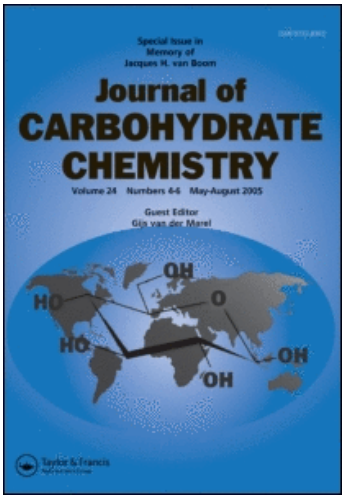

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Synthetic Approach Toward the Partial Sequences of Betaglycan in the Linkage Region on Solid Support and in Solution Phase

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Synthetic Approach Toward the Partial Sequences of Betaglycan in the Linkage Region on Solid Support and in Solution Phase

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We have synthesized, for the first time, the partial sequence of the betaglycan composed of the tetraosyl hexapeptide, which was directly usable as a probe for enzymatic glycosyl transfer. Stepwise elongation afforded the corresponding tetraosyl trichloroacetimidate. The common glycosyl dipeptide:[β-D-GlcA-(1 → 3)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → 4)-β-D-Xyl-(1 → O)-Ser-Gly] was synthesized by glycosylation of the corresponding tetraosyl trichloroacetimidate and Ser-Gly moiety. The glycosyl dipeptide was coupled with other core peptide parts in solution phase and on a solid support. These glycosyl hexapeptides were then transformed into the desired target compounds.

Keywords Proteoglycan, Glycosaminoglycan, Betaglycan, Heparin, Heparan sulfate

INTRODUCTION

Glycosaminoglycan (GAG) is the saccharide part of proteoglycans (PGs) covalently linked to the core peptide through the hydroxyl group of the serine residue. GAG is composed of two parts: the linkage (tetrasaccharide) region and repeating disaccharide region. Biochemically, GAG elongates by a stepwise addition with the help of UDP-sugars and the corresponding glycosyl transferases.[1] GAG is classified into two categories based on the

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type of the hexosamine residues in the repeating disaccharide region (Fig. 1). The first transfer of the hexosamine residue, α-GlcNAc or β-GalNAc, which is the fifth saccharide moiety from the reducing terminal, determines the type of GAG as either the heparin or chondroitin type. Most of the glycosyl transferases involved in the GAG biosynthesis have been found; however, the factors controlling the biosynthetic sorting mechanisms are still unclear.

To the best of our knowledge, two hypotheses on the sorting mechanisms, which are not consistent with each other, have been supported on the basis of the environment around the glycan and/or peptide part of PG. The common tetrasaccharide often possesses sulfates at specific positions. Sugahara and his coworkers advocated that no sulfate has been detected on the heparin type of GAG vs. that on the chondroitin type.[2] It seems that the sulfate in the linkage region of chondroitin might provide an important function for the β-GalNAc transferase. On the other hand, Esko's group reported that the hydrophobic and acidic amino acid clusters of the core peptide are often flanking the heparin type of GAG.[3] His group demonstrated the glycan elongation toward the heparin type of GAG using mutant peptides and glycosyl primers containing hydrophobic aglycons.[4] These results provided important evidence for the relationship between the selective biosynthesis of heparin/heparan sulfate and the specific character of the common tetrasaccharide and/or the core peptides; however, the transfer mechanism of the first hexosamine residue remains unclear.

Heparin/heparan sulfates are medically and pharmaceutically indispensable materials; therefore, the selective and effective biosynthesis of heparin/heparan sulfate is anticipated. These facts prompted us to elucidate the biosynthetic mechanisms of heparin/heparan sulfate from a chemical point of view. Figure 2 shows the partial structure of betaglycan, of which the core peptide contains acidic and hydrophobic amino acids. It is noteworthy that the Ser^{535} of the betaglycan predominantly possesses the heparin type of GAG.[5] We selected the target compounds (**1** and **2**), which are partial sequences of betaglycan containing the above core peptide. We now report

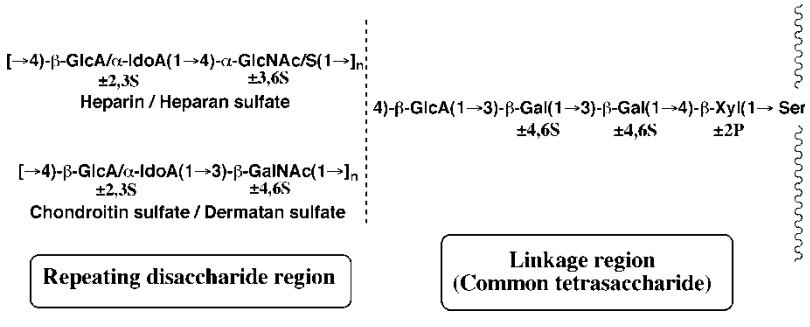


Figure 1: Structure of glycosaminoglycans.

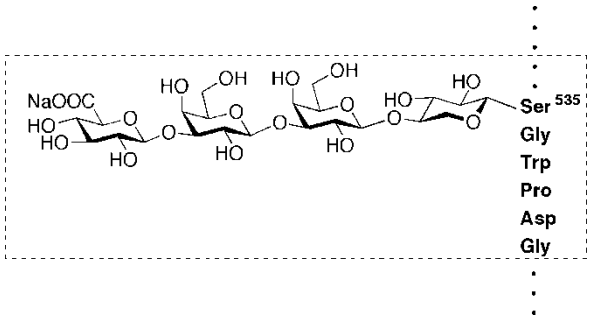


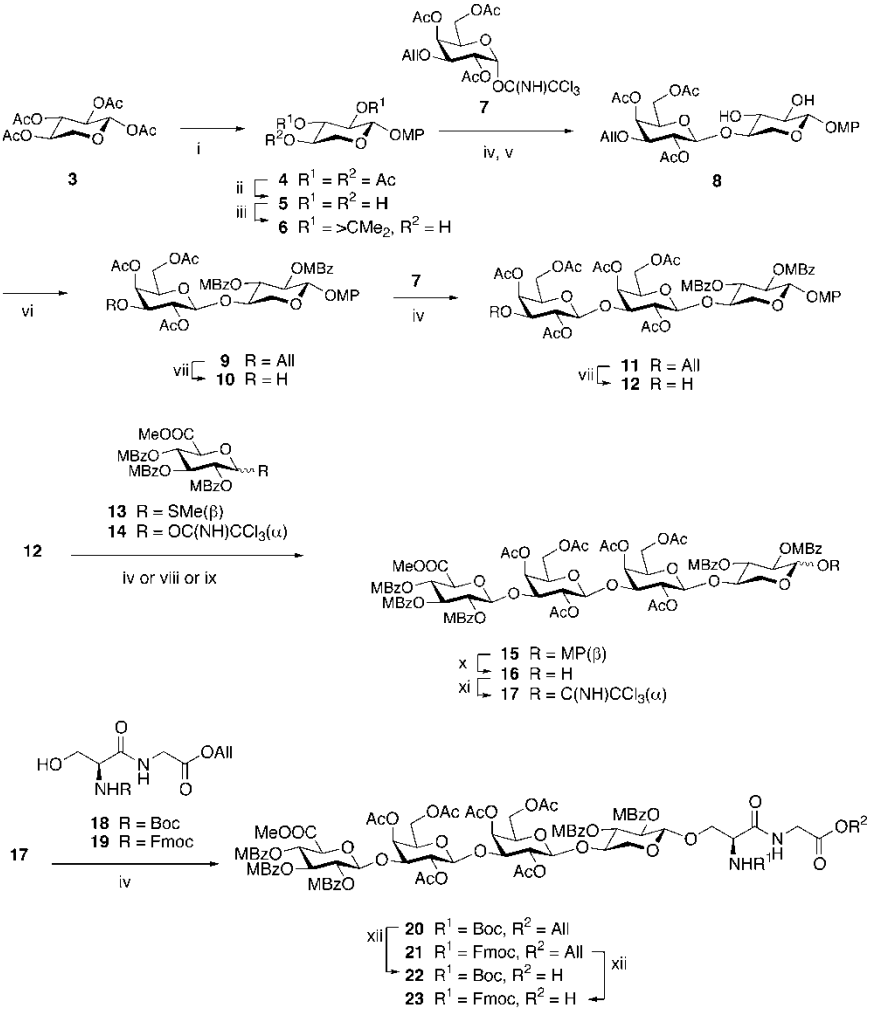
Figure 2: Target compounds. A partial sequence of betaglycan.

the detailed synthesis of these betaglycans, which can be the acceptors for the α-GlcNAc transfer.

RESULTS AND DISCUSSION

Based on a retrosynthetic analysis, we planned (1) the stepwise elongation by the coupling of each monosaccharide moiety from the reducing terminal, (2) the subsequent coupling of the obtained tetrasaccharide with the Ser-Gly moiety, (3) the coupling of the tetraosyl serylglycine with the tetrapeptide synthesized in the solution phase or on a solid support, and (4) the final deprotection to obtain the desired compounds.

As depicted in Scheme 1, in the presence of 4-methoxyphenol and TMSOTf, known tetraacetyl-β-D-xylose (**3**)[6] was converted into the corresponding 4-methoxyphenyl β-D-xyloside (**4**) in a 90% yield. The acetyl groups of **4** were removed with Et_3N to quantitatively give **5**. The hydroxyl groups of **5** at the 2 and 3 positions were isopropylidenated to afford **6** in a 75% yield. The galactosyl imidate (7) was converted from the known 4-methoxyphenyl 2,4,6-tri-O-acetyl-3-O-allyl-β-D-galactopyranoside[7] by the removal of the 4-methoxyphenyl group with CAN, then followed by trichloroacetimidation with CCl_3CN and DBU in 79% and 71% yields, respectively. The 4-OH of **6** was glycosylated with **7** in the presence of TMSOTf in CH_2Cl_2. The following methanolysis afforded the disaccharide (**8**) in an 82% yield in two steps. The diol **8** was acylated with 4-methylbenzoyl chloride to produce **9** in a 77% yield. The position of the newly formed glycosidic linkage was evident from the acylation shifts of the H-2 and H-3 of the xylosyl residue. The allyl group of **9** was isomerized using an iridium catalyst, and the I_2 treatment gave the disaccharide acceptor **10** in a 95% yield in two steps. The disaccharide acceptor **10** was stereoselectively coupled with 7 in the presence of TMSOTf in CH_2Cl_2 to afford the trisaccharide **11** in an 86% yield. The allyl group of **11** was removed in the same manner as above to give the trisaccharide acceptor **12** in an 85% yield in two steps.



Scheme 1: Reagents and conditions: i) 4-methoxyphenol, TMSOTf, MS4A, CH_2Cl_2, −20°C, 1 h; ii) Et_3N, MeOH, H_2O, rt, overnight; iii) 2-methoxypropene, camphorsulfonic acid, DMF, 60°C, 5 h; iv) TMSOTf, MSAW300, CH_2Cl_2, −20°C, 1 h; v) camphorsulfonic acid, MeOH, CH_2Cl_2, rt, 2 h; vi) 4-methylbenzoyl chloride, pyridine, rt, 1.5 h; vii) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate, THF, H_2, then I_2, H_2O, $NaHCO_3$, rt, 2.5 h; viii) $BF_3 \cdot OEt_2$, MSAW300, CH_2Cl_2, −20°C ~rt, overnight; ix) $CuBr_2$, AgOTf, *n*-Bu_4NBr, MS4A, CH_2Cl_2, 0°C ~rt, overnight; x) CAN, CH_3CN, H_2O, 0°C, 4 h; xi) CCl_3CN, DBU, CH_2Cl_2, 0°C, 2 h; xii) $(Ph_3P)_4Pd(0)$, *N*-methylaniline, THF, rt, 4 h.

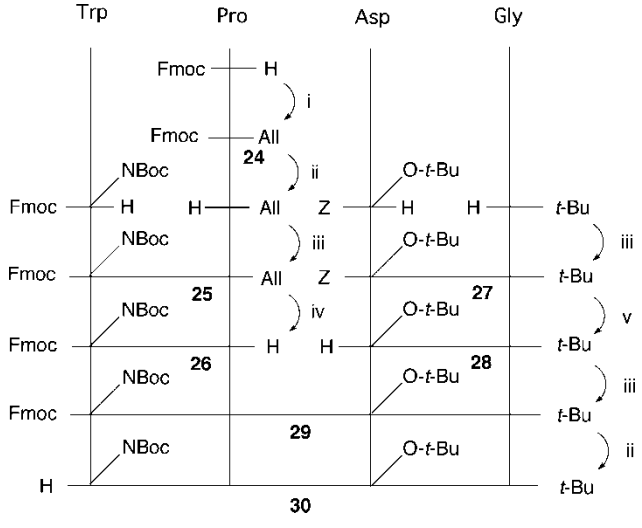
For the following coupling with the glucuronic acid moiety, we prepared two types of glycosyl donors: methyl thioglycoside **13**[8] and trichloroacetimidate **14**.[9] Although **13** was used to couple with **12** mediated by the NIS-TfOH system, no tetrasaccharide was obtained. However, AgOTf-$CuBr_2$-*n*-Bu_4NBr could afford the desired compound **15** in a 31% yield. The glycosylation of **12** with **14** using $BF_3 \cdot OEt_2$ also afforded **15** in a 22% yield with 69% of the

acceptor being recovered. When TMSOTf was used as the promoter, the reaction afforded **15** in a 32% yield, while the residual acceptor was completely decomposed.

The obtained tetrasaccharide was converted into the corresponding hemiacetal **16** with CAN in a 93% yield, and brought to **17** using CCl_3CN in the presence of DBU in an 89% yield. The imidate (**17**) was coupled with known serylglycine allyl esters (**18**[10] and **19**[10]) protected with Boc and Fmoc groups at their N-terminals, with the help of TMSOTf in CH_2Cl_2 at $-20^\circ C$ to give **20** and **21** in 72% and 74% yields, respectively. The allyl groups were removed with $Pd(PPh_3)_4$ and *N*-methylmorpholine to yield the corresponding carboxylic acids **22** and **23** in 90% and 76% yields, respectively.

Synthesis of the Peptide Part

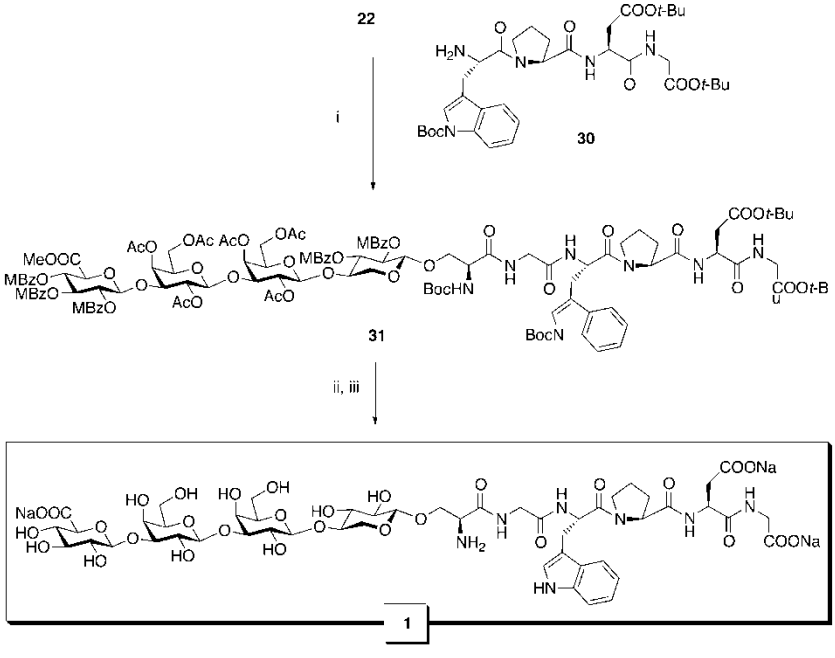
As shown in Scheme 2, the tetrapeptide Trp-Pro-Asp-Gly was synthesized in the conventional manner (liquid phase). Commercially available Fmoc-proline was converted into the corresponding allyl ester (**24**) in an 86% yield. The Fmoc group of **24** was removed with morpholine, and coupled with Fmoc-(N^i-Boc)-tryptophane using HOBt and HBTU in the presence of Hünig's base to give **25** in a 56% yield. The allyl ester was converted into the carboxylic acid **26** with $Pd(PPh_3)_4$ and *N*-methylmorpholine in a 63% yield. On the other hand, the Z-(O-*t*-Bu)-aspartic acid and the glycine *tert*-butyl ester, both of which were commercially available, were coupled with WSCD · HCl and HOBt in the presence of Et_3N to yield **27** in a 96% yield.



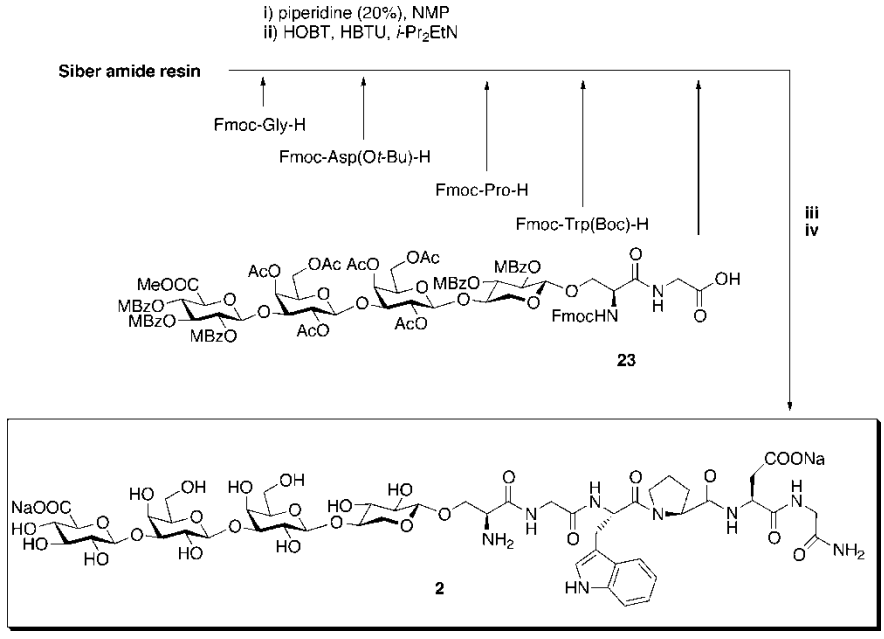
Scheme 2: Reagents and conditions: i) Cs_2CO_3, EtOH, rt, 1 h, then AllBr, DMF, rt, overnight; ii) Morpholine, CH_2Cl_2, rt, overnight; iii) HOBT, HBTU, CH_2Cl_2, $-20^\circ C$ ~rt, overnight; iv) $(Ph_3P)_4$ Pd(0), N-methylaniline, THF, rt, 15 h; v) Pd-C, H_2, MeOH, AcOH, rt, 3 h.

Hydrogenolysis of **27** was successfully performed to give the free amine **28** in a 93% yield. The following condensation of **26** and **28** were executed in the same manner as the synthesis of **27** to furnish the tetrapeptide **29** in an 85% yield. The Fmoc group of **29** was finally removed with morpholine to give the free amine **30**, which was coupled with **22** in the presence of HOBt and HBTU. As depicted in Scheme 3, the successful condensation afforded the tetraosyl hexapeptide **31** in a 63% yield. The following deprotection procedures—(1) treatment with a TFA cocktail and (2) saponification with M0.107 NaOMe in 50% MeOH—gave the desired compound **1** in an 83% yield after gel permeation chromatography.

Scheme 4 shows the synthesis of the corresponding glycopeptide on Sieber amide resin. The elongation was manually performed by the usual Fmoc procedure employing HOBt, HBTU, and *i*-Pr_2EtN in NMP. The Fmoc group was removed using piperidine in NMP. The *N*-terminal of the tetrapeptide on the resin was coupled with **23** in the same manner as the peptide elongation. The obtained glycosylated peptide on the resin was subjected to the TFA cocktail to give the corresponding tetraosyl hexapeptide. Careful saponification with diluted base (1M NaOH-MeOH 1:100, and the following 5 mM NaOH)[11] treatment afforded the fully deprotected glycosyl peptide **2** in the amide



Scheme 3: Reagents and conditions: i) HOBT, HBTU, *i*-Pr_2EtN, DMF, −20°C ∼rt, overnight; ii) TFA, thioanisole, phenol, H_2O, 1,2-ethanedithiol, triisopropylsilane, CH_2Cl_2, rt, 2 h; iii) NaOMe, MeOH, H_2O, rt, 3 h.



Scheme 4: Reagents and conditions: i) piperidine (20%), NMP, ii) HOBT, HBTU, i-Pr_2EtN, iii) TFA, thioanisole, phenol, H_2O, 1,2-ethanedithiol, triisopropylsilane, CH_2Cl_2, rt, 3 h; iv) NaOMe, MeOH, H_2O, rt.

form. Further purification was successfully performed by gel permeation chromatography and HPLC (C18). The final compounds **1** and **2** were in good agreement with the calculated value based on the mass spectrum.

It was revealed that compound **1** was a poor substrate for the chondroitin GalNAc T-1 and T-2[12] but a superior substrate for heparan sulfate elongation (EXT1/EXT2) as well as the disaccharide primer, β-D-GlcA(1 $\rightarrow$ 3) β-D-Gal(1 $\rightarrow$ O)C_2H_4NHZ having a hydrophobic aglycon.[13]

In summary, we synthesized the partial sequences in the linkage region of betaglycan as primers for the biosynthesis of heparan sulfate. Both synthetic strategies in the liquid phase as well as on a solid support are available for the synthesis of the target tetraosyl hexapeptides: βGlcA-βGal-βGal-βXyl-SerGlyTrpProAspGly.

EXPERIMENTAL

General Methods

Optical rotations were measured at 22 $\pm$ 3°C with a HORIBA polarimeter SEPA-200. ^{1}H NMR assignments were confirmed by two-dimensional HH-COSY experiments with a JEOL ECP 500 MHz spectrometer. Signal

assignments such as 1^{III} stand for a proton at C-1 of sugar residue III. MALDI-TOF and ESI mass spectra were measured by Daltonics Autoflex II spectrometer (Bruker) and Q-TOF2 (Micromass) instrument, respectively. Silica gel chromatography, analytical TLC, and preparative TLC (PTLC) were done on a column of Silica Gel 60 (Merck), Silica Gel 60 N (spherical neutral), (Kanto Kagaku) or glass plates coated with Silica Gel F_{254} (Merck), respectively. Gels for size-exclusion chromatography (Sephadex LH-20 and Biobeads S-X1) were purchased from Amersham Biosciences and BIO · RAD, respectively. Reversed phase short column (Bond Elut® C8) was purchased from Varian Inc. Molecular sieves (MS) were purchased from GL Science, Inc., and activated at 200°C under diminished pressure prior to use. All reactions in organic solvents were performed under dry Ar atmosphere. As a usual workup, the organic phase of the reaction mixture was washed with aq. $NaHCO_3$ and brine and dried over $MgSO_4$.

4-Methoxyphenyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (4)

To a suspension of 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose[6] (**3**, 15.5 g, 48.7 mmol) and 4-methoxyphenol (6.70 g, 54.0 mmol) in the presence of activated MS 4A (2.5 g) in 1,2-dichloroethane (100 mL) was added TMSOTf (4.1 mL, 2.3 mmol) at −20°C with stirring, and then the reaction temperature was gradually raised up to −14°C within 1 h. Et_3N (15 mL) and an excess amount of saturated $NaHCO_3$ were added. Insoluble materials were filtered on Celite. The organic phase was treated as described in the general methods. The crude materials were subjected to a column of silica gel (5:1–3:1 toluene:EtOAc) to give **4** (16.8 g) in 90% yield. A small amount of the product was recrystallized from *n*-hexane:EtOAc to give white powder: $[\alpha]_D$ −41.5 (*c* 0.66, $CHCl_3$); m.p. 150.4°C; ^{1}H NMR ($CDCl_3$): δ 6.96–6.94 (m, 2H, Ar H), 6.84–6.81 (m, 2H, Ar H), 5.23 (dd, 1H, $J_{2,3} = 8.29$ Hz, $J_{3,4} = 7.81$ Hz, H-3), 5.16 (dd, 1H, $J_{1,2} = 6.10$ Hz, H-2), 5.03 (d, 1H, H-1), 5.01 (m, 1H, H-4), 4.21 (dd, 1H, $J_{4,5eq} = 4.88$ Hz, $J_{gem} = 11.96$ Hz, H-5eq), 3.77 (s, 3H, OMe), 3.47 (dd, 1H, $J_{4,5ax} = 8.29$ Hz, H-5ax), 2.10, 2.08, 2.08 (3s, 3H × 3, 3MeCO). Anal. Calcd for $C_{18}H_{22}O_9$: C, 56.53; H, 5.81. Found: C, 56.51; H, 5.81.

4-Methoxyphenyl β-D-xylopyranoside (5)

Triacetate (**4**) (16.8 g, 44.0 mmol) was diluted with Et_3N (75 mL), MeOH (150 mL), and H_2O (75 mL) with stirring overnight. The volatiles were removed under diminished pressure and the residue was coevaporated with water and toluene. The residue was purified from a column of silica gel (1:1–1:5 *n*-hexane:EtOAc–100:1–40:1–20:1 EtOAc:MeOH) to give **5** quantitatively: $[\alpha]_D$ −26.7 (*c* 1.29, MeOH); ^{1}H NMR (CD_3OD): δ 6.91–6.89 (m, 2H, Ar H), 6.74–6.72 (m, 2H, Ar H), 4.61 (d, 1H, $J_{1,2} = 7.56$ Hz, H-1), 3.79 (dd, 1H, $J_{4,5a} = 5.37$ Hz, $J_{gem} = 11.47$ Hz, H-5a), 3.64 (s, 3H, OMe), 3.5–3.2 (m, 4H, H-2,3,4,5e). Anal. Calcd for $C_{12}H_{16}O_6$: C, 56.24; H, 6.31. Found: C, 56.19; H, 6.31.

4-Methoxyphenyl 2,3-O-isopropylidene β-D-xylopyranoside (6)

To a solution of **5** (15.2 g, 59.4 mmol) in DMF (70 mL) were added a camphor sulfonic acid (2.1 g, 9.0 mmol) and 2-methoxypropene (12.0 mL, 125 mmol) with stirring at 60°C. After 2 h, 6 mL of 2-methoxypropene was added for 30 minutes. Forty minutes later, the reaction mixture was cooled to rt and Et_3N (6.0 mL, 35 mmol) was added. The reaction mixture was extracted with EtOAc. The organic phase was treated as described in the general methods. The residue was eluted from a column of silica gel (5:1–4:1–1:1–1:5 toluene:EtOAc) to give **6** (13.2 g, 75%) as a syrup: $[\alpha]_D$ −32.3 (*c* 1.32, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.04–7.01 (m, 2H, Ar H), 6.84–6.81 (m, 2H, Ar H), 5.18 (d, 1H, $J_{1,2} = 5.86$ Hz, H-1), 4.15 (m, 1H, H-5eq), 4.11 (m, 1H, H-4), 3.77 (s, 3H, OMe), 3.62 (m, 2H, H-2,3), 3.38 (dd, 1H, $J_{4,5ax} = 6.34$ Hz, $J_{gem} = 11.47$ Hz, H-5ax), 2.49 (d, 1H, $J_{4,OH} = 3.91$ Hz, OH-4), 1.51, 1.49 (2s, 3H × 2, $2CH_3$). Anal. Calcd for $C_{15}H_{20}O_6 \cdot 0.2H_2O$: C, 60.06; H, 6.87. Found: C, 59.88; H, 6.90.

2,4,6-Tri-O-acetyl-3-O-allyl-α-D-galactopyranosyl trichloroacetimidate (7)

To a solution of 4-methoxyphenyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-galactopyranoside[7] (77.3 g, 171 mmol) in CH_3CN (400 mL) and H_2O (100 mL) was added cerium (IV) ammonium nitrate (CAN) (88.4 g, 161 mmol) at 0°C with stirring. After 3 h, additional CAN (175.57 g, 320.3 mmol) was supplied and the reaction mixture was stirred for another 2.5 h, then diluted with $CHCl_3$ and brine and extracted with $CHCl_3$. The organic phase was washed with brine and the residue was eluted from a column of silica gel (2:1–3:2–1:1–1:3 *n*-hexane:EtOAc) to give the corresponding hemiacetal (47.0 g, 79%) as a syrup. The hemiacetal was diluted with CH_2Cl_2 (350 mL). To the solution were added CCl_3CN (22 mL) and DBU (8.0 mL, 54 mmol) at 0°C. The reaction mixture was stirred for 1.5 h and directly subjected to a column of silica gel (3:1∼3:2∼1:1 *n*-hexane-EtOAc) to give **7** (47.4 g, 71%) as a syrup, which was used for the next reaction without further purification: ^1H NMR ($CDCl_3$): δ 8.63 (s, 1H, NH), 6.56 (s, 1H, H-1), 5.88–5.78 (m, 1H, CH═), 5.58 (d, 1H, $J_{3,4} = 2.54$ Hz, H-4), 5.31–5.17 (m, 3H, H-2, $=CH_2$), 4.35 (brt, 1H, $J = 6.47$ Hz, H-5), 4.23–3.97 (m, 5H, H-3, 6, CH_2O), 2.16, 2.05, 2.04 (3s, 3H × 3, 3MeCO).

4-Methoxyphenyl O-(2,4,6-tri-O-acetyl-3-O-allyl-β-D-galactopyranosyl)-(1 → 4)-β-D-xylopyranoside (8)

To a solution of **6** (321.0 mg, 1.083 mmol) and **7** (977.5 mg, 1.992 mmol) in CH_2Cl_2 (15 mL) was added MS AW300 (300 mg). This mixture was stirred for 30 min at rt and cooled to −15°C. To this solution was added a TMSOTf (70 μL, 0.38 mmol) with stirring and the reaction temperature was gradually raised to −10°C for 1 h. Then, Et_3N (110 μL, 0.79 mmol) and an excess

amount of aqueous $NaHCO_3$ were added. Insoluble materials were filtered on Celite. The organic phase was treated as described in the general methods. To a solution of the crude products in MeOH and CH_2Cl_2 (1:1, 46 mL) was added a camphor sulfonic acid (251.6 mg, 1.08 mmol) and the mixture stirred for 2 h and quenched with Et_3N (150 μL, 1.08 mmol). The volatiles were removed under diminished pressure and the residue was subjected to a column of silica gel (4:1–2:1–1:1–2:3 toluene:EtOAc) to give **8** (518.1 mg) in 82% yield as a syrup: $[\alpha]_D$ −3.9 (*c* 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.01–6.97 (m, 2H, Ar H), 6.85–6.82 (m, 2H, Ar H), 5.82–5.73 (m, 1H, CH═), 5.42 (d, 1H, $J_{3,4} = 3.42$ Hz, H-4^{II}), 5.34–5.17 (m, 2H, ═CH_2), 5.10 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{II}), 4.90 (d, 1H, $J_{1,2} = 6.59$ Hz, H-1^{I}), 4.48 (d, 1H, H-1^{II}), 4.24 (dd, 1H, $J_{5,6a} = 4.88$ Hz, $J_{gem} = 11.47$ Hz, H-6a^{II}), 4.14–4.07 (m, 1H, one of OCH_2), 4.09 (m, 1H, H-6b^{II}), 3.95 (dd, 1H, $J_{4,5eq} = 4.64$ Hz, $J_{gem} = 11.95$ Hz, H-5eq^{I}), 3.92–3.88 (m, 2H, H-5^{II}, one of CH_2O), 3.89 (m, 1H, OH-3^{I}), 3.78 (s, 3H, OMe), 3.78–3.73 (m, 1H, H-3^{I}), 3.67 (m, 2H, H-2^{I},4^{I}), 3.53 (dd, 1H, H-3^{II}), 3.41 (dd, 1H, $J_{4,5ax} = 8.54$ Hz, H-5ax^{I}), 2.78 (d, 1H, $J_{2,OH} = 3.66$ Hz, OH-2^{I}), 2.14, 2.12, 2.12, (3s, 3H × 3, 3MeCO). Anal. Calcd for $C_{27}H_{36}O_{14} \cdot 0.5\ H_2O$: C, 54.62; H, 6.30. Found: C, 54.68; H, 6.02.

4-Methoxyphenyl O-(2,4,6-tri-O-acetyl-3-O-allyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranoside (9)

To a solution of **8** (1.71 g, 2.92 mmol) in pyridine (10 mL) was added a 4-methylbenzoyl chloride (1.50 mL, 11.6 mmol) dropwise with stirring. After 1.5 h MeOH (0.5 mL) was added to the reaction mixture at 0°C and diluted with $CHCl_3$. The organic phase was treated as described in general methods. The crude materials were subjected to a column of silica gel (10:1–7:1–6:1–4:1–1:1 toluene:EtOAc) to give **9** (1.85 g, 77%) as a syrup: $[\alpha]_D$ +33.9 (*c* 0.945, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.97–7.95 (m, 4H, Ar H), 7.24–7.22 (m, 4H, Ar H), 6.98–6.96 (m, 2H, Ar H), 6.82–6.80 (m, 2H, Ar H), 5.84–5.71 (m, 1H, CH═), 5.68 (brt, 1H, $J = 6.30$ Hz, H-3^{I}), 5.43 (dd, 1H, $J_{1,2} = 5.04$ Hz, $J_{2,3} = 6.18$ Hz, H-2^{I}), 5.29 (brd, 2H, $J = 4.58$ Hz, H-1^{I},4^{II}), 5.23–5.14 (m, 2H, ═CH_2), 5.05 (dd, 1H, $J_{1,2} = 8.02$ Hz, $J_{2,3} = 10.08$ Hz, H-2^{II}), 4.57 (d, 1H, H-1^{II}), 4.23 (dd, 1H, $J_{4,5eq} = 3.90$ Hz, $J_{gem} = 12.37$ Hz, H-5eq^{I}), 4.10–4.06 (m, 1H, one of OCH_2), 3.98 (m, 1H, H-4^{I}), 3.87–3.84 (m, 1H, one of CH_2O), 3.83 (dd, 1H, $J_{5,6a} = 6.19$ Hz, $J_{gem} = 10.99$ Hz, H-6a^{II}), 3.76 (s, 3H, OMe), 3.71 (brt, 1H, $J = 6.53$ Hz, H-5^{II}), 3.65 (dd, 1H, $J_{4,5ax} = 6.65$ Hz, H-5ax^{I}), 3.59 (dd, 1H, $J_{5,6b} = 6.65$ Hz, H-6b^{II}), 3.46 (dd, 1H, $J_{3,4} = 3.44$ Hz, H-3^{II}), 2.40, 2.38 (2s, 3H × 2, 2PhMe), 2.03, 2.02, 1.96 (3s, 3H × 3, 3MeCO). Anal. Calcd for $C_{43}H_{48}O_{16} \cdot H_2O$: C, 61.56; H, 6.02. Found: C, 61.72; H, 5.87.

4-Methoxyphenyl O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranoside (10)

A suspension of a catalytic amount of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate in THF (10 mL) was stirred

under H_2 atmosphere, which was then replaced with argon. This manipulation was repeated a few times. Then, a solution of **9** (2.01 g, 2.45 mmol) in THF (20 mL) was added to the above solution of the iridium complex. After stirring for 2 h, H_2O (5 mL), $NaHCO_3$ (3.92 g, 46.7 mmol), and I_2 (1.72 g, 6.78 mmol) were added to the reaction mixture, and the stirring was continued for 2.5 h at 0°C. The reaction mixture was then diluted with $CHCl_3$. The organic phase was washed with aq. $NaHCO_3$, aq. $Na_2S_2O_3$, and brine. The volatiles were removed under diminished pressure, and the crude materials obtained were eluted from a column of silica gel (4:1–3:1–2:1–1:1–1:2 toluene:EtOAc) to give **10** (1.82 g, 95%) as a syrup: $[\alpha]_D$ +22.6 (*c* 1.54, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.94 (m, 4H, Ar H), 7.29–7.21 (m, 4H, Ar H), 6.98–6.96 (m, 2H, Ar H), 6.82–6.80 (m, 2H, Ar H), 5.69 (t, 1H, $J_{2,3} = J_{3,4} = 6.34$ Hz, H-3^{I}), 5.43 (dd, 1H, $J_{1,2} = 4.88$ Hz, H-2^{I}), 5.30 (d, 1H, H-1^{I}), 5.20 (d, 1H, $J_{3,4} = 3.42$ Hz, H-4^{II}), 4.90 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{II}), 4.60 (d, 1H, H-1^{II}), 4.26 (dd, 1H, $J_{4,5eq} = 3.66$ Hz, $J_{gem} = 12.20$ Hz, H-5eq^{I}), 4.00 (m, 1H, H-4^{I}), 3.85 (dd, 1H, $J_{5,6a} = 6.34$ Hz, $J_{gem} = 10.98$ Hz, H-6a^{II}), 3.80–3.73 (m, 2H, H-3^{II},5^{II}), 3.75 (s, 3H, OMe), 3.68 (dd, 1H, $J_{4,5ax} = 6.10$ Hz, H-5ax^{I}), 3.59 (dd, 1H, $J_{5,6b} = 6.83$ Hz, H-6b^{II}), 2.51, 2.49 (2s, 3H × 2, 2Ph*Me*), 2.06, 2.03, 1.94 (3s, 3H × 3, 3MeCO). Anal. Calcd for $C_{40}H_{44}O_{16} \cdot H_2O$: C, 60.14; H, 5.82. Found: C, 60.00; H, 5.53.

4-Methoxyphenyl O-(2,4,6-tri-O-acetyl-3-O-allyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranoside (11)

To a solution of **10** (7.40 g, 9.48 mmol) and **7** (7.12 g, 14.5 mmol) in CH_2Cl_2 (160 mL) was added MS AW300 (4.0 g). This mixture was stirred for 35 min at rt, and then cooled to −20°C. To this solution was added a TMSOTf (660 μL, 3.65 mmol) with stirring. After 1 h, the reaction was quenched with Et_3N (1.0 mL, 7.0 mmol) and excess amount of aq. $NaHCO_3$. The reaction was treated as described in the synthesis of **8**. The residue was subjected to a column of silica gel (5:1–4:1–3:1–2:1–1:2 *n*-hexane:EtOAc) to give **11** (9.06 g) in 86% yield as a syrup: $[\alpha]_D$ +51.5 (*c* 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.98–7.94 (m, 5H, Ar H), 7.24–7.23 (m, 3H, Ar H), 6.97 (m, 2H, Ar H), 6.82 (m, 2H, Ar H), 5.80–5.70 (m, 1H, =CH), 5.68 (t, 1H, $J_{2,3} = J_{3,4} = 6.10$ Hz, H-3^{I}), 5.42 (dd, 1H, $J_{1,2} = 4.88$ Hz, H-2^{I}), 5.37 (d, 1H, $J_{3,4} = 3.42$ Hz, H-4^{III}), 5.30 (d, 1H, H-1^{I}), 5.27 (d, 1H, $J_{3,4} = 3.42$ Hz, H-4^{II}), 5.19 (m, 2H, =CH_2), 5.16 (dd, 1H, $J_{1,2} = 7.81$ Hz, $J_{2,3} = 9.51$ Hz, H-2^{II}), 4.95 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{III}), 4.55 (d, 1H, H-1^{II}), 4.45 (d, 1H, H-1^{III}), 4.23 (dd, 1H, $J_{4,5eq} = 3.66$ Hz, $J_{gem} = 12.44$ Hz, H-5eq^{I}), 4.10 (m, 2H, OCH_2), 4.10 (dd, $J_{5,6b} = 5.12$ Hz, $J_{gem} = 12.69$ Hz, H-6b^{III}), 3.96 (m, 1H, H-4^{I}), 3.89–3.85 (m, 2H, H-6a^{III}, 6b^{II}), 3.84–3.75 (m, 3H, H-3^{II}, 5^{II}, 5^{III}), 3.64 (dd, 1H, $J_{4,5ax} = 6.10$ Hz, H-5ax^{I}), 3.51 (dd, 1H, $J_{5,6a} = 6.83$ Hz, H-6a^{II}), 3.41 (dd, 1H, H-3^{III}), 2.40, 2.39

(2s, 3H × 2, 2MePh), 2.14, 2.08, 2.06, 2.06, 2.04, 1.94 (6s, 3H × 6, 6MeCO). ESI-MS (positive) Calcd for $C_{55}H_{64}O_{24}Na$ $[M + Na]^+$, 1131.4. Found: 1130.9.

4-Methoxyphenyl O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranoside (12)

The iridium catalyst was suspended in THF and activated as described in the synthesis of **10**. Then, a solution of **11** (95.9 mg, 86.5 μmol) in THF (3 mL) was added to the iridium complex solution. After stirring for 45 min, H_2O (1 mL), $NaHCO_3$ (145.3 mg, 1.730 mmol), and I_2 (43.9 mg, 0.174 mmol) were added to the reaction mixture at 0°C. The solution was stirred for 45 min at 0°C and treated as described in the synthesis of **10**. The obtained crude material was eluted from a column of silica gel (9:1–7:1–5:1–3:1–2:1–1:5 toluene:EtOAc) to give **12** (78.5 mg, 85%) as a syrup: $[\alpha]_D$ +26.3 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.97–7.94 (m, 5H, Ar H), 7.24–7.22 (m, 3H, Ar H), 6.97 (m, 2H, Ar H), 6.81 (m, 2H, Ar H), 5.68 (t, 1H, $J_{2,3} = J_{3,4} = 6.10$ Hz, H-3^{I}), 5.43 (dd, 1H, $J_{1,2} = 4.89$ Hz, H-2^{I}), 5.30–5.27 (m, 3H, H-1^{I}, 4^{II}, 4^{III}), 5.16 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{II}), 4.83 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{III}), 4.58 (d, 1H, H-1^{II}), 4.47 (d, 1H, H-1^{III}), 4.23 (dd, 1H, $J_{4,5eq} = 3.66$ Hz, $J_{gem} = 12.44$ Hz, H-5eq^{I}), 4.17–4.08 (m, 2H, H-6^{III}), 3.97 (m, 1H, H-4^{I}), 3.86 (dd, 1H, $J_{5,6b} = 5.37$ Hz, $J_{gem} = 11.22$ Hz, H-6b^{II}), 3.79–3.71 (m, 3H, H-3^{II}, 3^{III}, 5^{II}), 3.64 (dd, 1H, $J_{4,5ax} = 6.34$ Hz, H-5ax^{I}), 3.51 (dd, 1H, $J_{5,6a} = 7.07$ Hz, H-6a^{II}), 2.41, 2.39 (2s, 3H × 2, 2MePh), 2.18, 2.12, 2.07, 2.04, 2.04, 1.94 (6s, 3H × 6, 6MeCO). TOF-MS (positive) Calcd for $C_{52}H_{60}O_{24}Na$ $[M + Na]^+$, 1091.3. Found: 1091.5.

4-Methoxyphenyl O-[methyl 2,3,4-tri-O-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranoside (15)

Method A: A suspension containing $CuBr_2$ (1.37 g, 6.14 mmol), AgOTf (1.58 g, 6.14 mmol), *n*-Bu_4NBr (327 mg, 1.01 mmol), and MS 4A (5.0 g) in CH_2Cl_2 (31 mL) was stirred in the dark at rt and cooled to 0°C after 30 min. Then, CH_2Cl_2 (60 mL) and a solution of **13** (798.3 mg, 1.347 mmol) and **12** (713.0 mg, 0.667 mmol) were added dropwise to the suspension and stirred overnight, gradually raised to rt. The reaction was quenched with aq. $NaHCO_3$ and brine. Then, the reaction mixture was diluted with EtOAc and filtered on Celite. The organic phase was treated as usual. The obtained crude materials were eluted from a column of silica gel (3:1–3:2–1:1–1:10 *n*-hexane:EtOAc) to give **15** (338.8 mg, 31%) as a syrup together with 288.6 mg (41%) of recovered **12**.

Method B: To a solution of **14** (1.11 g, 1.57 mmol) and **12** (675.1 mg, 0.631 mmol) in CH_2Cl_2 (28 mL) was added MS AW300 (300 mg). This

mixture was stirred for 10 min at rt, and then cooled to −20°C. To this solution was added a TMSOTf (85 μL, 0.47 mmol) with stirring, and the same amount of TMSOTf was added after 2 h. One hour later, the reaction was quenched with Et_3N (260 μL, 1.88 mmol) and an excess amount of aq. $NaHCO_3$ was added. The reaction was treated as described in the synthesis of **8**. The residue was purified as above to give **15** (323.7 mg) in 32% yield..

Method C: To a solution of **14** (147.9 mg, 209.2 μmol) and **12** (96.5 mg, 90.3 μmol) in toluene (17.5 mL) and CH_2Cl_2 (0.2 mL) was added MS AW300 (480 mg). This mixture was stirred for 30 min at rt, and then cooled to −20°C. To this solution was added a $BF_3 \cdot OEt_2$ (8 μL, 0.09 mmol) with stirring and the same amount of $BF_3 \cdot OEt_2$ was added after 1.5 h. The reaction mixture was stirred overnight while the temperature gradually was raised to rt. The reaction was worked up with an excess amount of aq. $NaHCO_3$ and treated as above to give **15** (31.4 mg) in 22% yield together with 66.4 mg (69%) of recovered **12**: $[\alpha]_D$ +26.9 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.97–7.93 (m, 4H, Ar H), 7.81–7.70 (m, 6H, Ar H), 7.23–7.07 (m, 10H, Ar H), 6.96 (m, 2H, Ar H), 6.81 (m, 2H, Ar H), 5.80 (t, 1H, $J_{2,3} = J_{3,4} = 9.27$ Hz, H-3^{IV}), 5.65 (t, 1H, $J_{2,3} = J_{3,4} = 6.10$ Hz, H-3^{I}), 5.64 (brt, 1H, $J = 9.76$ Hz, H-4^{IV}), 5.47 (d, 1H, $J_{3,4} = 3.45$ Hz, H-4^{III}), 5.41 (dd, 1H, $J_{1,2} = 4.88$ Hz, H-2^{I}), 5.37 (dd, 1H, $J_{1,2} = 7.56$ Hz, H-2^{IV}), 5.29 (d, 1H, H-1^{I}), 5.22 (d, 1H, $J_{3,4} = 3.42$ Hz, H-4^{II}), 5.08 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.00$ Hz, H-2^{II}), 5.02 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 10.24$ Hz, H-2^{III}), 4.91 (d, 1H, H-1^{IV}), 4.51 (d, 1H, H-1^{II}), 4.37 (d, 1H, H-1^{III}), 4.26 (d, 1H, $J_{4,5} = 10.01$ Hz, H-5^{IV}), 4.21 (dd, 1H, $J_{4,5eq} = 3.91$ Hz, $J_{gem} = 12.19$ Hz, H-5eq^{I}), 4.16 (dd, 1H, $J_{5,6b} = 5.85$ Hz, $J_{gem} = 11.71$ Hz, H-6b^{III}), 4.03 (dd, 1H, $J_{5,6a} = 6.34$ Hz, H-6a^{III}), 3.93 (m, 1H, H-4^{I}), 3.85–3.81 (m, 2H, H-6b^{II}, 3^{III}), 3.79–3.73 (m, 3H, H-3^{II}, 5^{II}, 5^{III}), 3.76 (s, 3H, MeOPh), 3.70 (s, 3H, COOMe), 3.61 (dd, 1H, $J_{4,5ax} = 5.61$ Hz, H-5ax^{I}), 3.51 (dd, 1H, $J_{5,6a} = 7.07$ Hz, $J_{gem} = 11.47$ Hz, H-6a^{II}), 2.40, 2.37, 2.37, 2.36, 2.30 (5s, 3H × 5, 5MePh), 2.16, 2.09, 2.02, 1.93, 1.89, 1.65 (6s, 3H × 6, 6MeCO). Anal. Calcd for $C_{83}H_{88}O_{33} \cdot 4H_2O$: C, 59.13; H, 5.57. Found: C, 59.01; H, 6.13. ESI-MS (positive) Calcd for $C_{83}H_{88}O_{33}Na$ $[M + Na]^+$, 1635.5. Found: 1635.3.

O-[Methyl 2,3,4-tri-O-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-α-D-xylopyranosyl trichloroacetimidate (17)

To a solution of **15** (445.0 mg, 0.276 mmol) in CH_3CN (36 mL) and H_2O (9 mL) was added CAN (453.6 mg, 0.827 mmol) at 0°C with stirring. After 3 h, additional CAN (150.0 mg, 0.274 mmol) was supplied. The reaction mixture was stirred for another 1.5 h and treated as the synthesis of **7**. The residue was eluted from a column of silica gel (3:1–2:1–1:1–1:5–1:8 *n*-hexane:EtOAc) to give **16** (385.8 mg, 93%) as a syrup. The obtained hemiacetal (**16**) was diluted with

CH_2Cl_2 (17 mL). To the solution were added CCl_3CN (260 μL) and 1 drop of DBU at 0°C. The reaction mixture was stirred for 2 h and directly subjected to a column of silica gel (3:1–1:1–1:3–1:4 *n*-hexane:EtOAc) to give **17** (377.2 mg, 89%) as a syrup, which was used for the glycosylation without further purification.

***N*-Butoxycarbonyl-*O*-{[methyl 2,3,4-tri-*O*-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-(4-methylbenzoyl)-β-D-xylopyranosyl}-L-serylglycine allyl ester (20)**

To a solution of **17** (409.7 mg, 248.0 μmol) and **18** (225.2 mg, 744.9 μmol) in CH_2Cl_2 (47 mL) was added MS AW300 (1.8 g). This mixture was stirred for 30 min at rt, and then cooled to −20°C. To this solution was added a TMSOTf (9 μL, 0.05 mmol) with stirring, and the same amount of TMSOTf was added after 1 h, and stirring was continued for another 1.5 h. Then, the reaction was treated as described in the synthesis of **8**. The residue was subjected to the columns of gel permeation (LH-20, 1:1 $CHCl_3$:MeOH) and silica gel (5:1–3:1–2:1–1:1–1:2 toluene:EtOAc) to give **20** (318.7 mg) in 72% yield: $[\alpha]_D$ +31.6 (*c* 1.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ7.80–7.62 (m, 10H, Ar H), 7.12–7.00 (m, 10H, Ar H), 6.82 [m, 1H, NH(Gly)], 5.80 (m, 1H, =CH), 5.72 (dd, 1H, $J_{2,3} = 9.03$ Hz, $J_{3,4} = 9.76$ Hz, H-3^{IV}), 5.57 (t, 1H, H-4^{IV}), 5.49 (t, 1H, $J_{2,3} = J_{3,4} = 7.81$ Hz, H-3^{I}), 5.40 (d, 1H, $J_{3,4} = 3.17$ Hz, H-4^{III}), 5.36 [m, 1H, NH(Ser)], 5.28 (dd, 1H, $J_{1,2} = 7.08$ Hz, H-2^{IV}), 5.20 (m, 2H, =CH_2), 5.14 (d, 1H, $J_{3,4} = 1.71$ Hz, H-4^{II}), 5.12 (dd, 1H, $J_{1,2} = 6.10$ Hz, H-2^{I}), 4.95 (dd, 1H, $J_{1,2} = 8.05$ Hz, $J_{2,3} = 7.81$ Hz, H-2^{II}), 4.92 (dd, 1H, $J_{1,2} = 7.81$ Hz, $J_{2,3} = 10.49$ Hz, H-2^{III}), 4.84 (d, 1H, H-1^{IV}), 4.65 (d, 1H, H-1^{I}), 4.46 (m, 2H, OCH_2), 4.37 (d, 1H, H-1^{II}), 4.29 (d, 1H, H-1^{III}), 4.19 (d, 1H, H-5^{IV}), 4.15 (m, 1H, Ser*α*), 4.09 (dd, 1H, $J_{5,6b} = 6.10$ Hz, $J_{gem} = 10.97$ Hz, H-6b^{III}), 4.06 (dd, 1H, $J_{4,5eq} = 5.61$ Hz, $J_{gem} = 13.17$ Hz, H-5eq^{I}), 3.97 (dd, 1H, $J_{5,6a} = 5.86$ Hz, H-6a^{III}), 3.89 (m, 1H, H-4^{I}), 3.89 (dd, 1H, $J_{a,NH} = 5.37$ Hz, $J_{gem} = 18.54$ Hz, Glya), 3.79 (dd, 1H, $J_{b,NH} = 5.37$ Hz, Glyb), 3.76 (m, 1H, H-3^{III}), 3.73 (dd, 1H, $J_{5,6b} = 5.85$ Hz, $J_{gem} = 11.22$ Hz, H-6b^{II}), 3.68 (brt, 1H, $J = 6.10$ Hz, H-5^{III}), 3.63 (m, 1H, H-3^{II}), 3.63 (s, 3H, COOMe), 3.57 (brt, 1H, $J = 6.10$ Hz, H-5^{II}), 3.48 (m, 2H, Ser*β*), 3.45 (m, 2H, H-5a^{I}, 6a^{II}), 2.29 (s, 6H, 2MePh), 2.28 (s, 6H, 2MePh), 2.22 (s, 3H, MePh), 2.07, 2.01, 1.94, 1.85, 1.83, 1.59 (6s, 3H × 6, 6MeCO), 1.36 (s, 9H, *tert*-Bu). Anal. Calcd for $C_{89}H_{102}N_2O_{37} \cdot H_2O$: C, 59.06; H, 5.80; N, 1.55. Found: C, 59.08; H, 5.97; N, 1.43.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-{[methyl 2,3,4-tri-*O*-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-(4-methylbenzoyl)-β-D-xylopyranosyl}-L-serylglycine allyl ester (21)**

To a solution of **17** (434.7 mg, 263.2 μmol) and **19** (335.1 mg, 789.5 μmol) in CH_2Cl_2 (50 mL) was added MS AW300 (2.3 g). This mixture was stirred

for 30 min at rt and then cooled to −20°C. To this solution was added a TMSOTf (15 μL, 83 μmol) with stirring. Additional TMSOTf (15 and 10 μL) were added after 45 and 100 min, respectively, and stirring was continued for another 1 h. The reaction was quenched and treated as described in the synthesis of **8**. The residue was subjected to the columns of gel permeation (LH-20, 1:1 $CHCl_3$:MeOH) and silica gel (3:1–1:2–1:4 *n*-hexane:EtOAc) to give **21** (427.3 mg) in 74% yield: $[\alpha]_D$ +37.1 (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.98–7.56 (m, 11H, Ar H), 7.70 (d, 2H, $J = 8.02$ Hz, Ar H), 7.57 (brt, 1H, $J = 7.45$ Hz, Ar H), 7.40 (brt, 1H, $J = 7.10$ Hz, Ar H), 7.31–7.22 (m, 2H, Ar H), 7.20–7.14 (m, 9H, Ar H), 7.08 (d, 2H, $J = 8.02$ Hz, Ar H), 6.91 [brs, 1H, NH(Gly)], 5.87–5.79 (m, 1H, =CH), 5.79 (brt, 1H, J=9.28 Hz, H-3^{IV}), 5.64 (t, 1H, $J_{3,4} = J_{4,5} = 9.63$ Hz, H-4^{IV}), 5.58 (t, 1H, $J_{2,3} = J_{3,4} = 8.02$ Hz, H-3^{I}), 5.51 [m, 1H, NH(Ser)], 5.47 (d, 1H, $J_{3,4} = 3.43$ Hz, H-4^{III}), 5.35 (m, 1H, H-2^{IV}), 5.31–5.23 (m, 2H, $=CH_2$), 5.21 (m, 2H, H-2^{I}, 4^{II}), 5.00 (m, 2H, H-2^{II}, 2^{III}), 4.91 (d, 1H, H-1^{IV}), 4.73 (m, 1H, H-1^{I}), 4.54–4.38 (m, 1H, Serα), 4.51 (m, 2H, OCH_2), 4.42 (m, 1H, H-1^{II}), 4.37–4.31 (m, 1H, Serβa), 4.36 (m, 1H, H-1^{III}), 4.26 (d, 1H, H-5^{IV}), 4.17 (m, 1H, H-$6b^{III}$), 4.12 (dd, 1H, $J_{4,5eq} = 7.10$ Hz, $J_{gem} = 14.44$ Hz, H-$5eq^{I}$), 4.06–3.68 [m, 3H, OCH_2 (Fmoc), H-9(Fmoc)], 4.04 (dd, 1H, $J_{5,6a} = 6.64$ Hz, $J_{gem} = 11.91$ Hz, H-$6a^{III}$), 3.99 (m, 1H, H-4^{I}), 3.93 (m, 1H, Glyb), 3.90–3.54 (m, 1H, Serβb), 3.89 (m, 1H, Glya), 3.82 (m, 1H, H-3^{III}), 3.77 (m, 1H, H-$6b^{II}$), 3.75 (brt, 1H, $J = 5.96$ Hz, H-5^{III}), 3.70 (s, 3H, COOMe), 3.68 (m, 1H, H-3^{II}), 3.63 (brt, 1H, $J = 6.30$ Hz, H-5^{II}), 3.53 (m, 2H, H-$5a^{I}$, $6a^{II}$), 2.36 (s, 12H, 4MePh), 2.29 (s, 3H, MePh), 2.15, 2.08, 2.05, 2.01, 1.92, 1.91 (6s, 3H × 6, 6MeCO). ESI-MS (positive) Calcd for $C_{99}H_{105}N_2O_{37}$ $[M + H]^+$, 1913.6. Found: 1913.2, Calcd for $C_{99}H_{104}N_2O_{37}Na$ $[M + Na]^+$, 1935.6. Found: 1935.1.

N-Butoxycarbonyl-O-{[methyl 2,3,4-tri-O-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranosyl}-L-serylglycine (22)

Tetrakis(triphenylphosphine)palladium(0) (22.0 mg, 19.0 μmol) and *N*-methylaniline (105 μL, 969 mmol) were added to the solution of **20** (173.5 mg, 96.6 μmol) in THF (4 mL) with stirring. Additional palladium catalysts (22 and 16 mg) were supplied to the solution after 2 and 3 h, respectively. One hour later, volatiles were removed under diminished pressure and the residue was subjected to the columns of gel permeation (LH-20, 10:10:1 $CHCl_3$:MeOH:AcOH) and silica gel (1:1–1:3 *n*-hexane:EtOAc) to give **22** (151.7 mg) in 90% yield. This compound was used for the next reaction without further purification.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-{[methyl 2,3,4-tri-*O*-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-*O*-(4-methylbenzoyl)-β-D-xylopyranosyl}-L-serylglycine (23)**

Tetrakis(triphenylphosphine)palladium(0) (45 mg, 39 μmol) and *N*-methylaniline (211 μL, 1.95 mmol) were added to the solution of **21** (427.3 mg, 195.1 μmol) in THF (8.2 mL) with stirring. Additional palladium catalyst (22 mg) was supplied to the solution after 1.5 h. One hour later, volatiles were removed under diminished pressure and the residue was subjected to the column of silica gel (1:1–1:2–1:3 *n*-hexane:EtOAc–100:1:0.3–50:1:0.3–30:1:0.3 EtOAc:MeOH:AcOH) to give **23** (320.0 mg) in 76% yield. This compound was used for the next reaction without further purification.

***N*-(9-Fluorenylmethoxycarbonyl)-proline allyl ester (24)**

To a solution of Fmoc-Pro-H (2.00 g, 5.93 mmol) in ethanol (100 mL) was added a 6 mL of aq. Cs_2CO_3 (0.965 g, 2.96 mmol) with stirring at 20°C. After 50 min, the reaction mixture was evaporated with toluene and dried in vacuo for 3 h. DMF (50 mL) and 3-bromopropene (0.57 mL, 6.75 mmol) were added to the residue and stirred overnight at rt. The reaction mixture was diluted with EtOAc and treated as described in general methods. The crude materials were subjected to the column of silica gel (11:1–7:1–5:1–4:1 *n*-hexane:EtOAc) to give **24** (1.93 g, 5.11 mmol) in 86% yield: $[\alpha]_D$ −54.4 (*c* 1.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.78–7.75 (m, 2H, Ar H), 7.64–7.54 (m, 2H, Ar H), 7.42–7.26 (m, 4H, Ar H), 5.89 (m, 1H, =CH), 5.28 (m, 2H, $=CH_2$), 4.65 (d, 2H, OCH_2), 4.41 (m, Proα), 4.44 [m, 1H, one of CH_2 (Fmoc)], 4.27 [m, 2H, one of CH_2 (Fmoc), H-9], 3.67 (m, 1H, Proδb), 3.55 (m, 1H, Proδa), 2.28 (m, 1H, Proβb), 2.05 (m, 1H, Proβa), 1.90 (m, 2H, Proγ). Anal. Calcd · for $C_{23}H_{23}NO_4 \cdot 0.3H_2O$: C, 72.21; H, 6.17; N, 3,66. Found: C, 72.64; H, 6.45; N, 3.64.

***N*-(9-Fluorenylmethoxycarbonyl)-N^i-(butoxycarbonyl)-L-tryptophanyl-L-proline allyl ester (25)**

Morpholine (4.9 mL) was added to a solution of **24** (429.4 mg, 1.137 mmol) in CH_2Cl_2 (20 mL) and stirred overnight. The crude mixture was evaporated to dryness with toluene and diluted with CH_2Cl_2 (22 mL). To this solution was added HOBt (278 mg, 2.08 mmol) with stirring. This solution was cooled to −20°C and HBTU (430 mg, 1.13 mmol) was added. The cooling bath was removed, and the reaction mixture was stirred at rt for 30 min. Then, Fmoc-Trp(Boc)-H (544.6 mg, 1.034 mmol) was added. The reaction mixture was stirred overnight and diluted with $CHCl_3$. Organic phase was washed with 1M HCl and aq. $NaHCO_3$, brine and dried over $MgSO_4$. The volatiles were removed under diminished pressure. The crude materials were subjected

to the column of silica gel (7:1–6:1–5:1–3:1–1:1 *n*-hexane:EtOAc) to give **25** (391.4 mg) in 56% yield: $[\alpha]_D$ −26.3 (*c* 1.12, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.14 (m, 1H, Ar H), 7.78–7.23 (m, 11H, Ar H), 5.93 (m, 1H, =CH), 5.65 [d, 1H, $J_{\alpha,NH} = 8.54$ Hz, NH(Trp)], 5.30 (m, 2H, $=CH_2$), 4.87 (ddd, $J_{\alpha,\beta a} = 7.08$ Hz, $J_{\alpha,\beta b} = 5.86$ Hz, Trpα), 4.66 (d, 2H, OCH_2), 4.55 (dd, $J_{\alpha,\beta a} = 4.88$ Hz, $J_{\alpha,\beta b} = 8.05$ Hz, Proα), 4.37–4.15 [m, 3H, CH_2 (Fmoc), 9H], 3.61 (m, 1H. Proδb), 3.25 (m, 1H, Proδa), 3.22 (dd, 1H, $J_{gem} = 14.64$ Hz, Trpβb), 3.11 (dd, 1H, Trpβa), 2.20 (m, 1H, Proβb), 1.97 (m, 1H, Proβa), 1.91 (m, 2H, Proγ), 1.63 (s, 9H, *t*-Bu). Anal. Calcd for $C_{39}H_{41}N_3O_7 \cdot 2.5H_2O$: C, 66.07; H, 6.55; N, 5.93. Found: C, 65.94; H, 6.17; N, 5.71. ESI-MS (positive) Calcd for $C_{39}H_{42}N_3O_7$ $[M + H]^+$, 664.3. Found: 664.5, Calcd for $C_{39}H_{41}N_3O_7Na$ $[M + Na]^+$, 686.3. Found: 686.4.

N-(9-Fluorenylmethoxycarbonyl)-N^i-(butoxycarbonyl)-L-tryptophanyl-L-proline (26)

Tetrakis(triphenylphosphine)palladium(0) (247.3 mg, 0.241 mmol) and *N*-methylaniline (2.3 mL, 21 mmol) were added to the solution of **25** (1.447 g, 2.135 mmol) in THF (18 mL) with stirring. After 15 h, volatiles were removed under diminished pressure and the residue was subjected to a column of silica gel (5:1 toluene:EtOAc–100:1:0.3–50:1:0.3 EtOAc:MeOH:AcOH) to give **26** (861.9 mg) in 63% yield. This compound was used for the next reaction without further purification: ^1H NMR ($CDCl_3$): δ 8.11–8.09 (d, 1H, Ar H), 7.74–7.21 (m, 11H, Ar H), 5.81 [d, 1H, $J_{\alpha,NH} = 8.78$ Hz, NH(Trp)], 4.85 (dd, $J_{\alpha,\beta a} = 7.32$ Hz, $J_{\alpha,\beta b} = 7.08$ Hz, Trpα), 4.57 (dd, $J_{\alpha,\beta a} = 3.66$ Hz, $J_{\alpha,\beta b} = 8.29$ Hz, Proα), 4.36–4.14 [m, 3H, CH_2 (Fmoc), 9H], 3.59 (m, 1H, Proδb), 3.16 (m, 3H, Proδa, Trpβ), 2.22 (m, 1H, Proβb), 2.03 (m, 1H, Proβa), 1.85 (m, 2H, Proγ), 1.62 (s, 9H, *t*-Bu).

N-(Benzyloxycarbonyl)-O-(tert-butyl)-L-aspartylglycine tert-butyl ester (27)

Z-Asp(*O*-*t*-Bu)-H (200.0 mg, 0.586 mmol) and H-Gly-*t*-Bu · HCl (98.2 mg, 0.586 mmol) were dissolved in CH_2Cl_2 (18 mL). To this was added Et_3N (81 μL, 0.59 mmol) and HOBt (158.4 mg, 1.172 mmol) with stirring. This solution was cooled to −20°C, and then, a solution of WSCD · HCl (146 mg, 0.762 mmol) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred overnight and gradually raised to rt, diluted with $CHCl_3$, and treated as described in the synthesis of **25**. The crude materials were subjected to a column of silica gel (5:1–1:2 *n*-hexane:EtOAc) to give **27** (246.1 mg) in 96% yield: $[\alpha]_D$ +15.0 (c 1.45, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.27 (m, 5H, Ar H), 6.96 [m, 1H, NH(Gly)], 5.98 [d, 1H, $J_{\alpha,NH} = 8.25$ Hz, NH(Asp)], 5.14 (s, 2H, $PhCH_2$), 4.57 (brs, 1H, Aspα), 3.92 (m, 2H, Gly), 2.92 (brdd, 1H, $J = 4.38$ and 16.95 Hz, Aspβa), 2.64 (dd, 1H, $J_{\alpha,\beta b} = 6.42$, $J_{gem} = 13.95$ Hz,

Aspβb), 1.46, 1.43 (2s, 9H × 2, 2*t*-Bu). Anal. Calcd. for $C_{22}H_{32}N_2O_7$: C, 60.54; H, 7.39; N, 6.42. Found: C, 60.31; H, 7.59; N, 6.27.

N-(9-Fluorenylmethoxycarbonyl)-N^i-(butoxycarbonyl)-L-tryptophanyl-L-prolyl-O-(tert-butyl)-L-aspartylglycine tert-butyl ester (29)

A solution of **27** (246.1 mg, 563.8 μmol) in MeOH (5 mL) and AcOH (0.1 mL) containing a catalytic amount of Pd-C was stirred vigorously for 3 h under H_2 atmosphere. The reaction mixture was filtered on Celite and the volatiles were removed under diminished pressure to afford **28** (191.2 mg, 93%), which was dissolved in CH_2Cl_2 (20 mL). To this solution were added **26** (404.1 mg, 633.6 μmol), Et_3N (73 μL, 0.53 mmol), and HOBt (171 mg, 1.27 mmol) with stirring. This solution was cooled to −20°C, and then a solution of WSCD · HCl (157 mg, 819 mmol) in CH_2Cl_2 (6 mL) was added. The reaction mixture was stirred overnight gradually raised to rt, diluted with $CHCl_3$, and treated as described in the synthesis of **25**. The crude materials were subjected to a column of silica gel (5:1–3:1–1:1–1:3–1:5 *n*-hexane:EtOAc) to give **29** (414.6 mg) in 85% yield: $[\alpha]_D$ −20.7 (*c* 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.79–7.21 [m, 13H, Ar H, NH (Asp)], 7.22 [m, 1H, NH (Gly)], 5.76 (d, 1H, $J_{\alpha,\beta} = 5.50$ and 8.02 Hz, Proα), 4.30 [dd, 1H, one of CH_2 (Fmoc)], 4.23 [dd, 1H, one of CH_2 (Fmoc)], 4.14 [m, 1H, H-9 (Fmoc)], 4.11 (m, 1H, Glya), 3.82 (m, 1H, Glyb), 3.79 (m, 1H, Proδb), 3.54 (m, 1H, Proδa), 3.28 (d, 2H, Trpβ), 3.00 (dd, 1H, $J_{\alpha,\beta a} = 4.35$ Hz, $J_{gem} = 16.95$ Hz, Aspβa), 2.59 (dd, 1H, $J_{\alpha,\beta b} =$ 5.96 Hz, Aspβb), 2.18 (m, 3H, Proβb, Trpβa, Proγb), 2.03 (m, 1H, Proγa), 1.66, 1.61, 1.46, (3s, 9H × 3, 3*t*-Bu). Anal. Calcd for $C_{50}H_{61}N_5O_{11} \cdot 3H_2O$: C, 62.41; H, 7.03; N, 7.28. Found: C, 62.22; H, 6.77; N, 7.07.

N-Butoxycarbonyl-O-{[methyl 2,3,4-tri-O-(4-methylbenzoyl)-β-D-glucopyranosyl uronate]-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3-di-O-(4-methylbenzoyl)-β-D-xylopyranosyl}-L-serylglycyl-N^i-(butoxycarbonyl)-L-tryptophanyl-L-prolyl-O-(tert-butyl)-L-aspartylglycine tert-Butyl Ester (31)

Morpholine (0.9 mL) was added to a solution of **29** (110.5 mg, 119.8 μmol) in CH_2Cl_2 (3.5 mL) and stirred overnight. The crude mixture was evaporated to dryness with toluene, and the residue was subjected to a column of silica gel (1:1 *n*-hexane:EtOAc–10:1:0.3 EtOAc:MeOH:Et_3N) to afford **30** (83.6 mg) quantitatively. A part of **30** (41.3 mg, 59.0 μmol) was dissolved in DMF (130 μL), and HOBt (7.9 mg, 58 μmol) was added to the solution with stirring. This solution was cooled to −20°C and HBTU (12.0 mg, 31.6 μmol) was added. The cooling bath was removed, and the reaction mixture was stirred at rt for 30 min. Then, **22** (47.8 mg, 27.2 μmol) and *i*-Pr_2EtN (10.3 μL, 59.1 μmol) in DMF (132 mL) were added. The reaction mixture was stirred overnight and diluted with EtOAc. Organic phase was treated as described

in the synthesis of **25**. The volatiles were removed under diminished pressure. The crude materials were subjected to a column of gel permeation (1:1 $CHCl_3$:MeOH) to give **31** (41.5 mg) in 63% yield: $[\alpha]_D$ +78 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.13–7.05 (m, 25H, Ar H), 7.45 [d, 1H, $J_{\alpha,NH} = 8.25$ Hz, NH (Asp)], 7.18 [m, 1H, NH (Gly)], 6.94 [br, 1H, NH (Gly)], 6.78 [br, 1H, NH (Trp)], 5.80 (t, 1H, $J_{2,3} = J_{3,4} = 9.39$ Hz, H-3^{IV}), 5.64 (brt, 1H, $J = 9.26$ Hz, H-4^{IV}), 5.53 (dd, 1H, $J_{2,3} = 7.79$ Hz, $J_{3,4} = 8.25$ Hz, H-3^{I}), 5.47 (d, 1H, $J_{3,4} = 2.75$ Hz, H-4^{III}), 5.37 [m, 1H, NH (Ser)], 5.36 (dd, 1H, $J_{1,2} = 7.10$ Hz, H-2^{IV}), 5.20 (d, 1H, $J_{3,4} = 3.21$ Hz, H-4^{II}), 5.17 (d, 1H, $J_{1,2} = 6.18$ Hz, H-2^{I}), 5.06 (m, 1H, Trpα), 4.99 (m, 2H, H-2^{II}, 2^{III}), 4.92 (d, 1H, H-1^{IV}), 4.79 (m, 1H, Aspα), 4.66 (d, 1H, H-1^{I}), 4.49 (t, 1H, $J = 6.42$ Hz, Proα), 4.40 (d, 1H, $J_{1,2} = 8.02$ Hz, H-$1^{III\ or\ II}$), 4.37 (d, 1H, $J_{1,2} = 8.02$ Hz, H-$1^{II\ or\ III}$), 4.28 (m, 1H, Serα), 4.27 (d, 1H, $J_{4,5} = 9.85$ Hz, H-5^{IV}), 4.16 (dd, 1H, $J_{5,6b} =$ 5.73 Hz, $J_{gem} = 11.62$ Hz, H-6b^{III}), 4.06 (m, 1H, H-5eq^{I}), 4.03 (dd, 1H, $J_{5,6a} = 5.27$ Hz, H-6a^{III}), 4.00 (m, 1H, Glya), 3.92 (m, 1H, H-4^{I}), 3.83 (dd, $J_{2,3} = 10.31$ Hz, H-3^{III}), 3.81 (m, 1H, Glyb), 3.8–3.5 (m, 2H, H-6^{II}), 3.76 (brt, $J = 6.56$ Hz, H-5^{III}), 3.75 (m, 2H, Gly), 3.70 (s, 3H, COOMe), 3.69 (m, 1H, H-3^{II}), 3.68 (m, 1H, Proδb), 3.61 (brt, 1H, $J = 7.10$ Hz, H-5^{II}), 3.55 (m, 2H, Serβ), 3.47 (dd, 1H, $J_{4,5ax} = 8.01$ Hz, $J_{gem} = 12.14$ Hz, H-5ax^{I}), 3.37 (m, 1H, Proδa), 3.24 and 3.15 (m, 2H, Trpβ), 2.90 (dd, 1H, $J_{\alpha,\beta a} = 4.81$ Hz, $J_{gem} = 16.96$ Hz, Aspβa), 2.62 (dd, 1H, $J_{\alpha,\beta b} = 5.95$ Hz, Aspβb), 2.36, 2.35 (2s, 3H × 2, 2MePh), 2.34 (s, 6H, 2MePh), 2.29 (s, 3H, MePh), 2.15 (s, 6H, 2MeCO), 2.12 (m, 2H, Proβ), 2.09, 2.02, 1.93, 1.91 (4s, each 3H, 4MeCO), 1.92 (m, 2H, Proγ), 1.66, 1.44, 1.40, 1.39 (4s, each 9H, 4*t*-Bu). ESI-MS (positive) Calcd. for $C_{121}H_{148}N_7O_{45}$ $[M + H]^+$, 2418.9. Found: 2418.8.

O-{β-D-Glucopyranuronosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-β-D-xylopyranosyl}-L-serylglycyl-L-tryptophanyl-L-prolyl-L-aspartylglycine (1)

To a solution of **31** (30.4 mg, 12.5 μmol) in CH_2Cl_2 (0.75 mL) was added a TFA cocktail (40.7:2.5:2.5:2.5:1.3:50 trifluoroacetic acid:thioanisole:phenol: H_2O:1,2-ethanedithiol:triisopropylsilane:CH_2Cl_2) (0.75 mL) and stirred for 2 h. Volatiles were removed with toluene under diminished pressure and the residue was eluted from Bond Elut® C8 (H_2O–1:1–1:2 H_2O:MeOH–MeOH–CH_2Cl_2). The crude product was diluted with 50% MeOH (3 mL) and 0.107M NaOMe (1.4 mL) was added to the solution during 3 h keeping less than pH 9. Then, the solution was neutralized with 50% AcOH and the volatiles were removed under diminished pressure. The crude materials were subjected to a column of gel permeation (LH-20, 1% AcOH) to give **1** (14.8 mg) in 83% yield. ^1H NMR (D_2O): δ (selected) 7.71–7.20 (m, 5H, Ar H), 4.96 (t, $J = 6.9$ Hz, Trpα), 4.75 (dd, $J = 6.0$ and 7.0 Hz, Aspα), 4.70 (d, $J_{1,2} = 7.9$ Hz, H-1^{IV}), 4.66 (dd, $J = 5.8$ and 7.8 Hz, Aspα'), 4.59 (d, $J_{1,2} = 7.9$ Hz, H-1^{III}), 4.57 (m, Trpα'), 4.56 (d, $J_{1,2} = 7.5$ Hz, H-1^{II}), 4.46 (d, $J_{1,2} = 7.8$ Hz, H-1^{I}), 4.42

(d, $J_{1,2} = 7.3$ Hz, H-1′ ^{I}), 4.41 (m, Proα, Serα), 4.33 (dd, $J = 5.0$ and 11.0 Hz, Serβa), 4.30 (t, $J = 4.1$ Hz, Serα'), 4.24 (m, Serβa′), 4.14 (s, H-4$^{\mathrm{IIorIII}}$), 4.10 (s, H-4$^{\mathrm{IIIorII}}$), 4.10 (m, Serβb), 4.04 (m, Serβb′), 3.78 (m, H-3$^{\mathrm{IIorIII}}$), 3.73 (m, H-2$^{\mathrm{II}}$, 2$^{\mathrm{III}}$, Proδa), 3.63 (m, H-3$^{\mathrm{IIIorII}}$), 3.42 (m, H-2$^{\mathrm{IV}}$), 3.37 (m, H-2^{I}, Proδb), 3.33 (m, H-2′ ^{I}), 3.31 (m, Trpβa), 3.30 (m, Proδb′), 3.14 (m, Trpβb), 2.94 (dd, $J_{gem} = 19.1$ Hz, Aspβa), 2.90 (dd, $J_{gem} = 19.1$ Hz, Aspβa′), 2.79 (dd, Aspβb), 2.77 (dd, Aspβb′), 2.22 (m, Proβa), 1.92 (m, Proβb, Proγ), 1.64, 1.44 (m, Proβ', Proγa′), 0.97 (m, Proγb′); FAB-MS (positive) Calcd. for $C_{50}H_{69}N_7O_{30}Na_3$ $[M + H]^+$, 1316.4. Found: 1316.3. Calcd. for $C_{50}H_{68}N_7O_{30}Na_4$ $[M + Na]^+$, 1338.4. Found: 1338.4.

General Procedure for Solid-Phase Synthesis

Glycopeptides were synthesized manually for 100 mg of Sieber amide resin (52 μmol) as follows. The Fmoc group was removed with 20% of piperidine/NMP (2 mL) (2 × 3 min and 1 × 20 min), and was monitored by Kaiser ninhydrin test. After washing with NMP (2.2 mL) (6 × 1 min) and CH_2Cl_2 (2.2 mL) (3 × 1 min), it was dried in vacuo. The *N*-terminal free resin or peptide-on-resin was swollen in NMP (2 mL) and shaken overnight with corresponding Fmoc amino acid (130 μmol), HOBt (130 μmol), HBTU (130 μmol), and *i*-Pr_2EtN (260 μmol). Coupling was monitored by Kaiser test. The resin was washed with NMP (2.2 mL) (3 × 1 min) and CH_2Cl_2 (2.2 mL) (3 × 1 min) and dried in vacuo.

O-{β-D-Glucopyranuronosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-β-D-xylopyranosyl}-L-serylglycyl-L-tryptophanyl-L-prolyl-L-aspartylglycineamide (2)

Fmoc-Gly-H (46.4 mg, 156 μmol), Fmoc-Asp (O-*t*-Bu)-H (64.1 mg, 156 μmol), Fmoc-Pro-H (52.6 mg, 156 μmol), and Fmoc-Trp(Boc)-H (82.1 mg, 156 μmol) were coupled in turn on Sieber amide resin (300 mg, 156 μmol) with HOBt (21.3 mg, 156 μmol), HBTU (53.3 mg, 156 μmol), and *i*-Pr_2EtN (55 μl, 312 μmol) in NMP (2.7 mL). A part of the *N*-terminal of H-Trp(Boc)-Pro-Asp(O-*t*-Bu)-Gly-resin (67.4 mg, 27 mmol) was coupled with **23** (116 mg, 54 μmol) as described in the general procedure. The resultant resin was exposed to TFA cocktail (3.5 mL) with shaking for 7.5 h and evaporated. The residue was eluted from Bond Elut® C8 (1:0–0:1 H_2O–MeOH) and subjected to a column of gel permeation (LH-20, 1% AcOH) to give crude glycopeptide (26.5 mg) still having acyl groups. Saponification was performed with 1 N NaOH-MeOH (1:100, 6.2 mL) overnight. The reaction mixture was diluted with H_2O (6 mL), neutralized with 0.1 N AcOH after 5 h, and evaporated to dryness. The residue was subjected to further saponification with 5 mM NaOH (5 mL) for 4 days. The reaction mixture was neutralized in the same manner. The crude products were purified by HPLC (C18, A∼B = 10%CH_3CN + 0.1%CF_3COOH∼

90%CH_3CN + 0.1%CF_3COOH) to give **2**. TOF-MS (positive) Calcd. for $C_{50}H_{71}N_8O_{29}Na$ $[M + Na]^+$, 1271.4. Found: 1271.4, Calcd. for $C_{50}H_{70}N_8O_{29}Na_2$ $[M-H + 2Na]^+$, 1293.4. Found: 1293.4.

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