Fmoc-Ile-OH (loading: 0.365 mmol g^{-1}), glycododecapeptide Boc-Leu-Gln(Trt)-Val-Ala-Ala-Leu-Asp(O*t*Bu)-Ala-Asn(βAc_3GlcNAc-βAc_3GlcNAc)-Gly-Ile-Ile-OH (**17**) was assembled using a peptide synthesizer applying Fmoc strategy (Fmoc deprotection: 30% piperidine in *N*-methylpyrrolidone; coupling reagents: HBTU,[23] HOBt, DIPEA) (Scheme 4). The glycosylated building block Fmoc-Asn(βAc_3GlcNAc-βAc_3GlcNAc)-OH (**16**) (3 equiv) was coupled manually using *O*-(7-aza-benzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate (HATU)[24]/*N*-hydroxy-7-azabenzotriazole (HOAt). After completion of the automated peptide synthesis, the glycopeptide was released from the solid support by two sequential treatments with two equivalents of tetrabutylammonium fluoride trihydrate (TBAF·3H_2O) in dichloromethane.

An aliquot was taken from the resin and analyzed by HR-MAS spectroscopy. The absence of the characteristic

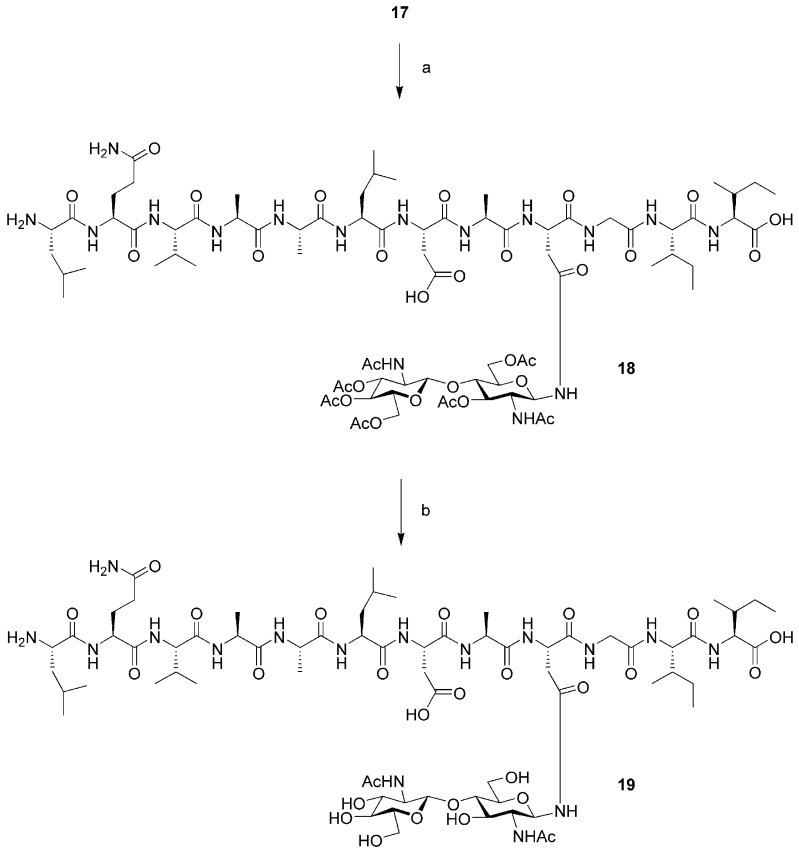


Scheme 3. Synthesis of chitobiosyl asparagine building block **16**. a) sat. HCl in acetyl chloride, RT, 48 h, 82%; b) NaN_3 (3.1 equiv), aliquat 336 (1.0 equiv), $CHCl_3/H_2O$, RT, 42 h, 70%; c) H_2, raney-nickel, dioxane/EtOH, RT, 3 h, quant.; d) TBTU (1.0 equiv), HOBt (1.0 equiv), DIPEA (2.0 equiv), DMF, RT, 40 h, 76%; e) $[Pd(PPh_3)_4]$ (0.06 equiv), sodium-*p*-toluenesulfinate (1.3 equiv), THF/MeOH, RT, 70 min, 57%.

signal corresponding to the trimethylsilyl group gave evidence for complete release of the glycopeptide from the resin. HPLC analysis of the crude material obtained from the described cleavage procedure showed high purity with almost no side products except for deletion sequences. In particular, formation of an aspartimide (the sequence Asp-Ala is well known to undergo this side reaction)[6b] was not observed. Final purification by preparative HPLC gave the protected glycopeptide **17** in 29% yield (based on the loading of polymer **9a**). The moderate yield is mostly due to secondary structure formation during the synthesis on the resin as was shown by 2D NMR spectroscopy (NOESY and ROESY experiments) of protected peptide **17**. In addition, the poor solubility of protected glycopeptide **17** in all common solvents such as dichloromethane, acetoni-



Scheme 4. Solid-phase synthesis of glycododecapeptide **17** from LI-cadherin (human). a) 2 × TBAF·3 H_2O (2 equiv), CH_2Cl_2, RT, 25 min, 29% (based on **9a**).

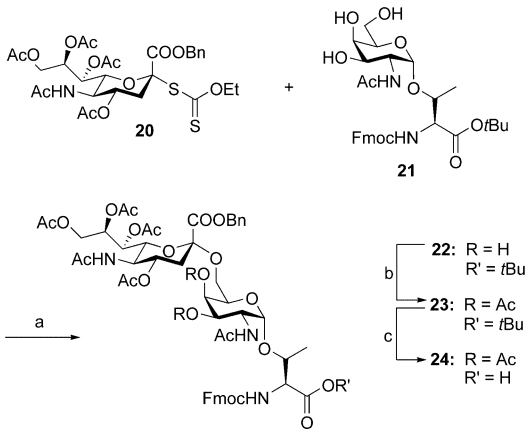


Scheme 5. Removal of protecting groups from **17**. a) TFA/TIS/H_2O (38:1:1), RT, 4 h, 97%; b) NaOMe, MeOH, pH 10, RT, 7 h, 79%.

trile and water has to be taken into account to explain the yield. The acid labile protecting groups of **17** were removed by treatment with a mixture of trifluoroacetic acid, triisopropylsilane and water (Scheme 5). In order to remove the O-acetyl protecting groups from the chitobiosyl unit, glycopeptide **18** was treated with sodium methanolate in methanol at pH 10 and efficiently yielded the completely deprotected glycopeptide **19** in 79% yield (22% yield based on the preloaded resin **9a**). Glycopeptide **19** was analyzed by 2D NMR spectroscopy, which showed clear evidence of a β-turn conformation for Asp, Ala, Asn(chitobiosyl) and Gly. The biological investigation of compound **19** and other peptides and glycopeptides from this series are ongoing and will be reported elesewhere.

Synthesis of a biotinylated O-glycododecapeptide from MUC1: The mucin MUC1[25] is a high molecular weight integral membrane glycoprotein located on the lumenal surface of ubiquitously distributed epithelial cells including those of the mammary gland ducts, the bladder, and the respiratory tract. The extracellular domain primarily consists of 20-amino acid tandem repeats. The main function of mucins is to provide lubrication and protection of epithelial cell surfaces. In contrast to healthy cells, the expression of MUC1 in epithelial tumors is dramatically increased. It is combined with an incomplete glycosylation pattern occurring due to a premature sialylation and resulting in additional exposed peptide epitopes of the protein backbone. These tumor-associated structure alterations constitute a promising basis for a selective immunological attack at certain epithelial cancer cells. Over the years, several T_N-, T- and sialyl-T_N-antigen-containing glycopeptides have been synthesized as potential vaccines, some of them showing interesting biological effects.[2,26] The biotinylated sialyl-T_N-glycododecapeptide described herein, which is derived from the tandem repeat unit of MUC1, is a promising candidate for the construction of tumor-selective immunostimulating antigens for the development of anticancer vaccines. The biotinylation facilitates immunological evaluation procedures. This glycoconjugate is also considered a challenging target molecule for a solid-phase synthesis using the PTMSEL-linker to demonstrate the beneficial properties of this novel technology even in the case of sensitive target molecules.

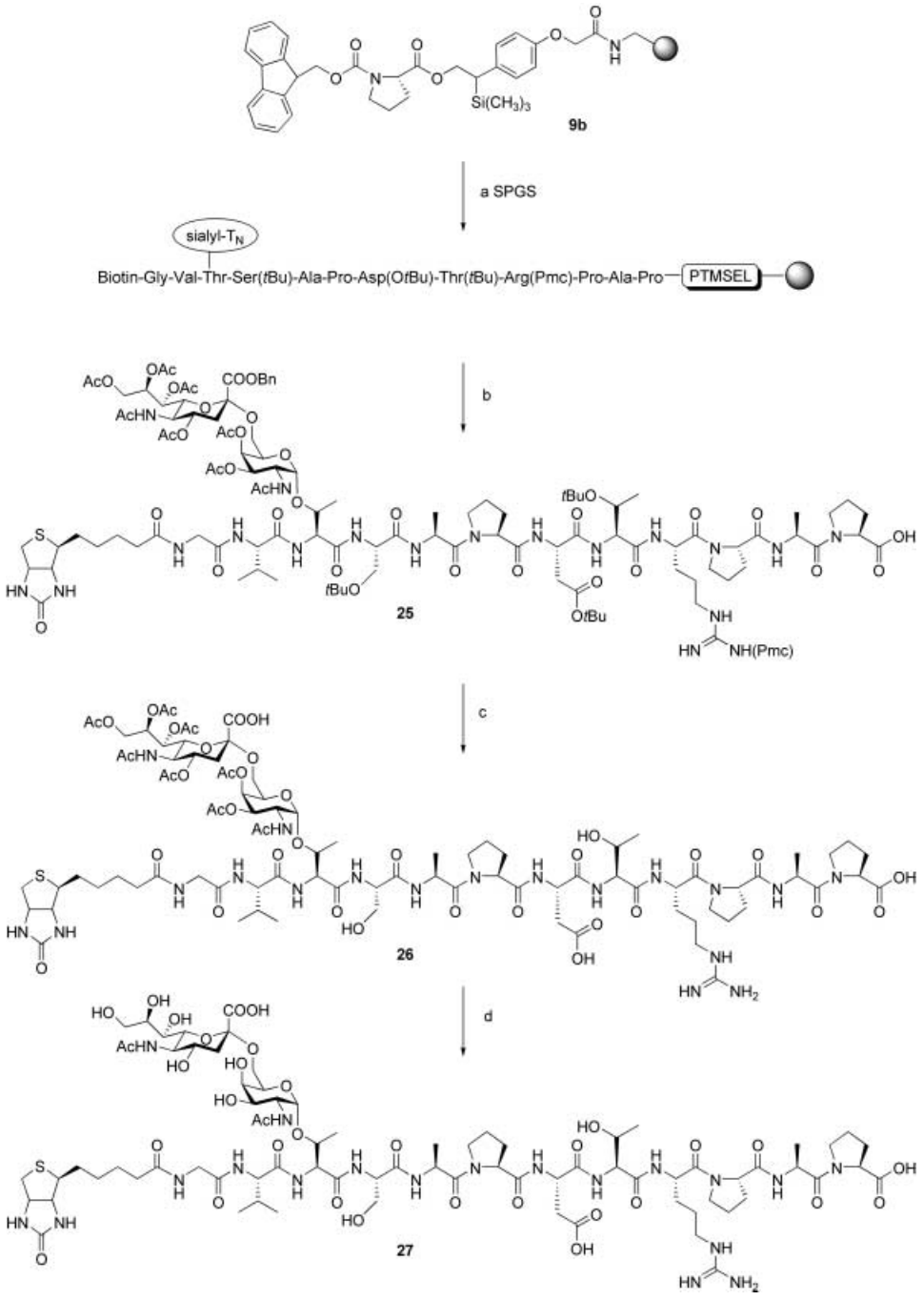
Synthesis of the sialyl-T_N-threonine building block: The pivotal step in the preparation of the Fmoc-protected sialyl-T_N threonine building block consists of the regio- and stereoselective sialylation of Fmoc-(GalNAc)-O*t*Bu (**21**)[27] at the reactive primary 6-hydroxy group.[28] (Scheme 6) This sialylation was accomplished employing benzyl ester protected sialyl xanthate **20**[28,29] activated by methyl sulfenyl triflate[30] (generated in situ from methyl sulfenyl bromide



Scheme 6. Synthesis of sialyl-T_N-threonine building block. a) methylsulfenyl bromide (1.6 M solution in 1,2-dichloroethane), CH_3CN/CH_2Cl_2 (2:1), −62 °C, 4 h, 57%; b) pyridine/Ac_2O (2:1), 4 h, 0 °C, 15 h, RT, 76%; c) CH_2Cl_2/TFA/anisole (10:10:1), 97%.

and silver triflate). Low temperature (−62 °C) and the use of acetonitrile/dichloromethane (2:1) favored the formation of the α-sialoside **22** in a kinetically controlled reaction. The desired α-sialyl-T_N conjugate **22** was obtained in a yield of 57 % (Scheme 6). Apart from the β-anomer (α/β 10:1) the glycal of the sialic acid benzyl ester was formed as the only by-product. Subsequent O-acetylation (**23**, 76 %) with acetic anhydride in pyridine and removal of the *tert*-butyl ester protecting group using a mixture of trifluoroacetic acid and anisole yielded the sialyl-T_N-threonine building block **24** (97 %) which was incorporated into the solid-phase synthesis of the sialyl-T_N-glycododecapeptide.

Solid-phase synthesis: Using Fmoc-Pro-PTMSEL preloaded NovaSyn Tg resin **9b** (loading: 0.228 mmol g^{-1}) the glycododecapeptide biotin-Gly-Val-Thr(α-Ac_4NeuNAcCOOBn-(2→6)-α-Ac_2GalNAc)-Ser(*t*Bu)-Ala-Pro-Asp(O*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Pro-Ala-Pro-OH (**25**) was prepared in an automated synthesis in analogy to the methodology described above. The sialyl-T_N-threonine conjugate **24** was introduced by a double coupling (2 × 1.3 equiv) using HATU,[24] HOAt and *N*-methylmorpholine (NMM) (Scheme 7). After N-terminal deprotection of the resin-linked glycopeptide, carboxy activated biotin (HBTU/HOBt, NMM) was attached in two consecutive coupling steps. The glycoconjugate was liberated from the resin by treatment with TBAF·3 H_2O in dichloromethane to furnish the selectively deblocked derivative **25** in a yield of 42 % after preparative HPLC (based on the preloaded resin **9b**). Only traces of side products were observed, and no aspartimide formation and rearrangements could be detected.



Scheme 7. Solid-phase synthesis of biotinylated sialyl-T_N-glycododecapeptide **27** from MUC1. a) iterative cycles: deprotection, coupling, capping; b) TBAF·3 H_2O, CH_2Cl_2, RT, 30 min, 42 % (with respect to **9b**); c) 1. Pd/C (10 %), MeOH, RT, 48 h; 2. CH_2Cl_2/TFA/thioanisole/1,2-ethanedithiol (10:10:1:1), RT, 2 h; d) NaOMe/MeOH, pH 8.5–9, RT, 15 h, 20 % (with respect to **25**).

The overall yield of 42 % is high considering not only the introduction of a complex glycosylated amino acid and an N-terminal biotin residue, but also that the Pro-Ala-Pro starting sequence[4] is very prone to diketopiperazine formation. The use of a sterically less hindered resin (allyl ester type) under identical conditions led to a high degree of diketopiperazine formation with almost no peptide remaining on the resin. The sialic acid benzyl ester of protected glycopeptide **25** was removed by hydrogenolysis using palladium on activated charcoal (10 %). Careful cleavage of the acid-labile side chain protecting groups by treatment with a mixture of trifluoroacetic acid, thioanisole and 1,2-ethanedithiol and subsequent mild methanolysis of the O-acetyl groups furnished the biotinylated MUC1 glycododecapeptide **27** in a yield of 20 % over three steps. Purification was accom-

plished by means of preparative HPLC. Currently, the glycoconjugate **27** is investigated in immunological experiments towards the induction of antibodies and the development of anticancer-vaccines based on tumor-associated glycopeptide antigens.

Conclusion

The solid-phase syntheses of complex glycopeptides from the LI-cadherin and mucin series have been accomplished using the novel (2-phenyl-2-trimethylsilyl)ethyl-(PTMSEL)-linker. Its cleavage under almost neutral conditions by tetrabutylammonium fluoride trihydrate in dichloromethane has been shown to have advantages over other common linkers: Most of the common protecting groups (e.g., Fmoc, Boc, Z, Aloc, *tert*-butyl, benzyl, allyl, trityl) are stable under the mild cleavage conditions of the PTMSEL linker, enabling the application of orthogonal, three dimensional protecting group strategies. Serious side reactions such as aspartimide formation and rearrangements can be decisively suppressed. Base sensitive glycosidic bonds, for example, sialyl-T_N-threonine, remain stable under the mild cleavage conditions. As was shown in the synthesis of the biotinylated O-glycododecapeptide from MUC1, the PTMSEL linker is sterically so demanding that even in sequences prone to diketopiperazine formation (Pro-Ala-Pro) this side reaction does not constitute a problem. Therefore, the novel PTMSEL-linker is particularly useful for the solid-phase synthesis of protected sensitive and complex peptides and glycopeptides.

Experimental Section

General: Solvents for moisture-sensitive reactions (THF, diethyl ether, methanol, dichloromethane) were distilled and dried according to standard procedures. DMF (amine free, for peptide synthesis) was purchased from Roth, acetic anhydride and pyridine in p.a. quality from ACROS. Reagents were purchased at highest available commercial quality and used without further purification unless outlined otherwise. Fmoc-protected amino acids were purchased from Novabiochem. As resins for solid-phase synthesis aminomethyl polystyrene (Advanced ChemTech) and aminomethylated tentagel (NovaSyn Tg amino resin, Novabiochem) were used. Reactions were monitored by thin-layer chromatography with precoated silica gel 60 F_{254} aluminium plates (Merck KGaA, Darmstadt). Flash column chromatography was performed with silica gel (40–63 μm) purchased form Merck KGaA, Darmstadt. Melting points were obtained on a Büchi-Tottoli apparatus and are uncorrected. Optical rotations $[\alpha]_D$ were measured with a Perkin–Elmer polarimeter 241. Elemental analyses were performed by the microanalytical laboratory of the Johannes Gutenberg-Universität, Mainz. RP-HPLC analyses were carried out on a Knauer HPLC system with Eurospher C8 (250×4 mm) and Vydac Peptide&Protein C18 columns (250×4 mm) at a pump rate of 1 mL min^{-1}. Preparative HPLC separations were performed on a Knauer HPLC system with a Eurospher C8 column (250×40 mm, 7 μm) and a pump rate of 20 mL min^{-1}. Semi-preparative HPLC separations were carried out on a Knauer HPLC system with a Vydac Peptide&Protein column 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 $CHCl_3$ (δ=7.24), DMSO (δ=2.49) or acetone (δ=2.05). Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). ^{13}C chemical shifts are reported relative to $CDCl_3$ (δ=77.0), DMSO (δ=39.5) or acetone (δ=29.84). Assignment of proton and carbon signals was achieved by COSY, TOCSY, HSQC, HMQC and HMBC experiments when noted. FD-mass spectra were recorded on a Finnigan MAT-95-spectrometer, MALDI-TOF mass spectra were aquired on a Micromass Tofspec E spectrometer. ESI-mass spectra were obtained on a ThemoQuest-Navigator spectrometer. HR-ESI mass spectra were recorded on a Micromass Q-TOF Ultima spectrometer.

2-Trimethylsilyl oxirane (3): The title compound was synthesized according to ref. [13] to yield a colorless liquid (8.69 g, 74%). B.p. (370 mbar): 75–79°C; $[\alpha]_D^{25}$ = 1.414; ^{1}H NMR (200 MHz, $CDCl_3$): δ=2.88 (t, 1H, β-CH, J=5.6 Hz), 2.50–2.56 (m, 1H, β-CH), 2.17 (t, 1H, α-CH, J=4.6 Hz), 0.04 (s, 9H, $Si(CH_3)_3$).

2-[4-(1-Ethoxyethyloxy)phenyl]-2-trimethylsilyl-ethanol (4): The title compound was synthesized in analogy to the procedure described for the corresponding phenyl derivative in ref. [8b]:

1) Lithiation: 1-(4-Bromophenoxy)-1-ethoxyethane (33.09 g, 135.0 mmol) was dissolved in dry diethyl ether (150 mL). At −45°C *n*-butyllithium (1.6 M in hexane) (84.37 mL, 135.0 mmol) was added within 30 min. The solution was stirred at −40°C for 20 min, warmed to +10°C within 45 min and stirred for further 90 min at this temperature.

2) Formation of cuprate and cuprate addition: In a 500 mL three-necked flask CuI (12.29 g, 65.5 mmol) was suspended in dry diethyl ether (40 mL). The solution containing the lithiated 1-phenoxy-1-ethoxyethane was transferred into a 500 mL dropping funnel (filled with argon) via a stainless steel tube and then added dropwise to the $Cu^{I}I$ suspension at 0°C within 70 min. The mixture was stirred for 30 min and then cooled to −50°C. At this temperature, 2-trimethylsilyl oxirane **3** (2.7 g, 23.2 mmol) was added dropwise via a syringe. The reaction mixture was stirred at −50°C for 5 h and overnight at −20°C. A sat. NH_4Cl solution (200 mL) was added and the resulting mixture was stirred at 0°C for 20 min, diluted with ethyl acetate (300 mL) and with additional sat. NH_4Cl (200 mL). The organic phase was washed twice with a sat. NH_4Cl solution (250 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (650 g silica gel; petrol ether/ethyl acetate 7:1, addition of 0.1% triethylamine). The product containing fractions were diluted with toluene and concentrated in vacuo to yield compound **4** (5.57 g, 85%) as a brown oil. R_f=0.23 (petrol ether/AcOEt 6:1); ^{1}H NMR (200 MHz, [D$_6$]acetone): δ=7.03 (d, 2H, 2 arom. H, J=8.8 Hz), 6.89 (d, 2H, 2 arom. H, J=8.3 Hz), 5.35 (q, 1H, O-C**H**-O, J=5.2 Hz), 3.92–4.07 (m, 2H, C**H$_2$**-OH), 3.65–3.77 (m, 1H, OH), 3.45–3.58 (m, 2H, CH_3-C**H$_2$**-O), 2.31–2.38 (dd, 1H, TMSC**H**, J_1=3.4 Hz, J_2=5.4 Hz), 1.39 (d, 3H, CH-C**H$_3$**, J=5.4 Hz), 1.13 (t, 3H, CH_2-C**H$_3$**, J=6.8 Hz), −0.04 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, [D$_6$]acetone, BB, DEPT): δ=155.42 (1C, $C_{ipso,Ar\text{-}O}$), 136.51 (1C, $C_{ipso,Ar\text{-}CH}$), 129.48 (2C, 2 arom. C), 118.02 (2C, 2 arom. C), 100.37 (1C, O-**C**H-O), 63.60 (1C, **C**H$_2$-OH), 61.79 (1C, CH_3-**C**H$_2$-O), 41.00 (1C, TMS-**C**H), 20.71 (1C, **C**H$_3$-CH), 15.52 (1C, **C**H$_3$-CH$_2$), −2.16 (3C, $Si(CH_3)_3$); elemental analysis calcd (%) for $C_{15}H_{26}O_3Si$: C 63.78 H 9.28; found: C 63.40 H 8.92.

2-(4-Hydroxyphenyl)-2-trimethylsilyl-ethanol (5): Under argon 2-[4-(1-ethoxyethyloxy)phenyl]-2-trimethylsilyl-ethanol (**4**; 2.55 g, 9.01 mmol) was dissolved in methanol (p.a.) (40 mL). A solution of pyridinium-*p*-toluenesulfonate (PPTS) (68.1 mg, 0.27 mmol, 0.03 equiv) in methanol (3 mL) was added and the mixture was stirred for 2 h at room temperature. The solvent was removed in vacuo and the residue purified by flash chromatography (140 g silica gel; petrol ether/ethyl acetate 4:1) to give the desired product **5** (1.71 g; 90%) as colorless crystals. R_f=0.18 (petrol ether/AcOEt 3:1); m.p. 138–141°C; ^{1}H NMR (200 MHz, [D$_6$]acetone): δ=7.94 (br s, 1H, OH phenol), 6.95 (d, 2H, 2 arom. H, J=8.8 Hz), 6.72 (d, 2H, 2 arom. H, J=8.8 Hz), 3.86–4.07 (m, 2H, C**H$_2$**-OH), 3.37 (t, 1H, OH, J=5.4 Hz), 2.28 (dd, 1H, TMS-C**H**, J_1=3.4 Hz, J_2=8.8 Hz); −0.05 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, [D$_6$]acetone, BB, DEPT): δ=155.53 (1C, $C_{ipso,Ar\text{-}OH}$), 133.74 (1C, $C_{ipso,Ar\text{-}CH}$), 129.60 (2C, 2 arom. C), 115.80 (2C, 2 arom. C), 63.85 (1C, **C**H$_2$-O), 41.00 (1C, TMS-**C**H), −2.11 (3C, $Si(CH_3)_3$); FD-MS calcd for $C_{11}H_{18}O_2Si$: 210.3; found: 210.4 $[M]^+$; elemental analysis calcd for $C_{11}H_{18}O_2Si$: C 62.81 H 8.63; found:C 62.79 H 8.52.

Allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]-phenoxyacetate (6): 2-(4-Hydroxyphenyl)-2-trimethylsilyl-ethanol (**5**; 1.55 g, 7.37 mmol) was dissolved in acetone (p.a.) (40 mL). Under argon K_2CO_3 (1.33 g, 9.59 mmol), KI (160.0 mg, 0.96 mmol) and allyl chloroacetate (1.03 mL 8.85 mmol) were added and the mixture was stirred at room temperature for 18 h. TLC monitoring (petrol ether/AcOEt 3:1) revealed 60% conversion. Further addition of K_2CO_3 (0.80 g, 5.75 mmol), KI (96.0 mg, 0.58 mmol) and allyl

chloroacetate (0.62 mL, 5.32 mmol) and stirring for another 20 h resulted in complete conversion. The mixture was filtered through Hyflo-Supercel and concentrated in vacuo. The brown oil was purified by flash chromatography (180 g silica gel, petrol ether/ethyl acetate 5:1) to afford the title compound **6** (1.76 g, 78%) as a colorless oil, which crystallized after drying in vacuo and storage at 4°C. $R_f = 0.29$ (petrol ether/AcOEt 3:1); m.p. 27–28°C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.01$ (d, 2H, 2 arom. H, $J = 8.3$ Hz), 6.83 (d, 2H, 2 arom. H, $J = 8.8$ Hz), 5.81–6.00 (m, 1H, C**H**=CH_2), 5.31 (dd, 1H, CH=C**Ha**Hb, $J = 16.3$ Hz), 5.24 (dd, 1H, CH=CHa**Hb**, $J = 10.5$ Hz), 4.69 (d, 2H, C**H$_2$**-CH=CH_2, $J = 5.5$ Hz), 4.61 (s, 2H, O-C**H$_2$**-C=O), 3.89–4.12 (m, 2H, CH-C**H$_2$**-OH), 2.37 (dd, 1H, TMSCH, $J_1 = 4.4$ Hz, $J_2 = 11.2$ Hz), 1.50 (brs, 1H, OH), −0.06 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$; BB, DEPT): $\delta = 168.80$ (1C, C=O), 155.74 (1C, $C_{ipso,Ar\text{-}O}$), 133.61 (1C, $C_{ipso,Ar\text{-}CH}$), 131.47 (1C, **C**H=CH_2), 128.82 (2C, 2 arom. C), 119.02 (1C, CH=**C**H$_2$), 115.03 (2C, 2 arom. C), 65.79, 65.61 (2C, CH_2=CH-**C**H$_2$-O, O=C-**C**H$_2$-O), 63.22 (1C, TMS-CH-**C**H$_2$-O), 40.81 (1C, TMS-**C**H), −2.11 (3C, Si(**C**H$_3$)$_3$); FD-MS: calcd for $C_{16}H_{24}O_4Si$: 308.4; found: 308.4 $[M]^+$; elemental analysis calcd for $C_{16}H_{24}O_4Si$: C 62.30 H 7.84; found: C 62.15 H 7.64.

Allyl 4-[2-(*N*-fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethylsilyl)-ethyl]-phenoxyacetate (7a): Allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]-phenoxyacetate (**6**; 0.83 g, 2.70 mmol) was added to a solution of Fmoc-Ile-OH (1.05 g, 2.97 mmol) in CH_2Cl_2 (20 mL). The solution was stirred under argon and cooled to 0°C. Successively DMAP[16] (20.0 mg, 0.16 mmol) and DCC (0.67 g, 3.26 mmol) were added. The mixture was stirred at 0°C for 3 h and filtered through Hyflo-Supercel. The filtrate was diluted with CH_2Cl_2 (70 mL), washed twice with a 5% $NaHCO_3$ (100 mL), once with water (100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (130 g silica gel, petrol ether/ethyl acetate 8.5:1) to afford the desired product **7a** (1.58 g, 91%) as a colorless oil. $R_f = 0.23$ (petrol ether/AcOEt 5:1); $[\alpha]_D^{24} = -7.01$ ($c = 1.00$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.74$ (d, 2H, H4-, H5-Fmoc, $J = 7.3$ Hz), 7.57 (d, 2H, H1-, H8-Fmoc, $J = 7.3$ Hz), 7.24–7.42 (m, 4H, H2-, H3-, H6-, H7-Fmoc), 6.93 (d, 2H, 2 arom. H PTMSEL, $J = 7.8$ Hz), 6.78 (d, 2H, 2 arom. H PTMSEL, $J = 8.3$ Hz), 5.81–6.00 (m, 1H, C**H**=CH_2), 5.17–5.31 (m, 3H, CH=C**H$_2$**, NH), 4.67 (d, 2H, C**H$_2$**-CH=CH_2, $J = 5.9$ Hz), 4.57 (s, 2H, O-C**H$_2$**-C=O), 4.52–4.77 (m, 1H, α-CH Ile), 4.33–4.37 (m, 2H, CH_2-Fmoc), 4.04–4.22 (m, 3H, TMS-CH-C**H$_2$**-O, H9-Fmoc), 2.47–2.55 (m, 1H, TMS-C**H**), 1.41–1.64 (m, 1H, β-CH Ile), 0.65–1.06 (m, 8H, γ-CH_2, γ-CH_3, δ-CH_3 Ile), −0.02 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$, BB, DEPT): $\delta = 172.20$ (1C, C=O ester Ile), 168.73 (1C, C=O allyl ester), 156.02, 155.74 (2C, C=O urethane, $C_{ipso,Ar\text{-}O}$), 143.98, 143.84 (2C, C4a-, C4b-Fmoc), 141.32 (2C, C8a-, C9a-Fmoc), 133.37 (1C, $C_{ipso,Ar\text{-}CH}$), 131.49 (1C, **C**H=CH_2), 128.39 (2C, 2 arom. C PTMSEL), 127.69 (2C, C3-, C6-Fmoc), 127.06 (2C, C2-, C7-Fmoc), 125.12 (2C, C1-, C8-Fmoc), 119.98 (2C, C4-, C5-Fmoc), 119.01 (1C, CH=**C**H$_2$), 114.73 (2C, 2 arom. C PTMSEL), 66.98, 66.31, 65.75, 65.59 (4C, CH_2=CH-**C**H$_2$-O, O=C-**C**H$_2$-O, CH_2-Fmoc, TMS-CH-**C**H$_2$-O), 58.33 (1C, α-CH Ile), 47.22 (1C, C9-Fmoc), 37.85 (1C, TMS-**C**H), 36.52 (1C, β-CH Ile), 24.7 (1C, γ-CH_2 Ile), 15.13 (1C, γ-CH_3 Ile), 11.56 (1C, δ-CH_3 Ile), −2.11 (3C, Si(**C**H$_3$)$_3$); ESI-MS: calcd for $C_{37}H_{45}NO_7SiNa$: 666.3; found: 666.4 $[M+Na]^+$; elemental analysis calcd for $C_{37}H_{45}NO_7Si$: C 69.02 H 7.04 N 2.18; found: C 69.53 H 6.71 N 2.01.

Allyl 4-[2-(*N*-fluorenylmethoxycarbonyl-L-prolyloxy)-1-(trimethylsilyl)-ethyl]-phenoxyacetate (7b): The preparation was carried out in analogy to **7a**, using allyl 4-[2-hydroxy-1-(trimethylsilyl)-ethyl]-phenoxyacetate (**6**; 0.83 g, 2.69 mmol), Fmoc-Pro-OH (1.00 g, 2.96 mmol) to yield **7b** as a colorless oil (1.57 g, 93%). $R_f = 0.18$ (PE/EE 5:1). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.75$ (d, 2H, H4-, H5-Fmoc, $J = 6.8$ Hz), 7.64–7.26 (m, 6H, H1-, H8-, H2-, H3-, H6-, H7-Fmoc), 6.98–6.63 (m, 4H, H_{ar} PTMSEL), 6.01–5.80 (m, 1H, C**H**=CH_2), 5.34–5.21 (m, 3H, CH=C**H$_2$**), 4.73–4.15 (m, 10H, C**H$_2$**-CH=CH_2, O-C**H$_2$**-C=O, TMS-CH-C**H$_2$**-O, CH_2-Fmoc, H9-Fmoc, α-CH Pro), 3.53–3.34 (m, 2H, γ-CH_2 Pro), 2.85–2.43 (m, 1H, TMS-CH), 2.09–1.86 (m, 1H, β-C**Ha**Hb Pro), 1.84–1.50 (m, 3H, β-CHa**Hb** Pro, δ-CH_2 Pro), −0.04 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$; BB, DEPT): $\delta = 172.80$ (1C, C=O-Pro), 168.75 (1C, C=O allyl ester), 155.54 (1C, C=O urethane), 154.80 (1C, $C_{ipso,Ar\text{-}O}$), 144.22, 143.98 (2C, C4a-, C4b-Fmoc), 141.30 (2C, C8a-, C9a-Fmoc), 133.71 (1C, $C_{ipso,Ar\text{-}CH}$), 131.48 (**C**H=CH_2), 128.50, 128.38 (2C, C_{ar} PTMSEL), 127.67, 127.04, 125.12, 119.93 (4C, C_{tert}-Fmoc), 118.99 (1C, CH=**C**H$_2$), 114.64, 114.55 (2C, C_{ar} PTMSEL), 67.54 (1C, **C**H$_2$-Fmoc), 66.03, 65.84, 65.51 (3C, CH_2=CH-**C**H$_2$-O, O=C-**C**H$_2$-O, TMS-CH-**C**H$_2$-O), 59.40 (1C, α-CH Pro), 47.27 (1C, C9-Fmoc), 46.85 (1C, γ-CH_2 Pro), 36.59 (TMS-**C**H), 29.87 (β-CH_2 Pro), 23.98 (1C, δ-CH_2 Pro), −2.65 (3C, Si(**C**H$_3$)$_3$); HR-ESI-TOF-MS: calcd for $C_{36}H_{41}NO_7SiNa$: 650.2550; found: 650.2528 $[M+Na]^+$.

4-[2-(*N*-Fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethyl-silyl)-ethyl]-phenoxy-acetic acid (8a): $[Pd(PPh_3)_4]$ (156.5 mg, 0.14 mmol) was added under argon to a stirred solution of allyl 4-[2-(*N*-fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethyl-silyl)-ethyl]-phenoxy-acetate (**7a**; 1.45 g, 2.26 mmol) in THF (p.a.) (20 mL) and a solution of sodium-*p*-toluenesulfinate (522.9 mg, 2.94 mmol) in MeOH (p.a.) (12 mL). The reaction mixture was stirred under argon at room temperature for 90 min, concentrated in vacuo and purified by flash chromatography (CH_2Cl_2/MeOH/AcOH/H_2O 97.5:2.5:0.25:0.25). Compound **8a** (1.36 g, quant.) was afforded as a colorless foam. $R_f = 0.11$ (CH_2Cl_2/MeOH/AcOH/H_2O 97.5:2.5:0.25:0.25); $[\alpha]_D^{24} = -6.09$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.32$ (brs, 1H, COOH), 7.75 (d, 2H, H4-, H5-Fmoc, $J = 7.3$ Hz), 7.58 (d, 2H, H1-, H8-Fmoc, $J = 7.3$ Hz), 7.14–7.52 (m, 4H, H2-, H3-, H6-, H7-Fmoc), 6.95 (d, 2H, 2 arom. H PTMSEL, $J = 7.3$ Hz), 6.80 (d, 2H, 2 arom. H PTMSEL, $J = 8.8$ Hz), 5.28 (t, 1H, NH), 4.54–4.78 (m, 1H, α-CH Ile), 4.58 (s, 2H, O-C**H$_2$**-C=O), 4.33–4.37 (m, 2H, CH_2-Fmoc), 4.16–4.22 (m, 3H, TMS-CH-C**H$_2$**-O, H9-Fmoc), 2.49–2.57 (m, 1H, TMS-C**H**), 1.55–1.81 (m, 1H, β-CH Ile), 0.66–1.06 (m, 8H, γ-CH_2, γ-CH_3, δ-CH_3 Ile), −0.01 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$, BB, DEPT): $\delta = 172.09$, 172.03 (2C, **C**OOH; C=O ester Ile), 156.08, 155.67 (2C, C=O urethane, $C_{ipso,Ar\text{-}O}$), 143.97, 143.81 (2C, C4a-, C4b-Fmoc), 141.31 (2C, C8a-, C9a-Fmoc), 132.20 (1C, $C_{ipso,Ar\text{-}CH}$), 127.04–129.01 (6C, 2 arom. C PTMSEL, C2-, C3-, C6-, C7-Fmoc), 125.26, 125.05 (2C, C1-, C8-Fmoc), 119.94 (2C, C4-, C5-Fmoc), 114.82 (2C, 2 arom. C PTMSEL), 67.02, 66.30, 65.25 (3C, O=C-**C**H$_2$-O, CH_2-Fmoc, TMS-CH-**C**H$_2$-O), 58.41 (1C, α-CH Ile), 47.22 (1C, C9-Fmoc), 37.82 (1C, TMS-**C**H), 36.62 (1C, β-CH Ile), 24.7 (1C, γ-CH_2 Ile), 15.21 (1C, γ-CH_3 Ile), 11.47 (1C, δ-CH_3 Ile), −2.72 (3C, Si(**C**H$_3$)$_3$); FD-MS: calcd for $C_{34}H_{41}NO_7Si$: 603.3; found: 604.1 $[M+H]^+$; elemental analysis calcd for $C_{34}H_{41}NO_7Si$: C 67.63 H 6.84 N 2.32; found: C 68.12 H 7.08 N 2.01.

4-[2-(*N*-Fluorenylmethoxycarbonyl-L-prolyloxy)-1-(trimethyl-silyl)-ethyl]-phenoxy-acetic acid (8b): The preparation was carried out in analogy to that of **8a** with **7b** (1.50 g, 2.39 mmol), $[Pd(PPh_3)_4]$ (166 mg, 0.143 mmol), sodium-*p*-toluenesulfinate (553 mg, 3.11 mmol) to yield **8b** as a colorless amorphous solid (1.38 g, 98%). $R_f = 0.34$ (CH_2Cl_2/MeOH/HOAc 40:1:0.05); $[\alpha]_D^{22} = -50.0$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.75$ (d, 2H, H4-, H5-Fmoc, $J = 6.8$ Hz), 7.64–7.26 (m, 6H, H1-, H8-, H2-, H3-, H6-, H7-Fmoc), 6.98–6.63 (m, 4H, H_{ar} PTMSEL), 4.78–4.12 (m, 8H, O-C**H$_2$**-C=O, TMS-CH-C**H$_2$**-O, CH_2-Fmoc, H9-Fmoc, α-CH Pro), 3.53–3.36 (m, 2H, γ-CH_2 Pro), 2.59–2.44 (m, 1H, TMS-CH), 2.10–1.89 (m, 1H, β-C**Ha**Hb), 1.81–1.53 (m, 3H, β-CHa**Hb** Pro, δ-CH_2 Pro), −0.04 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (50.3 MHz, $CDCl_3$; BB, DEPT): $\delta = 172.86$ (1C, COOH), 172.53 (1C, C=O-Pro), 155.18 (1C, C=O-urethane), 154.58 (1C, $C_{ipso,Ar\text{-}O}$), 144.14, 143.93 (2C, C4a-, C4b-Fmoc), 141.29 (2C, C8a-, C9a-Fmoc), 133.85 (1C, $C_{ipso,Ar\text{-}CH}$), 129.05, 128.56 (2C, C_{ar} PTMSEL), 127.69, 127.06, 125.15, 119.95 (4C, C_{tert}-Fmoc), 114.64, 114.56 (2C, C_{ar}), 67.61 (1C, CH_2-Fmoc), 66.09, 65.05 (2C, O=C-**C**H$_2$-O, TMS-CH-**C**H$_2$-O), 59.34 (1C, α-CH Pro), 47.21 (1C, C9-Fmoc), 46.86 (1C, γ-CH_2 Pro), 36.63 (1C, TMS-CH), 30.93 (1C, β-CH_2 Pro), 23.97 (1C, δ-CH_2 Pro), −2.72 (3C, Si(**C**H$_3$)$_3$); HR-ESI-TOF-MS: calcd for $C_{33}H_{36}NO_7SiNa_2$: 632.2056; found: 632.2037 $[M-H+2Na]^+$.

Preparation of loaded resin 9a: In a solid-phase synthesis reactor (100 mL flask with a frit in the bottom) AMPS (aminomethyl polystyrene) (ACT; 200–400 mesh; 1.00 mmol g^{-1}; 1.39 g, 1.39 mmol) was preswollen in CH_2Cl_2 (20 mL) for 30 min. 4-[2-(*N*-fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethylsilyl)-ethyl]-phenoxy-acetic acid (**8a**; 673.0 mg, 1.12 mmol) was dissolved in a mixture of CH_2Cl_2 (40 mL) and DMF (5 mL). To this solution sequentially HOBt (171.2 mg, 1.12 mmol), *N*-methylmorpholine (0.25 mL, 225.6 mg, 2.23 mmol) and TBTU (358.0 mg, 1.12 mmol) were added. After stirring for 20 min, the solution was transferred into the reactor containing the AMPS resin. The reaction mixture was shaken (orbitalshaking) for 18 h at room temperature and then filtered. The resin was washed three times with each DMF and CH_2Cl_2. Pyridine/acetic anhydride (3:1; 20 mL) was added and the mixture was shaken for 20 min. Subsequently, the resin was washed four times with DMF (20 mL), once with MeOH (20 mL), CH_2Cl_2 (20 mL),

MeOH (20 mL), CH_2Cl_2 (20 mL), MeOH (20 mL), CH_2Cl_2 (20 mL) and finally six times with diethyl ether (20 mL). The resin was dried in vacuo to afford the loaded polymer **9a** (2.33 g). The loading was determined by UV absorption of the fluorenylmethyl/piperidine adduct formed by treating the loaded resin (20 mg) with piperidine. Loading: $c = 0.365$ mmol g^{-1} (which corresponds to a coupling efficiency of 74%).

Preparation of resin 9b was performed in analogy to **9a** using Nova-Syn-Tg-amino-resin HL (Novabiochem; 110 μm beads, 0.43 mmol g^{-1}, 1.08 g, 0.464 mmol), **8b** (217 mg, 0.369 mmol), HOBt (57 mg, 0.372 mmol), *N*-methylmorpholine (75 mg, 82 μL, 0.745 mmol), TBTU (119 mg, 0.372 mmol); yield: 1.33 g loaded resin; loading: $c = 0.228$ mmol g^{-1} (which corresponds to a coupling yield of 81%).

βAc$_3$GlcNAc-βAc$_2$GlcNAc-N$_3$ (12): Chitobiosyl chloride (βAc$_3$GlcNAc-αAc$_2$GlcNAc-Cl, **11**)[22] (4.90 g, 7.50 mmol) and tricaprylmethylammoniumchloride (aliquat 336) (3.1 g, 7.71 mmol) were dissolved in $CHCl_3$ (100 mL). A solution of sodium azide (1.50 g, 23.07 mmol) in H_2O (25 mL) was added and the mixture was stirred vigorously at room temperature for 42 h. The organic layer was washed seven times with H_2O (25 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was extracted with methanol (14 mL) and the product was precipitated into diethyl ether (60 mL). After 1 h at 0°C the precipate was filtered off and washed with cold diethyl ether. The chitobiosyl azide **12** (3.45 g, 70%) was obtained as white crystals. $R_f = 0.41$ ($CHCl_3$/EtOH 9:1); m.p. 192°C, lit.:[22] m.p. 195°C; $[\alpha]_D^{22} = -49.0$ ($c = 1.00$ in $CHCl_3$), lit.:[22] $[\alpha]_D^{22} = -53.1$ ($c = 1.00$ in $CHCl_3$); ESI-MS: calcd for $C_{26}H_{37}N_5O_{15}Na$: 682.2; found: 682.3 $[M+Na]^+$.

βAc$_3$GlcNAc-βAc$_2$GlcNAc-NH$_2$ (13): In a three necked-flask chitobiosyl azide **12** (1.82 g, 2.73 mmol) was dissolved in dioxane (80 mL) and ethanol (20 mL). Under argon neutralized raney-nickel (1.00 g) was added. Hydrogen (1 atm) was bubbled through the solution. The mixture was stirred at room temperature for 3 h and filtered through Hyflo-Supercel. The filter residue was washed with dioxane (80 mL) and ethanol (100 mL). The combined filtrates were concentrated in vacuo to give the chitobiosyl amine **13** (1.73 g, quant.) as a colorless glass which was used without further purification and characterization for the synthesis of **15**. $R_f = 0.23$ ($CHCl_3$/EtOH 9:1).

Fmoc-Asn(βAc$_3$GlcNAc-βAc$_2$GlcNAc)-OAll) (15): Fmoc-Asp-OAll (**14**; 1.21 g, 3.06 mmol) was dissolved in DMF (20 mL). Sequentially HOBt(469.0 mg, 3.06 mmol), DIPEA (1.05 mL, 6.12 mmol) and TBTU (980.9 mg, 3.06 mmol) were added. After stirring for 10 min, a solution of chitobiosyl amine **13** (1.76 g, 2.78 mmol) in DMF (20 mL) was added. The mixture was stirred at room temperature under argon for 40 h. The solvent was distilled off in vacuo, the residue diluted was with dichloromethane (500 mL) and washed twice with a 5% $NaHCO_3$ (100 mL) and once with brine (50 mL). During the washing procedure the product precipitated. The precipitate was filtered off and washed with water and diethyl ether yielding the desired compound as colorless crystals (1.67 g). The organic layer was concentrated under reduced pressure and the residue extracted with water and diethyl ether to furnish further 0.46 g of **15**. In total, 2.13 g (76%) of **15** were obtained. $R_f = 0.18$ (CH_2Cl_2/MeOH/AcOH/H_2O 95:5:0.5:0.5); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 8.54$ (d, 1H, ω-NH Asn, $J = 9.0$ Hz), 7.97 (d, 1H, NH, $J = 9.0$ Hz), 7.87 (d, 2H, H4-, H5-Fmoc, $J = 7.9$ Hz), 7.81 (d, 1H, NH, $J = 9.4$ Hz), 7.67 (d, 2H, H1-, H8-Fmoc, $J = 7.4$ Hz), 7.24–7.41 (m, 4H, H2-, H3-, H6-, H7-Fmoc), 5.80–5.89 (m, 1H, C**H**=CH$_2$), 5.06–5.27 (m, 3H, CH=C**H$_2$**, H3″-Glc), 5.04 (t, 1H, H1′-Glc, $J = 9.5$ Hz), 4.93 (t, 1H, H3′-Glc, $J = 9.4$ Hz), 4.80 (t, 1H, H4″-Glc, $J = 9.8$ Hz), 4.65 (d, 1H, H1″-Glc, $J = 8.2$ Hz), 4.42–4.57 (m, 3H, α-CH Asn, C**H$_2$**-CH=CH$_2$), 4.18–4.32 (m, 5H, H6a″-, H6b″-, H6a′-Glc, CH$_2$-Fmoc), 3.81–3.96 (m, 2H, H6b′-Glc, H9-Fmoc), 3.66–3.78 (m, 3H, H5″-, H2′-, H4′-Glc), 3.43–3.55 (m, 2H, H2″-, H5′-Glc), 2.46–2.67 (m, 2H, β-CH$_2$ Asn), 2.03 (s, 3H, CH$_3$ OAc), 2.00 (s, 3H, CH$_3$ OAc), 1.94 (s, 3H, CH$_3$ OAc), 1.93 (s, 3H, CH$_3$ OAc), 1.89 (s, 3H, CH$_3$ OAc), 1.73 (s, 3H, CH$_3$ NHAc), 1.68 (s, 3H, CH$_3$ NHAc); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, BB, DEPT): $\delta = 171.09$ (1C, COOAll), 169.13–170.10 (8C, 5×C=O OAc, 3×C=O amide: NHAc′, NHAc″, ω-Asn), 155.82 (1C, C=O urethane), 143.74 (2C, C4a-, C4b-Fmoc), 140.71 (2C, C8a-, C9a-Fmoc), 132.31 (1C, **C**H=CH$_2$), 127.64 (2C, C3-, C6-Fmoc), 127.08 (2C, C2-, C7-Fmoc), 125.26 (2C, C1-, C8-Fmoc), 120.12 (2C, C4-, C5-Fmoc), 117.45 (1C, CH=**C**H$_2$), 100.16 (1C, C1″-Glc), 77.71 (1C, C1′-Glc), 75.81 (1C, C4′-Glc), 74.13 (1C, C3′-Glc), 73.22 (1C, C5′), 72.60 (1C, C3″-Glc), 70.59 (1C, C5′-Glc), 68.18 (1C, C4″-Glc), 66.18 (1C, CH$_2$-Fmoc), 66.04 (1C, C6″-Glc), 64.93 (1C, **C**H$_2$-CH=CH$_2$), 61.58 (1C, C6′-Glc), 54.00 (1C, C2″-Glc), 52.52 (1C, C2′-Glc), 50.06 (1C, α-CH Asn), 46.56 (1C, C9-Fmoc), 36.76 (1C, β-CH$_2$ Asn), 22.62, 22.57 (2C, 2×CH$_3$ NHAc), 20.78, 20.46, 20.48, 20.41, 20.32 (5C, 5×CH$_3$ OAc); ESI-MS: calcd for $C_{48}H_{58}N_4O_{20}Na$: 1033.3; found: 1033.2 $[M+Na]^+$.

Fmoc-Asn(β-Ac$_3$GlcNAc-β-Ac$_2$GlcNAc)-OH (16): Fmoc-Asn(βAc$_3$GlcNAc-βAc$_2$GlcNAc)-OAll (**15**; 1.62 g, 1.60 mmol) was dissolved in THF (120 mL) and methanol (42 mL). [Pd(PPh$_3$)$_4$] (111.1 mg, 0.10 mmol) and a solution of sodium-*p*-toluenesulfinate (371.2 mg, 2.08 mmol) in methanol (21 mL) were added. The mixture was stirred for 70 min under argon and protection from light. The solvent was evaporated in vacuo and the residue purified by flash chromatography (120 g silica gel, CH_2Cl_2/MeOH/AcOH/H_2O 92:8:0.8:0.8). Compound **16** (886 mg, 57%) was afforded as colorless crystals. Another compound (460 mg) could be isolated, which beared one acetyl-protecting group less than the desired product. **16**: $R_f = 0.33$ (CH_2Cl_2/MeOH/AcOH/H_2O 90:10:1:1); $[\alpha]_D^{22} = 3.63$ ($c = 0.63$, DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO, ^{1}H-COSY): $\delta = 8.50$ (d, 1H, ω-NH Asn, $J = 9.0$ Hz), 7.97 (d, 1H, NH, $J = 9.4$ Hz), 7.88 (d, 2H, H4-, H5-Fmoc, $J = 7.8$ Hz), 7.80 (d, 1H, NH, $J = 9.4$ Hz), 7.70 (d, 2H, H1-, H8-Fmoc, $J = 7.4$ Hz), 7.46 (d, 1H, NH, $J = 8.2$ Hz), 7.30–7.42 (m, 4H, H2-, H3-, H6-, H7-Fmoc), 5.12 (t, 1H, H3″-Glc, $J = 9.8$ Hz), 5.05 (t, 1H, H1′-Glc, $J = 9.8$ Hz), 4.94 (t, 1H, H3′-Glc, $J = 9.4$ Hz), 4.80 (m, 1H, H4″-Glc), 4.65 (d, 1H, H1″-Glc, $J = 8.2$ Hz), 4.18–4.32 (m, 6H, α-CH Asn, H6a″-, H6b″-, H6a′-Glc, CH$_2$-Fmoc), 3.81–3.98 (m, 2H, H6b′-Glc, H9-Fmoc), 3.71–3.81 (m, 2H, H5″-, H2′-Glc), 3.68 (t, 1H, H4′-Glc, $J = 9.4$ Hz), 3.49–3.55 (m, 2H, H2″-, H5′-Glc), 2.45–2.65 (m, 2H, β-CH$_2$ Asn), 2.04 (s, 3H, CH$_3$ OAc), 2.00 (s, 3H, CH$_3$ OAc), 1.95 (s, 3H, CH$_3$ OAc), 1.93 (s, 3H, CH$_3$ OAc), 1.89 (s, 3H, CH$_3$ OAc), 1.73 (s, CH$_3$ NHAc), 1.68 (s, 3H, CH$_3$ NHAc); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, BB, DEPT, HMQC): $\delta = 172.63$ (1C, COOH), 169.13–170.10 (8C, 5×C=O OAc, 3×C=O amide: NHAc′, NHAc″, ω-Asn), 155.82 (1C, C=O urethane), 143.77 (2C, C4a-, C4b-Fmoc), 140.70 (2C, C8a-, C9a-Fmoc), 127.64 (2C, C3-, C6-Fmoc), 127.08 (2C, C2-, C7-Fmoc), 125.26 (2C, C1-, C8-Fmoc), 120.12 (2C, C4-, C5-Fmoc), 100.16 (1C, C1″-Glc), 77.71 (1C, C1′-Glc), 75.81 (1C, C4′-Glc), 74.13 (1C, C3′-Glc), 73.22 (1C, C5′), 72.60 (1C, C3″-Glc), 70.59 (1C, C5″-Glc), 68.18 (1C, C4″-Glc), 66.18 (1C, CH$_2$-Fmoc), 66.04 (1C, C6″-Glc), 61.77 (1C, C6′-Glc), 54.00 (1C, C2″-Glc), 52.52 (1C, C2′-Glc), 50.06 (1C, α-CH Asn), 46.73 (1C, C9-Fmoc), 36.73 (1C, β-CH$_2$ Asn), 22.65, 22.57 (2C, 2×CH$_3$ NHAc), 20.81, 20.51, 20.48, 20.42, 20.34 (5C, 5×CH$_3$ Ac); ESI-MS: calcd for $C_{45}H_{54}N_4O_{20}$: 970.3; found: 993.2 $[M+Na]^+$, 1009.2 $[M+K]^+$, 1015.1 $[M+2Na]^+$.

Boc-Leu-Gln(Trt)-Val-Ala-Ala-Leu-Asp(O*t*Bu)-Ala-Asn(βAc$_3$GlcNAc-βAc$_2$GlcNAc)-Gly-Ile-Ile-OH (17): Starting from polymer **9a** loaded with Fmoc-Ile-OH (274.1 mg,0.1 mmol; loading: 0.365 mmol g^{-1}), glycododecapeptide Boc-Leu-Gln(Trt)-Val-Ala-Ala-Leu-Asp(O*t*Bu)-Ala-Asn(βAc$_3$GlcNAc-βAc$_3$GlcNAc)-Gly-Ile-Ile-OH (**17**) was assembled using a peptide synthesizer (Perkin–Elmer ABI 433 A). Fmoc deprotections were carried out by up to five four minute treatments with 30% piperidine in NMP. Peptide couplings were performed by reaction with 10 equiv Fmoc or Boc amino acids and coupling reagents (10 equiv HBTU, 10 equiv HOBt and 20 equiv DIPEA) within 35 min. The glycosylated building block Fmoc-Asn(βAc$_3$GlcNAc-βAc$_3$GlcNAc)-OH (291.2 mg, 3 equiv) was coupled manually using 3 equiv HATU/3 equiv HOAt and 6 equiv DIPEA (reaction time: 2 h). After each coupling step unreacted amino groups were capped with acetic anhydride/DIPEA/HOBt. After completion of the peptide synthesis, the resin was extensively washed with NMP (6×2 min) and CH_2Cl_2 (4×2 min), transferred to a reactor and dried in vacuo. The resin was washed three times with CH_2Cl_2 (10 mL). For the cleavage procedure the resin was treated with a solution of tetrabutylammonium fluoride trihydrate (63.1 mg, 0.2 mmol) in dry CH_2Cl_2 (10 mL) and shaken at room temperature for 25 min. After filtration, the resin was washed four times with CH_2Cl_2 (10 mL). The cleavage procedure was repeated once with a solution of TBAF·3H$_2$O (63.1 mg, 0.2 mmol) in CH_2Cl_2 (10 mL). The collected cleavage and washing filtrates of each cleavage were separately shaken with water (20 mL) which led to the precipitation of the target compound. The dichloromethane was distilled off and the remaining water was removed with a pasteur pipette. In this way, two product fractions were obtained: 1) cleavage: 78 mg colorless crystals, 2) cleavage: 41 mg colorless crystals. Both product fractions were analyzed by HPLC and identified to be of identical composition. Final purification was achieved by prepara-

tive RP-HPLC (30% MeCN in H_2O → 100% MeCN in 100 min). After lyophilysation the target compound (64.2 mg, 29%) was obtained as a colorless, amorphous solid. $R_f = 0.30$ (CH_2Cl_2/MeOH/AcOH/H_2O 90:10:1:1); HPLC: $t_R = 27.50$ min (Eurosphere C8, 30% MeCN in H_2O → 100% MeCN in 40 min); $[\alpha]_D^{23} = -14.3$ ($c = 0.38$ in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO, 300 K, ^{1}H-COSY, NOESY, TOCSY): $\delta = 8.55$ (s, 1H, NH ω-NH Gln), 8.52 (m, 1H, ω-NH Asn), 8.20 (m, 2H, NH Asn, Asp), 8.12 (d, 1H, NH Gln, $J = 5.6$ Hz), 7.75–8.05 (m, 8H, NH 2×Ala (8.20, 7.83), Gly (7.83), Leu (7.84), 2×Ile (7.83, 7.79), NHAc′ (7.78), NHAc″ (7.97)), 7.62 (d, 2H, NH Val, Ala, $J = 8.2$ Hz), 7.14–7.27 (m, 15H, Trt), 6.92 (d, 1H, NH Leu$_{N\text{-terminal}}$, $J = 8.2$ Hz), 5.14 (t, 1H, H3″-Glc, $J = 10.0$ Hz), 5.05 (t, 1H, H1′-Glc, $J = 9.4$ Hz), 4.96 (t, 1H, H3′-Glc, $J = 9.4$ Hz), 4.81 (t, 1H, H4″-Glc, $J = 9.7$ Hz), 4.65 (d, 1H, H1″-Glc, $J = 8.2$ Hz), 4.52–4.55 (m, 2H, α-CH Asp, Asn), 4.15–4.32 (m, 9H, 7×α-CH: 3×Ala (4.17, 4.24, 4.25), Ile (4.25), Leu (4.25), Val (4.15), Gln (4.25), H6a′-Glc (4.27), H6a″-Glc (4.27)), 4.08 (m, 1H, α-CH Ile), 3.92–3.97 (m, 2H, α-CH Leu$_{N\text{-terminal}}$, H6b′-Glc), 3.88 (d, 1H, H6b″-Glc, $J = 10.6$ Hz), 3.67–3.84 (m, 5H, H5″-Glc (3.81), H2′-Glc (3.77), α-CH$_2$ Gly (3.68), H4′-Glc (3.68)), 3.51–3.55 (m, 2H, H5′-Glc (3.51), H2″-Glc (3.53)), 2.40–2.70 (m, 4H, β-C**H**a**H**b Asp (2.64, 2.40), Asn (2.70, 2.44)), 2.31 (t, 2H, γ-CH$_2$ Gln, $J = 7.6$ Hz), 1.87–2.05 (m, 16H, 5×CH$_3$ OAc, β-CH Val), 1.65–1.81 (m, 10H, 2×CH$_3$ NHAc, β-CH 2×Ile, β-CH$_2$ Gln), 1.57 (m, 2H, γ-CH 2×Leu), 1.33–1.45 (m, 24H, 3×CH$_3$ *t*Bu, 3×CH$_3$ Boc, γ-C**H**aHb 2×Ile, β-CH$_2$ 2×Leu), 1.14–1.24 (m, 10H, β-CH$_3$ 3×Ala; γ-CHa**H**b Ile), 1.04 (m, 1H, γ-CHa**H**b Ile), 0.73–0.88 (m, 30H, γ-CH$_3$ 2×Ile, δ-CH$_3$ 2×Ile, γ-CH$_3$ 2×Val, δ-CH$_3$ 4×Leu); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO, 300 K, HSQC): $\delta = 128.47$ (3C, C$_{para}$-Trt), 127.23 (6C, C$_{ortho}$-Trt), 126.15 (6C, C$_{meta}$-Trt), 100.24 (1C, C1″-Glc), 77.76 (1C, C1′-Glc), 75.82 (1C, C4′-Glc), 74.40 (1C, C3′-Glc), 73.62 (1C, C5′-Glc), 72.33 (1C, C3″-Glc), 70.52 (1C, C5″-Glc), 68.19 (1C, C4″-Glc), 62.64 (1C, C6′-Glc), 61.82 (1C, C6″-Glc), 57.34 (1C, α-**C**H Val), 56.69 (1C, α-CH Ile), 56.43 (1C, α-CH Ile), 53.85 (1C, C2″-Glc), 53.07 (1C, α-CH Leu), 52.42 (1C, C2′-Glc), 52.30 (1C, α-CH Leu), 51.07 (1C, α-CH Gln), 49.46 (2C, α-CH Asn, Asp), 48.82, 48.42, 48.16 (3C, α-CH 3×Ala), 42.35 (1C, α-CH$_2$ Gly), 41.14, 40.68 (2C, β-CH$_2$ 2×Leu), 37.21 (1C, β-**C**H$_2$ Asn), 36.98 (1C, β-CH Ile), 36.91 (1C, β-CH$_2$ Asp), 36.30 (1C, β-CH Ile), 32.68 (1C, γ-CH$_2$ Gln), 30.83 (1C, β-CH Val), 28.21 (1C, β-CH$_2$ Gln), 27.98 (6C, 6×CH$_3$ *t*Bu, Boc), 24.91 (1C, γ-CH$_2$ Ile), 24.29 (2C, γ-CH 2×Leu), 24.21 (1C,γ-CH$_2$ Ile), 22.90 (2C, δ-CH$_3$ 2×Leu), 22.67 (2C, 2×CH$_3$ NHAc), 20.98, 20.44, 20.28 (5C, 5×CH$_3$ OAc), 19.34 (2C, δ-CH$_3$ 2 ×Leu), 18.13 (3C, β-CH$_3$ 3×Ala), 17.67 (2C, γ-CH$_3$ 2×Val), 15.43, 15.36 (2C, γ-CH$_3$ 2×Ile), 11.21, 11.05 (2C, δ-CH$_3$ 2×Ile), MALDI-TOF-MS: calcd for $C_{107}H_{158}N_{16}O_{34}$: 2211.1; found: 2235.0 [*M*+Na+]$^+$, 2256.9 [*M*+2Na]$^+$; ESI-MS found: 1129.1 ([*M*+2Na]$^+$/2), 1137.2 ([*M*+Na+K]$^+$/2), 1140.1 ([*M*+3Na]$^+$/2), 1148.1 ([*M*+2Na+K]$^+$/2), 1050.9 ([*M*+2Na−Boc−*t*Bu]$^+$/2), 1059.0 ([*M*+Na+K−Boc−*t*Bu]$^+$/2), 1062.0 ([*M*+3Na−Boc−*t*Bu]$^+$/2).

TFA·H-Leu-Gln-Val-Ala-Ala-Leu-Asp-Ala-Asn(βAc$_3$GlcNAc-βAc$_2$Glc-NAc)-Gly-Ile-Ile-OH) (18): Glycopeptide **17** (25.0 mg, 0.011 mmol) was stirred at room temperature for 4 h in a mixture containing trifluoroacetic acid (20 mL), triisopropylsilane (0.5 mL) and water (0.5 mL). Toluene (80 mL) was added and the solvent was removed under vacuo. The residue was extracted three times with diethyl ether (20 mL) and the ether solution was removed with a capillary. A colorless crystalline solid (20.5 mg, 97%) was isolated. The product was analyzed by analytical HPLC and shown to be almost pure. HPLC: $t_R = 28.90$ min (Eurosphere C8, 1% MeCN in H_2O → 60% MeCN in H_2O+0.1% TFA in 40 min); $t_R = 19.73$ min (Eurosphere C8, 1% MeCN in H_2O → 100% MeCN+0.1% TFA in 40 min); ESI-MS: calcd for $C_{79}H_{128}O_{32}N_{16}$: 1814.00; found: 929.9 ([*M*+2Na]$^+$/2), 937.8 ([*M*+Na+K]$^+$/2), 945.8 ([*M*+2K]$^+$/2), 948.8 ([*M*+2Na+K]$^+$/2), 952.0 ([*M*+4Na]$^+$/2).

TFA·H-Leu-Gln-Val-Ala-Ala-Leu-Asp-Ala-Asn(βGlcNAc-βGlcNAc)-Gly-Ile-Ile-OH) (19): Glycopeptide **18** (20.0 mg, 0.01 mmol) was dissolved in dry methanol (14 mL). A 0.1 molar solution of sodium methanolate in methanol (4 mL) was added (pH ≈10). The mixture was stirred for 7 h at room temperature. After completion of the deacetylation reaction (HPLC-MS analysis), the mixture was acidified by addition of concentrated acetic acid (pH ≈4) and the solvent was removed in vacuo. The residue was dissolved in water (20 mL) and lyophilized. The crude product was purified by preparative RP-HPLC (10% MeCN in H_2O → 40% MeCN in H_2O + 0.1% TFA in 100 min). After lyophilisation the title compound **19** (14 mg, 79%) was obtained as a colorless, amorphous solid. HPLC: $t_R = 23.48$ min (Eurospher C8, 1% MeCN in H_2O → 60% MeCN in H_2O + 0.1% TFA in 40 min): ^{1}H NMR (600 MHz,[D$_6$]DMSO, 300 K, ^{1}H-COSY, TOCSY, NOESY, ROESY): $\delta = 8.64$ (d, 1H, NH Gln, $J = 7.6$ Hz), 8.18–8.20 (m, 2H, NH Asp (8.19), ω-Asn (8.21)), 8.12 (d, 1H, NH Asn, $J = 5.9$ Hz), 8.07 (d, 1H, NH Ala$_{Val\text{-}\mathbf{Ala}\text{-}Ala}$, $J = 7.0$ Hz), 8.03 (d, 1H, NH Ile), 7.93–7.96 (m, 2H, NH Ala (7.94), Val (7.93), 7.87–7.89 (d, 2H, NH Leu (7.89), NHAc′ (7.87), $J = 7.6$ Hz), 7.69–7.80 (m, 4H, NH Ala (7.75), Ile (7.78), Gly (7.74), NHAc″ (7.72)), 7.27 (brs, 1H, ω-N**Ha**Hb Gln), 6.80 (brs, 1H, ω-NHa**Hb** Gln), 5.09 (d, 1H, OH4″-Glc, $J = 5.3$ Hz), 4.99 (d, 1H, OH3″-Glc, $J = 5.6$ Hz), 4.82 (t, 1H, H1′-Glc, $J = 9.4$ Hz), 4.75 (brs, 1H, OH), 4.70 (t, 1H, OH6″-Glc), 4.59 (t, 1H, OH6′-Glc), 4.48–4.50 (m, 2H, α-CH Asp (4.48), Asn (4.50), 4.37 (m, 1H, α-CH Gln), 4.33 (d, H1″-Glc, $J = 8.2$ Hz), 4.15–4.29 (m, 6H, α-CH: 3×Ala (4.29, 2×4.26), Ile (4.29), Leu (4.25), Val (4.16)), 4.09 (t, 1H, α-CH Ile, $J = 6.5$ Hz), 3.62–3.79 (m, 4H, α-CH Leu$_{N\text{-terminal}}$ (3.75), α-CH$_2$ Gly (3.71), **H6a**″H6b″-Glc (3.71)), 3.56–3.57 (d, 2H, H2′-Glc (3.57), **H6a**′H6b′-Glc (3.56)), 3.21–3.50 (m, 6H, H6a′**H6b**′-Glc (3.41), H6a″**H6b**″-Glc (3.35), H3′-Glc (3.48), H4′-Glc (3.28), H2″-Glc (3.42), H3″-Glc (3.26)), 3.17 (m, 1H, H5″-Glc), 3.11 (m, 2H, H3′-, H5′-Glc), 3.00 (m, 1H, H4″-Glc), 2.67 (m, 1H, β-C**Ha**Hb Asp), 2.40–2.61 (m, 3H, β-CHa**Hb** Asp (2.50), β-CH$_2$ Asn (2.40, 2.58), 2.09–2.18 (m, 2H, γ-CH$_2$ Gln (2.09, 2.16)), 1.96 (m, 1H, β-CH Val), 1.67–1.87 (m, 10H, CH$_3$ 2×NHAc (1.78, 1.81), β-CH$_2$ Gln (1.76, 1.83), β-CH 2×Ile (1.68, 1.75)), 1.36–1.64 (m, 8H, β-CH$_2$ 2×Leu (1.41, 1.54; 1.47, 1.51), γ-CH 2×Leu (1.53, 1.59), γ-C**H**aHb 2×Ile (1.39, 1.40)), 1.12–1.20 (m, 10H, β-CH$_3$ 3×Ala (1.17), γ-CHa**H**b Ile (1.15)), 1.03 (m, 1H, γ-CHa**H**b Ile), 0.75–0.90 (m, 3H, γ-CH$_3$ 2×Ile, δ-CH$_3$ 2×Ile, γ-CH$_3$ 2×Val, δ-CH$_3$ 4×Leu); ^{13}C NMR (150.9 MHz, [D$_6$]DMSO, 300 K, HSQC): $\delta = 102.22$ (1C, C1″-Glc), 81.46 (1C, C4′-Glc), 78.81 (1C, C1′-Glc), 77.09 (1C, C5″-Glc), 76.90 (1C, C5′-Glc), 74.13 (1C, C3″-Glc), 73.04 (1C, C3′-Glc), 70.91 (1C, C4″-Glc), 61.32 (1C, C6″-Glc), 60.04 (1C, C6′-Glc), 57.62 (1C, α-CH Val), 56.47, 56.58 (2C, α-CH 2×Ile), 55.61 (1C, C2″-Glc), 54.08 (1C, C2′-Glc), 52.44 (1C, α-CH Gln), 50.88 (2C, α-CH 2×Leu), 49.47 (2C, α-CH Asn, Asp), 48.26 (1C, α-CH Ala), 48.21 (2C, α-CH 2×Ala), 42.30 (1C, α-CH$_2$ Gly), 41.11 (1C, β-CH$_2$ Leu), 40.48 (1C, β-CH$_2$ Leu), 37.04 (1C, β-CH Ile), 36.08 (1C, β-CH Ile), 36.95 (1C, β-CH$_2$ Asp), 35.76 (1C, β-CH$_2$ Asn), 31.44 (1C, γ-CH$_2$ Gln), 30.72 (1C, β-CH Val), 28.01 (1C, β-CH$_2$ Gln), 24.40, 24.81 (2C, γ-CH$_2$ 2×Ile), 23.53, 24.25 (2C, γ-CH 2×Leu), 22.89, 23.21 (2C, CH$_3$ 2×NHAc), 21.77, 22.49 (4C, δ-CH$_3$ 4×Leu), 19.25 (2C, γ-CH$_3$ 2×Val), 18.17 (3C, β-CH$_3$ 3×Ala), 15.30, 15.61 (2C, γ-CH$_3$ 2×Ile), 11.14, 11.54 (2C, δ-CH$_3$ 2×Ile); MALDI-TOF-MS: calcd for $C_{69}H_{118}O_{27}N_{16}$: 1603.77; found: 1605.4 [*M*+H]$^+$, 1627.5 [*M*+Na]$^+$, 1649.4 [*M*+2Na]$^+$, 1671.4 [*M*+3Na]$^+$; ESI-MS found: 824.8 ([*M*+2Na]$^+$/2), 835.8 ([*M*+3Na]$^+$/2), 843.7 ([*M*+2Na+K]$^+$/2), 846.9 ([*M*+4Na]$^+$/2).

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-{2-acetamido-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-*glycero*-D-*gal-acto*-2-nonulopyranosyl)onat]-α-D-galactopyranosyl}-L-threonine-*tert*-butylester (22)**: Fmoc-Thr(α-GalNAc)-O*t*Bu (**21**)[27] (1.50 g, 2.50 mmol) and α-NeuAc$_4$NAcCOOBnXan (**20**)[28] (4.00 g, 5.95 mmol) were dissolved in a mixture of dry acetonitrile (50 mL) and dry dichloromethane (25 mL). The solution was stirred for 1 h in the presence of flame-dried molecular sieves (6.00 g, powder, 3 Å) under an argon atmosphere and the exclusion of moisture. Subsequently, dried silver triflate (1.53 g, 5.95 mmol) was added and the mixture was cooled to −65 °C. A pre-cooled (0 °C) solution (1.6 M) of methylsulfenyl bromide[30] in 1,2-dichloroethane (3.72 mL, 5.95 mmol) was added dropwise over a period of 25 minutes. The suspension was stirred for 4 h at −65 °C and was, after treatment with diisopropylamine (1.67 mL, 11.9 mmol), allowed to slowly warm to 10 °C. After dilution with dichloromethane (200 mL), the reaction mixture was filtered through Hyflo-Supercel. The filtrate was concentrated in vacuo and the crude residue was purified by flash chromatography (silica gel; ethyl acetate/ethanol 60:1 → 50:1 → 40:1) to yield the disaccharide **22** (1.64 g, 57%) as a colorless amorphous solid. $R_f = 0.22$ (AcOEt/EtOH 20:1); $[\alpha]_D^{22} = 11.8$ ($c = 1.00$ in $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.74$ (d, 2H, H4-, H5-Fmoc, $J = 7.4$ Hz), 7.59 (d, 2H, H1-, H8-Fmoc, $J = 7.3$ Hz), 7.40–7.26 (m, 9H, H2-, H3-, H6-, H7-Fmoc, H$_{arom.}$-Bn (5H)), 6.68 (d, 1H, NH-GalNAc, $J = 7.4$ Hz), 5.48 (d, 1H, NH-Thr, $J = 9.4$ Hz), 5.36–5.27 (m, 2H, H7′, H8′), 5.23 (d, 1H, OCH$_{2a}$-Bn, $J = 12.1$ Hz), 5.15 (d, 1H, OCH$_{2b}$-Bn, $J = 12.1$ Hz), 4.87–4.79 (m, 1H, H4′), 4.77 (d, 1H, H1-Gal, $J = 3.5$ Hz), 4.45 (d, 2H, CH$_2$-Fmoc, $J = 6.7$ Hz), 4.36–4.15 (m, 4H, H9′a H2-Gal, H9-Fmoc, α-CH Thr), 4.11–

4.00 (m, 4H, H6′,β-CH Thr, H5′, H9′b), 3.90 (dd, 1H, H6a-Gal, J_1= 7.0 Hz, J_2= 10.2 Hz), 3.73–3.60 (m, 3H, H5-, H3-, H4-Gal), 3.49 (dd, 1H, $H6_b$-Gal, J_1= 4.3 Hz, J_2= 10.2 Hz), 2.60 (dd, 1H, H3′e, J_1= 12.9 Hz, J_2= 4.7 Hz), 2.10, 2.09, 2.07, 1.99, (s, 15H, NHAc, OAc), 1.93 (t, 1H, H3″eq), 1.84 (s, 3H, OAc), 1.43 (s, 9H, $C(CH_3)_3$), 1.25 (d, 3H, γ-CH_3 Thr, J= 6.3 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 170.94, 170.79, 170.42, 170.35, 170.11 (C=O OAc, NHAc), 167.52 (C1′), 156.57 (C=O urethane), 143.71 (C4a-, C4b-Fmoc), 141.35 (C8a-, C9a-Fmoc), 134.82 (C_{ipso}-Bn), 128.90, 128.81, 128.58, 127.80, 127.13 (C-Bn, C2-, C3-, C6-, C7-Fmoc), 125.06 (C1-, C8-Fmoc), 120.04 (C4-, C5-Fmoc), 99.49 (C1-Gal), 98.80 (C2′), 83.24 (**C**$(CH_3)_3$), 72.90, 69.45, 69.33, 68.98, 68.43, 67.89, 67.60, 67.22 (C3-, C4-, C5-Gal, C4′, C6′, C7′, C8′, CH_2-Fmoc, OCH_2-Bn), 62.55 (C6-Gal), 59.11 (α-CH Thr), 49.30 (C5′), 47.91 (C2-Gal), 47.22 (C9-Fmoc), 37.44 (C3′), 28.12 (C(**C**$H_3)_3$), 23.13, 22.86, 21.10, 20.85, 20.78 (**C**H_3CO), 18.98 (γ-CH_3 Thr); ESI-MS: calcd for $C_{57}H_{71}N_3O_{22}$: 1149.45, found: 1172.5 [M+Na]$^+$, 1116.5 [M−tBu+Na]$^+$, 775.3 [M−Fmoc-Thr-OtBu−H+Na]$^+$.

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-{2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-*glycero*-D-*galacto*-2-nonulopyranosyl)onat]-α-D-galactopyranosyl}-L-threonine-*tert*-butylester (23)**: Fmoc-Thr(α-$NeuAc_4$NAcCOOBn-(2→6)-α-GalNAc)-OtBu (**22**; 1.24 g, 1.08 mmol) was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) at 0 °C. After stirring at 0 °C for 4 h, the solution was allowed to warm and kept at room temperature for 15 h. The mixture was diluted with dichloromethane (100 mL) and excess acetic anhydride was hydrolyzed by the addition of ice. The organic layer was washed with a sat. aq. $NaHCO_3$ solution (4×120 mL) and brine (150 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the resulting residue was performed by flash chromatography (silica gel; ethyl acetate) to furnish disaccharide **23** (1.00 g, 76%) as a colorless amorphous solid. $[\alpha]_D^{22}$ = 30.2 (c = 1.00 in $CHCl_3$); R_f = 0.33 (AcOEt); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.77 (d, 2H, H4-, H5-Fmoc, J = 7.3 Hz), 7.63 (d, 2H, H1-, H8-Fmoc, J = 7.3 Hz), 7.43–7.26 (m, 9H, H2-, H3-, H6-, H7-Fmoc, H2-, H3-, H4-, H5-, H6-Bn), 5.99 (d, 1H, NH-urethane, J = 8.1 Hz), 5.63 (d, 1H, NH-GalNAc, J = 9.3 Hz), 5.32–5.21 (m, 3H, H7′, H8′, OCH_2-Bn_a), 5.16–5.07 (m, 2H, H4, OCH_2-Bn_b), 4.96 (dd, 1H, H3-Gal, J = 2.9 Hz), 4.87–4.71 (m, 2H, H1-Gal, OCH_2-Bn_a), 4.82 (d, 1H, H4′, J = 3.4 Hz), 4.59–4.46 (m, 3H, CH_2-$Fmoc_b$, H2-Gal, β-CH Thr), 4.27–4.14 (m, 4H, H6′, H9′a, H9-Fmoc, α-CH Thr), 4.11–3.97 (m, 3H, H5-Gal, H5′, H9′b), 3.83 (dd, 1H, H6a-Gal, J_1 = 7.6 Hz, J_2 = 10.1 Hz), 3.10 (dd, 1H, H6b, J_1 = 4.4 Hz, J_2 = 10.0 Hz), 2.54 (dd, 1H, H3′e, J_1 = 12.7 Hz, J_2 = 4.4 Hz), 2.10, 2.02, 2.00, 1.98, 1.85 (s, 25H, NHAc, OAc, H-3′a), 1.43 (s, 9H, $C(CH_3)_3$), 1.23 (d, 3H, γ-CH Thr, J = 7.3 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, BB, DEPT): δ = 173.51, 173.38, 172.36, 172.14, 171.72, 170.24, 170.14, 169.68 (C=O OAc, C=O NHAc), 167.36 (C1′), 156.87 (C=O urethane), 143.74 (C4a-, C4b-Fmoc), 141.35 (C8a-, C9a-Fmoc), 134.73 (C_{ipso}-Bn), 128.87, 128.71, 127.80, 127.15 (C-Bn, C2-, C3-, C6-, C7-Fmoc), 125.07 (C1-, C8-Fmoc), 120.06 (C4-, C5-Fmoc), 100.03 (C1-Gal), 98.61 (C2′), 83.09 (**C**$(CH_3)_3$), 76.69 (β-CH Thr), 72.63, 68.91, 68.55, 68.10, 67.89, 67.62, 67.27, 67.14 (C3-, C4-, C5-Gal, C4′, C6′, C7′, C8′, CH_2-Fmoc, OCH_2-Bn), 63.90, 62.40 (C6-Gal, C9′), 59.16 (α-CH Thr), 49.20 (C5′), 47.38 (C2-Gal), 47.26 (C9-Fmoc), 37.31 (C3′), 28.11 (C(**C**$H_3)_3$), 23.19, 21.02, 20.82, 20.72, 20.65 (**C**H_3CO), 18.61 (γ-**C**H_3 Thr); ESI-MS: calcd for $C_{61}H_{75}N_3O_{24}$: 1233.47, found: 1256.6 [M+Na]$^+$, 1200.5 [M−tBu+Na]$^+$; HR-ESI-TOF-MS: calcd for $C_{61}H_{75}N_3O_{24}$Na: 1256.4638, found: 1256.4634 [M+Na]$^+$.

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-{2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-*glycero*-D-*galacto*-2-nonulopyranosyl) onat]-α-D-galactopyranosyl}-L-threonine (24)**: A solution of Fmoc-Thr(α-$NeuAc_4$NAcCOOBn-(2→6)-α-Ac_2-GalNAc)-OtBu (**23**; 0.983 g, 0.796 mmol) in dichloromethane (22 mL) was sequentially treated with anisole (2.2 mL) and trifluoroacetic acid (22 mL). The reaction mixture was stirred at room temperature for 3 h, diluted with toluene (75 mL) and concentrated in vacuo. The resulting residue was coevaporated with toluene (3×30 mL) and dichloromethane (3×30 mL). The crude product was purified by flash chromatography (silica gel; ethyl acetate/methanol 2:1) to give a colorless amorphous solid (0.91 g, 97%). R_f = 0.22 (AcOEt/MeOH 2:1); $[\alpha]_D^{22}$ = 38.3 (c = 1.00 in $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ = 7.88–7.81 (m, 2H, H4-, H5-Fmoc), 7.75–7.68 (m, 2H, H1-, H8-Fmoc), 7.48–7.33 (m, 9H, H2-, H3-, H6-, H7-Fmoc, $H_{arom.}$-Bn), 5.42–5.30 (m, 3H, H7′, H8′, OCH_2-Bn_a), 5.25–5.15 (m, 2H, H-4, OCH_2-Bn_b), 5.05 (dd, 1H, H-3, J_1 = 8.9 Hz, J_2 = 3.1 Hz), 4.90–4.83 (m, 2H, H1, H4′), 4.63 (dd, J_1 = 6.24, J_2 = 10.6 Hz, 1H, OCH_2-$Fmoc_a$), 4.48–4.37 (m, 2H, OCH_2-$Fmoc_b$, β-CH Thr), 4.35–4.18 (m, 5H, H-2, H-6′, H-9′a, H9-Fmoc, α-CH Thr), 4.13–3.97 (m, 3H, H5, H5′, H-9′b), 3.83 (dd, 1H, H6a, J_1 = 7.4 Hz, J_2 = 10.0 Hz), 3.15 (dd, 1H, H6b, J_1 = 4.7 Hz, J_2 = 10.4 Hz), 2.68 (dd, 1H, H3′e, J_1 = 12.7 Hz, J_2 = 4.3 Hz), 2.14, 2.12, 2.03, 2.02, 2.00, 1.98, 1.87 (s, 25H, NHAc, OAc), 1.43 (s, 9H, $C(CH_3)_3$), 1.22 (d, γ-CH_3, 3H, J = 6.2 Hz); ^{13}C NMR (100.6 MHz $CDCl_3$): δ = 173.57, 173.11, 172.24, 171.96, 171.75, 171.62, 171.40 (C=O OAc, NHAc), 168.68 (C1′), 159.04 (C=O urethane), 145.50, 145.18 (C4a-, C4b-Fmoc), 142.71 (C8a-, C9a-Fmoc), 136.60 (C_{ipso}-Bn), 129.99, 129.88, 128.85, 128.21 (C-Bn, C2-, C3-, C6-, C7-Fmoc), 126.20, 126.08 (C1-, C8-Fmoc), 120.96 (C4-, C5-Fmoc), 100.11 (C1-Gal), 99.91 (C2′), 77.75 (β-CH Thr), 73.37, 70.66, 70.04, 69.20, 68.96, 68.58, 67.57 (C3-, C4-, C5-Gal, C4′, C6′, C7′, C8′, CH_2-Fmoc, OCH_2-Bn), 65.10, 63.53 (C6-Gal, C9′), 61.24 (α-CH Thr), 49.20 (C5′), 38.89 (C3′), 28.11 (C(**C**$H_3)_3$), 23.05, 22.69, 21.42, 21.25, 20.95, 20.72 (**C**H_3CO), 19.35 (γ-CH_3 Thr); ESI-MS: calcd for $C_{57}H_{67}N_3O_{24}$: 1177.41, found: 1200.6 [M+Na]$^+$, 1216.7 [M+K]$^+$, 1222.7 [M−H+2Na]$^+$, 1238.7 [M−H+Na+K]$^+$; HR-ESI-TOF-MS: calcd for $C_{57}H_{66}N_3O_{24}Na_2$: 1222.3831, found: 1222.3821 [M−H+2Na]$^+$.

Biotinyl-Gly-Val-Thr(α-$NeuAc_4$NAcCOOBn-(2→6)-α-$GalAc_2$NAc)-Ser(*t*Bu)-Ala-Pro-Asp(O*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Pro-Ala-Pro-OH (25): The solid-phase glycopeptide synthesis was carried out according to the standard protocol described above employing a Perkin–Elmer A433 peptide synthesizer. Starting from Fmoc-Pro-PTMSEL preloaded NovaSyn Tg resin **9b** (439 mg, 0.1 mmol, loading: 0.228 mmol g^{-1}) the protected fragment Fmoc-Ser(*t*Bu)-Ala-Pro-Asp(*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Pro-Ala-Pro-PTMSEL-NovaSyn Tg was prepared by iterative coupling of the corresponding amino acids. Upon *N*-terminal deprotection by treatment with piperidine in NMP, the glycosylated building block **24** was introduced in a manual double coupling. To this end, a solution of Fmoc-Thr(α-$NeuAc_4$NAcCOOBn-(2→6)-α-$GalAc_2$NAc)-OH (165.0 mg, 0.14 mmol, 1.4 equiv), HATU (57.0 mg, 0.15 mmol, 1.5 equiv), HOAt (20.4 mg, 0.15 mmol, 1.5 equiv) and NMM (30.4 mg, 33.4 μL, 0.3 mmol, 3 equiv) in NMP (3 mL) was added twice to the resin which was then vortexed for 1 h. Resuming the standard protocol, the concluding two amino acids were attached. The resin was thoroughly washed with NMP and dichloromethane, removed from the reaction vessel and dried in vacuo to furnish 592 mg of loaded resin. The terminating biotinylation was carried out in the peptide synthesizer using 296 mg (max. 0.05 mmol) of the resin. After N-terminal deprotection (treatment with 30% piperidine in NMP), biotin was attached to the N-terminus by a manual double coupling. The resin was treated twice with a solution of D-(+)-biotin (122.0 mg, 0.5 mmol, 10 equiv), HBTU (208.5 mg, 0.55 mmol, 11 equiv), HOBt (84.2 mg, 0.55 mmol, 11 equiv) and NMM (111.2 mg, 120.94 μL, 1.1 mmol, 22 equiv) in NMP (4 mL; 1 h vortex each time). After filtration, the polymeric support was washed with NMP and dichloromethane. For the cleavage procedure the resin was placed into a reactor and treated with a solution of tetrabutylammonium fluoride trihydrate (31.5 mg, 0.1 mmol, 2 equiv) in CH_2Cl_2 (10 mL) and shaken for 30 min at room temperature. The mixture was filtered and the resin was washed four times with CH_2Cl_2 (10 mL). The cleavage process was repeated with a solution of TBAF·3H_2O (11.5 mg, 0.035 mmol) in dichloromethane (10 mL). Filtrates and washing solutions of both cleavage procedures were separately washed with water (3×20 mL), dried over anhydrous $MgSO_4$ and liberated from the solvents in vacuo to yield 69.1 mg and 9.1 mg, respectively, of a colorless crystalline solid. Both fractions were combined and purified by semi-preparative HPLC (Vydac Protein&Peptide C18, 1% MeCN in H_2O → 100% MeCN in H_2O in 100 min). Lyophilisation yielded the protected glycopeptide **25** as a colorless amorphous solid (56 mg, 42%). HPLC: t_R = 26.9 min (Phenomenex Luna C18, 5% MeCN in H_2O → 100% MeCN in H_2O in 42 min); ^{1}H NMR (400 MHz, $CDCl_3$,^{1}H-COSY): δ = 7.57–7.89 (m, 4H, 4×NH), 7.42–7.55 (m, 2H, 2×NH), 7.35 (s, 5H, arom. H Bn), 7.01–7.17 (m, 2H, 2×NH), 5.12–5.32 (m, 2H, H7′, H8′), 5.27 (s, 2H, OCH_2 Bn), 5.03–5.11 (m, 1H, H4′), 4.76–4.91 (m, 2H, H1-Gal, α-CH Asp), 4.57–4.71 (m, 4H, α-CH Arg, Thr_2, H2-Biot., Ala_a), 4.22–4.55 (m, 9H, α-CH Ala_b, Ser, H5-Biot., 3×Pro, Thr_1, Val, β-CH Thr_2), 3.93–4.10 (m, 3H, β-CH Thr_1, H6′, H2-Gal), 3.14–3.84 (m, 19H, H3-Gal, H4-Gal, H5-Gal, $H6_{a,b}$-Gal, H5′, $H9_{a,b}$′, α-CH_2 Gly, β-CH_2 Ser, 3×δ-CH_2 Pro, H4-Biot.), 2.98–3.06 (m, 2H, δ-CH_2 Arg), 2.80–2.96 (m, 3H, H3a-Biot., β-CH_2 Asp), 2.73 (d, 1H, H3b-Biot., J = 12.5 Hz), 2.54–2.64 (m, 3H, CH_2-Pmc, H3′e), 2.52 (s, 6H, *o*,*o*′-

CH_3-Pmc), 2.36 (m, 2H, H9$_{a,b}$-Biot.), 1.88–2.32 (m, 9H, 3×β-C**Ha**Hb Pro, 3×γ-C**Ha**Hb Pro, β-CH Val, CH_2-Pmc), 1.98, 2.00, 2.06, 2.07, 2.08, 2.10 (s, 27H, 2×NHAc, 6×OAc, *m*-CH_3-Pmc), 1.49–1.84 (m, 12H, 3×γ-CHa**Hb** Pro, β-CH_2 Arg, γ-CH_2 Arg, H3′a, H8$_{a,b}$-Biot., H6$_{a,b}$-Biot.), 1.11, 1.17, 1.29, 1.39 (s, 33H, 4×C(C**H**$_3$)$_3$, C(C**H**$_3$)$_2$-Pmc), 1.35–1.08 (m, 10H, H7$_{a,b}$-Biot., γ-CH_3 Thr$_2$, β-CH_3 2×Ala), 1.04 (d, 3H, γ-CH_3 Thr$_1$, J= 6.2 Hz), 0.95 (d, 6H, 2×γ-CH_3 Val, J=6.6 Hz); MALDI-TOF-MS: calcd for $C_{123}H_{185}N_{19}O_{42}S_2$: 2664.24, found: 2666.4 [M+H]$^+$, 2688.3 [M+Na]$^+$, 2704.5 [M+K]$^+$, 2710.2 [M−H+2Na]$^+$, 2726.6 [M−H+Na+K]$^+$, 2400.1 [M−Pmc+2×H]$^+$, 2422.0 [M−Pmc+H+Na]$^+$, 2438.0 [M−Pmc+H+K]$^+$.

Biotinyl-Gly-Val-Thr(α-NeuAc$_4$NAcCOOH-(2→6)-α-GalAc$_2$NAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-OH (26): Catalytic palladium on activated charcoal (10%; 0.1 mg) was added to a solution of biotinyl-Gly-Val-Thr(α-NeuAc$_4$NAcCOOBn-(2→6)-α-GalAc$_2$NAc)-Ser(*t*Bu)-Ala-Pro-Asp(O*t*Bu)-Thr(*t*Bu)-Arg(Pmc)-Pro-Ala-Pro-OH (**25**; 56 mg, 0.021 mmol) in methanol (15 mL) under argon. The reaction flask was purged with hydrogen and the solution was stirred under a hydrogen atmosphere for 48 h. The mixture was filtered through Hyflo-Supercel which was washed with methanol (3×25 mL) afterwards. After removal of the solvent in vacuo, the resulting colorless solid was dissolved in a mixture of dichloromethane/trifluoroacetic acid/thioanisole/1,2-ethanedithiol (5 mL, 10:10:1:1) and stirred for 2 h. The reaction mixture was concentrated in vacuo and coevaporated with toluene (3×25 mL). By addition of precooled (0°C) diethyl ether (20 mL) the product **26** was precipitated as a colorless crystalline solid, washed with diethyl ether (3× 20 mL), and dissolved in water (25 mL). Lyophilisation afforded 36 mg (crude: 80%) of glycopeptide **26** which was further deprotected without additional purification. HPLC: t_R=30.0 min (Phenomenex Luna C18, 214 nm, 10% MeCN in H_2O → 30% MeCN in H_2O+0.1% TFA in 40 min); MALDI-TOF-MS: calcd for $C_{90}H_{137}N_{19}O_{39}S$: 2139.90; found: 2232.0 [M−3H+4Na]$^+$, 2247.9 [M−3H+3Na+K]$^+$, 1682.8 [M−NeuAc$_4$-NAcCOOH+2H]$^+$, 1747.5 [M−NeuAc$_4$NAcCOOH−H+3Na]$^+$.

Biotinyl-Gly-Val-Thr(α-NeuNAcCOOH-(2→6)-α-GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-OH (27): A solution of sodium methanolate in methanol (0.1 M) was added dropwise until pH 8.5–9 was reached to a solution of crude glycopeptide **26** in methanol (15 mL). After stirring for 15 h the reaction mixture was neutralized by adding Amberlyst IR 120. The mixture was filtered and concentrated under reduced pressure. The residue was dissolved in water (15 mL), lyophilized and the crude product was purified by preparative RP-HPLC (Vydac Protein&Peptide C18, 1% MeCN in H_2O → 20% MeCN in H_2O+0.1% TFA in 110 min). Completely deprotected glycopeptide **27** was obtained as a colorless lyophilisate (8 mg, 20% over three steps). HPLC: t_R=13.2 min (Vydac Protein&Peptide C18, 10% MeCN in H_2O → 30% MeCN in H_2O+0.1% TFA in 40 min); $[\alpha]_D^{22}=-58.7$ (c=0.71, $CHCl_3$); ^{1}H NMR (600 MHz, D_2O, ^{1}H-COSY, TOCSY, HMQC): δ=4.82 (d, 1H, H1- Gal, J=3.8 Hz), 4.62 (t, 1H, α-CH Asp, J=6.6 Hz), 4.49–4.56 (m, 3H, α-CH Arg (4.54}, Thr$_2$ (4.52), H2-Biot.(4.50)), 4.43–4.48 (q, 1H, α-CH Ala$_a$, J=7.0 Hz), 4.40–4.34 (m, 2H, α-CH Ala$_b$ (4.37), Ser (4.36)), 4.26–4.34 (m, 4H, H5-Biot. (4.32), α-CH 3×Pro (4.30)), 4.16–4.23 (m, 3H, α-CH Thr$_1$ (4.22), Val (4.18), β-CH Thr$_2$ (4.19)), 4.08–4.13 (m, 1H, β-CH Thr$_1$), 4.00–4.04 (m, 1H, H6′), 3.96 (dd, 1H, H2-Gal, J_1=3.5 Hz, J_2=11.0 Hz), 3.82–3.89 (m, 3H, H4-Gal (3.87), H6$_a$-Gal, H5′), 3.47–3.79 (m, 17H, H3-Gal (3.77), H8′ (3.77), α-CH_2 Gly (3.75), β-C**Ha**Hb Ser (3.73), β-CHa**Hb** Ser (3.67), H4′ (3.60), δ-CH_2 3×Pro (3.56, 3.50), H6$_b$-Gal (3.53), H5-Gal, H9$_{a,b}$′), 3.43–3.47 (m, 1H, H7′), 3.18–3.25 (m, 1H, H4-Biot.), 3.04–3.15 (m, 2H, δ-CH_2 Arg), 2.84–2.90 (m, 2H, H3a-Biot. {2.87}, β-C**Ha**Hb Asp (2.85)), 2.77–2.83 (m, 1H, β-CHa**Hb** Asp), 2.65 (d, 1H, H3b-Biot., J=12.9 Hz), 2.59 (dd, 1H, H3′e, J_1=12.5 Hz, J_2=4.7 Hz), 2.13–2.29 (m, 5H, H9$_{a,b}$-Biot. (2.24), β-C**Ha**Hb 3×Pro (2.18)), 1.87–1.98 (m, 10H, β-CH Val (1.94), 2×CH_3 NHAc (1.92), γ-C**Ha**Hb 3×Pro (1.91)), 1.70–1.87 (m, 4H, γ-CHa**Hb** 3×Pro (1.79), β-C**Ha**Hb Arg (1.73)), 1.48–1.67 (m, 8H, β-CHa**Hb** Arg, γ-CH_2 Arg (1.63), H3′a (1.61), H8$_{a,b}$-Biot. (1.56), H6$_{a,b}$-Biot. (1.53)); 1.36–1.43 (m, 1H, H7$_a$-Biot.), 1.29–1.34 (m, 1H, H7$_b$-Biot.), 1.24–1.29 (m, 6H, β-CH_3 2×Ala), 1.20 (d, 3H, γ-CH_3 Thr$_2$, J=6.2 Hz), 1.08 (d, 3H, γ-CH_3 Thr$_1$, J=6.5 Hz), 0.85 (d, 6H, γ-CH_3 Val, J=6.8 Hz); MALDI-TOF-MS: calcd for $C_{78}H_{125}N_{19}O_{33}S$: 1887.84, found: 1890.4 [M+H]$^+$, 1912.3 [M+Na]$^+$, 1933.8 [M−H+2Na]$^+$, 1956.3 [M−2×H+3Na]$^+$, 1599.4 (M−NeuNAcCOOH$^-$+2H]$^+$, 1637.7 [M−NeuNAcCOOH+H+K]$^+$, 1659.6 [M−NeuNAcCOOH+Na+K]$^+$.

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