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Solid-phase synthesis of core 3 and core 6 *O*-glycan-linked glycopeptides by benzyl-protection method

Yuko Nakahara, Chinatsu Ozawa, Eriko Tanaka, Kazuki Ohtsuka, Yutaka Takano, Hironobu Hojo* and Yoshiaki Nakahara*

Department of Applied Biochemistry, Institute of Glycotechnology, Tokai University, 1117 Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan

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Abstract—Core 3 and core 6 *O*-glycoamino acids were prepared in a protected form suited for Fmoc solid-phase peptide synthesis (SPPS). An *N*-trichloroacetyllactosamine derivative (**2**) was used as a highly β-selective glycosyl donor in 3-O-glycosylation of acceptors **3/4** and in 6-O-glycosylation of acceptors **5/6**. Zn reduction of trisaccharides **7/8** and **13/14** was followed by acetylation to readily transform trichloroacetamido and azido groups to acetamido groups. Selective deprotection by Pd(0)-catalysis afforded core 3 *O*-glycan building blocks **11/12** and core 6 *O*-glycan building blocks **17/18**. Usefulness of these building blocks for SPPS was demonstrated by the syntheses of the core 3-linked MUC2 tandem repeat glycopeptide and the core 6-linked glycopeptide segment of MUC6. The synthetic glycopeptides detached from the resin were debenzylated under the 'low-acidity TfOH' conditions.

1. Introduction

Glycosylation of serine or threonine residue in proteins with an α-linked GalNAc residue is ubiquitous in eukaryotic cells. Further substitution on the carbohydrate with Gal, GlcNAc, Neu5Ac, and Fuc residues gives a structurally diverse family of *O*-glycans, which has now been classified into eight subclasses referred to as core 1–core 8 (Fig. 1).[1] *O*-Glycans are the principal component of mucin glycoproteins produced by epithelial cells that contributes to the hydrophilic and viscoelastic properties of mucus. The expressed O-glycosylation features on mucins are largely dependent on the organ and differentiation. On the other hand, underglycosylation or altered glycosylation in mucin often provides the immunogenic character of tumor cells. Although the importance of *O*-glycan has been widely recognized, the mechanism of interaction between *O*-glycan and its ligand in the biological system has not been understood in most cases. A major obstacle in the studies of glycoconjugates is ascribed to heterogeneity of the glycan structure in a molecule, particularly remarkable in the clustered O-glycosylation domain of mucin and mucin-like glycoprotein. Since structurally distinct samples are necessary for studying the function of glycans as well as for developing a precise method of identification, there has been a growing demand for synthetic oligosaccharides and their conjugate molecules. Recently, we have established an efficient procedure for the synthesis of *O*- and *N*-glycopeptide, in which a serine, threonine or asparagine derivative carrying benzyl- and benzylidene-protected glycan was used as a building block for the Fmoc solid-phase peptide synthesis (SPPS). The benzyl protecting groups of the synthetic glycopeptides were finally removed by a combination of strong acid and soft nucleophile with minimum scission of glycosidic linkages. Thus, *O*-glycopeptides for CD43,[2] MUC2[3] and MUC5AC,[4] and *N*-glycopeptide for emmprin[5] were successfully synthesized. In the synthesis of CD43 glycopeptide, core 2 tetrasaccharide **1** was constructed by using *N*-trichloroacetyllactosamine derivative **2**

core 1: Galβ1→3GalNAcα1→Ser/Thr

core 2: GlcNAcβ1→6(Galβ1→3)GalNAcα1→Ser/Thr

core 3: GlcNAcβ1→3GalNAcα1→Ser/Thr

core 4: GlcNAcβ1→6(GlcNAcβ1→3)GalNAcα1→Ser/Thr

core 5: GalNAcα1→3GalNAcα1→Ser/Thr

core 6: GlcNAcβ1→6GalNAcα1→Ser/Thr

core 7: GalNAcα1→6GalNAcα1→Ser/Thr

core 8: Galα1→3GalNAcα1→Ser/Thr

Figure 1. The core structure of *O*-glycan.

Keywords: Glycopeptide; Solid-phase synthesis; Core 3 *O*-glycan; Core 6 *O*-glycan; MUC2; MUC6.
* Corresponding authors. Tel./fax: +81 463 50 2075; e-mail addresses: hojo@keyaki.cc.u-tokai.ac.jp; yonak@keyaki.cc.u-tokai.ac.jp

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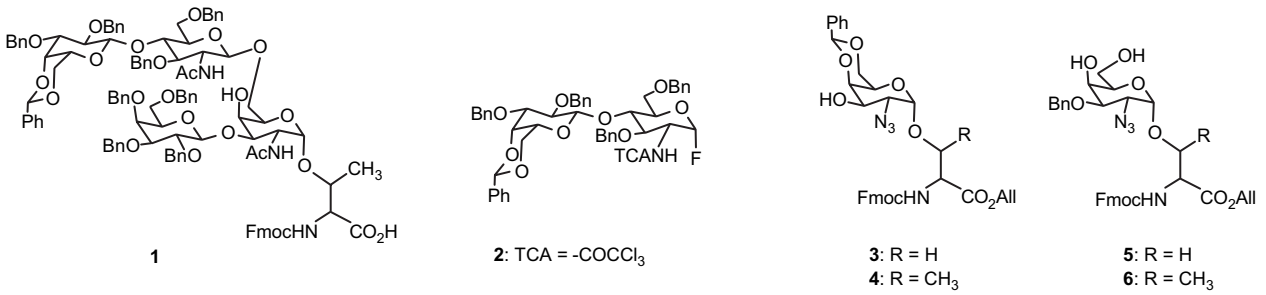


Figure 2. Structures of core 2 tetrasaccharide building block **1**, glycosyl donor **2**, and glycosyl acceptors **2–6**.

as a key glycosyl donor. The 2-trichloroacetamido group serving as a tool for β-stereoselective glycosylation was later reduced to a 2-acetamido group with Zn and AcOH (Fig. 2).

The *N*-acetylglucosamine substitution at the 3- or 6-position of GalNAc residue provides another core structure. Core 3 *O*-glycan biosynthetically forms by the action of core 3 β1,3-*N*-acetylglucosaminyl transferase as a result of competition with core 1 β1,3-galactosyltransferase,[6] whereas core 6 *O*-glycan is synthesized by virtue of core 6 β1,6-*N*-acetylglucosaminyl transferase or by possible enzymatic degradation of the core 2 glycan. These *O*-glycans have been identified in the human mucous fluids, but their specific functions such as biological signaling have remained to be elucidated.

In this paper we describe syntheses of the core 3 and core 6 *O*-glycan building blocks suitable for the Fmoc SPPS protocol, and application of them to the syntheses of MUC2 and MUC6 glycopeptides, respectively.[7,8]

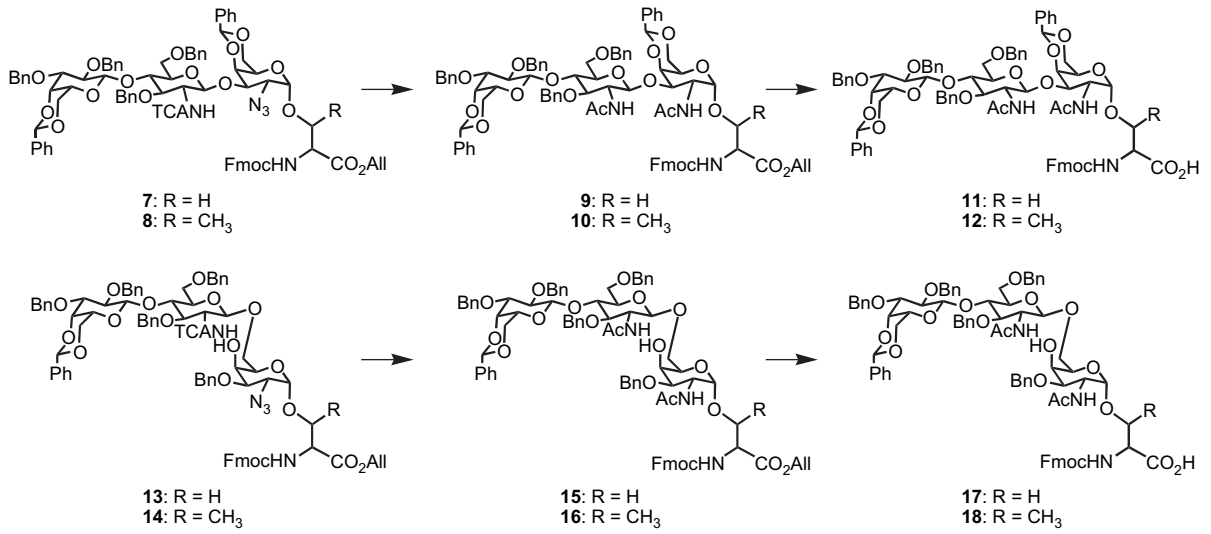
2. Syntheses of building blocks, 11, 12, 17, and 18

Core 3 building blocks **11** and **12** were synthesized using monosaccharide acceptors **3**[9] and **4**.[10] The highly reactive glycosyl donor (**2**) quantitatively gave trisaccharide **7** by reaction with **3** (1.3 equiv) and a specific promoter, $Cp_2Zr(ClO_4)_2$ (2 equiv),[11] at −15 °C in CH_2Cl_2 within 1 h. The newly formed glycosidic linkage was assigned to be β by the ^1H NMR spectrum. By reduction with Zn and AcOH, the trichloroacetamido group and the azido group were converted to an acetamido group and an amino group, respectively. The reduction product was acetylated with Ac_2O and pyridine to give **9** in 90% yield. Deallylation of the fully protected glycosyl serine derivative was performed with $Pd(Ph_3P)_4$ and 5,5-dimethyl-1,3-cyclohexanedione in THF to furnish building block **11** in 91% yield. Similarly, glycosylation of **4** with **2** followed by Zn reduction, acetylation, and deallylation afforded **12** in 82% overall yield.

Preparation of core 6 glycans was attained in somewhat lower efficiency. The dihydroxy compounds **5** and **6**, which had been reported as the intermediates for synthetic sialyl Tn antigen,[12] were chosen as the glycosyl acceptors. The $Cp_2Zr(ClO_4)_2$-promoted reaction of **2** and excess acceptor **5** (1.5 equiv) led to generation of some by-products in addition to trisaccharide **13** (72%). Undesired migration of the benzylidene group from glycosyl donor **2** or product **13** to acceptor **5** took place under the acidic reaction conditions to produce a benzylidenated monosaccharide (15%) and a debenzylidenated trisaccharide (6%). Glycosidation of **2** and threonine derivative **6** (1.2 equiv) was examined with a decreased amount of the promoter, $Cp_2Zr(ClO_4)_2$ (1.5 equiv). The desired **14** was obtained in 65% yield. The benzylidenated monosaccharide (12%) was also formed. In contrast, use of an excess of such reactive glycosyl donor as **2** caused another complication in the reaction with 4,6-dihydroxy type acceptor. The more detailed studies on the reactivity of **2** and its congener are discussed in the following papers for core 4 glycan synthesis. Through Zn reduction, acetylation, and deallylation, trisaccharides **13** and **14** were converted into building blocks **17** (84%) and **18** (78%), respectively (Scheme 1).

3. Solid-phase synthesis of glycopeptides 19 and 20

The core 3 *O*-glycans have been identified in human bronchial mucin,[13] colonic mucin,[14] and meconium glycoproteins.[15] MUC2 glycoprotein is a secreted mucin found in both trachea and intestine. In order to demonstrate usefulness of synthesized **12** as a building block for SPPS, a model glycopeptide (**19**), which represents a tandem repeat tricosapeptide of MUC2 with an appendage of core 3 *O*-glycan, was synthesized (Fig. 3). Starting with commercial Fmoc-CLEAR amide resin, 21 amino acids were assembled by using an automated peptide synthesizer under the *Fastmoc* program, where N-deprotection was performed with 20% piperidine/NMP, and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) were used to activate Fmoc amino acids in 1-methyl-2-pyrrolidinone (NMP). A part of the machine-made peptide resin (25 μmol) was subjected to the condensation with **12** (2 equiv). The reaction was performed overnight at an elevated temperature (50 °C) with HBTU/HOBt in NMP using a vortex mixer. The Fmoc group was then removed and the N-terminal proline residue was introduced to complete the MUC2 tandem repeat sequence. The resulting resin-bound glycopeptide was treated with reagent K (aq CF_3CO_2H, thioanisole, 1,2-ethanedithiol, and phenol) for 1 h at ambient temperature. The product and the resinous support were precipitated by an addition of ether. The precipitate separated by centrifugation was subjected to the low-acidity TfOH-promoted debenzylation reaction (3:5:1:1 DMS/TFA/*m*-cresol/TfOH) at −10 °C for 2 h. An HPLC elution profile of the crude product is shown in Figure 4a. The hydrophobic Fmoc group, which could be removed by piperidine, was retained for the easy analysis and separation by the reversed-phase HPLC. The major peak (1) corresponds to the desired glycopeptide (**19**), *m*/*z*



Scheme 1. Synthesis of core 3 building blocks **11** and **12**, and core 6 building blocks **17** and **18**.

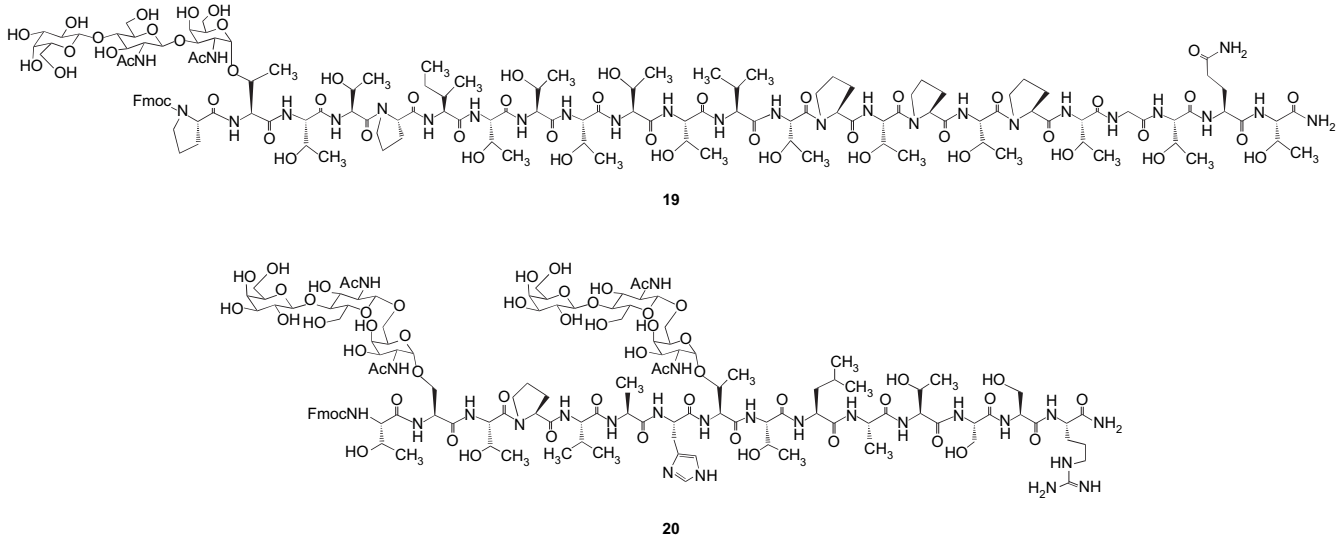


Figure 3. Structures of synthetic glycopeptides **19** and **20**.

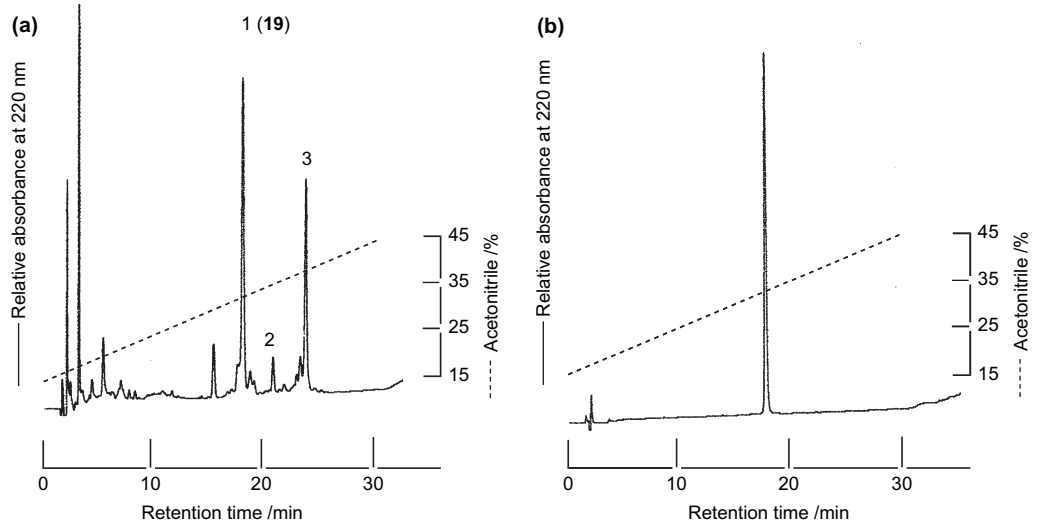


Figure 4. HPLC profiles of synthetic glycopeptides **19** (a) and isolated **19** (b). Conditions: column, Mightysil RP-18, 4.6×150 mm (5 μm); eluent A, distilled water containing 0.1% TFA; eluent B, acetonitrile containing 0.1% TFA; flow rate, 1 ml/min.

3127.20 ($M+Na^+$), and the second major peak (3) is assigned to the docosapeptide lacking the glycothreonine residue. The minor peak (2) at 22.0 min showed *m/z* 2762.77 ($M+Na^+$), suggesting that a small part of the sample has lost a Gal-GlcNAc moiety during the debenzylation process. The areas of peak 2 and peak 3 are 10% and 60% of peak 1, respectively. Other small peaks including the contaminants from the cleavage reagents were not assigned. The overall yield of **19** isolated by preparative HPLC (Fig. 4b) was estimated to be 12.2%, based on the calculation from the amino acid analysis data of the acid-hydrolyzed sample. Judging from this result, the yield of the desired glycopeptide is crucially dependent on the efficiency in the condensation of glycoamino acid **12**. Improved yield of **19** will be obtained by such a modified procedure as employed for the synthesis of core 6-linked glycopeptide (vide infra).

On the other hand, a core 6-linked glycopeptide (**20**) was synthesized as follows. The core 6 trisaccharide was identified in the oligosaccharides derived from human ovarian cyst glycoproteins as a core portion of blood-group determinants,[16] as well as in the oligosaccharides from desialylated human κ-casein.[17] Since MUC6 has been reported to be a major mucin of human ovarian cyst fluid,[18] building blocks **17** and **18** were utilized for the synthesis of glycopentadecapeptide **20** modeled after a part of MUC6 tandem repeat region consisting of 169 amino acids.[19] As mentioned for the synthesis of **19**, the automated synthesis of the C-terminal heptapeptide was performed by using commercial Fmoc-CLEAR amide resin, and the *N*-deprotected peptide resin (18 μmol) was then reacted with **18** (2 equiv) by a manual operation at 50 °C. The coupling procedure was doubled for this solid-phase reaction with **18**, since we knew that the single coupling with 2 equiv of glycoamino acid **12** only gave a moderate yield of the glycosylated MUC2 peptide (vide supra). Further elongation of the peptide chain was also manually conducted by the doubled procedure. Condensation reaction was run for Fmoc amino acids (4 equiv) at room temperature for 1 h and for glycoamino acid **17** (two times with 2 equiv) at 50 °C overnight to complete the solid-phase synthesis of the glycopentadecapeptide. The resulting resin was treated with reagent K and the benzylated glycopeptide was deprotected under the conditions of the low-acidity TfOH. Figure 5a shows an HPLC profile of the crude product. The major peak (1) at 15.3 min corresponds to the desired glycopeptide (**20**) [($M+H^+$): *m/z* 2887.19], whereas the peaks (2) and (3) exhibit the *m/z* values coinciding with the glycopeptide losing a Gal-GlcNAc component. The deprotective reaction run at the lowered temperature with an intent to suppress the disaccharide scission resulted in an additional incompleteness of debenzylation. Similar 6-*O*-disaccharide cleavage had previously been observed in the synthesis of core 2 tetrasaccharide-bound glycopeptide.[2b] The easier scission of GlcNAc glycoside in the core 6 glycan than in the core 3 glycan will be ascribed to the less stable nature of the 6-*O*-glycoside protonated in the acidic media. The acid-stability of the *O*-glycoside will be influenced not only by the concentration, temperature, and reaction period but also by the amino acid sequence of the sample. In order to obtain the optimum result in the debenzylation, further investigations will be necessary. In the minor fractions a glycopeptide lacking two Gal-GlcNAc (peak 4) and the glycopeptide(s) having one benzyl group (peak 5) were detected. The overall yield of isolated glycopeptide **20** (Fig. 5b) was 17.2% on the basis of the amino acid analysis data.

In conclusion, the benzyl- and benzylidene-protected core 3 and core 6 trisaccharide building blocks were synthesized via highly stereoselective glycosylation with glycosyl donor **2**. The building blocks were utilized in the solid-phase syntheses of core 3 *O*-glycan-linked MUC2 tandem repeat and the core 6 *O*-glycan-linked MUC6 glycopeptide. The benzyl protecting groups in the oligosaccharide moieties were finally removed under the low-acidity TfOH conditions with minimum damage to the *O*-glycan structures. Since the

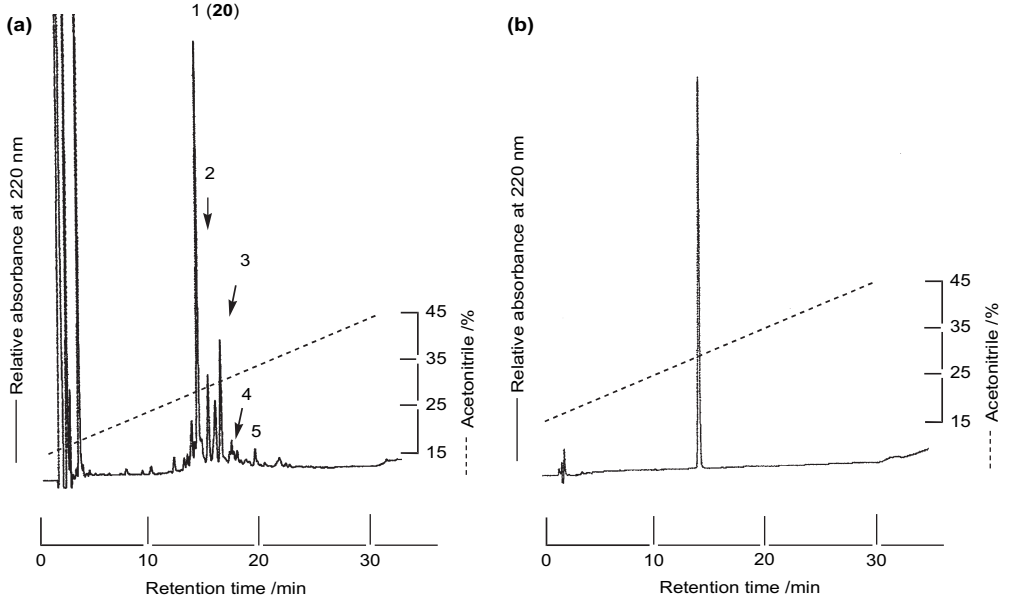
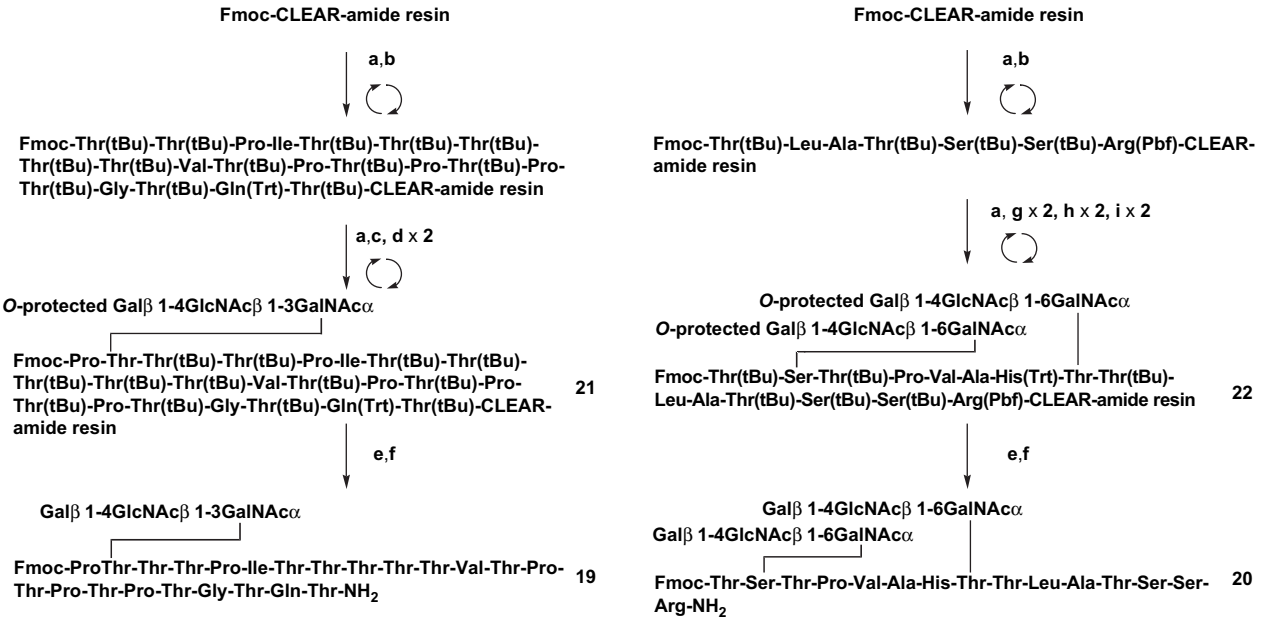


Figure 5. HPLC profiles of synthetic glycopeptides **20** (a) and isolated **20** (b). Conditions: column, Mightysil RP-18, 4.6×150 mm (5 μm); eluent A, distilled water containing 0.1% TFA; eluent B, acetonitrile containing 0.1% TFA; flow rate, 1 ml/min.



Scheme 2. Solid-phase synthesis of glycopeptides **19** and **20**. *Reaction conditions*: (a) 20% piperidine/NMP; (b) Fmoc amino acid, HBTU/HOBt/DMF, DIEA/NMP, synthesizer; (c) **12**, HBTU/HOBt/DMF, DIEA/NMP, 50 °C, overnight; (d) Fmoc-Pro-OH, HBTU/HOBt/DMF, DIEA/NMP, rt, 1 h, vortex; (e) reagent K, rt, 1 h; (f) DMS/TFA/*m*-cresol/TfOH, −15 °C, 2 h; (g) **18**, HBTU/HOBt/DMF, DIEA/NMP, 50 °C, overnight; (h) Fmoc amino acid, HBTU/HOBt/DMF, DIEA/NMP, rt, 1 h, vortex; and (i) **17**, HBTU/HOBt/DMF, DIEA/NMP, 50 °C, overnight.

N-acetyllactosamine-containing *O*-glycans of core 3 and core 6 are common pendants of mucin glycoproteins and can be enzymatically led to the LeX and SLeX epitopes, the method presented here will be used for preparation of a variety of glycopeptides of biological importance (Scheme 2).

4. Experimental

4.1. General

Optical rotation values were determined with a Jasco DIP-370 polarimeter at 20±2 °C for solutions in $CHCl_3$, unless noted otherwise. Column chromatography was performed on silica gel PSQ 100B (Fuji Silysia), while TLC and HPTLC were performed on silica gel 60 F_{254} (E. Merck). ^1H and ^{13}C NMR spectra were recorded with a Jeol AL400 spectrometer (^1H at 400 MHz and ^{13}C at 100 MHz). Chemical shifts are expressed in parts per million downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. With respect to the NMR data of the synthetic trisaccharides, a, b, and c are respectively used to represent GalNAc/$GalN_3$, GlcNAc/GlcNTCA, and Gal residue. MALDI TOF mass spectra were obtained with a PerSeptive Voyager-DE PRO spectrometer (2,5-dihydroxybenzoic acid was used as a matrix). Automated solid-phase peptide synthesis was performed with an Applied Biosystems 433A peptide synthesizer. Manual solid-phase reactions were undertaken in capped polypropylene test tubes equipped with a filter and three-way stopcock by stirring with an EYELA CM-1000 vortex mixer. HPLC was performed in Mightysil RP-18 (4.6×150 mm for analysis and 10×250 mm for preparation, Kanto Chemical Co.). Amino acids were analyzed by a Hitachi L-8500 amino acid analyzer. Fmoc-CLEAR amide resin was purchased from Peptide International Inc. The yield of glycopeptide was determined by amino acid analysis after a measured volume from the whole sample solution was hydrolyzed in a sealed tube with 20% HCl and 0.5% phenol at 150 °C for 2 h.

4.1.1. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 7. A mixture of Cp_2ZrCl_2 (81 mg, 0.28 mmol), $AgClO_4$ (115 mg, 0.55 mmol), and dried molecular sieves 4 Å (powder, 0.7 g) in anhydrous CH_2Cl_2 (7 ml) was stirred under Ar at room temperature for 1 h, then cooled at −15 °C. To the mixture, was added a mixture of **2** (135 mg, 0.14 mmol) and **3** (120 mg, 0.19 mmol) in anhydrous CH_2Cl_2 (5 ml). The reaction mixture was stirred at −15 °C for 1 h, before the reaction was quenched with aq $NaHCO_3$. The mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was successively washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene/EtOAc (4:1) to afford **7** (218 mg, 97%). $[\alpha]_D$ +68.0 (*c*, 1.1). R_f 0.45 (7:3 toluene/EtOAc). ^1H NMR: δ 7.75 (br d, 2H, *J*=7.6 Hz, Ar), 7.61 (br d, 2H, *J*=7.3 Hz, Ar), 7.49–7.11 (m, 35H, Ar, –N*H*), 5.95–5.84 (m, 2H, –CH_2CH=CH_2, –N*H*), 5.45 [s, 1H, PhC*H*$(O)_2$], 5.35 [s, 1H, PhC*H*$(O)_2$], 5.34 (br d, 1H, *J*=17.0 Hz, –CH=CH_2), 5.29 (d, 1H, *J*=7.3 Hz, H-1b), 5.27 (br d, 1H, *J*=10.0 Hz, –CH=CH_2), 5.25 (d, 1H, *J*=10.0 Hz, –CH_2Ph), 4.93 (d, 1H, *J*=3.0 Hz, H-1a), 4.87 (d, 1H, *J*=11.2 Hz, –CH_2Ph), 4.81 (d, 1H, *J*=11.2 Hz, –CH_2Ph), 4.73 (br s, 2H, –CH_2Ph), 4.68 (br d, 2H, *J*=5.8 Hz, –CH_2CH=CH_2), 4.62 (d, 1H, *J*=10.5 Hz, –CH_2Ph), 4.54 (m, 1H, Ser-αH), 4.46 (d, 1H, *J*=7.8 Hz, H-1c); ^{13}C NMR ($CDCl_3$): δ 92.3(–CO*C*Cl_3), 99.6 (C-1a), 99.8 (C-1b), 100.4 [Ph*C*H$(O)_2$], 101.3 [Ph*C*H$(O)_2$], 102.9 (C-1c). MALDI TOF MS: calcd for $C_{83}H_{82}N_5O_{19}Cl_3$ *m/z*

1557.47. Found 1580.18 (M+Na$^+$), 1596.17 (M+K$^+$). Anal. calcd for $C_{83}H_{82}N_5O_{19}Cl_3$: C, 63.91; H, 5.30; N, 4.49; Cl, 6.82. Found: C, 63.91; H, 5.07; N, 4.25; Cl, 6.81.

4.1.2. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 8. Condensation of **2** (130 mg, 0.14 mmol) and **4** (130 mg, 0.20 mmol) was performed using the same procedure as described for **7**. Chromatography of the crude product on silica gel gave **8** (191 mg, 92%). $[\alpha]_D$ +66.9 (*c*, 1.0). R_f 0.46 (7:3 toluene/EtOAc). ^{1}H NMR: δ 7.76 (br d, 2H, *J*=7.6 Hz, Ar), 7.64 (br d, 2H, *J*=7.1 Hz, Ar), 7.48–7.11 (m, 35H, Ar, –N*H*), 5.91 (m, 1H, –CH_2CH=CH_2), 5.74 (d, 1H, *J*=9.5 Hz, –N*H*), 5.45 [s, 1H, PhC*H*$(O)_2$], 5.36 [s, 1H, PhC*H*$(O)_2$], 5.39 (d, 1H, *J*=8.0 Hz, H-1b), 5.37 (dd, 1H, *J*=1.2, 17.4 Hz, –CH=CH_2), 5.29 (d, 1H, *J*=10.4 Hz, –CH_2Ph), 5.27 (dd, 1H, *J*=0.8, 10.2 Hz, –CH=CH_2), 5.01 (d, 1H, *J*=3.4 Hz, H-1a), 4.45 (d, 1H, *J*=7.8 Hz, H-1c), 1.30 (d, 3H, *J*=6.4 Hz, Thr-γH); ^{13}C NMR: δ 92.3 (–CO*C*Cl_3), 99.2 (C-1a), 99.3 (C-1b), 100.4 [Ph*C*H$(O)_2$], 101.2 [Ph*C*H$(O)_2$], 102.9 (C-1c). MALDI TOF MS: calcd for $C_{84}H_{84}N_5O_{19}Cl_3$ *m*/*z* 1572.48. Found 1595.38 (M+Na$^+$), 1611.35 (M+K$^+$). Anal. calcd for $C_{84}H_{84}N_5O_{19}Cl_3$: C, 64.10; H, 5.38; N, 4.45; Cl, 6.76. Found: C, 64.39; H, 5.44; N, 4.22; Cl, 7.05.

4.1.3. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 9. A mixture of **7** (160 mg, 0.10 mmol), powdered Zn (0.5 g), and AcOH (0.5 ml) was stirred at room temperature for 2 h. Then the same amount of Zn and AcOH was added to the mixture, which was stirred for further 2 h. This procedure was repeated three more times until the product gave a single spot on TLC. The mixture was diluted with $CHCl_3$ and the insoluble materials were filtered off through Celite. The filtrate was concentrated in vacuo with toluene to remove remaining AcOH. The residue was dissolved in CH_2Cl_2 (10 ml) and stirred with Ac_2O (0.1 ml) and pyridine (0.1 ml) at room temperature for 2 h. The mixture was washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with $CHCl_3$/MeOH (97:3) to give **9** (136 mg, 90%). $[\alpha]_D$ +58.2 (*c*, 1.2). R_f 0.61 (95:5 $CHCl_3$/MeOH). ^{1}H NMR: δ 6.18–6.09 (m, 3H, 3–N*H*), 5.88 (m, 1H, –CH_2CH=CH_2), 5.44 [s, 1H, PhC*H*$(O)_2$], 5.41 [s, 1H, PhC*H*$(O)_2$], 5.33 (br d, 1H, *J*=17.3 Hz, –CH=CH_2), 5.27 (br d, 1H, *J*=10.5 Hz, –CH=CH_2), 5.18 (br d, 1H, *J*=6.1 Hz, H-1b), 5.13 (br s, 1H, H-1a), 5.04 (d, 1H, *J*=11.4 Hz, –CH_2Ph), 4.84 (d, 1H, *J*=11.0 Hz, –CH_2Ph), 4.80 (d, 1H, *J*=11.0 Hz, –CH_2Ph), 4.72 (br s, 2H, –CH_2Ph), 4.65–4.63 (m, 3H, –CH_2Ph, –CH_2CH=CH_2), 4.43 (d, 1H, *J*=7.3 Hz, H-1c), 3.58 (br s, 1H, H-5a), 3.45 (dd, 1H, *J*=3.2, 9.5 Hz, H-3c), 3.37 (m, 1H, H-2b), 3.05 (br s, 1H, H-5c), 1.96 (s, 3H, Ac), 1.78 (s, 3H, Ac); ^{13}C NMR: δ 99.6 (C-1a), 100.7 [Ph*C*H$(O)_2$], 101.1 [C-1b, Ph*C*H$(O)_2$], 103.2 (C-1c). MALDI TOF MS: calcd for $C_{85}H_{89}N_3O_{20}$ *m*/*z* 1471.60. Found 1494.34 (M+Na$^+$), 1510.34 (M+K$^+$). Anal. calcd for $C_{85}H_{89}N_3O_{20}$: C, 69.33; H, 6.09; N, 2.85. Found: C, 69.32; H, 5.94; N, 2.70.

4.1.4. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 10. As described for **9**, compound **8** (140 mg, 0.09 mmol) was treated with Zn and AcOH to dechlorinate the trichloroacetamido group and to reduce the azido group. Subsequent acetylation followed by chromatography on silica gel with $CHCl_3$/MeOH (97:3) afforded **10** (120 mg, 91%). $[\alpha]_D$ +59.2 (*c*, 1.0). R_f 0.62 (95:5 $CHCl_3$/MeOH). ^{1}H NMR (DMSO-d_6): δ 7.88 (m, 2H, Ar), 7.71 (m, 2H, Ar), 7.45–7.17 (m, 34H, Ar,), 5.87 (m, 1H, –CH_2CH=CH_2), 5.64 [s, 1H, PhC*H*$(O)_2$], 5.46 [s, 1H, PhC*H*$(O)_2$], 5.32 (dd, 1H, *J*=1.5, 17.3 Hz, –CH=CH_2), 5.22 (dd, 1H, *J*=1.2, 10.5 Hz, –CH=CH_2), 4.88 (d, 1H, *J*=8.3 Hz, H-1b), 4.70 (br, 1H, H-1a), 1.84 (s, 3H, Ac), 1.75 (s, 3H, Ac), 1.10 (d, 3H, *J*=6.4 Hz, Thr-γH); ^{13}C NMR ($CDCl_3$): δ 99.7 (C-1a), 100.7 [Ph*C*H$(O)_2$], 100.9 (C-1b), 101.1 [Ph*C*H$(O)_2$], 103.2 (C-1c). MALDI TOF MS: calcd for $C_{86}H_{91}N_3O_{20}$ *m*/*z* 1485.62. Found 1508.62 (M+Na$^+$), 1525.56 (M+K$^+$). Anal. calcd for $C_{86}H_{91}N_3O_{20}$: C, 69.48; H, 6.17; N, 2.83. Found: C, 69.39; H, 6.50; N, 2.58.

4.1.5. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-serine 11. A mixture of **9** (127 mg, 0.09 mmol), 5,5-dimethyl-1,3-cyclohexanedione (240 mg, 1.71 mmol), and Pd$(Ph_3P)_4$ (5 mg, 4.3 μmol) in anhydrous THF (12 ml) was stirred under Ar at room temperature for 1 h, and concentrated in vacuo. The residue was chromatographed on silica gel with $CHCl_3$/MeOH (95:5) to elute excess 5,5-dimethyl-1,3-cyclohexanedione and the less polar adduct. Then the less mobile **11** (112 mg, 91%) was eluted with $CHCl_3$/MeOH/AcOH (92:8:1). $[\alpha]_D$ +69.8 (*c*, 0.9). R_f 0.20 (95:5:1 $CHCl_3$/MeOH/AcOH). ^{1}H NMR (DMSO-d_6): δ 5.64 [s, 1H, PhC*H*$(O)_2$], 5.41 [s, 1H, PhC*H*$(O)_2$], 5.04 (d, 1H, *J*=11.0 Hz, –CH_2Ph), 4.80–4.70 (m, 5H, H-1b, H-1a, 3×–CH_2Ph), 4.61 (d, 1H, *J*=12.2 Hz, –CH_2Ph), 4.55–4.52 (m, 2H, H-1c, –CH_2Ph), 1.82 (s, 3H, Ac), 1.75 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$): δ 100.4 (C-1a), 101.1 [Ph*C*H$(O)_2$, C-1b], 103.2 (C-1c). MALDI TOF MS: calcd for $C_{82}H_{85}N_3O_{20}$ *m*/*z* 1431.57. Found 1454.63 (M+Na$^+$), 1470.61 (M+K$^+$). Anal. calcd for $C_{82}H_{85}N_3O_{20}\cdot 0.5H_2O$: C, 68.32; H, 6.01; N, 2.91. Found: C, 68.14; H, 5.74; N, 2.79.

4.1.6. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine 12. Compound **10** (85 mg, 0.06 mmol) was deallylated as described for **11**. Chromatography of the crude product gave **12** (81 mg, 98%). $[\alpha]_D$ +59.0 (*c*, 1.0). R_f 0.20 (95:5:1 $CHCl_3$/MeOH/AcOH). ^{1}H NMR (DMSO-d_6, 50 °C): δ 5.61 [s, 1H, PhC*H*$(O)_2$], 5.45 [s, 1H, PhC*H*$(O)_2$], 4.89 (d, 1H, *J*=8.1 Hz, H-1b), 4.78 (br, 1H, H-1a), 4.55 (d, 1H, *J*=7.6 Hz, H-1c), 4.26 (m, 1H, Thr-αH), 1.85 (s, 3H, Ac), 1.74 (s, 3H, Ac), 1.12 (d, 3H, *J*=6.1 Hz, Thr-γH); ^{13}C NMR ($CDCl_3$): δ 100.5 (C-1a), 101.0 [Ph*C*H$(O)_2$, C-1b], 103.2 (C-1c). MALDI

TOF MS: calcd for $C_{83}H_{87}N_3O_{20}$ *m/z* 1445.59. Found 1468.52 ($M+Na^+$), 1484.47 ($M+K^+$). Anal. calcd for $C_{83}H_{87}N_3O_{20}$: C, 68.91; H, 6.06; N, 2.90. Found: C, 68.71; H, 6.22; N, 2.75.

4.1.7. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 13. Condensation of **2** (140 mg, 0.15 mmol) and **5** (145 mg, 0.23 mmol) was performed using Cp_2ZrCl_2 (87 mg, 0.30 mmol) and $AgClO_4$ (123 mg, 0.59 mmol) in anhydrous CH_2Cl_2 (10 ml) as described for the synthesis of **7**. The crude product was chromatographed on silica gel with toluene/EtOAc (7:3) to afford **13** (169 mg, 72%). The benzylidenated acceptor (17 mg, 0.02 mmol) and the debenzylated trisaccharide (12 mg, 6%) were isolated in a less polar fraction (R_f 0.53, 7:3 toluene/EtOAc) and in a more polar fraction (R_f 0.38, 2:3 toluene/EtOAc), respectively. $[\alpha]_D$ +41.2 (*c*, 1.1). R_f 0.34 (7:3 toluene/EtOAc). ^1H NMR: δ 7.71 (d, 2H, *J*=7.4 Hz, Ar), 7.61 (d, 1H, *J*=7.3 Hz, Ar), 7.56 (d, 1H, *J*=7.3 Hz, Ar), 7.49–7.14 (m, 35H, Ar, –N*H*), 5.89 (m, 1H, –CH_2CH=CH_2), 5.75 (d, 1H, *J*=7.6 Hz, –N*H*), 5.45 [s, 1H, PhC*H*$(O)_2$], 5.32 (br d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.23 (br d, 1H, *J*=10.5 Hz, –CH=CH_2), 5.19 (d, 1H, *J*=10.5 Hz, –CH_2Ph), 4.94 (d, 1H, *J*=7.8 Hz, H-1b), 4.82 (br s, 1H, H-1a), 3.38 (dd, 1H, *J*=3.7, 9.5 Hz, H-3c), 2.98 (br s, 1H, H-5c); ^{13}C NMR ($CDCl_3$): δ 92.4 (–CO*C*Cl_3), 98.3 (C-1a), 99.2 (C-1b), 101.2 [Ph*C*H$(O)_2$], 102.8 (C-1c). MALDI TOF MS: calcd for $C_{83}H_{84}N_5O_{19}Cl_3$ *m/z* 1559.48. Found 1582.05 ($M+Na^+$), 1598.00 ($M+K^+$). Anal. calcd for $C_{83}H_{84}N_5O_{19}Cl_3$: C, 63.82; H, 5.42; N, 4.48; Cl, 6.81. Found: C, 63.86; H, 5.27; N, 4.11; Cl, 7.08.

4.1.8. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)-2-azido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 14. Glycosylation of **6** (143 mg, 0.22 mmol) with **2** (170 mg, 0.18 mmol) was performed using Cp_2ZrCl_2 (80 mg, 0.27 mmol) and $AgClO_4$ (113 mg, 0.55 mmol) in anhydrous CH_2Cl_2 (10 ml) as described for the synthesis of **7**. Chromatography of the crude product on silica gel with toluene/EtOAc (85:15) gave **14** (186 mg, 65%). $[\alpha]_D$ +30.5 (*c*, 1.4). R_f 0.34 (7:3 toluene/EtOAc). ^1H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.62 (d, 1H, *J*=7.3 Hz, Ar), 7.47–7.18 (m, 34H, Ar), 6.98 (d, 1H, *J*=7.7 Hz, –N*H*), 5.93 (m, 1H, –CH_2CH=CH_2), 5.65 (d, 1H, *J*=9.0 Hz, –N*H*), 5.45 [s, 1H, PhC*H*$(O)_2$], 5.35 (br d, 1H, *J*=16.1 Hz, –CH=CH_2), 5.25 (br d, 1H, *J*=10.2 Hz, –CH=CH_2), 5.17 (d, 1H, *J*=10.5 Hz, –CH_2Ph), 4.94 (d, 1H, *J*=7.8 Hz, H-1b), 4.89 (d, 1H, *J*=3.4 Hz, H-1a), 3.38 (dd, 1H, *J*=3.7, 9.8 Hz, H-3c), 2.99 (br s, 1H, H-5c) 1.29 (d, 3H, *J*=6.3 Hz, Thr-γH); ^{13}C NMR ($CDCl_3$): δ 92.4 (–CO*C*Cl_3), 99.2 ($^1J_{CH}$=170.0 Hz, C-1a), 99.6 ($^1J_{CH}$=161.8 Hz, C-1b), 101.2 [Ph*C*H$(O)_2$], 102.8 ($^1J_{CH}$=165.9 Hz, C-1c). MALDI TOF MS: calcd for $C_{84}H_{86}N_5O_{19}Cl_3$ *m/z* 1573.49. Found 1596.38 ($M+Na^+$), 1617.36 ($M+K^+$). Anal. calcd for $C_{84}H_{86}N_5O_{19}Cl_3$: C, 64.02; H, 5.50; N, 4.44; Cl, 6.75. Found: C, 64.12; H, 5.48; N, 4.06; Cl, 6.58.

4.1.9. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 15. As described for **9**, compound **13** (139 mg, 0.09 mmol) was treated with Zn and AcOH to dechlorinate the trichloroacetamido group and to reduce the azido group. The crude product was stirred with Ac_2O (0.3 ml) in a mixture of CH_2Cl_2 (10 ml) and MeOH (5 ml) at room temperature for 1 h. The mixture was concentrated in vacuo. The residue was dissolved in $CHCl_3$, washed with satd $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product chromatographed on silica gel with $CHCl_3$/MeOH (97:3) afforded **15** (116 mg, 88%). $[\alpha]_D$ +43.3 (*c*, 0.5). R_f 0.45 (95:5 $CHCl_3$/MeOH). ^1H NMR: δ 7.72 (d, 2H, *J*=7.3 Hz, Ar), 7.61 (br t, 2H, *J*=6.5 Hz, Ar), 7.47 (m, 2H, Ar), 7.34–7.18 (m, 32H, Ar), 6.22 (d, 1H, *J*=7.8 Hz, –N*H*), 5.98 (d, 1H, *J*=7.1 Hz, –N*H*), 5.85 (m, 1H, –CH_2CH=CH_2), 5.43 [s, 1H, PhC*H*$(O)_2$], 5.39 (d, 1H, *J*=9.3 Hz, –N*H*), 5.29 (br d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.20 (br d, 1H, *J*=10.0 Hz, –CH=CH_2), 1.89 (s, 3H, Ac), 1.80 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$): δ 98.5 (C-1a), 100.3 (C-1b), 101.2 [Ph*C*H$(O)_2$], 102.9 (C-1c). MALDI TOF MS: calcd for $C_{85}H_{91}N_3O_{20}$ *m/z* 1473.62. Found 1496.59 ($M+Na^+$), 1512.62 ($M+K^+$). Anal. calcd for $C_{85}H_{91}N_3O_{20}$: C, 69.23; H, 6.22; N, 2.85. Found: C, 69.07; H, 6.18; N, 2.74.

4.1.10. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 16. As described for **9**, compound **14** (175 mg, 0.11 mmol) was treated with Zn and AcOH to dechlorinate the trichloroacetamido group and to reduce the azido group. Subsequent acetylation in CH_2Cl_2/MeOH and purification by chromatography on silica gel with $CHCl_3$/MeOH (97:3) afforded **16** (130 mg, 79%). $[\alpha]_D$ +36.1 (*c*, 1.5). R_f 0.45 (95:5 $CHCl_3$/MeOH). ^1H NMR: δ 7.75 (d, 2H, *J*=7.1 Hz, Ar), 7.60 (br d, 2H, *J*=7.3 Hz, Ar), 7.36 (m, 2H, Ar), 7.31–7.21 (m, 32H, Ar), 5.84 (m, 2H, –N*H*, –CH_2CH=CH_2), 5.53 (d, 1H, *J*=9.3 Hz, –N*H*), 5.46 (d, 1H, *J*=9.3 Hz, –N*H*), 5.43 [s, 1H, PhC*H*$(O)_2$], 5.29 (br d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.23 (br d, 1H, *J*=10.2 Hz, –CH=CH_2), 1.94 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.24 (d, 3H, *J*=6.3 Hz, Thr-γH); ^{13}C NMR ($CDCl_3$): δ 99.9 (C-1a), 100.5 (C-1b), 101.2 [Ph*C*H$(O)_2$], 103.0 (C-1c). MALDI TOF MS: calcd for $C_{86}H_{93}N_3O_{20}$ *m/z* 1487.63. Found 1510.57 ($M+Na^+$), 1526.64 ($M+K^+$). Anal. calcd for $C_{86}H_{93}N_3O_{20}$: C, 69.39; H, 6.30; N, 2.82. Found: C, 69.26; H, 6.26; N, 2.87.

4.1.11. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-serine 17. Compound **15** (102 mg, 0.07 mmol) was deallylated with $Pd(PPh_3)_4$ (5 mg, 4.3 μmol) and 5,5-dimethyl-1,3-cyclohexanedione (200 mg, 1.43 mmol), and in anhydrous THF (10 ml) as described for **11**. Chromatography of the crude product on silica gel with $CHCl_3$/MeOH (95:5) and then with $CHCl_3$/MeOH/AcOH (92:8:1) gave **17** (94 mg, 95%). $[\alpha]_D$ +42.9 (*c* 0.5). R_f 0.09 (95:5:1

$CHCl_3$/MeOH/AcOH). ^{1}H NMR (DMSO-d_6): δ 12.86 (br, 1H, –CO_2H), 7.87 (d, 2H, J=7.6 Hz, Ar), 7.70 (d, 2H, J=7.3 Hz, Ar), 7.61–7.17 (m, 37H, Ar, N*H*), 5.64 [s, 1H, PhC*H*(O)$_2$], 5.09 (d, 1H, J=11.0 Hz, –CH_2Ph), 1.83 (s, 3H, Ac), 1.80 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$/CD_3OD): δ 98.1 (C-1a), 100.8 (C-1b), 100.9 [Ph*C*H(O)$_2$], 102.5 (C-1c). MALDI TOF MS: calcd for $C_{82}H_{87}N_3O_{20}$ *m*/*z* 1433.58. Found 1456.47 (M+Na$^+$). Anal. calcd for $C_{82}H_{87}N_3O_{20}$: C, 68.65; H, 6.11; N, 2.93. Found: C, 68.35; H, 6.01; N, 2.94.

4.1.12. *N*-(Fluorenylmethoxycarbonyl)-*O*-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2-acetamido-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl]-L-threonine 18. Compound **16** (125 mg, 0.08 mmol) was deallylated with $Pd(PPh_3)_4$ (5 mg, 4.3 μmol) and 5,5-dimethyl-1,3-cyclohexanedione (200 mg, 1.43 mmol), and in anhydrous THF (10 ml) as described for **11**. Chromatography of the crude product on silica gel with $CHCl_3$/MeOH (95:5) and then with $CHCl_3$/MeOH/AcOH (92:8:1) gave **18** (122 mg, 99%). $[\alpha]_D$ +48.5 (*c* 1.0). R_f 0.12 (95:5:1 $CHCl_3$/MeOH/AcOH). ^{1}H NMR (DMSO-d_6): δ 12.88 (br, 1H, –CO_2H), 7.93–7.87 (m, 3H, –N*H*, Ar), 7.72 (d, 2H, J=7.3 Hz, Ar), 7.58–7.19 (m, 36H, Ar, N*H*), 5.64 [s, 1H, PhC*H*(O)$_2$], 5.09 (d, 1H, J=11.0 Hz, –CH_2Ph), 4.80 (d, 1H, J=4,6 Hz, H-1a), 1.86 (s, 3H, Ac), 1.82 (s, 3H, Ac), 1.12 (d, 3H, J=6.1 Hz, Thr-γH). MALDI TOF MS: calcd for $C_{83}H_{89}N_3O_{20}$ *m*/*z* 1447.60. Found 1470.28 (M+Na$^+$), 1486.28 (M+K$^+$). Anal. calcd for $C_{83}H_{89}N_3O_{20}\cdot H_2O$: C, 67.97; H, 6.25; N, 2.87. Found: C, 68.06; H, 6.08; N, 2.81.

4.1.13. Glycopeptide 19. Commercial Fmoc-CLEAR amide resin (2.08 g, 0.1 mmol) was subjected to an automated synthesis of the peptide to produce a henicosapeptide (TTPITTTTTTVTPTPTPTGTQT)-resin by the *Fastmoc* program of the synthesizer, using 20% piperidine/NMP for N-deprotection and HBTU/HOBt as the condensing agent. *t*-Bu and Trt groups were employed for the protection of Thr and Gln, respectively. A part of the peptide resin (25 μmol) was transferred into a polypropylene test tube, *N*-deprotected by stirring with a vortex mixer with 20% piperidine/NMP (1.5 ml) for 10 min, and then thoroughly washed with NMP. This procedure was conducted several times to complete the N-deprotection. Glycoamino acid **12** (72 mg, 0.05 mmol) was pre-activated with 0.45 M HBTU/HOBt/DMF (1:1 HBTU/HOBt, 111 μl, 50 μmol) and 2 M DIEA/NMP (26 μl, 52 μmol) in NMP (0.3 ml) at room temperature for 10 min, and added to the reaction vessel of the *N*-deprotected peptide resin with NMP (0.5 ml). The mixture was stirred overnight with the vortex mixer at 50 °C in an oven. The resin was washed with NMP and *N*-deprotected with 20% piperidine/NMP as already mentioned. The *N*-terminal proline residue was manually attached with Fmoc-Pro-OH (34 mg, 100 μmol). Condensation was performed at room temperature for 1 h with the same activating agents. Since the ninhydrin test showed incomplete attachment of the proline, the manual procedure was repeated. The resin was successively washed with NMP and CH_2Cl_2, and dried in vacuo to afford **21** (137 mg). Experiments to isolate the synthetic glycopeptide were undertaken with part of resin **21** (72 mg). To the resin in a polypropylene tube was added reagent K (33:2:2:2:1 TFA/phenol/water/thioanisole/ethanedithiol, 1.5 ml), and the mixture was stirred with the vortex mixer at room temperature for 1 h. Then the volatile materials in the mixture were evaporated in a stream of N_2. Ether was added to the residue to precipitate the product, which was separated by centrifugation. The precipitate was washed several times by suspending in ether and then centrifuging to give a mixture of the resin and the crude product, to which was added a mixture of TFA/DMS/*m*-cresol (5:3:1, 1.08 ml), and the mixture was cooled at −10 °C. TfOH (120 μl) was added to the mixture and the plastic vessel was shaken in the cooling bath. The reaction mixture was left at −10 °C for 2 h, before the reaction was terminated by the addition of ether. The mixture was centrifuged to separate the debenzylated product, which was washed three times with ether and centrifuged as already mentioned to give a precipitate. The crude product was dissolved in distilled water, separated from the resin by filtration through a membrane filter, and purified by preparative HPLC. The major fraction was collected and lyophilized to afford glycopeptide **19** (1.4 μmol, 12.2% overall yield based on the amino acid analysis). MALDI TOF MS: calcd for $C_{136}H_{213}N_{27}O_{55}$ *m*/*z* 3104.47. Found 3127.20 (M+Na$^+$). Amino acid analysis: $Thr_{12.6}Glu_{1.21}Pro_{4.29}Gly_{1.00}Val_{1.34}Ile_{0.78}$. MS for peak 2: calcd for $C_{122}H_{190}N_{26}O_{45}$, *m*/*z* 2739.34. Found 2762.77 (M+Na$^+$). MS for peak 3: calcd for $C_{110}H_{170}N_{24}O_{38}$, *m*/*z* 2435.21. Found 2458.15 (M+Na$^+$).

4.1.14. Glycopeptide 20. The heptapeptide (TLATSSR)-linked resin was synthesized from Fmoc-CLEAR amide resin (2.08 g, 0.1 mmol) with an automated synthesizer, as mentioned for **19**. A part of the peptide resin (18 μmol) was used for the condensation with **18** (59 mg, 41 μmol) in the presence of 0.45 M HBTU/HOBt/DMF (96 μl, 43 μmol) and 2 M DIEA/NMP (26 μl, 52 μmol) in NMP (0.8 ml) at 50 °C overnight. The soluble materials in the mixture were filtered off. After being thoroughly washed with NMP and CH_2Cl_2, the resulting resin was again reacted with **18** (59 mg) in the same manner. The following five amino acids were also manually introduced at room temperature for 1 h by the successive reactions two times with Fmoc-His(Trt)-OH (45 mg, 72 μmol), Fmoc-Ala-OH (22 mg, 72 μmol), Fmoc-Val-OH (24 mg, 72 μmol), Fmoc-Pro-OH (24 mg, 72 μmol), and Fmoc-Thr(*t*-Bu)-OH (29 mg, 72 μmol). Then glycoserine **17** (60 mg, 42 μmol) was attached in a similar procedure as used for the reaction with **18**. Finally, N-terminal threonine residue was condensed by reaction with Fmoc-Thr(*t*-Bu)-OH (29 mg, 72 μmol) to give glycopeptide-resin **21** (117 mg). Resin **21** (57 mg) was stirred with reagent K (1 ml) at room temperature for 1 h before evaporating the volatile materials in a stream of N_2. The product and resin were precipitated by addition of ether and centrifugation. The precipitate was washed three times with ether and then dried in vacuo. To the precipitate was added a mixture of TFA/DMS/*m*-cresol (5:3:1, 0.63 ml). The mixture was cooled at −10 °C for 30 min, then shaken with TfOH (70 μl) and left for 2 h at that temperature. The reaction was worked up as described for the preparation of **19**, and the product was purified by preparative HPLC to afford **20** (1.5 μmol, 17.2% overall yield based on the amino acid analysis). MALDI TOF MS: calcd for $C_{122}H_{191}N_{25}O_{55}$ *m*/*z* 2886.29. Found 2887.19 (M+H$^+$). Amino acid analysis: $Thr_{4.67}Ser_{2.77}Pro_{0.93}Ala_{2.02}Val_{1.55}Leu_{1.00}His_{1.00}Arg_{1.01}$. MS

for peak 2: calcd for $C_{108}H_{168}N_{24}O_{45}$ *m/z* 2521.16. Found 2522.26 ($M+H^+$). MS for peak 3: calcd for $C_{108}H_{168}N_{24}O_{45}$ *m/z* 2521.16. Found 2522.29 ($M+H^+$). MS for peak 4: calcd for $C_{94}H_{145}N_{23}O_{35}$ *m/z* 2156.03. Found 2157.04 ($M+H^+$). MS for peak 5: calcd for $C_{129}H_{197}N_{25}O_{55}$ *m/z* 2976.34. Found 2977.37 ($M+H^+$).

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