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Synthesis of biantennary LacNAc-linked *O*-glycan (core 4) and glycopeptide thioester by benzyl protection strategy: rapid zinc reduction of GlcNTCA to GlcNAc by microwave irradiation

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Abstract—A synthetic method for the core 4 *O*-glycan-linked Ser and Thr was developed. Highly stereoselective 3-O- and 6-O-glycosylation was achieved by using two distinctively protected *N*-trichloroacetyllactosaminyl fluorides (**3** and **12**). Microwave-assisted Zn reduction rapidly and efficiently converted *N*-trichloroacetylglucosamine (GlcNTCA) to *N*-acetylglucosamine (GlcNAc). In order to demonstrate the usefulness of the protected core 4 *O*-glycan a segment (Gly^{34}-Gly^{58}) of emmprin (extracellular matrix metalloproteinase inducer), a cancer metastasis-related glycoprotein, was synthesized by the solid-phase method, utilizing the pentasaccharyl Thr (**2**) to introduce an *O*-glycan in place of the native *N*-glycan at Asn^{44}.

1. Introduction

Mucins and their *O*-glycans are of great importance and interest in a number of biological processes. Aberrant features of neoplastic mucins, such as overexpression and altered glycosylation, have attracted particular attention in connection with metastasis.[1] However, only limited knowledge of the biological roles of the alteration in mucins has been obtained so far. By considering the inaccessibility of a homogeneous mucin sample from natural sources, we have studied a synthetic approach to the glycoproteins with *O*-glycan, and recently established an original protocol using the benzyl-protected glycoamino acid building blocks in solid-phase glycopeptide synthesis.[2] In a previous study, we have synthesized the core 3 and core 6 oligosaccharides by glycosylating either the 3- or 6-hydroxyl group of the core *N*-acetylgalactosamine precursor with an *N*-trichloroacetyllactosaminyl glycosyl donor of high reactivity and β-selectivity. Usefulness of the synthetic *O*-glycan building blocks was demonstrated by the synthesis of MUC2 and MUC6 related glycopeptides.[3]

The *N*-acetylglucosaminyl substitution at both 3- and 6-position gives another core class *O*-glycan, known as core 4, which has been identified in the oligosaccharides from human bronchial mucins of cystic fibrosis patients,[4] secreted mucins of a human colonic cancer cell line,[5] human meconium mucins,[6] and sheep gastric mucins.[7] The core 4 oligosaccharides bearing the *N*-acetyllactosamine branches are of particular interest regarding an unanswered question, whether their physical, structural, and biological properties are different from those of the complex-type *N*-glycan as well as those of the core 2 *O*-glycan having an extension of *N*-acetyllactosamine to the core galactose residue.[6,8] To this end our investigations were directed to the synthesis of a glycopeptide with core 4 *O*-glycan. In this paper, we describe preparation of the core 4 glycoserine and glycothreonine building blocks, **1** and **2**, and performance of the solid-phase glycopeptide synthesis with **2** according to the established protocol.[9]

2. Synthesis of the building blocks 1 and 2

We first attempted selective di-O-glycosylation of 3,4,6-*O*-unmasked $GalN_3$–Thr derivative **4**[10] with known *N*-trichloroacetyllactosaminyl fluoride **3**[11] (2.2 equiv) by using $Cp_2ZrCl_2/AgClO_4$ as the promoter[12] in CH_2Cl_2 at −15 °C, since the 4-hydroxyl group of the $GalN_3$ residue was hardly glycosylated in many cases (Fig. 1).[3,10,11] This simple strategy, however, was unsuccessful and gave a complex mixture of a heptasaccharide and three pentasaccharides each in 5–14% yield after consuming the glycosyl fluoride for 3 h. As the second attempt, we reacted **3** and 6-*O*-silylated

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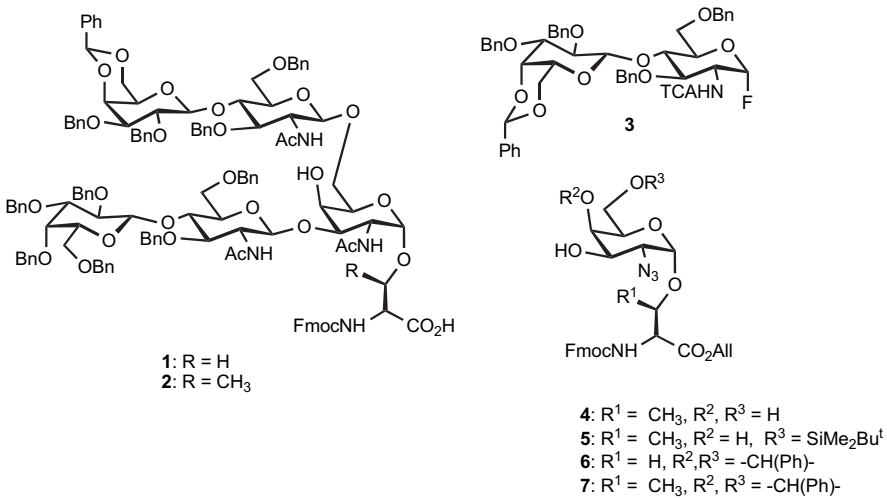
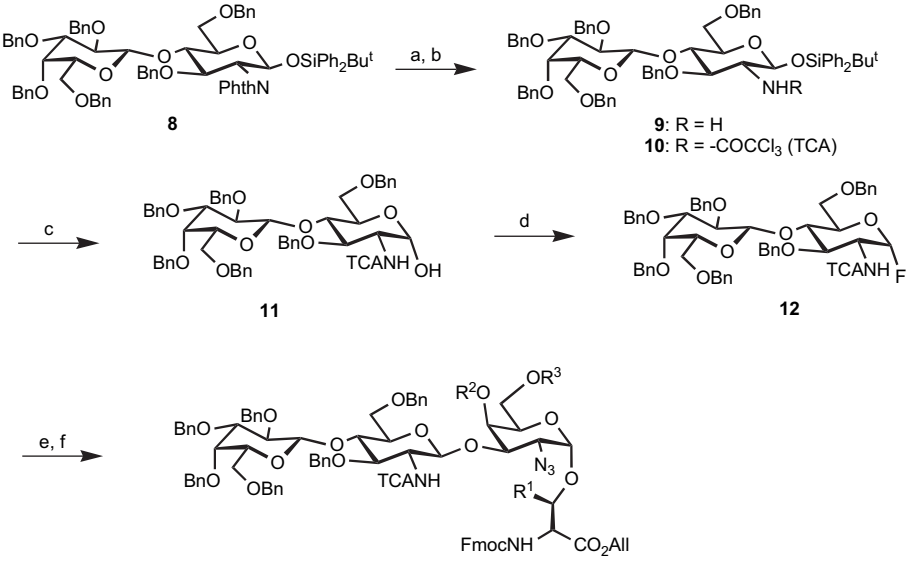


Figure 1. Structures of the protected core 4 pentasaccharyl Ser/Thr (**1** and **2**) and the known intermediates (**3**–**7**).

acceptor **5**[10] in expectation of attaining selective 3-O-glycosylation. But a pentasaccharide (12%) derived by 3,4-di-O-glycosylation was produced along with the desired trisaccharide (13%). In this reaction, an additional complication arose from the departure of the acid-labile silyl group of **5** under the reaction conditions. Thus, we were convinced that the side reaction on the 4-hydroxyl group was unavoidable, when this reactive glycosyl donor was used with the 3,4-unprotected glycosyl acceptors. In order to secure mono 3-O-glycosylation, we decided to use 4,6-*O*-benzylidene $GalN_3$–Ser/Thr derivatives, **6**[13] and **7**,[10] as the glycosyl acceptors, and instead needed a benzylidene group-free glycosyl donor that allowed selective deprotection of the 6-O position of the $GalN_3$ residue at a later stage. Thus, a perbenzylated *N*-trichloroacetyllactosaminyl fluoride was synthesized as an alternative to glycosyl donor **3**. Known lactosamine derivative **8**[14] was heated with ethylenediamine in *n*-BuOH to remove the *N*-phthaloyl group (Scheme 1). The resulting amine **9** was reacted with trichloroacetyl chloride in pyridine to give **10** (81% in two steps). Desilylation of **10** with *n*-Bu_4NF in THF in the presence of excess AcOH afforded hemiacetal **11**, which upon treatment with Et_2NSF_3 gave fluoride **12** (82% in two steps) as a mixture of anomers (α/β=19/1). Fluoride **12** seemed more reactive than **3**, and reacted with glycosyl serine **6** within 0.5 h by activation with $Cp_2Zr(ClO_4)_2$ at −15 °C to afford trisaccharide **13** as



Scheme 1. Synthesis of hexabenzylated glycosyl fluoride **12** and trisaccharyl serine/threonine, **15** and **16**. *Reaction conditions*: (a) 1,2-diaminoethane, *n*-BuOH, 90 °C, 2 days, 96%; (b) trichloroacetyl chloride, pyridine, 0 °C, 1.5 h, 84%; (c) *n*-Bu_4NF, AcOH, THF, room temperature, overnight, 89%; (d) diethylaminosulfur trifluoride, THF, 0 °C, 1 h, 92%; (e) **6** or **7**, Cp_2ZrCl_2, $AgClO_4$, CH_2Cl_2, −15 °C, 1 h, **13** (75%), **14** (80%); (f) 80% aq TFA, CH_2Cl_2, 94% (**15**), 83% (**16**).

the sole coupling product in 75% yield. Similarly, trisaccharyl threonine **14** was obtained from **7** and **12** in 80% yield. Stereochemistry of the newly generated linkage was evidenced by the $^{1}J_{CH}$ value (168.4 Hz for **13** and 165.9 Hz for **14**). Removal of the benzylidene group of **13** and **14** with 80% TFA led to 4,6-dihydroxy compounds, **15** and **16**.

Selective 6-O-glycosylation was first tested with glycosyl donor **12** and diol **16**. The reaction, however, again gave an unsatisfactory result. Two pentasaccharides were produced, when donor **12** and excess acceptor **16** (**12**/**16**=0.83) were reacted in CH_2Cl_2 at −15 °C for 1 h. 4-*O*-Glycosylated product **18** was obtained in a considerable amount (30%) along with the desired **17** (44%). The structures of **17** and **18** were determined after acetylation of the product samples (Fig. 2). The characteristic lower field shift of the H-4 signal ($GalN_3$) was observed at 5.36 ppm for the acetate derivative of **17**. The altered condition with the lowered temperature or with other solvents was ineffective for suppressing the 4-O-glycosylation. On the other hand, we had achieved 6-O-selective glycosylation to the desired extent by using glycosyl donor **3** in the synthesis of core 2 and core 6. Thus, condensation of **3** and **16** was next investigated. When **3** was reacted with excess **16** (**3**/**16**=0.83) at −20 °C for 2.5 h, the desired pentasaccharide **20** was produced in a moderate yield (40%) and acceptor **16** (54%) was recovered. In contrast, the use of excess donor (**3**/**16**=1.2) promoted better conversion of **16**, but resulted in formation of the byproducts, **22** and **24**. Through several attempts to reduce the side reactions, an improved yield (71%) of **20** was attained under the reaction of **3** and **16** (**3**/**16**=1.1) at −40 to −15 °C for 2 h. However, the formation of **22** (9%) and **24** (8%) could not be suppressed. Structures of the products were assigned by ^{1}H and ^{13}C NMR. The same reaction conditions were applied to the condensation of **3** and serine derivative **15** to produce **19** (71%), **21** (6%), and **23** (4%). With no further optimization, synthesis was forwarded using

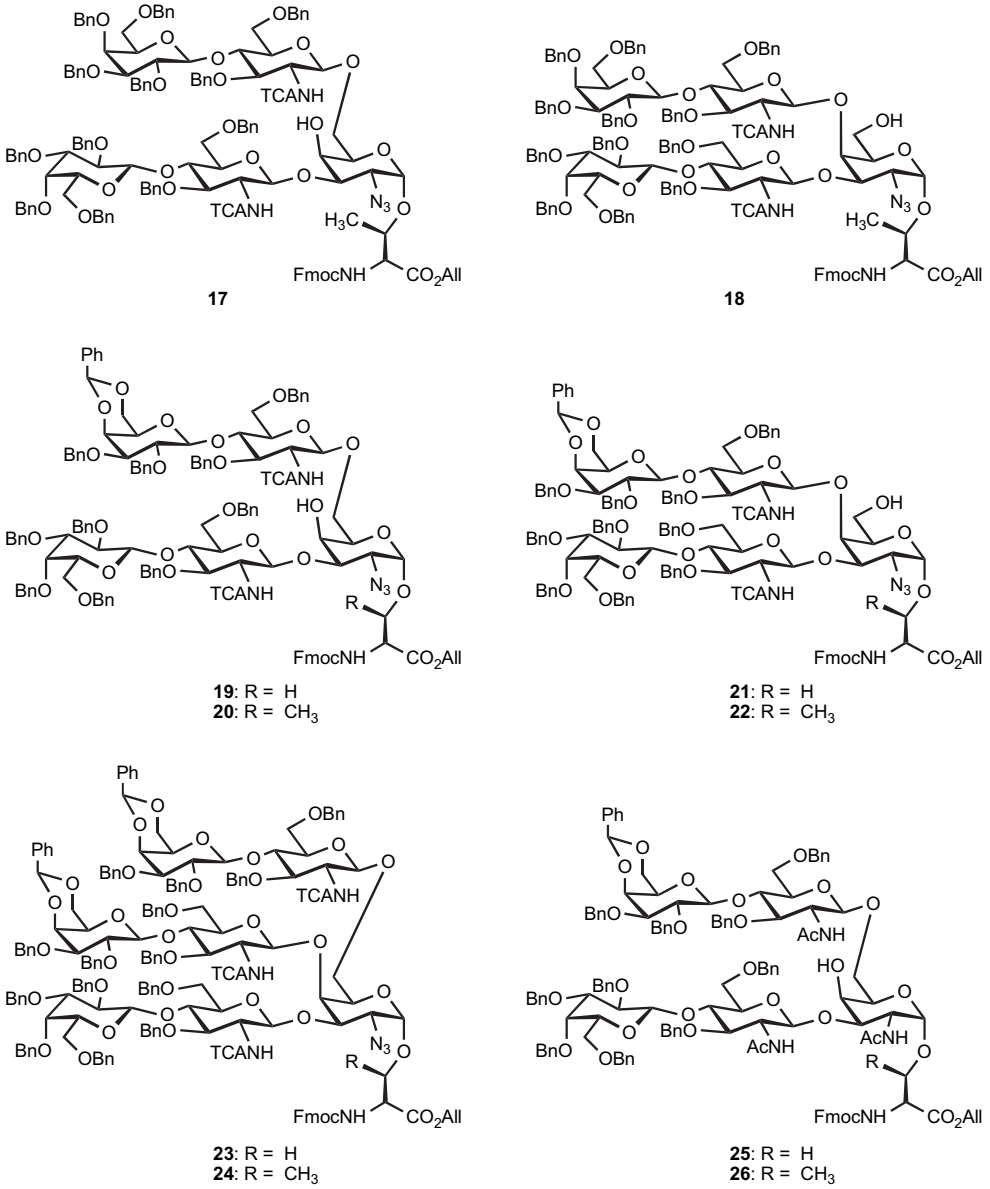


Figure 2. Structures of glycosylation products.

the obtained pentasaccharides **19** and **20**. In the previous studies, we have accomplished dechlorination of the trichloroacetamido group and simultaneous reduction of the azido group with large excess of Zn and AcOH. Despite giving a fairly good yield, the tedious procedure of the reaction was a drawback. Intermittent additions of Zn powder and AcOH to the reactant in CH_2Cl_2 were necessary at 3–5 h intervals during 1–2 days until completion of the dechlorination. In addition, $Zn(OAc)_2$ resulted in a large quantity, which gave some difficulty in the following acetylation of the crude reduction product. In recent years, acceleration of reactions by microwave irradiation has received considerable attention.[15] The apparent effectiveness reported for the reactions in a heterogeneous system prompted us to investigate the Zn reduction under microwave. We examined several conditions by varying the amount of the reductant, microwave power, reaction time, and solvent with a model monosaccharide as well as **19**. After optimization, the best result was obtained with a reduced amount of Zn by microwave heating gently at refluxing temperature in EtOAc for 30 min. The reduction products derived from **19** and **20** were then acetylated with Ac_2O in CH_2Cl_2/MeOH to give **25** (81%) and **26** (80%), respectively. To fulfill the synthesis of the building blocks suitable for the solid-phase synthesis of glycopeptide, **25** and **26** were converted to carboxylic acids **1** (93%) and **2** (92%) by deallylation with $Pd(PPh_3)_4$ and 5,5-dimethyl-1,3-cyclohexanedione in THF.

3. Solid-phase synthesis of glycopeptide

As a suitable platform that allows us to test the effectiveness of the benzyl and benzylidene-protected core 4 building blocks in the solid-phase synthesis, the chosen model glycopeptide was a glycosylated pentacosapeptide thioester that mimics a segment (Gly^{34}-Gly^{58}) of the extracellular matrix metalloproteinase inducer (emmprin). Emmprin is produced by tumor cells and is thought to function in the early stage of metastasis. The native emmprin carries an *N*-linked oligosaccharide at the Asn^{44} site, and the first Ig domain (Gly^{34}-Met^{94}) (Fig. 3) including the *N*-glycan is responsible for the inducer activity. Recently, we have established an efficient synthetic route to the first Ig domain and its extended peptide bearing chitobiosyl or core pentasaccharyl motif of the *N*-glycan by taking advantage of the thioester method of segment condensation.[16] In the present study, building block **2** was introduced into the significant segment in place of the *N*-glycan at the Asn^{44} position. The synthetic imitation would be helpful to explore the structural requirement of the oligosaccharide attached to the bioactive Ig domain, since the biantennary LacNAc in the core 4 *O*-glycan pentasaccharide resembles the peripheral structure of the complex-type *N*-glycan.

Glycopentacosapeptide **27**, which carries a thioester functionality necessary for the segment condensation in the Ig domain synthesis, was synthesized from commercial Fmoc-CLEAR-amide resin (Scheme 2). Attachment of Fmoc–Gly–$SCH_2CH_2CO_2H$ to the *N*-deprotected resin was followed by removal of the Fmoc group using a cocktail of 1-methylpyrrolidine (25%), hexamethyleneimine (2%), and 1-hydroxybenzotriazole (2%) in NMP/DMSO (1:1).[17] According to the reported procedure,[16b] the tripeptide-resin was prepared by coupling first with N^6-benzyloxycarbonyl-N^2-triisopropylsilyloxycarbonyl-lysine pentafluorophenyl ester [(Tsoc)–Lys(*Z*)–OPfp] and then with Fmoc–Leu–F in the presence of tetrabutylammonium fluoride. This two-step protocol is indispensable to get rid of the side reaction caused by a diketopiperazine formation in the early stage of thioester synthesis. Further elongation with 11 amino acids was performed with an automated peptide synthesizer using Fmoc amino acids activated with HBTU, HOBt, and DIEA. All the N-deprotection steps were carried out with the above cocktail under the automated program. Benzyloxycarbonyl group (*Z*), *tert*-butoxycarbonyl group (Boc), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl group (Pbf), and triphenylmethyl group (Trt) were employed for protection of the functional side chain amino groups of

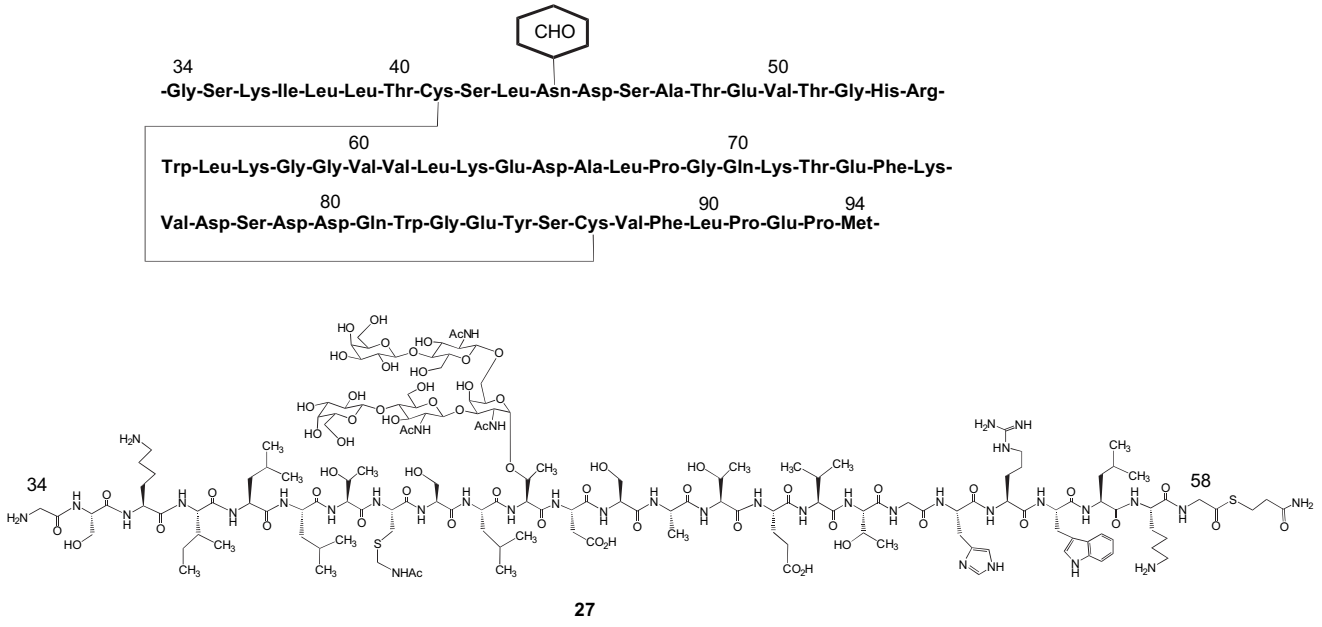
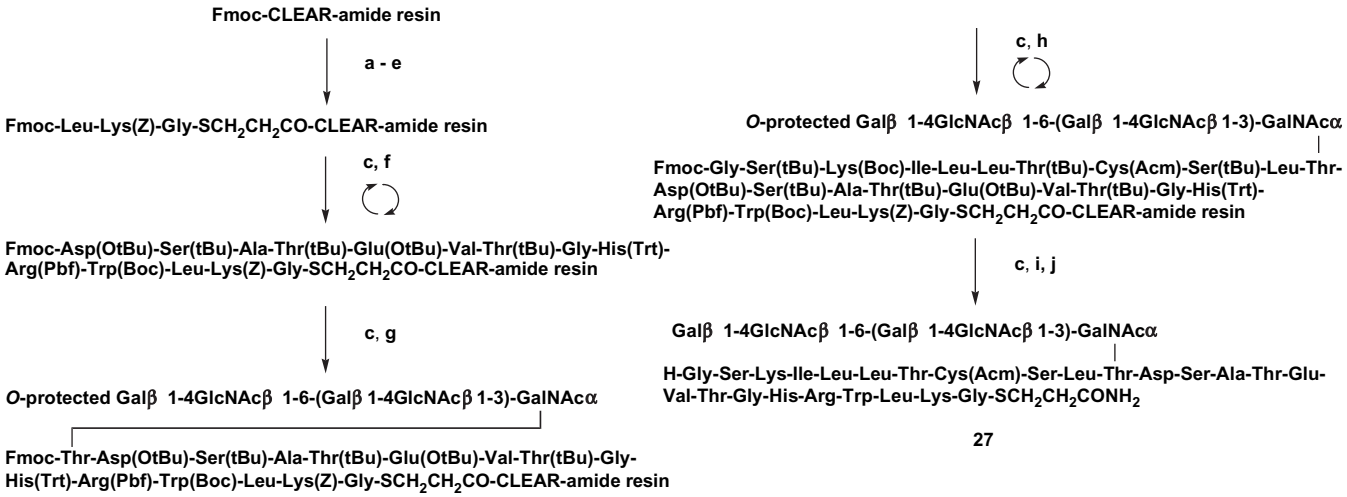


Figure 3. Structures of the emmprin Ig domain (Gly34-Met94) and synthetic glycopeptide **27**.



Scheme 2. Solid-phase synthesis of glycopeptide **27**. *Reaction conditions*: (a) 20% piperidine/NMP; (b) Fmoc–Gly–$SCH_2CH_2CO_2H$, DCC/HOBt/NMP; (c) 1-methylpyrrolidine/hexamethyleneimine/1-hydroxybenzotriazole/NMP/DMSO; (d) Tsoc–Lys(Z)–OPfp; (e) Fmoc–Leu–F, TBAF, CH_2Cl_2; (f) Fmoc-amino acid, HBTU, HOBt, DIEA, ABI 433A peptide synthesizer, FastMoc program; (g) **2**, DCC/HOBt/NMP, 50 °C, 3 h; (h) Fmoc amino acid, DCC/HOBt/NMP, 50 °C; (i) TFA/phenol/thioanisole/EDT/H_2O, room temperature, 2 h; (j) DMS/TFA/*m*-cresol, −15 °C, then TfOH, 2 h.

Lys, Trp, Arg, and His, respectively, whereas the *tert*-butyl group (^{t}Bu) was used for masking the hydroxyl groups of Ser and Thr, and the carboxyl groups of Glu and Asp residues. These protecting groups are readily removed under acidic conditions. The *N*-deprotected tetradecapeptide-resin (25 μmol) was coupled with **2** (2 equiv) by manual operation using DCC and HOBt at 50 °C for 3 h in a polypropylene vessel with stirring by a vortex mixer. Among the tested conditions including other condensing agents, the combination of DCC and HOBt gave the most acceptable coupling efficiency. The coupling was monitored by HPLC and MS analyses of the glycopeptide detached from the resin sample. The glycopeptide chain was further elongated manually to complete the pentacosapeptide synthesis. For persistent protection of the thiol group of Cys, an acetamidomethyl group (Acm) was employed. According to our standard protocol for isolation of synthetic glycopeptide, a series of deprotection procedures were conducted. The resin was treated with reagent K (aq CF_3CO_2H, thioanisole, 1,2-ethanedithiol, and phenol)[18] for 2 h to split the glycopeptide from the resin. The crude product was thoroughly debenzylated under the 'low-acidity TfOH' conditions[19] at −15 °C for 2 h and then purified by HPLC. Figure 4 shows that the desired product **27** was obtained as the major product (peak 1). The structure of the synthesized glycopeptide thioester was evidenced by MALDI TOF MS [*m*/*z* 3764.95 ($M+H^+$)]. A byproduct eluted in the less mobile fraction (peak 2) was assigned as a glycopeptide lacking a LacNAc moiety [*m*/*z* 3399.62 ($M+H^+$)]. Although the resulting glycan structure of the latter glycopeptide has not been elucidated, it is probable that an acid-labile 6-*O*-glycosidic linkage might have been cleaved as observed in the synthetic glycopeptide carrying core 6 *O*-glycans.[3] The overall yield of purified **27** was deduced to be 2.2% from the data of amino acid analysis of the acid-hydrolyzed sample, and the value is comparable to that obtained previously for the synthetic *N*-glycan-linked glycopeptide.[16b] The LacNAc-missing glycopeptide was also isolated in 1.3% overall yield. The isolated **27** (total

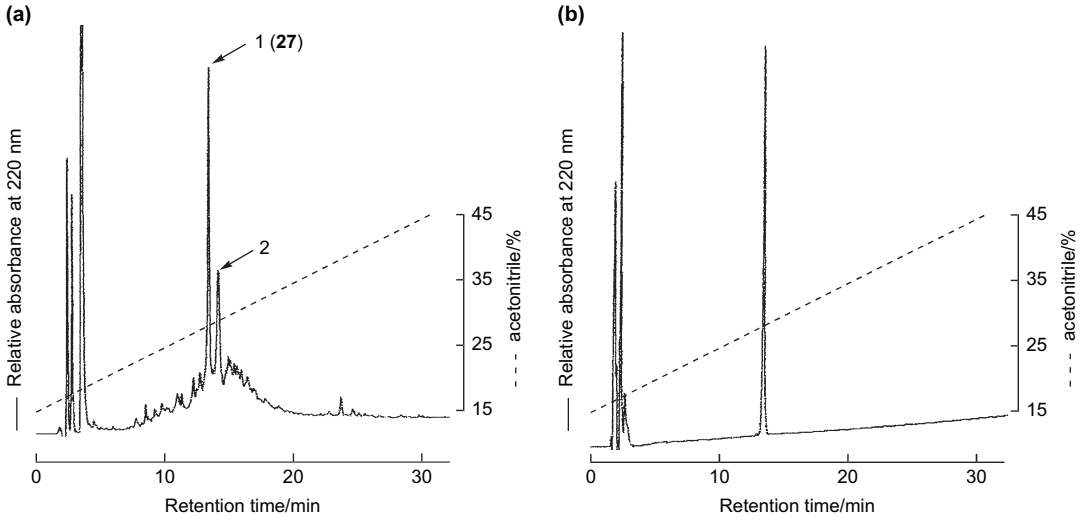


Figure 4. HPLC profiles of synthetic glycopeptide **27** (a) and isolated **27** (b). Conditions: column, Mightysil RP-18, 4.6×150 mm (5 μm); eluant A, distilled water containing 0.1% TFA; eluant B, acetonitrile containing 0.1% TFA; flow rate, 1 ml/min.

0.6 μmol, Fig. 4) is a useful intermediate to synthesize an *O*-glycan analog of the emmprin first Ig domain, through *N*-*tert*-butoxycarbonylation, condensation with the known segment (Gly^{59}-Met^{94}), and disulfide formation.

In summary, the biantennary LacNAc-linked *O*-glycosyl Ser and Thr were synthesized both in a benzyl and benzylidene-protected form by using two distinctively protected LacNTCA glycosyl donors. The dechlorination of the trichloroacetamido group by Zn/AcOH reduction was greatly accelerated by employing microwave irradiation. By the solid-phase synthetic procedure suited for the labile Fmoc peptide thioester, the glycothreonine derivative was successfully introduced into a glycopeptide, which mimics the glycosylated segment in the metastasis-related emmprin first Ig domain. The benzyl-protecting groups of the glycan moiety were readily removed under the low-acidity TfOH conditions to complete the desired glycopeptide thioester. Synthesis toward the 61 amino acid structure for the Ig domain and bioassay with the glycan-mutated sample will be reported in due course.

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). 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 (400 MHz) and ^{13}C (100 MHz)]. Chemical shifts are expressed in parts per million downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. For assignment of the signals of pentasaccharides, the reducing terminal residue GalNAc is described as a. GlcNAc and Gal residues in the 3-*O*-substituent of GalNAc are described as b and c, respectively, while GlcNAc and Gal residues in the 6(or 4)-*O*-substituent of GalNAc are shown as d and e. For heptasaccharides **23** and **24**, GlcNAc and Gal residues in the 6-*O*-substituent of GalNAc are described as f and g, respectively. MALDI TOF mass spectra were obtained with a PerSeptive Voyager-DE PRO spectrometer (2,5-dihydroxybenzoic acid was used as a matrix). Microwave irradiation was carried out with a CEM *Discover* microwave reactor. Automated solid-phase peptide synthesis was performed with Applied Biosystems Model 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 with 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. Due to the thermolabile azide group, N analysis values of compounds **13**, **14**, and **15** showed some inconsistency. 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. *tert*-Butyldiphenylsilyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 9. A mixture of **8** (703 mg, 0.56 mmol) and 1,2-diaminoethane (0.75 ml, 11.2 mmol) in *n*-BuOH (25 ml) was heated at 90 °C with stirring for 2 days under Ar, before being concentrated in vacuo. The residue was extracted with $CHCl_3$, successively washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with $CHCl_3$ to give **9** (603 mg, 96%). $[\alpha]_D$ −5.3 (*c* 1.0). R_f 0.43 (1:1 hexane/EtOAc). ^1H NMR: δ 7.71–7.67, 7.49–7.10 (m, 40H, Ar), 5.16 (d, 1H, *J*=10.5 Hz, –C*H*$_2$Ph), 4.97 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.76–4.69 (m, 4H, 4× –C*H*$_2$Ph), 4.58–4.47 (m, 2H, 2× –C*H*$_2$Ph), 4.45 (d, 1H, *J*=7.8 Hz, H-1b), 4.39 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.37 (d, 1H, *J*=7.1 Hz, H-1a), 4.36 (d, 1H, *J*=12.2 Hz, –C*H*$_2$Ph), 4.26 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.20 (d, 1H, *J*=12.2 Hz, –C*H*$_2$Ph), 4.01 (t, 1H, *J*=9.3 Hz, H-4a), 3.91 (d, 1H, *J*=2.4 Hz, H-4b), 3.72 (t, 1H, *J*=8.1 Hz, H-2b), 3.67 (dd, 1H, *J*=2.9, 11.2 Hz, H-6a), 3.53 (m, 1H, H-6a), 3.43–3.37 (m, 3H, H-3b, H-5b, and H-6b), 3.26–3.21 (m, 2H, H-3a and H-6b), 2.91–2.98 (m, 2H, H-2a and H-5a), 1.10 (s, 9H, ^{t}Bu). ^{13}C NMR: δ 102.4 (C-1b), 98.8 (C-1a). MALDI TOF MS calcd for $C_{70}H_{77}NO_{10}Si$ *m*/*z*: 1119.53. Found: 1142.75 (M+Na^+). Anal. Calcd for $C_{70}H_{77}NO_{10}Si$: C, 75.04; H, 6.93; N, 1.25. Found: C, 75.01; H, 6.83; N, 1.14.

4.1.2. *tert*-Butyldiphenylsilyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside 10. To an ice-cooled solution of **9** (3.43 g, 3.06 mmol) in pyridine (40 ml) was added CCl_3COCl (513 μl, 4.06 mmol). The mixture was stirred for 1.5 h at 0 °C, and then concentrated in vacuo. The residue was extracted with $CHCl_3$, washed successively with water and brine, dried over Na_2SO_4, and concentrated in vacuo. Chromatography of the crude on silica gel with hexane/EtOAc (4:1) afforded **10** (3.23 g, 84%). $[\alpha]_D$ −3.3 (*c* 1). R_f 0.66 (1:1 hexane/EtOAc). ^1H NMR: δ 7.69–7.62, 7.39–7.10 (m, 40H, Ar), 6.88 (d, 1H, *J*=7.8 Hz, –N*H*), 4.95 (d, 1H, *J*=10.5 Hz, –C*H*$_2$Ph), 4.94 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.89 (d, 1H, *J*=7.1 Hz, H-1a), 4.76–4.66 (m, 4H, 4× –C*H*$_2$Ph), 4.54 (d, 1H, *J*=10.5 Hz, –C*H*$_2$Ph), 4.52 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.44 (d, 1H, *J*=7.6 Hz, H-1b), 4.37 (d, 1H, *J*=12.2 Hz, –C*H*$_2$Ph), 4.33 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.23 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.22 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.04 (t, 1H, *J*=8.8 Hz, H-4a), 3.89 (d, 1H, *J*=2.4 Hz, H-4b), 3.83 (t, 1H, *J*=7.8 Hz, H-3a), 3.77 (dd, 1H, *J*=7.8, 9.8 Hz, H-2b), 3.71 (dd, 1H, *J*=7.8, 9.8 Hz, H-2b), 3.66 (dd, 1H, *J*=3.4, 11.2 Hz, H-6a), 3.41 (dd, 1H, *J*=2.9, 9.8 Hz, H-3b), 3.40–3.37 (m, 1H, H-5b), 3.33–3.29 (m, 2H, H-6a and H-6b), 3.07–3.05 (m, 1H, H-5a), 1.05 (s, 9H, ^{t}Bu). ^{13}C NMR: δ 161.2 (Cl_3C*C*ONH), 102.7 (C-1b), 94.8 (C-1a), 92.5 (–*C*Cl_3). MALDI TOF MS calcd for $C_{72}H_{76}Cl_3NO_{11}Si$ *m*/*z*: 1263.43. Found: 1286.26 (M+Na^+). Anal. Calcd for $C_{72}H_{76}Cl_3NO_{11}Si$: C, 68.32; H, 6.05; N, 1.11. Found: C, 68.51; H, 5.84; N, 1.01.

4.1.3. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranose 11. To an ice-cooled mixture of **10** (2.03 g, 1.60 mmol) and AcOH (912 μl, 16.0 mmol) in freshly distilled THF (20 ml) was added 1 M *n*-Bu_4NF/THF (6.41 ml,

6.41 mmol). Then the mixture was stirred at room temperature overnight and concentrated in vacuo. The residue was extracted with $CHCl_3$, successively washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with hexane/EtOAc (4:1–2:1) to give **11** as a mixture of anomers (1.46 g, 89%, α/β>10). R_f 0.50 (1:1 hexane/EtOAc). ^{1}H NMR: δ 7.70–7.69, 7.44–7.11 (m, 30H, Ar), 6.88 (d, 1H, *J*=7.8 Hz, –N*H*), 5.33 (br t, 1H, *J*=3.4 Hz, H-1a), 5.02 (d, 1H, *J*=11.0 Hz, –C*H*$_2$Ph), 4.94 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.83 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.78 (d, 1H, *J*=11.0 Hz, –C*H*$_2$Ph), 4.71 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.68 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.53 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.50 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.32 (d, 1H, *J*=8.1 Hz, H-1b), 4.30 (d, 1H, *J*=12.6 Hz, –C*H*$_2$Ph), 4.29 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.19 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.14–4.08 (m, 1H, H-2a), 4.02–3.93 (m, 2H, H-4a and H-5a), 3.89 (d, 1H, *J*=2.4 Hz, H-4b), 3.82–3.74 (m, 3H, H-2b, H-3a, and H-6a), 3.59 (br d, 1H, *J*=9.8 Hz, H-6a), 3.43–3.34 (m, 4H, H-3b, H-5b, H-6b, O*H*), 3.32–3.28 (m, 1H, H-6b). ^{13}C NMR: δ 161.4 (Cl_3C*C*ONH), 102.9 (C-1b), 92.5 (–*C*Cl_3), 90.8 (C-1a, α-anomer). MALDI TOF MS calcd for $C_{56}H_{58}Cl_3NO_{11}$ *m*/*z*: 1025.31. Found: 1048.27 (M+Na$^+$). Anal. Calcd for $C_{56}H_{58}Cl_3NO_{11}$: C, 65.46; H, 5.69; N, 1.36. Found: C, 65.73; H, 5.72; N, 1.24.

4.1.4. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl fluoride 12. To a stirred solution of **11** (635 mg, 0.62 mmol) in freshly distilled THF (10 ml) was added Et_2NSF_3 (121 μl, 0.93 mmol) at 0 °C. The mixture was stirred for 1 h before the reaction was quenched with MeOH and concentrated in vacuo. The residue was extracted with EtOAc, successively washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene/EtOAc (9:1) to give **12** as a mixture of anomers (582 mg, 92%, α/β=19/1). R_f 0.40 (9:1 toluene/EtOAc). ^{1}H NMR: δ 7.36–7.14 (m, 30H, Ar), 6.54 (d, 1H, *J*=8.1 Hz, −N*H*), 5.75 (dd, 1H, *J*=2.4, 53.7 Hz, H-1a), 5.03 (d, 1H, *J*=11.0 Hz, −C*H*$_2$Ph), 4.96 (d, 1H, *J*=11.5 Hz, −C*H*$_2$Ph), 4.84 (d, 1H, *J*=11.2 Hz, −C*H*$_2$Ph), 4.76 (d, 1H, *J*=11.2 Hz, −C*H*$_2$Ph), 4.72–4.67 (m, 2H, –C*H*$_2$Ph), 4.66 (d, 1H, *J*=8.3 Hz, H-1b), 4.63 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.56 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.54 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.38–4.36 (m, 4H, –C*H*$_2$Ph), 4.24 (d, 1H, *J*=11.3 Hz, –C*H*$_2$Ph), 4.15 (t, 1H, *J*=9.5 Hz, H-2a), 3.92–3.89 (m, 2H, H-4b and H-5a), 3.86 (br d, 1H, *J*=11.4 Hz, H-2b), 3.79–3.72 (m, 2H, H-3a and H-6a), 3.56 (dd, 1H, *J*=1.2, 11.0 Hz, H-6a), 3.52–3.43 (m, 1H, H-6b), 3.38–3.32 (m, 3H, H-4a, H-5b, and H-6b). ^{13}C NMR: δ 161.5 (Cl_3C*C*ONH), 105.3 (d, J_{CF}=220.1 Hz, C-1a, α-F), 102.4 (C-1b), 91.9 (–*C*Cl_3). MALDI TOF MS calcd for $C_{56}H_{57}Cl_3FNO_{10}$ *m*/*z*: 1027.30. Found: 1050.39 (M+Na$^+$). Anal. Calcd for $C_{56}H_{57}Cl_3FNO_{10}$: C, 65.34; H, 5.58; N, 1.36. Found: C, 65.45; H, 5.58; N, 1.33.

4.1.5. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-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 13. A mixture of Cp_2ZrCl_2 (315 mg, 1.08 mmol), $AgClO_4$ (447 mg, 2.16 mmol), and dried molecular sieves 4 Å powder (1 g) in anhydrous CH_2Cl_2 (3 ml) was stirred at room temperature under Ar for 30 min and then cooled at −15 °C. To the mixture was added a mixture of **6** (415 mg, 0.65 mmol) and **12** (554 mg, 0.54 mmol) in anhydrous CH_2Cl_2 (7 ml). The mixture was stirred at −15 °C for 1 h, before the reaction was quenched with aq $NaHCO_3$. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was successively washed with satd $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene/EtOAc (88:12) to give **13** (670 mg, 75%). $[\alpha]_D$ +62.9 (*c* 1). R_f 0.51 (4:1 toluene/EtOAc). ^{1}H NMR: δ 7.76 (d, 2H, *J*=7.6 Hz, Ar), 7.62 (d, 2H, *J*=7.3 Hz, Ar), 7.61–7.06 (m, 40H, Ar and Cl_3CCON*H*), 5.98–5.85 (m, 2H, –CH_2C*H*=CH_2, FmocN*H*), 5.351 [s, 1H, PhC*H*(O)$_2$], 5.35 (d, 1H, *J*=17.1 Hz, –CH=C*H*$_2$), 5.28 (d, 1H, *J*=10.7 Hz, –CH=C*H*$_2$), 5.26 (d, 1H, *J*=7.1 Hz, H-1b), 5.04 (d, 1H, *J*=10.3 Hz, –C*H*$_2$Ph), 4.97 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.95 (d, 1H, *J*=3.5 Hz, H-1a), 4.85 (d, 1H, *J*=11.6 Hz, –C*H*$_2$Ph), 4.82 (d, 1H, *J*=11.6 Hz, –C*H*$_2$Ph), 4.74 (d, 1H, *J*=12.2 Hz, –C*H*$_2$Ph), 4.73–4.68 (m, 1H, –C*H*$_2$Ph), 4.69 (d, 2H, *J*=5.9 Hz, –C*H*$_2$C=CH_2), 4.55 (d, 1H, *J*=10.3 Hz, –C*H*$_2$Ph), 4.54 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 3.92 (d, 1H, *J*=2.7 Hz, H-4c), 3.83 (dd, 1H, *J*=3.5, 10.7 Hz, H-2a), 3.73–3.67 (m, 1H, H-5b), 3.59 (br s, 1H, H-4a), 3.45 (dd, 1H, *J*=2.7, 9.8 Hz, H-3c), 3.39 (dd, 1H, *J*=4.9, 7.6 Hz, H-5c), 3.32 (dd, 1H, *J*=4.9, 8.5 Hz, H-6c). ^{13}C NMR: δ 169.2 (–*C*O$_2$), 161.7 (Cl_3C*C*ONH), 155.7 (O*C*ONH), 131.1 (–*C*H=), 119.0 (=*C*H$_2$), 103.1 ($^1J_{CH}$=160.1 Hz, C-1c), 100.4 [Ph*C*H(O)$_2$], 99.8 ($^1J_{CH}$=168.4 Hz, C-1b, $^1J_{CH}$=171.8 Hz, C-1a), 92.3 (–*C*Cl_3). MALDI TOF MS calcd for $C_{90}H_{90}N_5O_{19}Cl_3$ *m*/*z*: 1649.53. Found: 1672.56 (M+Na$^+$), 1688.34 (M+K$^+$). Anal. Calcd for $C_{90}H_{90}N_5O_{19}Cl_3$: C, 65.43; H, 5.49; N, 4.24. Found: C, 65.61; H, 5.64; N, 3.90.

4.1.6. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-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 14. Condensation of **7** (368 mg, 0.56 mmol) and **12** (481 mg, 0.47 mmol) was performed in a similar manner as described for **13**. Chromatography of the crude product on silica gel with toluene/EtOAc (88:12) gave **14** (622 mg, 80%). $[\alpha]_D$ +58.8 (*c* 1). R_f 0.34 (7:1 toluene/EtOAc). ^{1}H NMR: δ 7.77 (d, 2H, *J*=7.6 Hz, Ar), 7.64 (d, 2H, *J*=7.3 Hz, Ar), 7.52–7.06 (m, 40H, Ar and Cl_3CCON*H*), 5.92 (ddt, 1H, *J*=6.1, 10.5, 17.1 Hz, –CH_2C*H*=CH_2), 5.72 (d, 1H, *J*=9.5 Hz, FmocN*H*), 5.36 [s, 1H, PhC*H*(O)$_2$], 5.35 (dd, 1H, *J*=1.2, 17.1 Hz, –CH=C*H*$_2$), 5.33 (d, 1H, *J*=7.8 Hz, H-1b), 5.25 (dd, 1H, *J*=1.2, 10.5 Hz, –CH=C*H*$_2$), 5.05 (d, 1H, *J*=10.3 Hz, –C*H*$_2$Ph), 5.01 (d, 1H, *J*=3.7 Hz, H-1a), 4.97 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.85 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.82 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.74 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.70 (d, 1H, *J*=12.0 Hz, –C*H*$_2$Ph), 4.66 (m, 2H, –C*H*$_2$C=CH_2), 4.54 (d, 2H, *J*=11.2 Hz, –C*H*$_2$Ph), 4.49–4.39 (m, 4H, Thr–βH, H-6a, and –OC*H*$_2$CHAr$_2$), 4.40 (d, 1H, *J*=7.8 Hz, H-1c), 4.33 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.32 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.31–4.27 (m, 3H, H-3b, H-6a, and –CH_2C*H*Ar$_2$), 4.22 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.18 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.11–4.04 (m, 2H, H-6b and H-3a), 3.92 (d, 1H,

J=2.7 Hz, H-4c), 3.93–3.78 (m, 2H, H-4b and H-5a), 3.85 (dd, 1H, J=3.7, 10.7 Hz, H-2a), 3.80 (dd, 1H, J=7.8, 9.3 Hz, H-2c), 3.76–3.72 (m, 2H, H-5b and Thr–αH), 3.63 (br d, 1H, J=12.0 Hz, H-6b), 3.58 (br s, 1H, H-4a), 3.52–3.45 (m, 3H, H-2b, H-6c, and H-3c), 3.41 (dd, 1H, J=5.1, 7.8 Hz, H-5c), 3.32 (dd, 1H, J=5.1, 8.8 Hz, H-6c), 1.30 (d, 3H, J=6.3 Hz, Thr–γH). ^{13}C NMR: δ 169.7 (–*C*O$_2$), 161.8 (Cl$_3$C*C*ONH), 156.6 (O*C*ONH), 131.1 (–*C*H=), 119.2 (=*C*H$_2$), 103.1 ($^1J_{CH}$=160.1 Hz, C-1c), 100.5 [Ph*C*H(O)$_2$], 99.6 ($^1J_{CH}$=165.9 Hz, C-1b), 99.3 ($^1J_{CH}$=173.4 Hz, C-1a), 92.3 (–*C*Cl$_3$). MALDI TOF MS calcd for $C_{91}H_{92}N_5O_{19}Cl_3$ *m/z*: 1663.55. Found: 1686.85 (M+Na$^+$), 1702.75 (M+K$^+$). Anal. Calcd for $C_{91}H_{92}N_5O_{19}Cl_3$: C, 65.60; H, 5.57; N, 4.20. Found: C, 65.84; H, 5.74; N, 3.87.

4.1.7. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 15. To an ice-cooled solution of **13** (266 mg, 0.16 mmol) in CH_2Cl_2 (8 ml) was added 80% aq CF_3CO_2H (4 ml) with stirring. The mixture was stirred at 0 °C for 30 min, neutralized by addition of satd $NaHCO_3$, and extracted with EtOAc. The extract was successively washed with satd $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene/EtOAc (2:1) to give **15** (237 mg, 94%). [α]$_D$ +41.9 (*c* 1). R_f 0.18 (3:2 toluene/EtOAc). ^{1}H NMR: δ 7.74 (d, 2H, J=7.6 Hz, Ar), 7.62 (d, 2H, J=7.3 Hz, Ar), 7.57–7.06 (m, 35H, Ar and Cl$_3$CCON*H*), 6.09 (d, 1H, J=8.3 Hz, FmocN*H*), 5.90 (ddt, 1H, J=5.9, 10.5, 16.8 Hz, –CH$_2$C*H*=CH$_2$), 5.33 (d, 1H, J=16.8 Hz, –CH=C*H*$_2$), 5.25 (1H, d, J=10.5 Hz, –CH=C*H*$_2$), 5.21 (1H, d, J=7.8 Hz, H-1b), 5.02 (1H, d, J=10.5 Hz, –C*H*$_2$Ph), 4.97 (d, 1H, J=11.5 Hz, –C*H*$_2$Ph), 4.85 (d, 1H, J=11.0 Hz, –C*H*$_2$Ph), 4.84 (m, 1H, H-1a), 4.79 (d, 1H, J=11.0 Hz, –C*H*$_2$Ph), 4.74 (d, 1H, J=12.0 Hz, –C*H*$_2$Ph), 4.71 (d, 1H, J=12.0 Hz, –C*H*$_2$Ph), 4.69–4.63 (m, 2H, –C*H*$_2$CH=CH$_2$), 4.55 (d, 1H, J=10.5 Hz, –C*H*$_2$Ph), 4.54 (d, 1H, J=11.5 Hz, –C*H*$_2$Ph), 4.54 (m, 1H, Ser–αH), 4.44–4.28 (m, 6H, H-1c, –NCO$_2$C*H*$_2$CH, –C*H*$_2$Ph×3), 4.23 (d, 1H, J=11.5 Hz, –C*H*$_2$Ph), 4.10 (br s, 1H, H-4a), 3.34 (dd, 1H, J=4.9, 8.5 Hz, H-6c), 3.24 (br s, 1H, –O*H*), 2.41 (br s, 1H, –O*H*). ^{13}C NMR: δ 169.3 (–*C*O$_2$), 161.9 (Cl$_3$C*C*ONH), 155.6 (O*C*ONH), 131.1 (–*C*H=), 118.9 (=*C*H$_2$), 103.1 ($^1J_{CH}$=160.9 Hz, C-1c), 99.3 ($^1J_{CH}$=170.9 Hz, C-1a), 98.8 ($^1J_{CH}$=167.6 Hz, C-1b), 92.1 (–*C*Cl$_3$). MALDI TOF MS calcd for $C_{83}H_{86}N_5O_{19}Cl_3$ *m/z*: 1561.50. Found: 1584.27 (M+Na$^+$), 1600.24 (M+K$^+$). Anal. Calcd for $C_{83}H_{86}N_5O_{19}Cl_3$: C, 63.74; H, 5.54; N, 4.48. Found: C, 63.42; H, 5.72; N, 4.09.

4.1.8. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 16. Compound **14** (403 mg, 0.24 mmol) was debenzylidenated in a similar manner as described for **15**. The crude product was purified by column chromatography on silica gel with toluene/EtOAc (2:1–3:2) to give **16** (319 mg, 83%). [α]$_D$ +34.5 (*c* 1). R_f 0.50 (1:1 toluene/EtOAc). ^{1}H NMR: δ 7.76 (d, 2H, J=7.3 Hz, Ar), 7.63 (d, 2H, J=7.3 Hz, Ar), 7.41–7.12 (m, 35H, Ar and Cl$_3$CCON*H*), 5.92 (ddt, 1H, J=5.9, 10.5, 17.1 Hz, –CH$_2$C*H*=CH$_2$), 5.66 (d, 1H, J=9.3 Hz, FmocN*H*), 5.34 (br d, 1H, J=17.1 Hz, –CH=C*H*$_2$), 5.29 (d, 1H, J=7.6 Hz, H-1b), 5.25 (d, 1H, J=10.5 Hz, –CH=C*H*$_2$), 5.02 (d, 1H, J=10.5 Hz, –C*H*$_2$Ph), 4.97 (d, 1H, J=11.5 Hz, –C*H*$_2$Ph), 4.95 (d, 1H, J=5.1 Hz, H-1a), 4.85 (d, 1H, J=11.0 Hz, –C*H*$_2$Ph), 4.79 (d, 1H, J=11.0 Hz, –C*H*$_2$Ph), 4.75 (d, 1H, J=12.0 Hz, –C*H*$_2$Ph), 4.71 (d, 1H, J=12.0 Hz, –C*H*$_2$Ph), 4.65 (d, 2H, J=5.9 Hz, –C*H*$_2$CH=CH$_2$), 4.54 (d, 2H, J=11.2 Hz, –C*H*$_2$Ph×2), 4.48–4.20 (m, 1H, H-1c), 4.15 (br s, 1H, H-4a), 4.01 (dd, 1H, J=2.7, 10.5 Hz, H-3a), 3.94 (d, 1H, J=2.4 Hz, H-4c), 3.35 (dd, 1H, J=4.9, 8.5 Hz, H-6c), 3.25 (br s, 1H, –OH), 2.30 (br s, 1H, –OH), 1.30 (d, 3H, J=6.3 Hz, Thr–γH); ^{13}C NMR: δ 169.8 (–*C*O$_2$), 162.1 (Cl$_3$C*C*ONH), 156.6 (O*C*ONH), 131.2 (–*C*H=), 119.2 (=*C*H$_2$), 103.2 ($^1J_{CH}$=160.1 Hz, C-1c), 98.9 ($^1J_{CH}$=175.1 Hz, C-1a), 98.4 ($^1J_{CH}$=161.8 Hz, C-1b), 92.1 (–*C*Cl$_3$). MALDI TOF MS calcd for $C_{84}H_{88}N_5O_{19}Cl_3$ *m/z*: 1575.51. Found: 1598.69 (M+Na$^+$). Anal. Calcd for $C_{84}H_{88}N_5O_{19}Cl_3$: C, 63.94; H, 5.62; N, 4.44. Found: C, 64.11; H, 5.46; N, 4.15.

4.1.9. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 17 and *N*-(9-fluorenylmethoxycarbonyl)-*O*-{2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→4)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 18. Condensation of **12** (29 mg, 28 μmol) and **16** (54 mg, 34 μmol) was performed at −15 °C for 1 h in a similar manner as described for **13**. Chromatography of the crude product on silica gel with toluene/EtOAc (2:1) gave **17** (32 mg, 44%) and isomer **18** (22 mg, 30%). Unconsumed **16** (11 mg, 21%) was also recovered.

Compound **17**: [α]$_D$ +20.1 (*c* 1.7). R_f 0.39 (3:1 toluene/EtOAc). ^{1}H NMR: δ 7.75 (d, 2H, J=7.6 Hz, Ar), 7.62 (d, 1H, J=6.8 Hz, Ar), 7.60 (d, 1H, J=6.8 Hz, Ar), 7.45–7.06 (m, 65H, Ar and Cl$_3$CCON*H*), 7.01 (d, 1H, J=7.8 Hz, Cl$_3$CCON*H*), 5.91 (ddd, 1H, J=17.1, 10.7, 5.9 Hz, –C*H*=CH$_2$), 5.61 (d, 1H, J=9.3 Hz, FmocN*H*), 5.34 (dd, 1H, J=17.1, 1.2 Hz, –CH=C*H*$_2$), 5.24 (d, 1H, J=10.7 Hz, –CH=C*H*$_2$), 5.23 (d, 1H, J=6.8 Hz, H-1b), 5.01 (d, 1H, J=10.5 Hz, –C*H*$_2$Ph), 4.97 (d, 1H, J=11.4 Hz, –C*H*$_2$Ph), 4.95 (d, 1H, J=11.2 Hz, –C*H*$_2$Ph), 4.88 (d, 1H, J=7.1 Hz, H-1d), 4.87 (d, 1H, J=3.2 Hz, H-1a), 4.81 (d, 1H, J=11.2 Hz, –C*H*$_2$Ph), 4.80 (d, 1H, J=11.2 Hz, –C*H*$_2$Ph), 4.78 (d, 1H, J=11.2 Hz, –C*H*$_2$Ph), 3.02 (br s, 1H, –O*H*), 1.30 (d, 3H, J=6.3 Hz, Thr–γH). ^{13}C NMR: δ 169.9 (–*C*O$_2$), 162.1 (Cl$_3$C*C*ONH), 161.4 (Cl$_3$C*C*ONH), 156.6 (O*C*ONH), 119.3 (=*C*H$_2$), 103.02 ($^1J_{CH}$=165.9 Hz, C-1c or C-1e), 102.96 ($^1J_{CH}$=165.9 Hz, C-1c or C-1e), 99.9 ($^1J_{CH}$=167.6 Hz, C-1b or C-1d), 99.1 ($^1J_{CH}$=174.3 Hz, C-1a), 98.6 ($^1J_{CH}$=160.9 Hz, C-1b or C-1d), 92.5 (–*C*Cl$_3$), 92.2 (–*C*Cl$_3$), 18.8 (Thr–γC). MALDI TOF MS calcd for $C_{140}H_{144}N_6O_{29}Cl_6$ *m/z*: 2582.81. Found: 2606.11 (M+Na$^+$).

Anal. Calcd for $C_{140}H_{144}N_6O_{29}Cl_6 \cdot H_2O$: C, 64.54; H, 5.65; N, 3.23. Found: C, 64.61; H, 5.67; N, 2.93.

Compound **18**: $[\alpha]_D$ +35.5 (*c* 1.4). R_f 0.35 (3:1 toluene/EtOAc). ^{1}H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.64 (d, 2H, *J*=7.3 Hz, Ar), 7.48–7.01 (m, 65H, Ar and Cl_3CCONH), 6.96 (d, 1H, *J*=8.5 Hz, Cl_3CCONH), 5.90 (ddd, 1H, *J*=17.1, 10.5, 5.9 Hz, –C*H*=CH_2), 5.64 (d, 1H, *J*=9.5 Hz, FmocN*H*), 5.33 (d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.32 (d, 1H, *J*=8.1 Hz, H-1b or H-1d), 5.24 (d, 1H, *J*=10.5 Hz, –CH=CH_2), 5.14 (d, 1H, *J*=8.3 Hz, H-1b or H-1d), 5.05 (d, 1H, *J*=10.0 Hz, –CH_2Ph), 4.93 (d, 2H, *J*=11.2 Hz, –CH_2Ph×2), 4.85 (d, 1H, *J*=3.7 Hz, H-1a), 4.81 (d, 1H, *J*=10.5 Hz, –CH_2Ph), 4.80 (d, 1H, *J*=11.2 Hz, –CH_2Ph), 1.29 (d, 3H, *J*=6.3 Hz, Thr–γH). ^{13}C NMR: δ 169.8 (–*C*O_2), 162.0 (Cl_3CCONH), 161.5 (Cl_3CCONH), 156.7 (O*C*ONH), 119.3 (=*C*H_2), 103.3 ($^1J_{CH}$=159.2 Hz, C-1c or C-1e), 102.7 ($^1J_{CH}$=159.2 Hz, C-1c or C-1e), 100.4 ($^1J_{CH}$=162.5 Hz, C-1b or C-1d), 100.3 ($^1J_{CH}$=162.5 Hz, C-1b or C-1d), 98.9 ($^1J_{CH}$=172.5 Hz, C-1a), 92.6 (–*C*Cl_3), 92.2 (–*C*Cl_3), 19.1 (Thr–γC). MALDI TOF MS calcd for $C_{140}H_{144}N_6O_{29}Cl_6$ *m*/*z*: 2582.81. Found: 2606.11 (M+Na^+), 2622.05 (M+K^+).

4.1.10. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-serine allyl ester 19. A mixture of Cp_2ZrCl_2 (89 mg, 0.30 mmol), $AgClO_4$ (127 mg, 0.61 mmol), and dried molecular sieves 4 Å powder (700 mg) in anhydrous CH_2Cl_2 (5 ml) was stirred at room temperature under Ar for 30 min and then cooled at −40 °C. To the stirred mixture was added a mixture of **3** (143 mg, 0.15 mmol) and **15** (218 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (15 ml). Then the temperature was raised to −15 °C for 30 min and stirring was continued for further 1.5 h, before the reaction was quenched with aq $NaHCO_3$. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was successively washed with satd $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene/EtOAc (4:1 to 3:1) to give **19** (233 mg, 68%). Heptasaccharide **23** (20 mg, 4%) and isomer **21** (20 mg, 6%) were obtained in the less polar and the more polar fractions, respectively. Unconsumed **15** (13 mg, 6%) was also recovered. Compound **19**: $[\alpha]_D$ +28.5 (*c* 1). R_f 0.71 (3:2 toluene/EtOAc). ^{1}H NMR ($CDCl_3$): δ 7.71 (d, 1H, *J*=6.6 Hz, Ar), 7.70 (d, 1H, *J*=6.8 Hz, Ar), 7.61 (d, 1H, *J*=7.6 Hz, Ar), 7.55 (d, 1H, *J*=7.3 Hz, Ar), 7.54–7.09 (m, 61H, Ar and Cl_3CCONH×2), 5.90 (m, 1H, –CH_2C*H*=CH_2), 5.80 (d, 1H, *J*=7.3 Hz, FmocN*H*), 5.46 [s, 1H, PhC*H*$(O)_2$], 5.31 (d, 1H, *J*=16.6 Hz, –CH=CH_2), 5.22 (br d, 2H, *J*=10.3 Hz, –CH=CH_2, –CH_2Ph), 5.13 (d, 1H, *J*=7.6 Hz, H-1b), 5.01 (d, 1H, *J*=10.5 Hz, –CH_2Ph), 4.97 (d, 1H, *J*=11.5 Hz, –CH_2Ph), 4.95 (d, 1H, *J*=7.8 Hz, H-1d), 4.80 (d, 1H, *J*=5.1 Hz, H-1a), 4.84 (d, 1H, *J*=11.5 Hz, –CH_2Ph), 2.98 (br s, 1H, –O*H*), 2.95 (s, 1H, H-4e). ^{13}C NMR: δ 169.0 (–*C*O_2), 161.8 (Cl_3CCONH), 161.4 (Cl_3CCONH), 155.5 (O*C*ONH), 131.1 (–*C*H=), 118.9 (=*C*H_2), 102.9 ($^1J_{CH}$=160.1 Hz, C-1c or C-1e), 102.6 ($^1J_{CH}$=160.9 Hz, C-1c or C-1e), 101.2 [Ph*C*H$(O)_2$], 99.3 ($^1J_{CH}$=163.4 Hz, C-1d), 99.0 ($^1J_{CH}$=169.3 Hz, C-1b), 97.5 ($^1J_{CH}$=173.4 Hz, C-1a), 92.4 (–*C*Cl_3), 92.1 (–*C*Cl_3). MALDI TOF MS calcd for $C_{132}H_{134}N_6O_{29}Cl_6$ *m*/*z*: 2476.73. Found: 2499.82 (M+Na^+). Anal. Calcd for $C_{132}H_{134}N_6O_{29}Cl_6$: C, 63.90; H, 5.44; N, 3.39. Found: C, 63.68; H, 5.47; N, 3.20.

Compound **21**: $[\alpha]_D$ +43.7 (*c* 1.4). R_f 0.36 (3:2 toluene/EtOAc). ^{1}H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.63 (d, 1H, *J*=7.8 Hz, Ar), 7.61 (d, 1H, *J*=7.8 Hz, Ar), 7.56–7.01 (m, 61H, Ar and Cl_3CCONH×2), 5.97–5.81 (m, 2H, –C*H*=CH_2 and FmocN*H*), 5.43 [s, 1H, PhC*H*$(O)_2$], 5.32 (d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.25 (br d, 2H, *J*=9.3 Hz, –CH=CH_2 and H-1b or H-1d), 5.12 (d, 1H, *J*=8.3 Hz, H-1b or H-1d), 5.05 (d, 1H, *J*=10.2 Hz, –CH_2Ph), 5.02 (d, 1H, *J*=10.7 Hz, –CH_2Ph), 4.94 (d, 1H, *J*=11.7 Hz, –CH_2Ph), 4.36 (d, 1H, *J*=8.3 Hz, H-1c or H-1e), 2.99 (br s, 1H, H-4e). ^{13}C NMR: δ 169.5 (–*C*O_2), 162.0 (Cl_3CCONH), 161.6 (Cl_3CCONH), 156.0 (O*C*ONH), 119.2 (=*C*H_2), 103.4 ($^1J_{CH}$=162.2 Hz, C-1c or C-1e), 102.8 ($^1J_{CH}$=159.7 Hz, C-1c or C-1e), 101.1 [Ph*C*H$(O)_2$], 100.7 ($^1J_{CH}$=166.3 Hz, C-1b or C-1d), 100.3 ($^1J_{CH}$=168.8 Hz, C-1b or C-1d), 99.2 ($^1J_{CH}$=170.5 Hz, C-1a), 92.6 (–*C*Cl_3), 92.3 (–*C*Cl_3). MALDI TOF MS calcd for $C_{132}H_{134}N_6O_{29}Cl_6$ *m*/*z*: 2476.73. Found: 2499.74 (M+Na^+). Anal. Calcd for $C_{132}H_{134}N_6O_{29}Cl_6 \cdot H_2O$: C, 63.44; H, 5.48; N, 3.36. Found: C, 63.68; H, 5.54; N, 3.07.

Compound **23**: $[\alpha]_D$ +26.5 (*c* 0.7). R_f 0.56 (2:1 toluene/EtOAc). ^{1}H NMR: δ 7.71 (d, 2H, *J*=7.3 Hz, Ar), 7.57 (d, 1H, *J*=7.8 Hz, Ar), 7.54 (d, 1H, *J*=7.3 Hz, Ar), 7.51–6.97 (m, 88H, Ar and Cl_3CCONH×3), 5.95–5.76 (m, 2H, –C*H*=CH_2 and FmocN*H*), 5.43 [s, 2H, PhC*H*$(O)_2$×2], 5.30 (d, 1H, *J*=17.1 Hz, –CH=CH_2), 5.25–5.09 (m, 5H, –CH=CH_2, GlcNTCA H-1×2, –CH_2Ph×2), 5.04 (d, 1H, *J*=10.2 Hz, –CH_2Ph), 4.92 (d, 1H, *J*=11.2 Hz, –CH_2Ph), 4.87 (d, 1H, *J*=2.9 Hz, H-1a), 2.94 (br s, 1H, H-4e or H-4g), 2.84 (br s, 1H, H-4e or H-4g). ^{13}C NMR: δ 169.6 (–*C*O_2), 162.0 (Cl_3CCONH), 161.8 (Cl_3CCONH), 161.4 (Cl_3CCONH), 156.0 (O*C*ONH), 119.2 (=*C*H_2), 103.1 ($^1J_{CH}$=159.7 Hz), 102.8 ($^1J_{CH}$=163.9 Hz), and 102.5 ($^1J_{CH}$=159.7 Hz) (C-1c, C-1e, and C-1g), 101.3 [Ph*C*H$(O)_2$], 101.26 [Ph*C*H$(O)_2$], 100.9 ($^1J_{CH}$=158.9 Hz), 99.8 ($^1J_{CH}$=162.2 Hz), and 99.4 ($^1J_{CH}$=164.7 Hz) (C-1b, C-d, and C-1f), 98.0 ($^1J_{CH}$=170.5 Hz, C-1a), 92.7 (–*C*Cl_3), 92.6 (–*C*Cl_3), 92.3 (–*C*Cl_3). MALDI TOF MS calcd for $C_{182}H_{184}N_7O_{39}Cl_9$ *m*/*z*: 3391.97. Found: 3431.09 (M+K^+). Anal. Calcd for $C_{181}H_{182}N_7O_{39}Cl_9 \cdot 2H_2O$: C, 63.30; H, 5.46; N, 2.85. Found: C, 63.21; H, 5.40; N, 2.62.

4.1.11. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 20. In a similar manner as described for **19**, condensation of **3** (204 mg, 0.22 mmol) and **16** (312 mg, 0.20 mmol) was performed with Cp_2ZrCl_2 (127 mg, 0.43 mmol), $AgClO_4$ (181 mg, 0.87 mmol), and dried molecular sieves 4 Å powder (1 g) in anhydrous CH_2Cl_2 (30 ml) at −40 to −15 °C for 2 h. Chromatography of the crude product on silica gel with

toluene/EtOAc (88:12 to 80:20) afforded **20** (351 mg, 71%), **22** (43 mg, 9%), **24** (55 mg, 8%), and unreacted **16** (13 mg, 4%).

Compound **20**: $[\alpha]_D$ +21.7 (*c* 1). R_f 0.54 (2:1 toluene/EtOAc). ^{1}H NMR: δ 7.74 (d, 2H, *J*=7.3 Hz, Ar), 7.60 (t, 2H, *J*=7.3 Hz, Ar), 7.60–7.05 (m, 60H, Ar and Cl_3CCONH), 6.95 (d, 1H, *J*=7.3 Hz, Cl_3CCONH), 5.90 (m, 1H, $-CH_2CH{=}CH_2$), 5.59 (d, 1H, *J*=9.5 Hz, FmocN*H*), 5.45 [s, 1H, PhC*H*(O)$_2$], 5.33 (d, 1H, *J*=17.1 Hz, $-CH{=}CH_2$), 5.24 (d, 1H, *J*=10.3 Hz, $-CH{=}CH_2$), 5.23 (d, 1H, *J*=7.6 Hz, H-1b), 5.19 (d, 1H, *J*=10.5 Hz, $-CH_2Ph$), 5.00 (d, 1H, *J*=10.5 Hz, $-CH_2Ph$), 4.96 (d, 1H, *J*=11.5 Hz, $-CH_2Ph$), 4.88 (d, 1H, *J*=8.8 Hz, H-1d), 4.87 (d, 1H, *J*=4.1 Hz, H-1a), 4.84 (d, 1H, *J*=11.5 Hz, $-CH_2Ph$), 4.49–4.28 (m, 10H, H-1c, H-1e, Thr–αH, $-CH_2CHAr_2$, Thr–βH, and $-CH_2Ph\times4$), 2.99 (br s, 1H, –O*H*), 2.90 (s, 1H, H-4e), 1.29 (d, 3H, *J*=6.3 Hz, Thr–γH). ^{13}C NMR: δ 169.6 ($-CO_2$), 161.9 (Cl_3CCONH), 161.3 (Cl_3CCONH), 156.5 (O*C*ONH), 131.1 (–*C*H=), 119.1 (=*C*H$_2$), 102.9 ($^1J_{CH}$=160.1 Hz, C-1c or C-1e), 102.5 ($^1J_{CH}$=160.1 Hz, C-1c or C-1e), 101.2 [Ph*C*H(O)$_2$], 99.9 ($^1J_{CH}$=165.9 Hz, C-1d), 99.0 ($^1J_{CH}$=172.5 Hz, C-1a), 98.6 ($^1J_{CH}$=169.2 Hz, C-1b), 92.4 ($-CCl_3$), 92.1 ($-CCl_3$). MALDI TOF MS calcd for $C_{133}H_{136}N_6O_{29}Cl_6$ *m*/*z*: 2490.75. Found: 2514.13 (M+Na$^+$). Anal. Calcd for $C_{133}H_{136}N_6O_{29}Cl_6$: C, 64.02; H, 5.49; N, 3.37. Found: C, 64.02; H, 5.32; N, 3.22.

Compound **22**: $[\alpha]_D$ +38.0 (*c* 1.8, $CHCl_3$). R_f 0.21 (2:1 toluene/EtOAc). ^{1}H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.63 (d, 2H, *J*=7.3 Hz, Ar), 7.57–7.00 (m, 61H, Ar and $Cl_3CCONH\times2$), 5.90 (ddd, 1H, *J*=16.6, 10.7, 5.9 Hz, $-CH{=}CH_2$), 5.67 (d, 1H, *J*=9.8 Hz, FmocN*H*), 5.43 [s, 1H, PhC*H*(O)$_2$], 5.33 (d, 1H, *J*=16.6 Hz, $-CH{=}CH_2$), 5.30 (d, 1H, *J*=7.8 Hz, H-1b or H-1d), 5.24 (d, 1H, *J*=10.2 Hz, $-CH_2Ph$), 5.13 (d, 1H, *J*=8.3 Hz, H-1b or H-1d), 5.06 (d, 1H, *J*=10.2 Hz, $-CH_2Ph$), 5.00 (d, 1H, *J*=11.2 Hz, $-CH_2Ph$), 4.93 (d, 1H, *J*=11.7 Hz, $-CH_2Ph$), 4.85 (d, 1H, *J*=3.4 Hz, H-1a), 4.81 (d, 1H, *J*=11.2 Hz, $-CH_2Ph$), 4.80 (d, 1H, *J*=11.2 Hz, $-CH_2Ph$), 4.78 (d, 1H, *J*=11.2 Hz, $-CH_2Ph$), 2.98 (br s, 1H, H-4e), 1.29 (d, 3H, *J*=6.3 Hz, Thr–γH). ^{13}C NMR: δ 170.0 ($-CO_2$), 162.1 (Cl_3CCONH), 161.6 (Cl_3CCONH), 156.8 (O*C*ONH), 119.4 (=*C*H$_2$), 103.4 ($^1J_{CH}$=158.9 Hz, C-1c or C-1e), 102.8 ($^1J_{CH}$=158.9 Hz, C-1c or C-1e), 101.1 [Ph*C*H(O)$_2$], 100.5 ($^1J_{CH}$=169.6 Hz, C-1b or C-1d), 100.4 ($^1J_{CH}$=169.6 Hz, C-1b or C-1d), 99.1 ($^1J_{CH}$=173.0 Hz, C-1a), 92.6 ($-CCl_3$), 92.2 ($-CCl_3$), 18.9 (Thr–γC). MALDI TOF MS calcd for $C_{133}H_{136}N_6O_{29}Cl_6$ *m*/*z*: 2490.75. Found: 2513.67 (M+Na$^+$). Anal. Calcd for $C_{133}H_{136}N_6O_{29}Cl_6$: C, 64.02; H, 5.49; N, 3.37. Found: C, 63.89; H, 5.58; N, 3.18.

Compound **24**: $[\alpha]_D$ +25.9 (*c* 1.0). R_f 0.64 (2:1 toluene/EtOAc). ^{1}H NMR: δ 7.73 (d, 2H, *J*=7.3 Hz, Ar), 7.61 (d, 1H, *J*=7.3 Hz, Ar), 7.57 (d, 1H, *J*=9.3 Hz, Ar), 7.55–6.99 (m, 87H, Ar and $Cl_3CCONH\times2$), 6.97 (d, 1H, *J*=8.3 Hz, Cl_3CCONH), 5.95–5.81 (m, 1H, $-CH{=}CH_2$), 5.59 (d, 1H, *J*=9.8 Hz, FmocN*H*), 5.43 [s, 1H, PhC*H*(O)$_2$], 5.42 [s, 1H, PhC*H*(O)$_2$], 5.31 (d, 1H, *J*=16.6 Hz, $-CH{=}CH_2$), 5.27–5.08 (m, 5H, $-CH{=}CH_2$, GlcNTCA H-1×2, $-CH_2Ph\times2$), 5.04 (d, 1H, *J*=10.2 Hz, $-CH_2Ph$), 4.92 (d, 1H, *J*=11.2 Hz, $-CH_2Ph$), 4.87 (d, 1H, *J*=2.9 Hz, H-1a), 2.96 (br s, 1H, H-4c, H-4e, or H-4g), 2.80 (br s, 1H, H-4c, H-4e, or H-4g), 1.27 (d, 3H, *J*=7.3 Hz, Thr–γH). ^{13}C NMR: δ 169.9 ($-CO_2$), 162.1 (Cl_3CCONH), 161.6 (Cl_3CCONH), 161.4 (Cl_3CCONH), 156.7 (O*C*ONH), 119.6 (=*C*H$_2$), 103.0 ($^1J_{CH}$=158.1 Hz), 102.8 ($^1J_{CH}$=162.2 Hz), and 102.5 ($^1J_{CH}$=160.6 Hz) (C-1c, C-1e, and C-1g), 101.3 [Ph*C*H(O)$_2$], 101.2 [Ph*C*H(O)$_2$], 100.8 ($^1J_{CH}$=163.9 Hz), 100.2 ($^1J_{CH}$=164.7 Hz), and 99.4 ($^1J_{CH}$=165.5 Hz) (C-1b, C-1d, and C-1f), 99.0 ($^1J_{CH}$=172.1 Hz, C-1a), 92.6 ($-CCl_3$), 92.6 ($-CCl_3$), 92.3 ($-CCl_3$), 18.6 (Thr–γC). MALDI TOF MS calcd for $C_{182}H_{184}N_7O_{39}Cl_9$ *m*/*z*: 3405.98. Found: 3444.23 (M+K$^+$). Anal. Calcd for $C_{182}H_{184}N_7O_{39}Cl_9$: C, 64.06; H, 5.44; N, 2.87. Found: C, 63.97; H, 5.40; N, 2.70.

4.1.12. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-serine allyl ester 25. A mixture of **19** (254 mg, 102.4 μmol), powdered Zn (1 g, 15.3 mmol), and AcOH (1 ml, 17.5 mmol) in EtOAc (10 ml) was placed in a round-bottom flask equipped with a reflux condenser. The atmosphere was replaced with a balloon of Ar. The reaction mixture was stirred under microwave irradiation at 150 W for 30 min. The microwave machine was controlled so as to continuously irradiate the flask during this period. 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 residue was dissolved in a mixture of CH_2Cl_2 (8 ml) and MeOH (2 ml), and stirred with Ac_2O (1 ml) at room temperature for 1 h. The mixture was concentrated in vacuo to the residue, which was chromatographed on silica gel with toluene/EtOAc (1:4) and then with $CHCl_3$/MeOH (95:5) to afford **25** (200 mg, 85%). $[\alpha]_D$ +26.7 (*c* 1.1). R_f 0.59 (9:1 $CHCl_3$/MeOH). ^{1}H NMR (DMSO-d_6): δ 7.85 (d, 3H, *J*=7.8 Hz, Ar), 7.75 (d, 2H, *J*=8.3 Hz, Ar), 7.70–7.67 (m, 2H, Ar), 7.56–7.02 (m, 60H, Ar and N*H*×4), 5.87 (m, 1H, $-CH_2CH{=}CH_2$), 5.64 [s, 1H, PhC*H*(O)$_2$], 5.29 (d, 1H, *J*=17.3 Hz, $-CH{=}CH_2$), 5.18 (d, 1H, *J*=10.5 Hz, $-CH{=}CH_2$), 5.09 (d, 1H, *J*=11.0 Hz, $-CH_2Ph$), 4.88 (d, 1H, *J*=10.7 Hz, $-CH_2Ph$), 4.84 (d, 1H, *J*=11.7 Hz, $-CH_2Ph$), 1.83 (s, 3H, Ac), 1.77 (s, 6H, Ac×2). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.1, 170.0, 169.7 ($CH_3CONH\times3$, $-CO_2$ All), 155.8 (O*C*ONH), 131.1 (–*C*H=), 118.8 (=*C*H$_2$), 102.8 ($^1J_{CH}$=160.9 Hz, C-1c or C-1e), 102.5 ($^1J_{CH}$=165.1 Hz, C-1c or C-1e), 101.0 [Ph*C*H(O)$_2$], 100.3 ($^1J_{CH}$=165.9 Hz, C-1b and C-1d), 97.8 ($^1J_{CH}$=172.6 Hz, C-1a), 23.41, 23.36, 23.2 ($CH_3CO\times3$). MALDI TOF MS calcd for $C_{134}H_{144}N_4O_{30}$ *m*/*z*: 2288.99. Found: 2312.28 (M+Na$^+$), 2328.31 (M+K$^+$). Anal. Calcd for $C_{134}H_{144}N_4O_{30}\cdot H_2O$: C, 69.71; H, 6.37; N, 2.43. Found: C, 69.78; H, 6.41; N, 2.37.

4.1.13. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 26. Reduction of **20** (46 mg, 18.4 μmol) was performed with Zn (180 mg,

2.75 mmol) and AcOH (0.2 ml, 3.49 mmol) in EtOAc (2 ml) under microwave irradiation for 30 min, according to the procedure described above. The crude product was acetylated and purified by column chromatography. Toluene/EtOAc (1:4) followed by $CHCl_3$/MeOH (95:5) eluted **26** (33 mg, 80%). $[\alpha]_D$ +29.5 (*c* 1). R_f 0.61 (9:1 $CHCl_3$/MeOH). ^{1}H NMR (DMSO-d_6): δ 7.91–7.05 (m, 67H, Ar and N*H*×4), 5.87 (m, 1H, –CH_2CH=CH_2), 5.64 [s, 1H, PhC*H*$(O)_2$], 5.33 (d, 1H, *J*=16.8 Hz, –CH=C*H*$_2$), 5.22 (d, 1H, *J*=10.5 Hz, –CH=C*H*$_2$), 4.89 (d, 1H, *J*=12.9 Hz, –C*H*$_2$Ph), 4.85 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.84 (d, 1H, *J*=11.0 Hz, –C*H*$_2$Ph), 4.81–4.73 (m, 4H, H-1b and –C*H*$_2$Ph×3), 4.70 (d, 1H, *J*=12.2 Hz, –C*H*$_2$Ph), 4.69 (d, 1H, *J*=11.2 Hz, –C*H*$_2$Ph×2), 1.89 (s, 3H, Ac), 1.81 (s, 3H, Ac), 1.80 (s, 3H, Ac), 1.13 (d, 3H, *J*=4.9 Hz, Thr–γH). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.34, 170.26, 169.8 (CH_3*C*ONH×3, –*C*O_2 All), 156.3 (O*C*ONH), 130.9 (–*C*H=), 119.3 (=*C*H_2), 103.0 ($^1J_{CH}$=160.9 Hz, C-1c or C-1e), 102.7 ($^1J_{CH}$=165.1 Hz, C-1c or C-1e), 101.1 [Ph*C*H$(O)_2$], 100.5 ($^1J_{CH}$=163.4 Hz, C-1b or C-1d), 100.3 ($^1J_{CH}$=167.6 Hz, C-1b or C-1d), 99.0 ($^1J_{CH}$=170.1 Hz, C-1a), 23.6, 23.4 (CH_3*C*O×3), 18.5 (Thr C-4). MALDI TOF MS calcd for $C_{135}H_{146}N_4O_{30}$ *m*/*z*: 2303.00. Found: 2325.96 (M+Na^+), 2341.92 (M+K^+). Anal. Calcd for $C_{135}H_{146}N_4O_{30}\cdot H_2O$: C, 69.81; H, 6.42; N, 2.43. Found: C, 69.84; H, 6.16; N, 2.30.

4.1.14. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-serine 1. A mixture of **25** (49 mg, 0.02 mmol), 5,5-dimethyl-1,3-cyclohexanedione (60 mg, 0.43 mmol), and $Pd(PPh_3)_4$ (3 mg, 2.6 μmol) in freshly distilled THF (5 ml) was stirred under Ar at room temperature for 30 min, before concentrated in vacuo. The residue was chromatographed on silica gel with $CHCl_3$/MeOH (95:5) to elute less polar 5,5-dimethyl-1,3-cyclohexanedione and the byproducts. Then addition of AcOH (2%) to the eluant afforded **1** (44 mg, 93%). $[\alpha]_D$ +31.1 (*c* 1). R_f 0.46 (9:1 $CHCl_3$/MeOH, 2% AcOH). ^{1}H NMR (DMSO-d_6): δ 7.85 (d, 2H, *J*=7.6 Hz, Ar), 7.80–7.03 (m, 65H, Ar and N*H*×4), 5.63 [s, 1H, PhC*H*$(O)_2$], 5.09 (d, 1H, *J*=10.7 Hz, –C*H*$_2$Ph), 4.87 (d, 1H, *J*=11.0 Hz, –C*H*$_2$Ph), 4.84 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 1.85 (s, 3H, Ac), 1.79 (s, 3H, Ac), 1.77 (s, 3H, Ac). ^{13}C NMR (DMSO-d_6): δ 171.5 (–*C*O_2H), 169.0, 168.8, 168.7 (CH_3*C*ONH×3), 155.8 (O*C*ONH), 102.0 (C-1c and C-1e), 101.8, 101.4 (C-1b and C-1d), 99.7 [PhCH$(O)_2$], 97.7 (C-1a), 23.0 (CH_3*C*O×3). MALDI TOF MS calcd for $C_{131}H_{140}N_4O_{30}$ *m*/*z*: 2248.96. Found: 2271.87 (M+Na^+), 2287.85 (M+K^+). Anal. Calcd for $C_{131}H_{140}N_4O_{30}\cdot H_2O$: C, 69.36; H, 6.31; N, 2.47. Found: C, 69.34; H, 6.33; N, 2.47.

4.1.15. *N*-(9-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)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 2. Compound **26** (102 mg, 44.3 μmol) was deallylated according to the procedure described for the synthesis of **1**. Chromatography of the crude product gave **2** (92 mg, 92%). $[\alpha]_D$ +32.2 (*c* 1). R_f 0.48 (9:1 $CHCl_3$/MeOH, 2% AcOH). ^{1}H NMR (DMSO-d_6): δ 7.93–7.77, 7.77–7.55, 7.48–7.03 (m, 67H, Ar and N*H*×4), 5.64 [s, 1H, PhC*H*$(O)_2$], 5.08 (d, 1H, *J*=10.7 Hz, –C*H*$_2$Ph), 4.88 (d, 1H, *J*=10.7 Hz, –C*H*$_2$Ph), 4.84 (d, 1H, *J*=11.7 Hz, –C*H*$_2$Ph), 4.82–4.73 (m, 5H, H-1b, and –C*H*$_2$Ph×4), 4.70 (d, 1H, *J*=11.5 Hz, –C*H*$_2$Ph), 4.69 (d, 3H, *J*=12.7 Hz, –C*H*$_2$Ph×3), 4.63 (d, 1H, *J*=12.5 Hz, –C*H*$_2$Ph), 4.61 (d, 2H, *J*=12.0 Hz, –C*H*$_2$Ph×2), 1.91 (s, 3H, Ac), 1.794 and 1.790 (2s, 3H×2, Ac×2), 1.11 (d, 3H, *J*=6.1 Hz, Thr–γH). ^{13}C NMR (DMSO-d_6): δ 171.4 (–*C*O_2H), 169.0, 168.8, and 168.4 (CH_3*C*ONH×3), 156.3 (O*C*ONH), 102.0 ($^1J_{CH}$=161.8 Hz, C-1c and C-1e), 101.3 ($^1J_{CH}$=157.6 Hz, C-1b and C-1d), 99.7 [PhCH$(O)_2$], 98.5 ($^1J_{CH}$=168.4 Hz, C-1a), 23.03, and 22.97 (CH_3*C*O×3), 18.8 (Thr C-4). MALDI TOF MS calcd for $C_{132}H_{142}N_4O_{30}$ *m*/*z*: 2262.97. Found: 2285.75 (M+Na^+), 2301.72 (M+K^+). Anal. Calcd for $C_{131}H_{140}N_4O_{30}\cdot H_2O$: C, 69.46; H, 6.36; N, 2.45. Found: C, 69.22; H, 6.22; N, 2.35.

4.2. Synthesis of glycopeptide 27

Commercial Fmoc-CLEAR-amide resin (435 mg, 0.2 mmol) was stirred with 20% piperidine/NMP (5 ml) in a polypropylene tube by a vortex mixer for 5 min. After filtration the resin was again stirred with 20% piperidine/NMP (5 ml) for 15 min to complete N-deprotection. Then the resin was repeatedly washed with NMP to remove piperidine and reacted for 1 h with Fmoc–Gly–SCH_2CH_2COOBt prepared from the carboxylic acid (154 mg, 0.4 mmol) by activation with 1 M DCC in NMP (0.4 ml, 0.4 mmol) and 1 M HOBt in NMP (0.4 ml, 0.4 mmol). The Fmoc group was removed by stirring with a mixture of 1-methylpyrrolidine (25%), hexamethyleneimine (2%), and 1-hydroxybenzotriazole (4.8%) in NMP/DMSO (1:1, 3 ml) for 2 min and again with the same amount of the mixture for 15 min. After washing the resin with NMP, Tsoc–Lys(*Z*)–OPfp (259 mg, 0.4 mmol) in THF (2.5 ml) was reacted with it for 30 min. The coupling reaction was repeated with the same amount of Tsoc–Lys(*Z*)–OPfp. After washing with THF and dichloromethane, Fmoc–Leu–F (142 mg, 0.4 mmol) dissolved in CH_2Cl_2 (2.5 ml) was added to the resin. Then 1 M Bu_4NF in THF (20 μl, 0.02 mmol) was added and the resin was stirred for 1 h before washing with THF and NMP. The resulting tripeptide-resin was subjected to the automated synthesis by ABI 433A peptide synthesizer using FastMoc protocol. The Fmoc deprotection protocol was modified so that the premixed reagent was introduced to the reaction vessel without dilution. The deprotection time was 5 and 20 min. After the synthesis of tetradecapeptide (Asp^{45}-Gly^{58}) was completed, an eighth part of the resin placed in a propylene tube was stirred with the Fmoc deblocking reagent (1 ml) for 5 min and then for 20 min as already mentioned. The resin was washed with NMP and reacted with **2** (113 mg, 50 μmol), 1 M DCC in NMP (100 μl, 100 μmol), and 1 M HOBt in NMP (100 μl, 100 μmol) in NMP (75 μl) at 50 °C for 3 h by using a vortex mixer. The remaining sequence was also manually introduced using Fmoc amino acid (100 μmol), DCC (150 μmol), and HOBt (150 μmol) in NMP to give glycopeptide (Gly^{34}-Gly^{58})-resin (108 mg). The experiment for deprotection was performed with the resin (27 mg), which was treated with the Fmoc deblocking reagent as mentioned

above. The resulting *N*-deprotected glycopeptide-resin was stirred with reagent K (TFA/phenol/water/thioanisole/ethanedithiol, 33:2:2:2:1, 500 μl) by the vortex mixer at room temperature for 2 h. 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 crude product, which was dissolved in a mixture of TFA/DMS/*m*-cresol (5:3:1, 180 μl) and cooled at −15 °C. TfOH (20 μl) was added to the mixture and the plastic vessel was shaken in the cooling bath. The reaction mixture was left at −15 °C for 2 h, before the reaction was terminated by the addition of ether. The mixture was centrifuged to precipitate the debenzylated product, which was washed three times with ether as mentioned above. The crude product was purified by HPLC to give **27** (0.14 μmol, 2.2% overall yield based on the amino group on the initial resin). MALDI TOF MS calcd for $C_{158}H_{262}N_{38}O_{63}S_2$ *m/z*: 3763.79. Found: 3764.95 ($M+H^+$). Amino acid analysis: $Asp_{1.00}Thr_{3.26}Ser_{2.42}Glu_{0.94}Gly_3Ala_{1.14}Val_{1.36}Ile_{0.94}Leu_{3.74}Lys_{2.01}His_{0.97}Arg_{1.25}$.

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