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Synthesis and evaluation of a radioiodinated trisaccharide derivative as a synthetic substrate for a sensitive *N*-acetylglucosaminyltransferase V radioassay

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ABSTRACT

N-acetylglucosaminyltransferase V (GnT-V) is one of the most relevant glycosyltransferases to tumor invasion and metastasis. Based on previous findings of molecular recognition between GnT-V and synthetic substrates, we designed and synthesized a *p*-iodophenyl-derivatized trisaccharide, 2-(4-iodophenyl)ethyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (IPGMG, **1**) and its radiolabeled form, [^{125}I]IPGMG ([^{125}I]**1**), for use in assays of GnT-V activity *in vitro*. The tributyltin derivative, 2-[4-(*n*-tributylstannyl)phenyl]ethyl 6-*O*-[2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**21**), was synthesized as a precursor for the preparation of [^{125}I]**1**. The iododestannylation of **21** using hydrogen peroxide as an oxidant followed by deacetylation yielded [^{125}I]**1**. When [^{125}I]**1** was incubated in GnT-V-expressing cells with a UDP-GlcNAc donor, the production of β1–6GlcNAc-bearing IPGMG (IPGGMG, **2**) was confirmed by radio-HPLC. In kinetic analysis, **1** was found to be a good substrate with a K_m of 23.7 μM and a V_{max} of 159 pmol/h. μg protein. [^{125}I]**1** would therefore be a useful synthetic substrate for the quantitative determination of GnT-V activity.

1. Introduction

The sugar chains of glycoproteins on cell surfaces, which are constructed by glycosyltransferases and glycosidases, change during particular biological events including development, carcinogenesis, and malignant transformation. Numerous studies conducted in the last two decades have demonstrated that *N*-acetylglucosaminyltransferase V (GnT-V) is relevant to tumor invasion and metastasis. Since Cummings first reported its expression in mouse lymphoma cells in 1982,[1] GnT-V has been shown to be highly expressed in various cancer cell lines and tissues from the early stages of tumorigenesis.[2–8] In addition, studies using GnT-V-deficient mice have directly demonstrated an essential role of GnT-V in tumor growth and metastasis.[9,10]

GnT-V catalyzes the transfer of an *N*-acetylglucosamine (GlcNAc) residue from a donor, UDP-GlcNAc, to an acceptor, the mannose α1–6 side chain of the trimannosyl core of *N*-glycans, to form a GlcNAcβ1–6Man linkage. This activity has been assessed predominantly using radiolabeled donors or fluorescent-labeled acceptors. In the former case, the production of sugar chains, the non-reducing ends of which are modified with radiolabeled GlcNAc by the enzymatic reaction, is measured through the use of a UDP-[^{3}H]GlcNAc or UDP-[^{14}C]GlcNAc donor.[2,11,12] However, there is a problem with specificity since UDP-GlcNAc functions as a substrate for many glycosyltransferases. In the latter case, a GnT-specific acceptor, GlcNAcβ1-2Manα1-3-(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc, is labeled with 2-aminopyridine to yield a fluorescent substrate (Gn,Gn-bi-PA). This acceptor is obtained from the carbohydrate moiety of human transferrin through laborious and time-consuming processes, repeated digestion with various enzymes, chemical modifications, and purification.[13–15] Moreover, the PA-oligosaccharide could still be a substrate of GnT-III.

Thus, we planned and produced a GnT-V-specific radioiodinated substrate for use in assays of GnT-V activity *in vitro*. First, to ensure a high degree of specificity, GlcNAcβ1-2Manα1-6Glc[12,16–18] was

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employed as the saccharide moiety on the basis of previous findings; (i) it could not be a substrate of GnT-I and GnT-IV because of the lack of an acceptor site for GnT-I and GnT-IV, that is, a Man residue linked to the bisecting Man with an α1–3 linkage, (ii) it is a poor substrate of GnT-III because of the replacement of the acceptor residue, that is, bisecting Man, with Glc, and (iii) it already carries an GlcNAc residue that should be transferred by GnT-II. Second, the 1-hydroxyl of the reducing terminal Glc, which was adopted as a substitute of the bisecting Man, was conjugated with a hydrophobic phenethyl group without loss of activity.[11,12,16–18] Finally, the radioiodine ^{125}I was considered to be incorporated into the *para*-position of the phenyl ring immediately before the biological assay because the *para*-substitution of iodine is effective in preventing deiodination *in vivo*.[19] The *p*-iodophenyl-derivatized trisaccharide, 2-(4-iodophenyl)ethyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside, was expected to behave as a substrate for *in vitro* assays of GnT-V activity.

Herein, we report synthesis of an unlabeled IPGMG (**1**), a ^{125}I-labeled IPGMG ([^{125}I]**1**), and the *N*-acetylglucosaminylated product β1-6GlcNAc-bearing IPGMG (IPGGMG, **2**). The reaction kinetics of [^{125}I]**1** were evaluated in GnT-V-expressing cells and its recognition by GnT-V was compared with that of Gn,Gn-bi-PA. From these results, the usefulness of [^{125}I]**1** for the GnT-V assay was assessed (Fig. 1).

2. Results and discussion

2.1. Chemistry

For the synthesis of **1** and [^{125}I]**1**, glycosylation of 3,4,6-tri-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl bromide (**3**) and 2-(4-iodophenyl) ethyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**4**) was used as a key reaction (Scheme 1).

Synthesis of the bromide **3** began with the condensation, which was promoted by silver trifluoromethanesulfonate and collidine, of benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**5**)[20] and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**6**)[21] to afford disaccharide **7** (57%). Subsequent treatment of **7** with hydrazine hydrate followed by acetylation yielded **8** (80%, two steps). Debenzylidenation of **8** in 70% AcOH and subsequent acetylation yielded intermediate **9** (80%, two steps), whose hydrogenolysis and acetylation afforded octaacetate **10** (86%, two steps). Subsequent treatment of **10** with 30% HBr–AcOH afforded **3**, which was used as the glycosyl donor for the next reaction (Scheme 2).

Synthesis of glycosyl acceptor **4** was achieved by the coupling of 2-(4-bromophenyl)ethyl alcohol and 2,3,4-tri-*O*-acetyl-6-*O*-allyloxycarbonyl-α-D-glucopyranosyl trichloroacetimidate (**13**) prepared from 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (**11**)[22] in three steps: protection of the 6-OH group with an allyloxycarbonyl group that afforded **12**, anomerical deacetylation of **12** with piperidine, and reaction with CCl_3CN in the presence of DBU that afforded the imidate **13**. (Scheme 3).

Glycosylation of 2-(4-bromophenyl)ethyl alcohol with imidate **13** led to 2-(4-bromophenyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-allyloxycarbonyl-β-D-glucopyranoside (**14**) (72%), which was subjected to palladium-catalyzed deallylation to afford **15** (94%). **15** was reacted with ethyl vinyl ether in the presence of a catalytic amount of pyridinium *p*-toluenesulfonate to form **16** (99%). At this point, replacement of the bromo group with an iodo group was investigated before developing the route to **1** and [^{125}I]**1**. The intermediate, 2-[4-(tributylstannyl)phenyl]ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(1-ethoxyethyl)-β-D-glucopyranoside (**17**), was prepared from **16** using a bromo-to-tributyltin exchange reaction catalyzed by Pd(0) (47%). The tributyltin derivative **17** was readily converted to 2-(4-iodophenyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(1-ethoxyethyl)-β-D-glucopyranoside (**18**) by reaction with iodine (94%). Treatment of **17** and **18** with pyridinium *p*-toluenesulfonate in methanol yielded **19** (95%) and **4** (98%), respectively (Scheme 3).

The treatment of glycosyl donor **3** with glycosyl acceptor **4** in the presence of silver carbonate and silver perchlorate in dichloromethane and toluene yielded **20** (67%), which was deacetylated to furnish the target compound **1** (59%) (Scheme 4). Next, synthesis of the precursor of [^{125}I]**1**, **22**, was attempted from which the tributyltin group was expected to be facilely replaced with a radioactive iodo group by iododestannylation to yield [^{125}I]**1**. However, deacetylation of **21**, which was obtained by the condensation of **3** and **19** in the presence of silver perchlorate and collidine (43%), did not yield **22**. Therefore, the tributyltin derivative **21** was transformed into the fully protected and radiolabeled trisaccharide [^{125}I]**20** by an iododestannylation reaction using hydrogen peroxide as the oxidant. Conventional deacetylation of [^{125}I]**20** afforded the desired radioiodinated compound [^{125}I]**1** (Scheme 4). It was anticipated that no-carrier-added preparation would result in a final product bearing a theoretical specific activity similar to that of ^{125}I. The radiochemical identity of [^{125}I]**1** was verified by co-injection with nonradioactive **1** and comparison of their HPLC profiles. The final radioiodinated compound [^{125}I]**1** showed a single peak of radioactivity at the same retention time as that of nonradioactive **1**. The radioiodinated product was obtained in 50% radiochemical yield with a radiochemical purity of >95% after HPLC.

Finally, 2-(4-iodophenyl)ethyl 6-*O*-[2,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (IPGGMG, **2**), the *N*-acetylglucosaminylated product of **1**, was synthesized starting with removal of the benzyliden group of **7** with 70% AcOH to afford **23** (83%). Selective silylation of 6-OH of the mannose unit with *tert*-butyldimethylsilyl chloride yielded **24** (94%), the remaining hydroxyl group of which was acetylated to afford **25** (99%). Treatment of **25** with pyridinium *p*-toluenesulfonate afforded **26** (75%), whose glycosylation with the bromide **6**

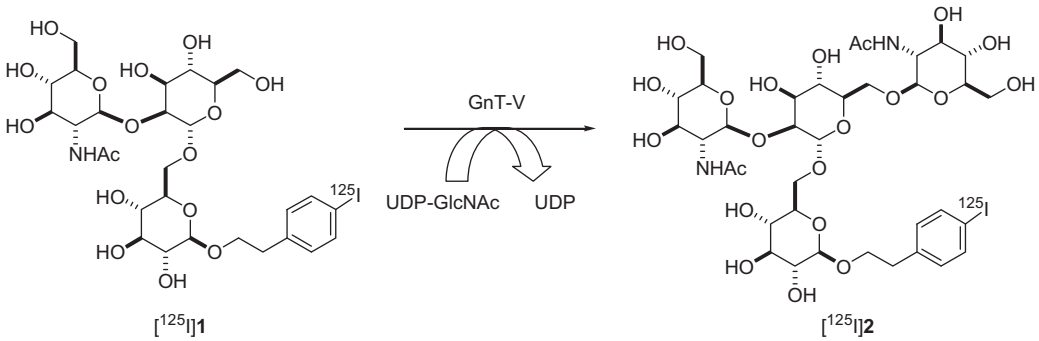
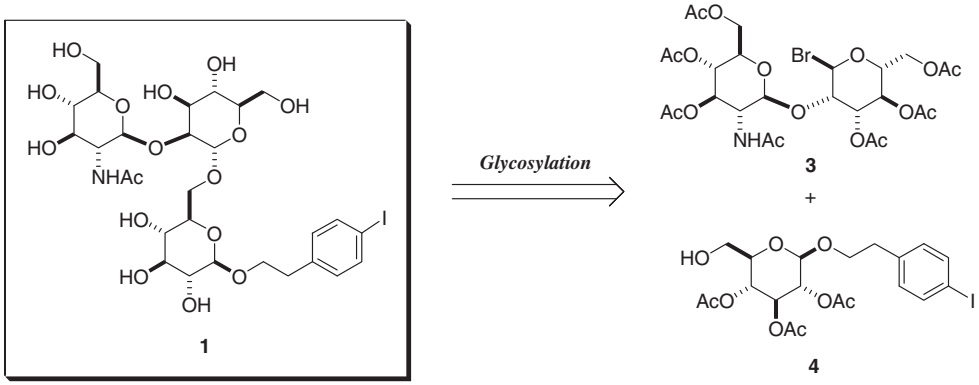
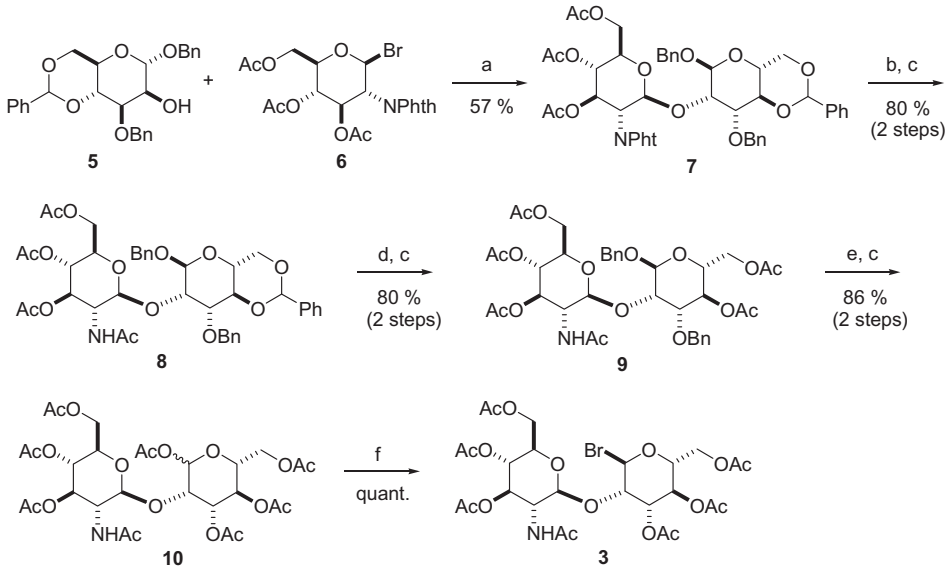


Figure 1. Glycosylation of [^{125}I]**1** by GnT-V.



Scheme 1. Synthesis of trisaccharide **1**.



Scheme 2. Synthesis of disaccharide **3**. Reagents and condition: (a) AgOTf, collidine, CH_3NO_2, (b) $NH_2NH_2 \cdot H_2O$, EtOH, (c) Ac_2O, pyridine, (d) 70% AcOH, (e) 10% Pd-C, H_2, EtOH, (f) 30% HBr-AcOH.

promoted by silver trifluoromethanesulfonate and collidine yielded **27** (40%). Removal of the *N*-phthalimido groups with hydrazine hydrate and subsequent acetylation yielded **28** (93%, two steps). Cleavage of the benzyl groups of **28** by hydrogenolysis followed by acetylation yielded **29** (72%, two steps). Treatment of **29** in acetic anhydride with HBr yielded bromide **30**. Glycosylation of **4** with bromide **30** promoted by silver carbonate and silver perchlorate yielded **31** (7%, two steps), which was sequentially deacetylated to furnish the target compound **2** (Scheme 5).

2.2. Biological studies

A typical HPLC profile of the GnT-V-reaction products of [^{125}I]**1** is shown in Figure 2. The reaction products showed peaks at retention times of 21 and 30 min, respectively, identical to those of the nonradioactive compounds **2** and **1**.

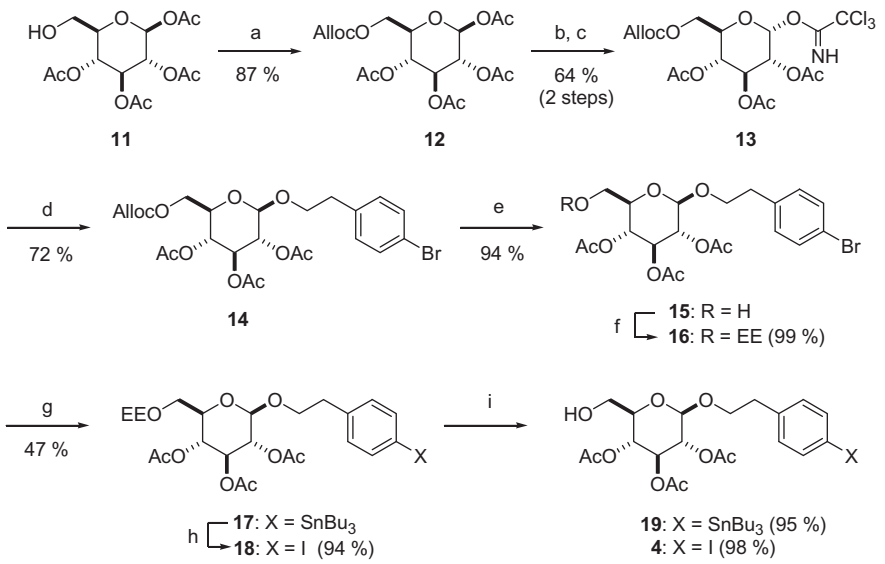
This result indicates that GnT-V catalyzes the transfer of a GlcNAc residue from UDP-GlcNAc to [^{125}I]**1** to form a β1–6 linkage, providing evidence that [^{125}I]**1** acts as a substrate for GnT-V.

The kinetics of the conversion of **1** to **2** by GnT-V were determined using the IPGMG solution containing [^{125}I]**1**. This radioiodinated substrate exhibited saturation kinetics at high substrate concentrations (Fig. 3A). The data satisfied the Michaelis–Menten equation, originally developed for enzymatic kinetics. The kinetic parameters were K_m = 23.7 μM and V_{max} = 159 pmol/h·μg protein, determined from the double-reciprocal Lineweaver–Burk plot (Figure 3B). The K_m value is one order of magnitude lower than that for a fluorescent substrate, Gn,Gn-bi-PA.

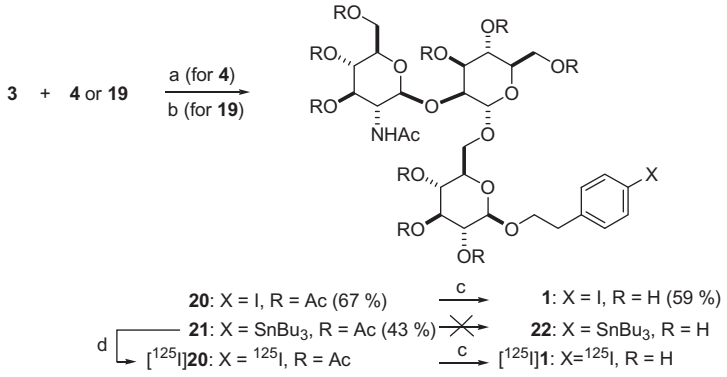
The novel radioiodinated substrate [^{125}I]**1** was used to assay GnT-V activity in crude extracts of various tissues in normal rats. To compare the findings with previous results obtained using Gn,Gn-bi-PA as a substrate, we assayed the GnT-V activity under similar experimental conditions.[13] The results are summarized in Table 1. This enzyme was uniformly distributed in rat tissues with relatively high activity levels in the small intestine and serum. A similar tendency was observed in a previous investigation using Gn,Gn-bi-PA.[13] The reaction velocity using [^{125}I]**1** was 3- to 22-fold greater than that using Gn,Gn-bi-PA. These findings demonstrate that **1** serves as a better substrate for GnT-V than Gn,Gn-bi-PA.

3. Conclusions

We successfully designed and synthesized a novel radioiodinated trisaccharide derivative, [^{125}I]**1**, as a synthetic substrate for



Scheme 3. Synthesis of glucose derivative **4**. Reagents and condition: (a) AllocCl, pyridine, THF, (b) piperidine, THF, (c) CCl_3CN, DBU, CH_2Cl_2, (d) 2-(4-bromophenyl)ethyl alcohol, $BF_3 \cdot Et_2O$, CH_2Cl_2, (e) $Pd(PPh_3)_4$, PPh_3, HCO_2H, THF, (f) ethyl vinyl ether, PPTS, CH_2Cl_2, (g) $Pd(PPh_3)_4$, PPh_3, (n-$Bu_3Sn)_2$, DMF, (h) I_2, CH_2Cl_2, (i) PPTS, MeOH.



Scheme 4. Synthesis of **1** and [^{125}I]**1**. Reagents and condition: (a) $AgClO_4$, Ag_2CO_3, CH_2Cl_2, toluene, (b) $AgClO_4$, collidine, CH_2Cl_2, toluene, (c) NaOMe, MeOH, (d): [^{125}I]NaI, 5% H_2O_2 aq, 0.1 % HCl aq.

GnT-V. [^{125}I]**1** exhibited higher affinity than a fluorescent substrate, Gn,Gn-bi-PA, suggesting it to be the preferred acceptor for this enzyme. The present findings contribute to the development of sensitive *in vitro* GnT-V assays.

4. Experimental sections

4.1. Synthetic methods

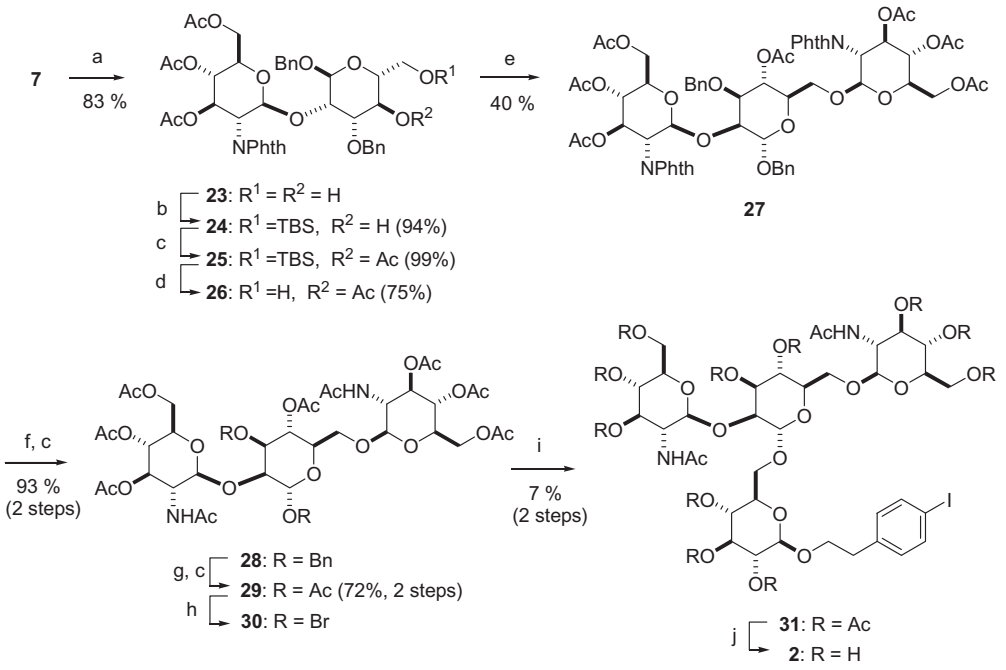
All reagents were commercial products and used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on a JEOL JNM EX-270, JNM AL-300, and Varian Unity INOVA 400 spectrometer. Signals are given in ppm using tetramethylsilane as an internal standard. MS spectra were determined on a JEOL JMX-SX102A QQ and Shimadzu LCMS-QP8000α mass spectrometer.

4.1.1. Benzyl 3-*O*-benzyl-4,6-*O*-benzylidiene-2-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-manno pyranoside (7)

To a mixture of **5** (255 mg, 0.57 mmol), silver trifluoromethanesulfonate (263 mg, 1.02 mmol), and collidine (135 μL, 1.02 mmol) in nitromethane (1 mL) was added a nitromethane (2 mL) solution of **6** (426 mg, 0.86 mmol) at −20 °C. The reaction mixture was stirred at −20 °C for 10 min and room temperature for 1.5 h before being filtered through Celite® and concentrated *in vacuo*. The residue was purified by silica gel chromatography (toluene/diethyl ether = 3:1) to afford **7** (280 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.93, 2.08 and 2.09 (each s, 3H), 3.20 (t, J = 10.1 Hz, 1H), 3.52–3.65 (m, 1H), 3.71–3.76 (m, 1H), 3.88–3.96 (m, 3H), 4.15–4.17 (m, 1H), 4.25 (d of ABd, J_{AB} = 12.2 Hz, J = 2.3 Hz, 1H), 4.33 (ABd, J_{AB} = 11.7 Hz, 1H), 4.34 (d of ABd, J_{AB} = 12.2 Hz, J = 4.7 Hz, 1H), 4.52 (ABd, J_{AB} = 11.7 Hz, 1H), 4.53 (dd, J = 10.8 and 8.4 Hz, 1H), 4.64 (s, 1H), 4.67–4.76 (m, 2H), 5.22 (t, J = 9.6 Hz, 1H), 5.44 (d, J = 8.4 Hz, 1H), 5.50 (s, 1H), 5.92 (dd, J = 10.7 and 9.1 Hz, 1H), 7.20–7.48 (m, 15H), 7.77–7.82 (m, 2H), 7.88–7.91 (m, 2H); MS FAB(+) m/z 866 [(M+H)$^+$, 26]; HR-MS calcd for $C_{47}H_{47}NO_{15}Na$ [(M+Na)$^+$] 888.2843, found: 888.2845.

4.1.2. Benzyl 3-*O*-benzyl-4,6-*O*-benzylidiene-2-*O*-[3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-manno pyranoside (8)

To an ethanol (50 mL) solution of **7** (900 mg, 1.04 mmol) was added hydrazine·hydrate (80% in water, 1.5 mL). The reaction mixture was heated at reflux for 4.5 h before being filtered and concentrated in vacuo. The residue was purified by silica gel chroma



Scheme 5. Synthesis of tetrasaccharide **2**. Reagents and condition: (**a**) 70% AcOH, (**b**) TBSCl, imidazole, DMF, (**c**) Ac_2O, pyridine, (**d**) PPTS, CH_2Cl_2, (**e**) **6**, AgOTf, collidine, CH_3NO_2, (**f**) NH_2NH_2 H2O, EtOH, (**g**) 10% Pd-C, H_2, EtOH, (**h**) 30% HBr-AcOH, Ac_2O, (**i**) **4**, $AgClO_4$, Ag_2CO_3, CH_2Cl_2, toluene, (**j**) NaOMe, MeOH.

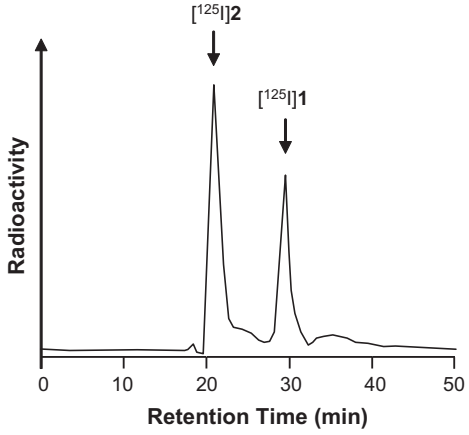


Figure 2. Chromatogram of reaction products of [^{125}I]**1.**

tography (chloroform/methanol = 3:1) to afford the dephthaloyl product (500 mg, 95%). A mixture of the dephthaloyl product (500 mg, 0.82 mmol) and acetic anhydride (2.5 mL) in pyridine (5 mL) was stirred overnight at room temperature. The mixture was poured into ice water, and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 3:1) to afford **8** (625 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.73, 2.01, 2.02 and 2.02 (each s, 3H) 3.46 (ddd, J = 9.9, 8.3 and 7.6 Hz, 1H), 3.70–3.78 (m, 1H), 3.82–3.86 (m, 2H), 4.01–4.04 (m, 1H), 4.11–4.24 (m, 5H), 4.49 (ABd, J_{AB} = 11.7 Hz, 1H), 4.70 (ABd, J_{AB} = 11.4 Hz, 1H), 4.71 (ABd, J_{AB} = 11.7 Hz, 1H), 4.80 (ABd, J_{AB} = 11.4 Hz, 1H), 4.86 (d, J = 1.6 Hz, 1H), 5.00 (dd, J = 10.1 and 9.3 Hz, 1H), 5.12 (d, J = 8.4 Hz, 1H), 5.52 (d, J = 7.5 Hz, 1H), 5.64 (s, 1H), 5.67 (dd, J = 10.6 and 9.3 Hz, 1H), 7.27–7.40 (m, 13H), 7.49–7.52 (m, 2H); MS FAB(+) m/z 778 [$(M+H)^+$, 5]; HR-MS calcd for $C_{41}H_{48}NO_{14}$ [$(M+H)^+$] 778.3075, found: 778.3070.

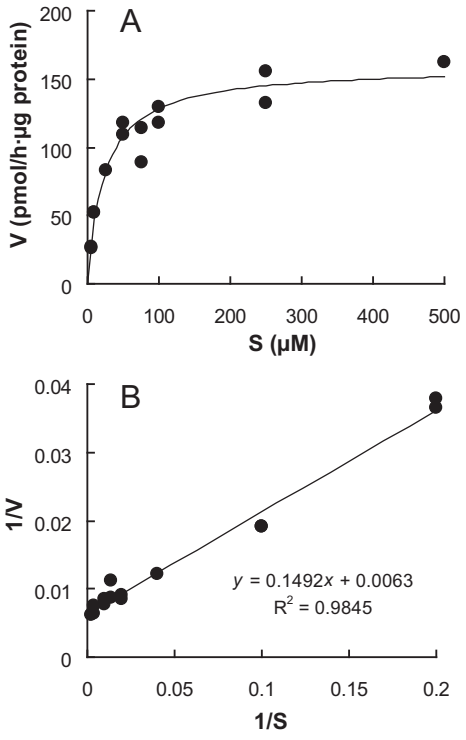


Figure 3. (A) Michaelis–Menten's curve of the GnT-V-catalyzed reaction: (B) Lineweaver–Burk plot of the GnT-V-catalyzed reaction.

4.1.3. Benzyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-α-D-mannopyranoside (9)

A solution of **8** (160 mg, 0.21 mmol) and 70% acetic acid (2 mL) was heated to 55 °C for 6.5 h. The mixture was poured into ice water, neutralized with sodium hydrogencarbonate,

Table 1
GnT-V activity in rat tissues determined with [^{125}I]**1** or Gn,Gn-bi-PA as a substrate.

Tissue	Activity (pmol/h mg protein)			
	[^{125}I]IPGMG [a]		Gn,Gn-bi-PA [b]	
Heart	31	± 1	10	±1
Liver	48	± 5	8.2	±0.8
Stomach	116	± 65	25	±1
Lung	179	± 6	38	±4
Spleen	244	± 117	21	±2
Brain	261	± 18	38	±1
Kidney	671	± 240	30	±6
Intestine	774	± 67	68	±11
Serum	1284	± 223	112	±53

[a] Each value represents the mean ± SD for three samples.
[b] Results were reported previously.[13]

and extracted with chloroform. The organic layer was washed with a saturated sodium hydrogencarbonate solution, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 10:1) to afford the diol compound (125 mg, 91%). A mixture of the diol compound (138 mg, 0.20 mmol) and acetic anhydride (0.5 mL) in pyridine (1 mL) was stirred overnight at room temperature. The mixture was poured into ice water, neutralized with sodium hydrogencarbonate, and extracted with chloroform. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to afford **9** (137 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.82 (s, 3H), 2.00 (s, 6H), 2.01, 2.05 and 2.08 (each s, 3H), 3.29 (ddd, J = 9.9, 8.2 and 7.5 Hz, 1H), 3.71–3.75 (m, 1H), 3.85–3.90 (m, 2H), 4.03 (d of ABd, J_{AB} = 12.1 Hz, J = 2.4 Hz, 1H), 4.10–4.20 (m, 2H), 4.24 (d of ABd, J_{AB} = 12.1 Hz, J = 6.0 Hz, 1H), 4.49 (ABd, J_{AB} = 11.4 Hz, 1H), 4.51 (ABd, J_{AB} = 11.6 Hz, 1H), 4.68 (ABd, J_{AB} = 11.4 Hz, 1H), 4.71 (ABd, J_{AB} = 11.6 Hz, 1H), 4.92 (d, J = 2.0 Hz, 1H), 4.97 (dd, J = 10.1 and 9.2 Hz, 1H), 5.24 (t, J = 9.6 Hz, 1H), 5.27 (d, J = 8.2 Hz, 1H), 5.57 (d, J = 7.1 Hz, 1H), 5.74 (dd, J = 10.5 and 9.2 Hz, 1H), 7.28–7.41 (m, 10H); MS FAB(+) m/z 774 [(M+H)$^+$, 9]; HR-MS calcd for $C_{38}H_{48}NO_{16}$ [(M+H)$^+$] 774.2973, found: 774.2979.

4.1.4. 1,3,4,6-Tetra-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyrosyl)-D-mannopyranose (10)

A mixture of **9** (1.13 g, 1.46 mmol) and PD-C (10%, *ca.* 600 mg) in a mixed solvent of ethanol (35 mL) and acetic acid (12 mL) was stirred overnight at 50 °C under atmospheric pressure of H_2. The catalysts were filtered, and the filtrate was evaporated. The residue was chromatographed on a silica gel column (chloroform/methanol = 15:2) to give the diol compound (740 mg, quant.). A mixture of the diol compound (225 mg, 0.38 mmol) and acetic anhydride (2 mL) in pyridine (4 mL) was stirred overnight at room temperature. The solvent was removed, and the residue was purified by silica gel chromatography (chloroform/methanol = 10:1) to give **10** (257 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96, 2.01, 2.02, 2.04, 2.05, 2.08, 2.12 and 2.15 (each s, 3H), 3.28 (ddd, J = 7.1, 5.0 and 2.2 Hz, 1H), 3.87 (ddd, J = 10.6, 8.6 and 8.4 Hz, 1H), 3.96 (ddd, J = 7.3, 4.9 and 2.4 Hz, 1H), 4.02 (d of ABd, J_{AB} = 12.3 Hz, J = 2.2 Hz, 1H), 4.07 (d of ABd, J_{AB} = 12.5 Hz, J = 2.4 Hz, 1H), 4.20 (dd, J = 3.3 and 2.4 Hz, 1H), 4.22 (d of ABd, J_{AB} = 12.5 Hz, J = 5.0 Hz, 1H), 4.25 (d of ABd, J_{AB} = 12.3 Hz, J = 5.0 Hz, 1H), 4.80 (d, J = 8.4 Hz, 1H), 5.04 (dd, J = 10.3 and 3.8 Hz, 1H), 5.06 (dd, J = 10.1 and 1.8 Hz, 1H), 5.33 (dd, J = 10.8 and 4.2 Hz, 1H), 5.36 (dd, J = 10.8 and 4.0 Hz, 1H), 5.79 (d, J = 8.6 Hz, 1H), 5.98 (d, J = 2.0 Hz, 1H); MS FAB(+) *m/z* 678 [(M+H)$^+$, 6]; HR-MS calcd for $C_{28}H_{40}NO_{18}$ [(M+H)$^+$] 678.2245, found: 678.2241.

4.1.5. 3,4,6-Tri-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyrosyl)-α-D-mannopyranosyl bromide (3)

To a solution of **10** (136 mg, 0.20 mmol) and acetic anhydride (1.5 mL) was added hydrogen bromide (30% in acetic acid, 3 mL). The reaction mixture was stirred at room temperature for 4 h under a nitrogen atmosphere, and hydrogen bromide (30% in acetic acid, 3 mL) was added. The mixture was stirred overnight at room temperature, poured into ice water, and extracted with chloroform. The organic layer was washed with water and a saturated sodium hydrogencarbonate solution, dried over sodium sulfate, filtered, and evaporated to give **3** (137 mg, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95, 2.02, 2.03, 2.05, 2.08, 2.09 and 2.11 (each s, 3H), 3.60 (dt, J = 10.8 and 8.2 Hz, 1H), 3.71–3.77 (m, 1H), 4.00–4.03 (m, 1H), 4.08–4.14 (m, 2H), 4.24–4.28 (m, 2H), 4.48 (dd, J = 3.3 and 1.7 Hz, 1H), 5.01 (dd, J = 9.9 and 9.3 Hz, 1H), 5.08 (d, J = 8.2 Hz, 1H), 5.35–5.52 (m, 3H), 5.87 (d, J = 7.9 Hz, 1H), 6.29 (d, J = 1.3 Hz, 1H); MS FAB(+) *m/z* 698 (M$^+$, 7); HR-MS calcd for $C_{26}H_{36}BrNO_{16}$ (M$^+$) 698.1296, found: 698.1301.

4.1.6. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-allyloxycarbonyl-β-D-glucopyranose (12)

To a tetrahydrofuran (30 mL) solution of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (**11**) (1.38 g, 4.31 mmol) in an ice bath were added allyl chloroformate (1.37 mL, 12.9 mmol) and pyridine (1.39 mL, 17.2 mmol). The reaction mixture was stirred at room temperature for 2 h under a nitrogen atmosphere, poured into aqueous ammonium chloride (15 mL), and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to afford **12** (1.63 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01, 2.03, 2.04 and 2.10 (each s, 3H), 3.84 (m, 1H), 4.22–4.32 (m, 2H), 4.61–4.63 (m, 2H), 5.06–5.16 (m, 2H), 5.23–5.39 (m, 3H), 5.74 (d, J = 7.7 Hz, 1H), 5.86–5.99 (m, 1H); MS FAB(+) m/z 455 [(M+Na)$^+$, 100]; HR-MS calcd for $C_{18}H_{24}O_{12}Na$ [(M+Na)$^+$] 455.1166, found: 455.1171.

4.1.7. 2,3,4-Tri-*O*-acetyl-6-*O*-allyloxycarbonyl-α-D-glucopyranosyl trichloroacetimidate (13)

To a tetrahydrofuran (50 mL) solution of **12** (3.45 g, 11.4 mmol) was added piperidine (2.26 mL, 22.8 mmol). The reaction mixture was stirred at room temperature for 20 h under a nitrogen atmosphere. After the reaction was completed, the mixture was diluted with ethyl acetate. The organic layer was washed with 10% hydrochloric acid and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to give a crude compound (2.41 g). To a solution of the crude compound (2.41 g) in dichloromethane (30 mL) were added trichloroacetonitrile (1.23 mL, 12.3 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (46 μL, 0.31 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 3:1) to afford **13** (2.73 g, 64%, 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.01, 2.04 and 2.06 (each s, 3H), 4.22–4.33 (m, 3H), 4.62 (dt, J = 1.3 and 5.9 Hz, 2H), 5.12 (dd, J = 3.7 and 10.2 Hz, 1H), 5.19 (t, J = 9.6 Hz, 1H), 5.28 (qd, J = 1.3 and 10.5 Hz, 1H), 5.36 (qd, J = 1.5 and 17.2 Hz, 1H), 5.58 (t, J = 9.6 Hz, 1H), 5.92 (ddt, J = 5.9, 10.5 and 17.2 Hz, 1H), 6.57 (d, J = 3.7 Hz, 1H), 8.69 (s, 1H); MS FAB(+) *m/z* 556 [(M+Na)$^+$, 11]; HR-MS calcd for $C_{18}H_{22}O_{11}Cl_3NNa$ [(M+Na)$^+$] 556.0156, found: 556.0152.

4.1.8. 2-(4-Bromophenyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-allyloxycarbonyl-β-D-glucopyranoside (14)

To a dichloromethane (20 mL) solution of **13** (904 mg, 1.69 mmol) and 2-(4-bromophenyl)ethyl alcohol (197 μL, 1.41 mmol) was added boron trifluoride diethyl etherate (36 μL,

0.28 mmol). The reaction mixture was stirred at −20 °C for 2 h, and a saturated sodium hydrogencarbonate solution was added. Following extraction with chloroform, the organic phase was dried over sodium sulfate and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (toluene/diethyl ether = 3:1) to afford **14** (584 mg, 72%). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.89, 1.99 and 2.03 (each s, 3H), 2.80–2.86 (m, 2H), 3.58–3.75 (m, 2H), 4.06–4.29 (m, 3H), 4.46 (d, J = 7.9 Hz, 1H), 4.62 (dt, J = 1.3 and 5.9 Hz, 2H), 4.93–5.05 (m, 2H), 5.14–5.40 (m, 3H), 5.85–5.99 (m, 1H), 7.04–7.08 (m, 2H), 7.37–7.40 (m, 2H); MS FAB(+) m/z 595 [(M+Na)$^+$, 54]; HR-MS calcd for $C_{24}H_{29}O_{11}BrNa$ [(M+Na)$^+$] 595.0791, found: 595.0787.

4.1.9. 2-(4-Bromophenyl)ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (15)

To a tetrahydrofuran (30 mL) solution of **14** (2.12 g, 3.70 mmol) were added tetrakis(triphenylphosphine)palladium (0) (214 mg, 0.18 mmol), triphenylphosphine (291 mg, 1.11 mmol), and formic acid (279 mL, 7.39 mmol). The reaction mixture was stirred at room temperature for 6 h under a nitrogen atmosphere. After the reaction was completed, the mixture was diluted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 1:1) to afford **15** (1.70 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89, 2.00 and 2.05 (each s, 3H), 2.81–2.86 (m, 2H), 3.46–3.52 (m, 2H), 3.55–3.67 (m, 3H), 3.71–3.75 (m, 1H), 4.13 (ddd, J = 9.5, 6.1 and 5.9 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.96 (dd, J = 9.7 and 7.9 Hz, 1H), 5.01 (dd, J = 9.7 and 9.5 Hz, 1H), 5.21 (t, J = 9.5 Hz, 1H), 7.05–7.08 (m, 2H), 7.38–7.41 (m, 2H); MS FAB(+) m/z 511 [(M+Na)$^+$, 99]; HR-MS calcd for $C_{20}H_{25}O_9BrNa$ [(M+Na)$^+$] 511.0580, found: 511.0573.

4.1.10. 2-(4-Bromophenyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(1-ethoxyethyl)-β-D-glucopyranoside (16)

To a dichloromethane (3 mL) solution of **15** (173 mg, 0.35 mmol) were added ethyl vinyl ether (51 μL, 0.53 mmol), and pyridinium p-toluenesulfonate (9 mg, 0.035 mmol). The reaction mixture was stirred at room temperature for 1 h under a nitrogen atmosphere. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to afford **16** (197 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17 (t, J = 7.1 Hz, 1.5H), 1.18 (t, J = 7.1 Hz, 1.5H), 1.28 (d, J = 5.3 Hz, 1.5H), 1.29 (d, J = 5.3 Hz, 1.5H), 1.89 (s, 1.5H), 1.89 (s, 1.5H), 1.99 (s, 3H), 2.02 (s, 3H), 2.80–2.85 (m, 2H), 3.40–3.70 (m, 6H), 4.06–4.16 (m, 1H), 4.46 (d, J = 7.9 Hz, 1H), 4.70 (q, J = 5.3 Hz, 0.5H), 4.72 (q, J = 5.3 Hz, 0.5H), 4.96 (dd, J = 9.7 and 7.9 Hz, 1H), 5.03 (dd, J = 9.7 and 9.5 Hz, 1H), 5.16 (t, J = 9.5 Hz, 1H), 7.05–7.07 (m, 2H), 7.37–7.40 (m, 2H); MS FAB(+) m/z 583 [(M+Na)$^+$, 34]; HR-MS calcd for $C_{24}H_{33}O_{10}BrNa$ [(M+Na)$^+$] 583.1155, found: 583.1151.

4.1.11. 2-[4-(Tributylstannyl)phenyl]ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(1-ethoxyethyl)-β-D-glucopyranoside (17)

To a *N*,*N*-dimethylformamide (5 mL) solution of **16** (161 mg, 0.29 mmol) were added tetrakis(triphenylphosphine)palladium (0) (33 mg, 0.029 mmol), triphenylphosphine (15 mg, 0.057 mmol), and bis(tributyltin) (289 μL, 0.57 mmol). The reaction mixture was stirred at 100 °C for 4 h under a nitrogen atmosphere. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 5:1 to 2:1) to afford **17** (103 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (t, J = 7.3 Hz, 9H), 1.02 (t, J = 8.1 Hz, 6H), 1.17 (t, J = 7.2 Hz, 1.5H), 1.18 (t, J = 7.2 Hz, 1.5H), 1.27–1.36 (m, 9H), 1.47–1.57 (m, 6H), 1.89 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 2.86 (t, J = 6.8 Hz, 2H), 3.43–3.71 (m, 6H), 4.08–4.15 (m, 1H), 4.48 (d, J = 8.1 Hz, 1H), 4.70–4.72 (m, 1H), 4.98 (t, J = 8.1 Hz, 1H), 5.04 (t, J = 9.5 Hz, 1H), 5.17 (t, J = 9.5 Hz, 1H), 7.13–7.16 (m, 2H), 7.35–7.37 (m, 2H); MS FAB(+) m/z 795 [(M+Na)$^+$, 43]; HR-MS calcd for $C_{36}H_{60}O_{10}SnNa$ [(M+Na)$^+$] 795.3106, found: 795.3111.

4.1.12. 2-(4-Iodophenyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(1-ethoxyethyl)-β-D-glucopyranoside (18)

To a dichloromethane (10 mL) solution of **17** (305 mg, 0.40 mmol) was added iodine (201 mg, 0.79 mmol). The reaction mixture was stirred at 0 °C for 2 h under a nitrogen atmosphere, and a saturated sodium hydrogencarbonate and sodium thiosulfate solution was added. Following extraction with chloroform, the organic phase was dried over sodium sulfate and filtered. The solvent was removed, and the residue was purified by silica gel chromatography (toluene/diethyl ether = 3:1) to afford **18** (226 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17 (t, J = 7.0 Hz, 1.5H), 1.17 (t, J = 7.0 Hz, 1.5H), 1.27 (d, J = 5.5 Hz, 1.5H), 1.29 (d, J = 5.1 Hz, 1.5H), 1.89 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.79–2.84 (m, 2H), 3.42–3.69 (m, 6H), 4.10 (dt, J = 9.5 and 5.9 Hz, 1H), 4.45 (d, J = 7.7 Hz, 1H), 4.70 (q, J = 5.1 Hz, 0.5H), 4.71 (q, J = 5.1 Hz, 0.5H), 4.95 (dd, J = 9.7 and 7.7 Hz, 1H), 5.03 (dd, J = 9.7 and 9.5 Hz, 1H), 5.16 (t, J = 9.5 Hz, 1H), 6.92–6.95 (m, 2H), 7.57–7.59 (m, 2H); MS FAB(+) m/z 631 [(M+Na)$^+$, 16]; HR-MS calcd for $C_{24}H_{33}O_{10}INa$ [(M+Na)$^+$] 631.1016, found: 631.1011.

4.1.13. 2-[4-(Tributylstannyl)phenyl]ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (19)

A mixture of **17** (310 mg, 0.40 mmol), pyridinium p-toluenesulfonate (10 mg, 0.040 mmol), and methanol (10 mL) was stirred at room temperature for 2 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 3:1 to 2:1) to afford **19** (268 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (t, J = 7.2 Hz, 9H), 1.02 (t, J = 8.1 Hz, 6H), 1.33 (sextet, J = 7.2 Hz, 6H), 1.53 (tt, J = 8.1 and 7.2 Hz, 6H), 1.89 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.12 (t, J = 6.1 Hz, 1H), 2.86 (dd, J = 7.2 and 6.6 Hz, 2H), 3.46–3.52 (m, 1H), 3.55–3.61 (m, 1H), 3.63–3.76 (m, 2H), 4.13 (dt, J = 9.5 and 6.1 Hz, 1H), 4.53 (d, J = 8.1 Hz, 1H), 4.98 (dd, J = 9.7 and 8.1 Hz, 1H), 5.02 (dd, J = 9.7 and 9.5 Hz, 1H), 5.22 (t, J = 9.5 Hz, 1H), 7.14–7.16 (m, 2H), 7.35–7.38 (m, 2H); MS FAB(+) m/z 723 [(M+Na)$^+$, 100]; HR-MS calcd for $C_{32}H_{52}O_9NaSn$ [(M+Na)$^+$] 723.2531, found: 723.2533.

4.1.14. 2-(4-Iodophenyl)ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (4)

To a methanol (10 mL) solution of **18** (222 mg, 0.41 mmol) was added pyridinium p-toluenesulfonate (10 mg, 0.036 mmol). The reaction mixture was stirred at 55 °C for 2 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 1:1) to afford **4** (192 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89, 2.00 and 2.05 (each s, 3H), 2.20 (br.t, J = Hz, 1H), 2.80–2.84 (m, 2H), 3.46–3.52 (m, 1H), 3.55–3.77 (m, 3H), 4.12 (dt, J = 9.5 and 5.9 Hz, 1H), 4.49 (d, J = 7.7 Hz, 1H), 4.96 (dd, J = 9.5 and 7.7 Hz, 1H), 5.01 (t, J = 9.5 Hz, 1H), 5.21 (t, J = 9.5 Hz, 1H), 6.93–6.95 (m, 2H), 7.58–7.60 (m, 2H); MS FAB(+) m/z 559 [(M+Na)$^+$, 100]; HR-MS calcd for $C_{20}H_{25}O_9NaI$ [(M+Na)$^+$] 559.0441, found: 559.0447.

4.1.15. 2-(4-Iodophenyl)ethyl 6-*O*-[2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (20)

To a solution of **4** (214 mg, 0.40 mmol), dichloromethane (10 mL), and toluene (10 mL) was added silver carbonate (121 mg, 0.44 mmol). The reaction mixture was stirred at room temperature for 1 h under a nitrogen atmosphere, and silver perchlorate (12 mg, 0.060 mmol) was added. After stirring for 20 min at room temperature, a solution of **3** (334 mg, 0.48 mmol), dichloromethane (10 mL), and toluene (10 mL) was added and the mixture was stirred

for another 15 h. After the reaction was completed, the mixture was filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 4:1 to ethyl acetate only) to afford **20** (310 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.87, 1.93, 1.94, 2.00, 2.01, 2.01, 2.03, 2.05, 2.09 and 2.09 (each s, 3H), 2.82–2.88 (m, 2H), 3.36–3.45 (m, 1H), 3.51–3.54 (m, 1H), 3.61–3.78 (m, 4H), 3.87–3.92 (m, 1H), 4.00–4.09 (m, 2H), 4.13–4.20 (m, 3H), 4.26–4.32 (m, 1H), 4.47 (d, J = 7.7 Hz, 1H), 4.70 (s, 1H), 4.89–5.05 (m, 4H), 5.08 (d, J = 8.1 Hz, 1H), 5.18 (t, J = 9.5 Hz, 1H), 5.21 (t, J = 9.9 Hz, 1H), 5.60 (t, J = 9.2 Hz, 1H), 5.61 (d, J = 9.2 Hz, 1H), 6.94–6.97 (m, 2H), 7.56–7.59 (m, 2H); MS FAB(+) *m/z* 1154 [(M+H)$^+$, 3]; HR-MS calcd for $C_{46}H_{61}O_{25}NI$ [(M+H)$^+$] 1154.2578, found: 1154.2588.

4.1.16. 2-[4-(Tributylstannyl)phenyl]ethyl 6-*O*-[2-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (21)

To a solution of **19** (30 mg, 0.043 mmol), dichloromethane (1 mL), and toluene (1 mL) was added collidine (14 μL, 0.10 mmol). The reaction mixture was stirred at room temperature for 20 min under a nitrogen atmosphere, and silver perchlorate (11 mg, 0.052 mmol) was added. After stirring for 20 min at room temperature, a solution of **3** (36 mg, 0.052 mmol), dichloromethane (2 mL), and toluene (2 mL) was added and the mixture was stirred for another 5 h. After the reaction was completed, the mixture was filtered. The filtrate was washed with 1% hydrochloric acid, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (ethyl acetate) to afford **21** (24 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (t, J = 7.3 Hz, 9H), 1.01 (t, J = 8.2 Hz, 6H), 1.32 (sextet, J = 7.3 Hz, 6H), 1.52 (tt, J = 8.2 and 7.3 Hz, 6H), 1.85 (s, 3H), 1.90 (s, 3H), 1.93 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.85–2.91 (m, 2H), 3.38–3.48 (m, 1H), 3.52–3.55 (m, 1H), 3.66–3.80 (m, 4H), 3.90–3.95 (m, 1H), 4.00–4.04 (m, 2H), 4.13–4.21 (m, 3H), 4.26–4.32 (m, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.69 (s, 1H), 4.91–5.07 (m, 5H), 5.16–5.25 (m, 2H), 5.51–5.62 (m, 2H), 7.13–7.16 (m, 2H), 7.33–7.36 (m, 2H); MS FAB(+) *m/z* 1318 [(M+H)$^+$, 18]; HR-MS calcd for $C_{58}H_{88}O_{25}NSn$ [(M+H)$^+$] 1318.4668, found: 1318.4672.

4.1.17. 2-(4-Iodophenyl)ethyl 6-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (1)

To a methanol (10 mL) solution of **20** (188 mg, 0.16 mmol) was added sodium methoxide (28% in methanol, 332 μL, 1.63 mmol) and the mixture was stirred at room temperature for 2 h. After the reaction was completed, the mixture was neutralized with Amberlyst® 15 (ca. 1.0 g) and filtered. The filtrate was concentrated in vacuo and the residue was purified by a recycle HPLC (LC-908, Japan Analytical Industry Co. Ltd. Tokyo, Japan) on a reversed phase column (methanol/distilled water = 7:3) to afford **1** (74 mg, 59%). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.99 (s, 3H), 2.89–2.92 (m, 2H), 3.16–4.04 (m, 21H), 4.28 (d, J = 7.3 Hz, 1H), 4.46 (d, J = 7.7 Hz,1H), 7.06–7.09 (m, 2H), 7.59–7.62 (m, 2H); MS FAB(+) *m/z* 776 [(M+H)$^+$, 10]; HR-MS calcd for $C_{28}H_{43}O_{16}NI$ [(M+H)$^+$] 776.1627, found: 776.1635.

4.1.18. Benzyl 3-*O*-benzyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-mannopyranoside (23)

A mixture of **7** (1.45 g, 1.67 mmol) and 70% acetic acid (10 mL) was stirred at 55 °C for 2 h. The mixture was poured into ice water, neutralized with sodium hydrogencarbonate, and extracted with chloroform. The organic layer was washed with a saturated sodium hydrogencarbonate solution, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to afford **23** (1.07 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.26 (br.t, J = 6.6 Hz, 1H), 1.89 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.28 (br.s, 1H), 3.12–3.16 (m, 1H), 3.37–3.46 (m, 2H), 3.68–3.69 (m, 2H), 3.84–3.90 (m, 1H), 4.13 (br.s, 1H), 4.20 (d of ABd, J_{AB} = 12.4 Hz, J = 2.4 Hz, 1H), 4.27 (d of ABd, J_{AB} = 12.4 Hz, J = 4.8 Hz, 1H), 4.30 (ABd, J_{AB} = 11.7 Hz, 1H), 4.38 (ABd, J_{AB} = 11.0 Hz, 1H), 4.41 (dd, J = 10.6 and 8.4 Hz, 1H), 4.49 (ABd, J_{AB} = 11.7 Hz, 1H), 4.62 (d, J = 1.5 Hz, 1H), 4.75 (ABd, J_{AB} = 11.0 Hz, 1H), 5.17 (t, J = 9.5 Hz, 1H), 5.36 (d, J = 8.4 Hz, 1H), 5.87 (dd, J = 11.0 and 9.2 Hz, 1H), 7.18–7.21 (m, 2H), 7.28–7.34 (m, 8H), 7.76–7.80 (m, 2H), 7.85–7.88 (m, 2H); MS FAB(+) *m/z* 800 [(M+Na)$^+$, 53]; HR-MS calcd for $C_{40}H_{43}O_{15}NNa$ [(M+Na)$^+$] 800.2530, found: 800.2527.

4.1.19. Benzyl 3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-mannopyranoside (24)

To a *N*,*N*-dimethylformamide (10 mL) solution of **23** (1.07 g, 1.38 mmol) were added *tert*-butyldimethylsilyl chloride (311 mg, 2.06 mmol) and imidazole (281 mg, 4.13 mmol). The reaction mixture was stirred at room temperature for 16 h under a nitrogen atmosphere, poured into aqueous ammonium chloride (20 mL), and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to afford **24** (1.15 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: -0.13 (s, 3H), -0.12 (s, 3H), 0.78 (s, 9H), 1.86 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.64 (br.s, 1H), 3.14–3.20 (m, 1H), 3.41–3.49 (m, 2H), 3.60–3.71 (m, 2H), 3.82–3.87 (m, 1H), 4.13–4.26 (m, 3H), 4.31 (ABd, J_{AB} = 11.4 Hz, 1H), 4.40 (d, J = 9.5 Hz, 1H), 4.45 (ABd, J_{AB} = 11.2 Hz, 1H), 4.56 (ABd, J_{AB} = 11.4 Hz, 1H), 4.64 (s, 1H), 4.78 (ABd, J_{AB} = 11.2 Hz, 1H), 5.16 (t, J = 9.7 Hz, 1H), 5.44 (d, J = 8.1 Hz, 1H), 5.78 (dd, J = 10.6 and 9.2 Hz, 1H), 7.21–7.38 (m, 10H), 7.71–7.74 (m, 2H), 7.82–7.84 (m, 2H); MS FAB(+) *m/z* 914 [(M+Na)$^+$, 62]; HR-MS calcd for $C_{46}H_{57}O_{15}NSiNa$ [(M+Na)$^+$] 914.3395, found: 914.3398.

4.1.20. Benzyl 4-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-mannopyranoside (25)

A mixture of **24** (1.14 g, 1.28 mmol) and acetic anhydride (5 mL) in pyridine (5 mL) was stirred at room temperature for 2 h and methanol (20 mL) was added. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 2:1) to give **25** (1.18 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: -0.19 (s, 3H), -0.18 (s, 3H), 0.77 (s, 9H), 1.85 (s, 3H), 1.95 (s, 3H), 2.01 (s, 6H), 3.22 (d of ABd, J_{AB} = 11.3 Hz, J = 7.2 Hz, 1H), 3.40 (d of ABd, J_{AB} = 11.3 Hz, J = 2.6 Hz, 1H), 3.55–3.62 (m, 1H), 3.75–3.81 (m, 2H), 4.11–4.24 (m, 3H), 4.32 (ABd, J_{AB} = 11.6 Hz, 1H), 4.44 (ABd, J_{AB} = 11.7 Hz, 1H), 4.44 (dd, J = 10.8 and 8.6 Hz, 1H), 4.60 (ABd, J_{AB} = 11.6 Hz, 1H), 4.69 (d, J = 2.4 Hz, 1H), 4.70 (ABd, J_{AB} = 11.7 Hz, 1H), 4.88 (t, J = 9.1 Hz, 1H), 5.15 (t, J = 9.5 Hz, 1H), 5.53 (d, J = 8.4 Hz, 1H), 5.74 (dd, J = 10.7 and 9.1 Hz, 1H), 7.22–7.35 (m, 10H), 7.68–7.71 (m, 2H), 7.80–7.83 (m, 2H); MS FAB(+) *m/z* 956 [(M+Na)$^+$, 45]; HR-MS calcd for $C_{48}H_{59}O_{16}NSiNa$ [(M+Na)$^+$] 956.3501, found: 956.3503.

4.1.21. Benzyl 4-*O*-acetyl-3-*O*-benzyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-α-D-mannopyranoside (26)

A mixture of **25** (1.17 g, 1.25 mmol), pyridinium *p*-toluenesulfonate (32 mg, 0.13 mmol), and methanol (10 mL) was stirred at 50 °C for 2 h. The solvent was removed, and the residue was purified by silica gel chromatography (hexane/ethyl acetate = 1:1) to

afford **26** (765 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.44 (br.dd, J = 9.4 and 4.4 Hz, 1H), 1.88(s, 3H), 1.95 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 3.03–3.10 (m, 1H), 3.17–3.25 (m, 1H), 3.39–3.46 (m, 1H), 3.80–3.84 (m, 2H), 4.10–4.11 (m, 1H), 4.16–4.21 (m, 1H), 4.22–4.28 (m, 1H), 4.35 (ABd, J_{AB} = 11.7 Hz, 1H), 4.42–4.48 (m, 1H), 4.47 (ABd, J_{AB} = 11.9 Hz, 1H), 4.51 (ABd, J_{AB} = 11.7 Hz, 1H), 4.68 (ABd, J_{AB} = 11.9 Hz, 1H), 4.71 (d, J = 2.0 Hz, 1H), 4.93 (t, J = 9.6 Hz, 1H), 5.17 (t, J = 9.6 Hz, 1H), 5.45 (d, J = 8.4 Hz, 1H), 5.82 (dd, J = 10.7 and 9.1 Hz, 1H), 7.17–7.21 (m, 2H), 7.26–7.35 (m, 8H), 7.71–7.74 (m, 2H), 7.83–7.86 (m, 2H); MS FAB(+) *m/z* 842 [(M+Na)$^+$, 100]; HR-MS calcd for $C_{42}H_{45}O_{16}NNa$ [(M+Na)$^+$] 842.2636, found: 842.2639.

4.1.22. Benzyl 2,6-di-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-4-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranoside (27)

To a mixture of **26** (564 mg, 0.69 mmol), silver trifluoromethanesulfonate (318 mg, 1.24 mmol), and collidine (164 μL, 1.24 mmol) in nitromethane (5 mL) was added a nitromethane (5 mL) solution of **6** (514 mg, 1.03 mmol) at −20 °C. The reaction mixture was stirred at −20 °C for 30 min and room temperature for 2 h before being filtered through Celite® and concentrated in vacuo. The residue was purified by silica gel chromatography (1st; hexane/ethyl acetate = 1:1, 2nd; chloroform/methanol = 20:1) to afford **27** (337 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.83, 1.86, 1.91, 2.00, 2.01, 2.03 and 2.07 (each s, 3H), 2.87–2.94 (m, 1H), 3.55–3.74 (m, 6H), 4.00–4.01 (m, 1H), 4.07–4.41 (m, 7H), 4.35 (ABd, J_{AB} = 12.0 Hz, 1H), 4.47 (d, J = 2.0 Hz, 1H), 4.59 (ABd, J_{AB} = 12.0 Hz, 1H), 4.73 (t, J = 9.4 Hz, 1H), 5.00 (d, J = 8.3 Hz, 1H), 5.06 (t, J = 9.4 Hz, 1H), 5.12 (t, J = 9.4 Hz, 1H), 5.42 (d, J = 8.6 Hz, 1H), 5.71 (dd, J = 10.7 and 9.1 Hz, 1H), 7.01–7.04 (m, 2H), 7.18–7.29 (m, 8H), 7.61–7.76 (m, 6H), 7.83–7.86 (m, 2H); MS FAB(+) *m/z* 1259 [(M+Na)$^+$, 20]; HR-MS calcd for $C_{62}H_{64}N_2O_{25}Na$ [(M+Na)$^+$] 1259.3696, found: 1259.3704.

4.1.23. Benzyl 2,6-di-*O*-[3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-4-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranoside (28)

To an ethanol (10 mL) solution of **27** (157 mg, 0.13 mmol) was added hydrazine·hydrate (80% in water, 200 μL). The reaction mixture was heated at reflux for 4 h before being concentrated in vacuo. The residue was dissolved in pyridine (2 mL) and acetic anhydride (2 mL) was added. The mixture was stirred overnight at room temperature and methanol (20 mL) was added. The solvent was removed, and the residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to give **28** (125 mg, 93%, 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.94, 1.98, 2.02, 2.02, 2.04 and 2.06 (each s, 3H), 2.07 (s, 6H), 2.07 (s, 3H), 2.98 (br dt, J = 7.1 and 9.5 Hz, 1H), 3.13 (br d, J = 10.6 Hz, 1H), 3.56 (br d, J = 9.8 Hz, 1H), 3.64 (ddd, J = 2.6, 4.7 and 9.5 Hz, 1H), 3.79 (br dt, J = 3.0 and 10.1 Hz, 1H), 3.87 (dd, J = 3.7 and 9.8 Hz, 1H), 4.09–4.15 (m, 5H), 4.21–4.32 (m, 4H), 4.51 (ABd, J_{AB} = 11.8 Hz, 1H), 4.65 (ABd, J_{AB} = 11.8 Hz, 1H), 4.73 (d, J = 11.4 Hz, 1H), 4.96 (t, J = 9.2 Hz, 1H), 5.00 (t, J = 9.3 Hz,1H), 5.03–5.11 (m, 2H), 5.30 (t, J = 9.8 Hz, 1H), 5.54 (d, J = 8.4 Hz, 1H), 5.98 (dd, J = 9.2 and 10.7 Hz, 1H), 6.63 (d, J = 9.8 Hz, 1H), 7.24–7.39 (m, 11H); MS FAB(+) *m/z* 1061 [(M+H)$^+$, 5]; HR-MS calcd for $C_{50}H_{65}N_2O_{23}Na$ [(M+H)$^+$] 1061.3978, found: 1061.3981.

4.1.24. 2,6-Di-*O*-[3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-1,3,4-tri-*O*-acetyl-α-D-mannopyranose (29)

To an ethanol (10 mL) solution of **28** (118 mg, 0.11 mmol) was added Pd-C (10%, ca. 100 mg) and ammonium formate (140 mg). The reaction mixture was refluxed overnight, filtered through Celite® and concentrated in vacuo. The residue was dissolved in pyridine (2 mL) and acetic anhydride (2 mL) was added. The mixture was stirred overnight at room temperature and methanol (20 mL) was added. The solvent was removed, and the residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to give **29** (78 mg, 72%, 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.97, 2.00, 2.01, 2.02, 2.05, 2.06, 2.06, 2.09, 2.11 and 2.12 (each s, 3H), 2.97–3.05 (m, 2H), 3.13–3.16 (m, 1H), 3.62–3.66 (m, 1H), 3.75–3.79 (m, 2H), 3.96–4.01 (m, 1H), 4.10–4.29 (m, 6H), 4.92 (t, J = 9.7 Hz, 1H), 5.06–5.10 (m, 4H), 5.45 (t, J = 9.7 Hz, 1H), 5.46 (d, J = 9.2 Hz, 1H), 5.90 (dd, J = 10.6 and 9.2 Hz, 1H, 6.05 (d, J = 1.8 Hz, 1H), 6.41 (d, J = 9.5 Hz, 1H), 7.23 (d, J = 3.9 Hz, 1H); MS FAB(+) *m/z* 965 [(M+H)$^+$, 5]; HR-MS calcd for $C_{40}H_{57}N_2O_{25}$ [(M+H)$^+$] 965.3250, found: 965.3255.

4.1.25. 2-(4-Iodophenyl)ethyl 6-*O*-[2,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-3,4-di-*O*-acetyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (31)

To a solution of **29** (70 mg, 0.073 mmol) and acetic anhydride (1.5 mL) was added hydrogen bromide (30% in acetic acid, 3 mL). The mixture was stirred overnight at room temperature, poured into ice water, and extracted with chloroform. The organic layer was washed with water and a saturated sodium hydrogencarbonate solution, dried over sodium sulfate, filtered, and evaporated to give **30** (67 mg). To a solution of **4** (30 mg, 0.055 mmol), dichloromethane (1 mL), and toluene (1 mL) was added silver carbonate (17 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h under a nitrogen atmosphere, and silver perchlorate (2 mg, 0.0082 mmol) was added. After stirring for 30 min at room temperature, a solution of **30** (65 mg, 0.066 mmol), dichloromethane (2 mL), and toluene (2 mL) was added and the mixture was stirred for another 15 h. After the reaction was completed, the mixture was filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel chromatography (chloroform/methanol = 20:1) to afford **31** (5 mg, 7%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.87, 1.94, 1.96, 1.97, 1.99, 2.00, 2.03, 2.04, 2.05, 2.06, 2.08, 2.09, and 2.09 (each s, 3H), 2.82–2.96 (m, 3H), 3.06–3.09 (m, 1H), 3.55–3.58 (m, 1H), 3.63–3.73 (m, 5H), 3.77–3.82 (m, 1H), 3.98–4.01 (m, 1H), 4.09–4.32 (m, 8H), 4.45 (d, J = 8.1 Hz, 1H), 4.82 (d, J = 1.5 Hz, 1H), 4.88–4.98 (m, 3H), 5.02–5.10 (m, 3H), 5.17 (t, J = 9.5 Hz, 1H), 5.35 (t, J = 9.6 Hz, 1H), 5.38 (d, J = 8.2 Hz, 1H), 5.92 (dd, J = 10.8 and 9.2 Hz, 1H), 6.43 (d, J = 9.9 Hz, 1H), 6.95–6.97 (m, 2H), 7.30 (d, J = 7.0 Hz, 1H), 7.56–7.59 (m, 2H); MS FAB(+) *m/z* 1463 [(M+Na)$^+$, 5]; HR-MS calcd for $C_{58}H_{77}N_2O_{32}I$ [(M+Na)$^+$] 1463.3402, found: 1463.3394.

4.1.26. 2-(4-Iodophenyl)ethyl 6-*O*-[2,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (IPGGMG, 2)

To a methanol (1 mL) solution of **31** (13 mg, 0.009 mmol) was added sodium methoxide (28% in methanol, 10 μL, 0.050 mmol) and the mixture was stirred at room temperature for 30 min. After the reaction was completed, the mixture was neutralized with Amberlyst® 15 (ca. 100 mg) and filtered. The filtrate was concentrated in vacuo to afford **2** (5 mg, 57%). ^{1}H NMR (400 MHz, CD_3OD) δ: 2.02, 2.06 (each s, 3H), 2.85–2.93 (m, 2H), 3.12–3.19 (m, 1H), 3.24–3.38 (m, 8H), 3.45–3.76 (m, 11H), 3.84–3.89 (m, 3H), 3.96 (br s, 1H), 4.01–4.10 (m, 2H), 4.28 (d, J = 7.7 Hz, 1H), 4.45 (d, J = 8.2 Hz, 1H), 4.58 (d, J = 7.7 Hz, 1H), 4.80 (s, 1H), 7.06–7.09 (m, 2H), 7.59–7.61 (m, 2H); MS FAB(+) *m/z* 1001 [(M+Na)$^+$, 2]; HR-MS calcd for $C_{36}H_{55}O_{21}N_2INa$ [(M+Na)$^+$] 1001.2240, found: 1001.2245.

4.2. Iododestannylation Reaction

The radiolabeled compound [^{125}I]**20** was prepared from the corresponding tributyltin derivative **21** by iododestannylation. Briefly, to initiate the reaction, 10 μL of H_2O_2 (5%) was added to a mixture of a tributyltin derivative (100 μg/100 μL MeOH), 0.2 mCi sodium [^{125}I]iodide, and 20 μL of 0.1 N HCl in a sealed vial. The reaction was allowed to proceed at room temperature for 30 min. The reaction mixture was neutralized with sodium bicarbonate, and extracted with ethyl acetate. The extract was evaporated and methanol (100 μL) was added. To the solution was added sodium methoxide (28% in methanol, 1.6 μL). The reaction mixture was stirred at room temperature for 45 min, and neutralized with Amberlyst® 15. The residue was purified by HPLC on a Cosmosil 5C18 AR-300 column (4.6×150 mm) with an isocratic solvent of H_2O/acetonitrile (83/17) at a flow rate of 1.0 mL/min.

4.3. Enzymatic Reactions of Radioiodinated IPGMG

GnT-V-transfected WiDr cells were used as the enzyme source. The protein concentration was measured by the Lowry method.[23] Lysate of GnT-V-overexpressing WiDr cells was incubated with MES buffer (125 mM, pH 6.25) containing 40 mM UDP-GlcNAc, 200 mM GlcNAc, 0.5% Triton X-100, and 10 mM EDTA for 10 min at 37 °C. [^{125}I]IPGMG was then added, and the incubation continued for an additional 4 h. The reaction was stopped by heating at 100 °C for 1 min. Enzymatic products were analyzed by HPLC on a TSK-GEL ODS-80TM column (4.6×150 mm) with an isocratic solvent of H_2O/acetonitrile (85/15) at a flow rate of 1.0 mL/min.

4.4. GnT-V Activities in Rat Tissues

Animal experiments were conducted in accordance with our institutional guidelines, and the experimental procedures were approved by the Kyoto University Animal Care Committee. Various tissues from male Wistar rats weighing 240–260 g were homogenized in 4 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. After centrifugation at 900 × g for 10 min, the supernatants were collected and used as crude enzyme preparations. The GnT-V activity and protein concentration were determined according to the procedures described above.

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