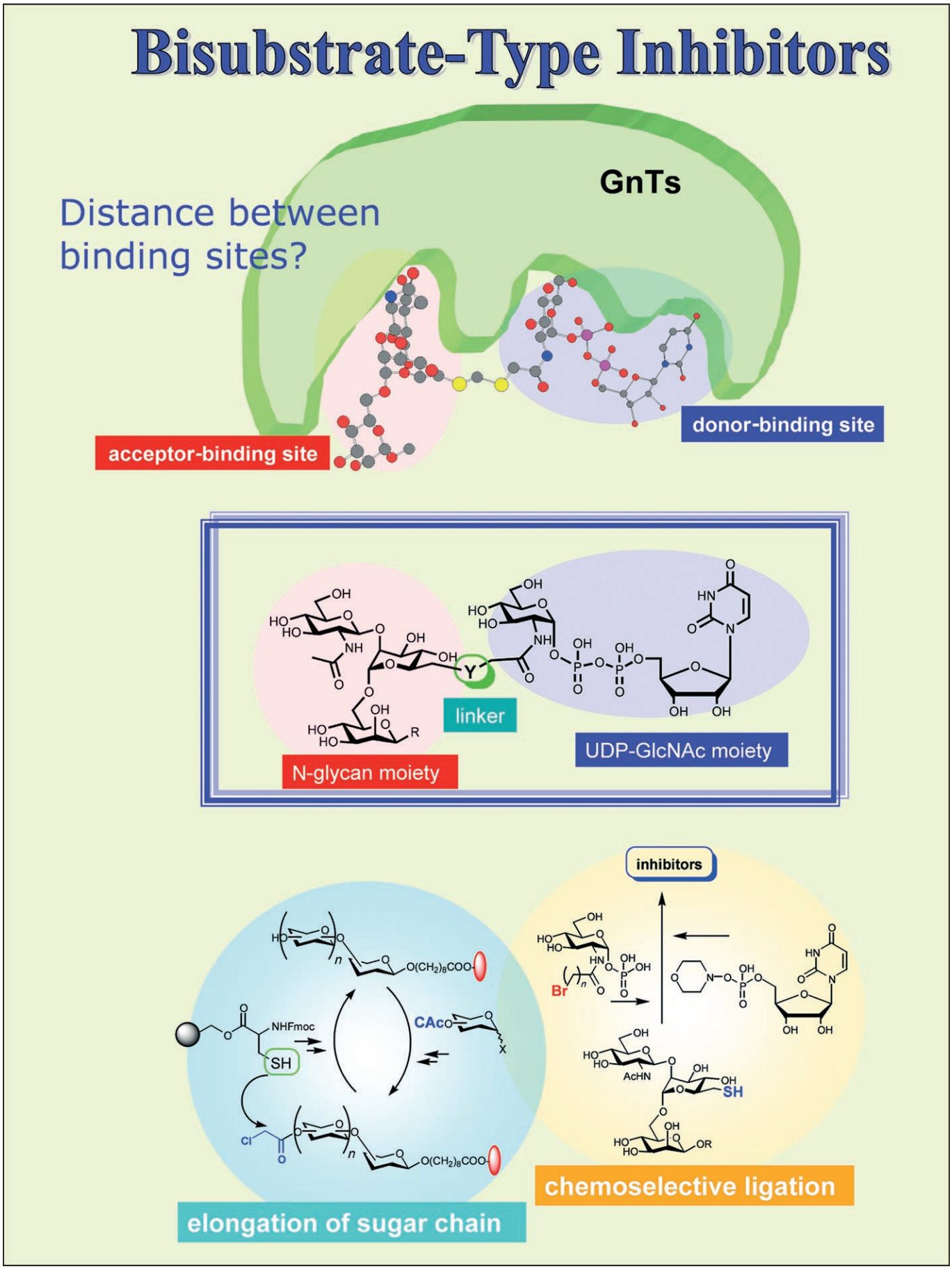

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Systematic Synthesis of Bisubstrate-Type Inhibitors of *N*-Acetylglucosaminyltransferases

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Abstract: Bisubstrate-type inhibitors for *N*-acetylglucosaminyltransferase (GnT)-V and -IX were designed and synthesized. These compounds carry both an acceptor trisaccaride and an UDP–GlcNAc component tethered by a linker of variable length. The acceptor trisaccharide unit was constructed using a combination of a polymer support and a resin capture–release strategy. Namely, starting with a β-mannoside bound to low molecular weight monomethyl PEG (MPEG), successive glycosylations with donors having chloroacetyl group produced the trisaccharide, which was subjected to the capture–release purification using cysteine loaded resin. UDP–GlcNAc units carrying phosphate moieties were separately synthesized from the bromoacetamide-containing glucosamine derivative. Ligation between the acceptor thiol and each alkyl bromide on the donor unit readily proceeded, and produced the coupling product. The introduction of the UMP component gave target compounds. All of the synthesized compounds had significant activities to GnT-V and -IX. Their potencies were dependent upon the linkers length. GnT-IX was more sensitive to these inhibitors and optimum linker length was clearly different between these GnTs. The most potent inhibitor of GnT-V had $K_i = 18.3$ μM, while that of GnT-IX had $K_i = 4.7$ μM.

Keywords: glycoproteins · glycosylation · glycosyltransferase · inhibitors · solid-phase synthesis

Introduction

Glycosyltransferases are a group of enzymes responsible for the biosynthesis of glycoconjugate oligosaccharides.[1] Mammalian *N*-acetylglucosaminyltransferases (GnTs) are of particular importance, because they are key enzymes in the production of highly diverse and variously branched complex-type asparagine (Asn)-linked (N-linked) glycoproteins. They share the ability to transfer an *N*-acetylglucoamine (GlcNAc) residue to the appropriate hydroxyl group of glycans or serine/threonine residues using uridine-5′-diphosphate-α-D-*N*-acetylglucosamine (UDP-GlcNAc) as a donor substrate. Among a variety of glycan structures made by GnTs, the characteristic branch produced by GnT-V, in which a GlcNAc residue is linked via a β1,6-linkage to the core α1,6-mannose (Man) arm on complex type N-glycan (Figure 1a), is highly intriguing, because of its relationship with various biomedically important phenomena (see below).[2] The more recently discovered GnT-IX is a closely related homologue of GnT-V, being exclusively expressed in brain.[3] It has a broader substrate specificity than GnT-V and transfers a GlcNAc residue to both α1,3- and α1,6-linked mannose structures, as well as to O-linked GlcNAcβ1,2-Man.[4]

Elevated levels of GnT-V and the GlcNAcβ1,6-Man-branched glycans produced by this enzyme correlate with the malignant transformation and metastatic potential of tumor cells.[5,6a–h] Therefore, the suppression of GnT-V might have clinical potential in the treatment of cancer.[7] In addition, GnT-V affects T-cell activation and angiogenesis.[6f,g] The ubiquitous expression of GnT-V is in sharp con-

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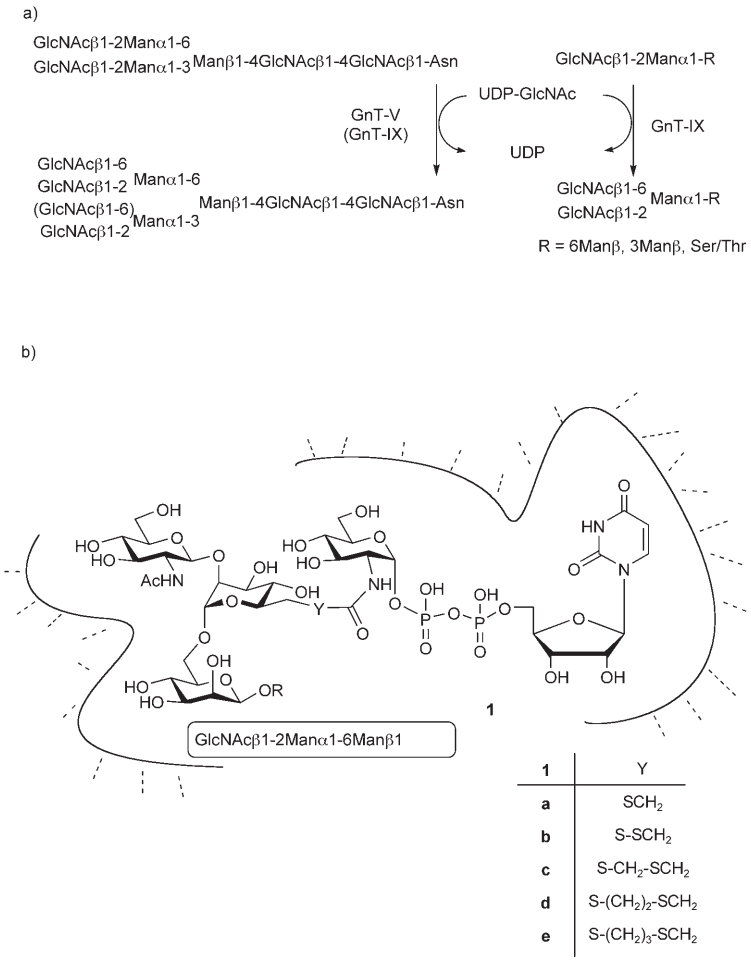


Figure 1. a) Substrate specificities of GnT-V and GnT-IX; b) bisubstrate-type inhibitors introduced various lengths of linkers, consisting of acceptor trisaccharide and UDP-GlcNAc moieties.

trast to the restricted distribution of GnT-IX in brain, suggesting that GnT-IX plays an important role in neural development and functions.[8]

Several GnT-V inhibitors have been reported, the design of which has relied upon the modification of the acceptor substrate oligosaccharide.[9] Because the incorporation of a donor component into an acceptor mimetic is expected to augment the binding and result in a potent and specific inhibitor of glycosyltransferases, we turned our attention to bisubstrate-type derivatives, **1a–e** (Figure 1b).[10] these compounds were designed to include both donor (UDP-GlcNAc) and acceptor units, taking into consideration the proposed mechanism of inverting GnTs.[11] This design enabled us to alter the lengths of linkers, in order to define the optimal distance between these components. These compounds are expected to help provide an insight into the molecular basis of the glycosyl transfer mechanism and specificity of GnT-V and -IX.

As an acceptor component, GlcNAcβ1,2-Manα1,6-Manβ was chosen a priori, because this trisaccharide was reported to be an efficient acceptor substrate of GnT-V.[12] In order to achieve conjugation with a donor (UDP-GlcNAc) component, we planned to incorporate GlcN-α-phosphate using a thiol-based ligation strategy, because of its technical simplicity and chemoselectivity, which was to be followed by coupling with uridine-5′-monophosphate (UMP).

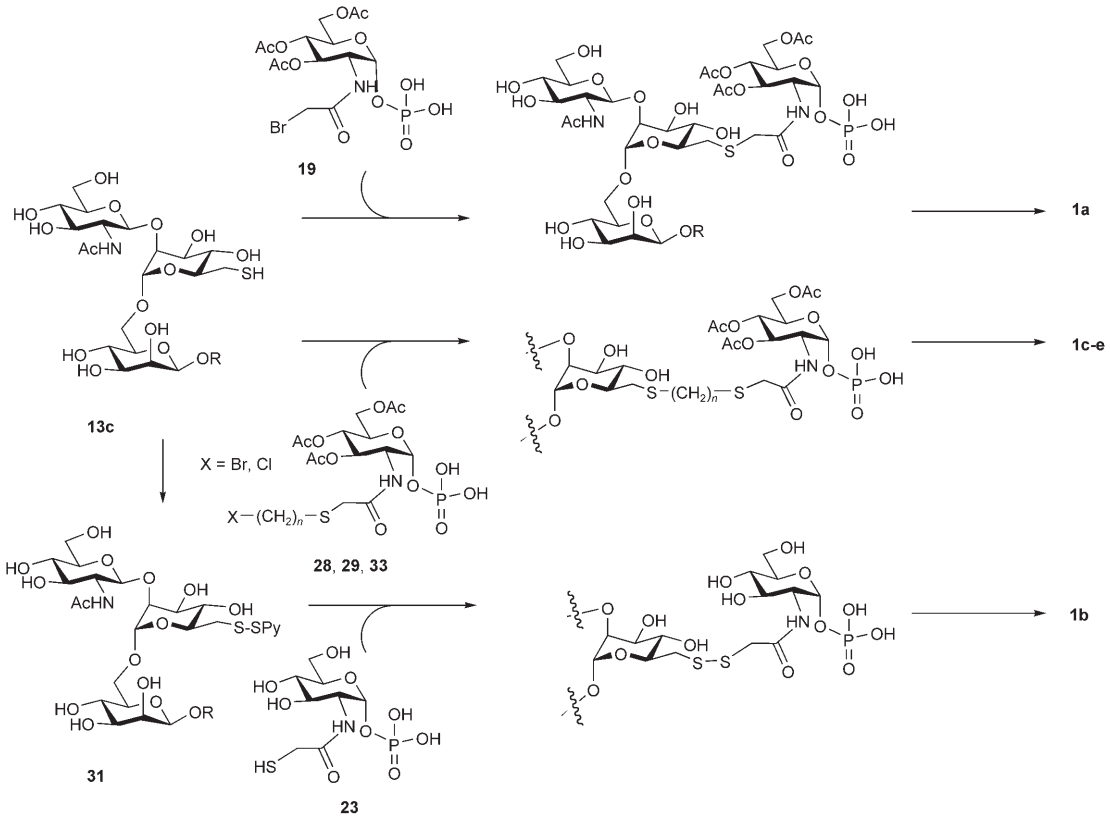
With our approach, the acceptor trisaccharide unit and GlcNAc unit are constructed separately, and coupled together by chemoselective ligation (Scheme 1). The acceptor trisaccharide unit was synthesized using a soluble polymer support.[13] Preliminary results on the synthesis of **1a** and evaluation of its inhibitory activity against GnT-V and -IX were reported previously.[14] We now wish to fully disclose the further systematic preparation and inhibitory activities of a series of derivatives (**1a–e**) toward GnT-V and -IX.

Co-crystallization and multidimensional NMR spectroscopy are direct and powerful methods of elucidating the substrate-binding structure of enzymes.[15] By contrast, the results obtained with tunable synthetic probes in combination with a traditional inhibition assay can not be directly visualized. However, they should provide reliable information on active site structures.

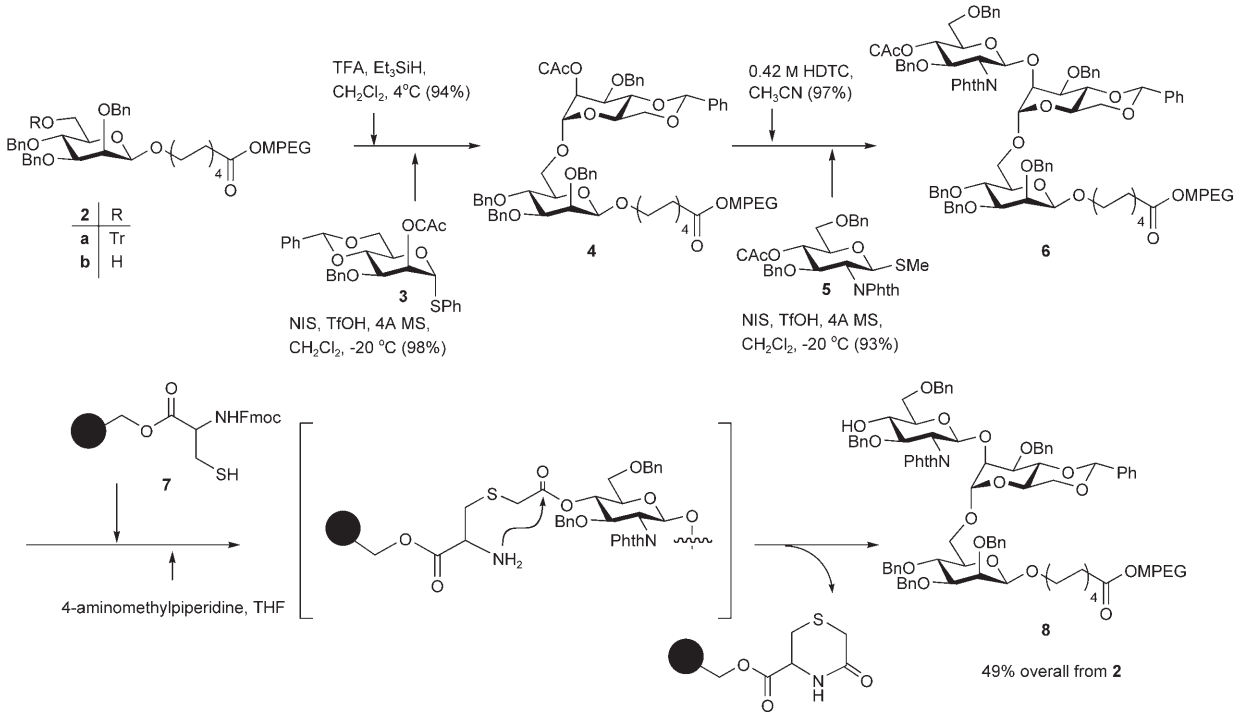
Results and Discussion

The acceptor trisaccharide was synthesized based on a solution-phase polymer-support strategy, which was combined with resin capture–release purification, as depicted in Scheme 2.[14] As described previously, monomethyl polyethyleneglycol (MPEG: average $M_W \approx 750$ Da) was used as a support, which functioned as a polar tag; the product could be readily isolated from the reaction mixture by passage through a short silica gel column.[13,16] Namely, non MPEG-containing fractions (e.g. excess donor) were first eluted with ethyl acetate (hexane/EtOAc). With subsequent elution with a more polar solvent such as EtOAc/MeOH, MPEG-bound materials can be retrieved.

To start with, the 6-*O*-trityl (Tr)-protected monosaccharide **2a**[13d] was synthesized using Kovác's anomer locked β-mannosylation strategy.[17] After the removal of Tr with trifluoroacetic acid (TFA) and Et_3SiH, the liberated alcohol



Scheme 1. Route for the synthesis of bisubstrate-type derivatives **1a**–**e**.



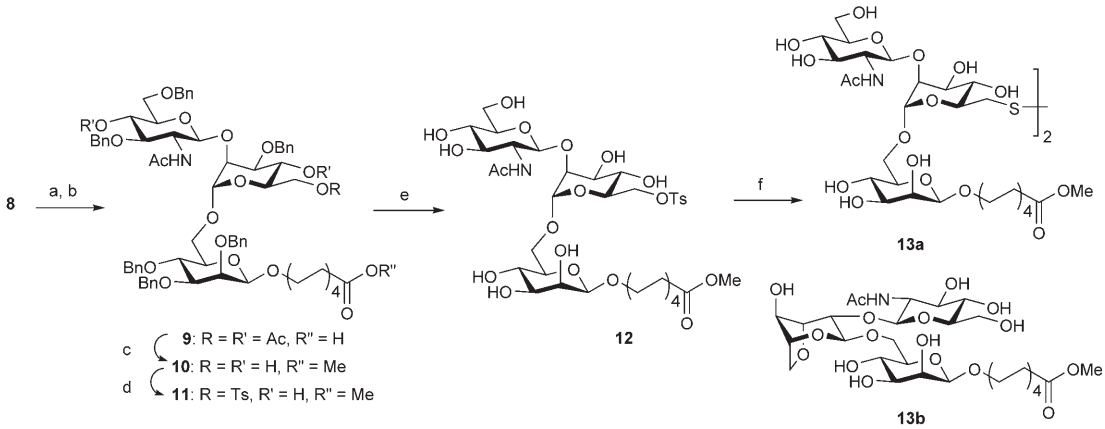
Scheme 2. Polymer-resin hybrid-based synthesis of trisaccharide **8**.

2b was glycosylated with phenylthio mannoside **3** by the action of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH, 0.7 equiv) to afford disaccharide **4**.[18,19] The crude mixture was capped with Ac_2O/pyridine so that any unreacted **2b** would not be carried over to the next glycosylation. Cleavage of the chloroacetyl group[20] and further

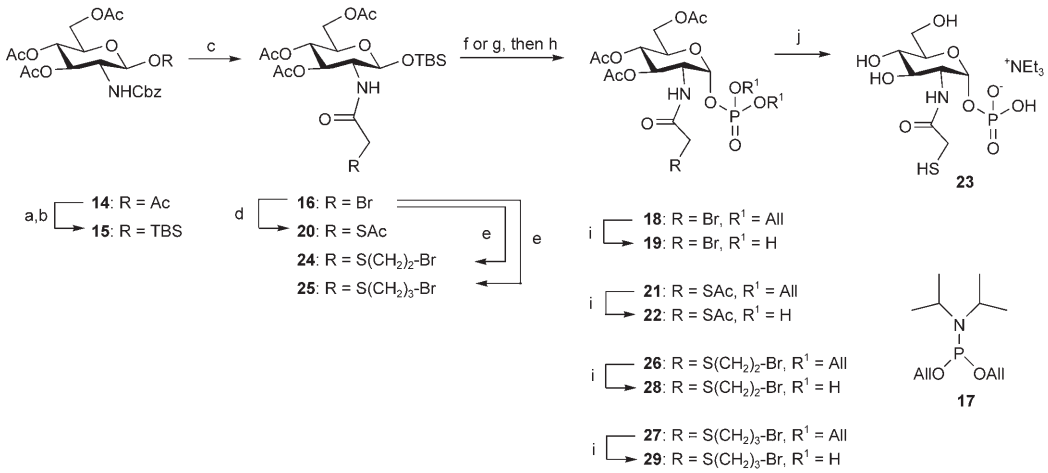
glycosylation with **5** provided trisaccharide **6**.[21] At this stage, the product was subjected to capture–release purification. Namely, **6** was captured with cysteine-conjugated Wang resin **7** through a selective reaction between chloroacetyl and thiol groups. Liberation of an amino group by Fmoc deprotection with 4-aminomethylpiperidine[22] initiated cyclization and trisaccharide **8** was obtained in 49% overall yield from **2**.

Further deprotection and introduction of a thiol group were conducted as depicted in Scheme 3. Acidic cleavage of the benzylidene acetal, dephthaloylation with ethylenediamine, and acetylation afforded **9**. Subsequent deacetylation and esterification gave **10** in 85% yield. Tosylation of the primary hydroxy group was performed using 4-dimethylaminopyridine (DMAP) as a base to afford **11**; removal of the benzyl groups gave **12**. The tosyl group was substituted with thioacetate by using AcSK in DMF at 70°C.[23,24] Deacetylation under mildly basic conditions using Et_3N in MeOH/H_2O caused a spontaneous oxidation to provide disulfide **13a**.

Glucosamine-1-phosphate derivatives **19**, **22**, **23**, **28**, and **29** carrying various linkers were prepared as depicted in Scheme 4. To begin with, the common starting material **14** was transformed into **15** in a standard manner. Namely, the anomeric position was selectively deacetylated and reprotected with a *tert*-butyldimethylsilyl (TBS) group to provide **15**. After hydrogenolytic removal of the benzyloxycarbonyl (Cbz) group, a reactive bromoacetoamide group was introduced to furnish **16**. For the synthesis of the glucosamine phosphate unit **19**, **16** was desilylated and reacted with the amidite **17**[25] to introduce a phosphite group, which was oxidized to the phosphate **18**.[26] Deprotection of the allyl ester using $[Pd(PPh_3)_4]$ furnished **19** in 85% yield.



Scheme 3. Synthesis of disulfide **13a**. a) 60% AcOH/H_2O, 60°C, 77%; b) i) 1M KOH, EtOH/THF, reflux, ii) ethylenediamine, 1-BuOH, 100°C, iii) Ac_2O, pyridine, 70%; c) i) 0.05M NaOMe/MeOH, ii) $TMSCHN_2$, PhH, MeOH, 85%; d) *p*-TsCl, DMAP, CH_2Cl_2, 60%; e) H_2, 20% $Pd(OH)_2/C$, AcOH, MeOH, 88%; f) i) AcSK, DMF 70°C, ii) Et_3N, MeOH, H_2O, 75%.



Scheme 4. Synthesis of GlcN phosphates. a) H_2NNH_2·AcOH, THF, 94%; b) TBSCl, imidazole, DMF, 95%; c) i) H_2, 10% Pd/C, EtOAc, ii) $(BrAc)_2O$, pyridine, CH_2Cl_2, 98%; d) AcSH, *i*Pr_2NEt, CH_3CN, 4°C, 93%; e) i) $HO(CH_2)_nSH$, *i*Pr_2NEt, CH_3CN, ii) NBS, Ph_3P, CH_2Cl_2, **24**: 96%, **25**: 95%; f) 47% HFaq, CH_3CN, from **16**: 93%; from **24**: 70%; from **25**: 90%; g) HF-pyridine, THF, from **20**: 78%; h) i) **17**, 1*H*-tetrazole, CH_2Cl_2, −10°C, ii) TBHP, Me_2S, −40 → 0°C, **18**: 83%; **21**: 77%; **26**: 68%; **27**: 78%; i) $[Pd(PPh_3)_4]$, Et_3SiH, AcOH, toluene, **19**: 85%; **22**: 51%; **28**: 86%, **29**: 56%; j) Et_3N, MeOH, H_2O.

In order to convert bromoacetamide **16** to the mercaptoacetamide **23**, compound **16** was treated with thioacetic acid and Hünig's base to give **20**. It was then desilylated, phosphitylated, and oxidized to afford **21**. Deprotection of the allyl ester gave **22**, which was deacetylated to afford **23**. On the other hand, for the preparation of compounds **28** and **29**, 2-mercaptoethanol and 3-mercaptopropanol were introduced into **16**. Subsequent treatment with *N*-bromosuccinimide (NBS) and Ph_3P gave **24** and **25**. These compounds were transformed to **28** and **29** in moderate overall yield, via **26** and **27**, respectively.

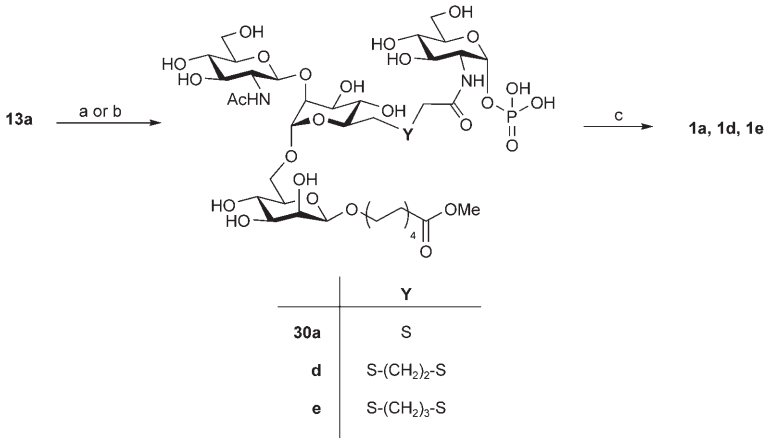
Three components, namely acceptor trisaccharide (**13a**), linker-conjugated GlcNAc-phosphate (**19**, **28**, **29**) and UMP were sequentially coupled as shown in Scheme 5. For instance, disulfide **13a** was first reduced with triscarboxyethylphosphine (TCEP) in MeOH/H_2O to liberate thiol, which was subjected to chemoselective ligation with **19** having a bromoacetamide linker. It proceeded smoothly to give the coupled product that was deacetylated to afford **30a** in 58% yield. Finally, the UMP unit was introduced into **30a** by using UMP–morpholidate[27] in pyridine to afford **1a** in 78% yield.

For the synthesis of **30d** and **30e**, nBu_3P was used for reducing the disulfide bond instead of TCEP. After the removal of excess phosphine and phosphine oxide by ether extraction, ligation between thiol and bromide **28** or **29** was conducted in the presence of Cs_2CO_3 in dry DMF.[28] This was followed by the removal of acetyl groups with Et_3N to afford **30d** and **30e** in 37 and 63% yield, respectively. Coupling with the UMP unit readily produced **1d** and **1e** in moderate yield.

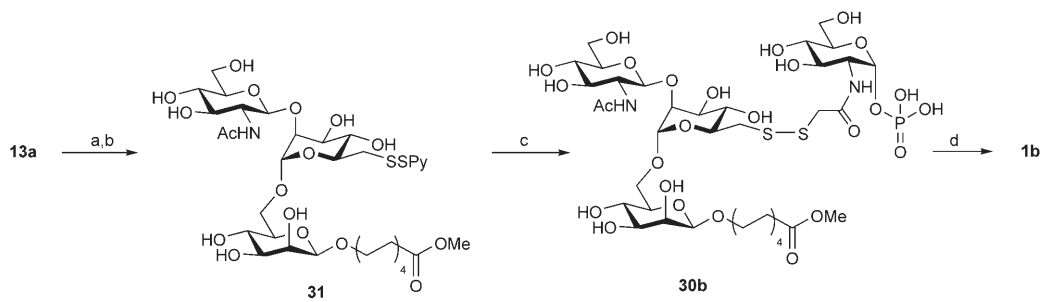
Synthesis of the disulfide-linked congener **1b** was achieved as shown in Scheme 6. The thiol derived from **13a** (nBu_3P) was converted to pyridyl disulfide under acidic conditions[29] to afford **31**. A reaction with mercaptoacetamide **23** in MeOH/1 M NH_4OAc[30] smoothly produced the mixed disulfide **30b** in 47% yield from **13**, which was coupled with UMP to give **1b**.

The synthesis of **1c** called for the introduction of a C_1 linker, which was less straightforward, because of the instability of the halomethylthio moiety. This task was achieved by the route depicted in Scheme 7. Namely, compound **20** was selectively deacetylated to give **32** and chloromethylated by using a large excess of bromochloromethane[31] in the presence of iPr_2NEt to give **33**.

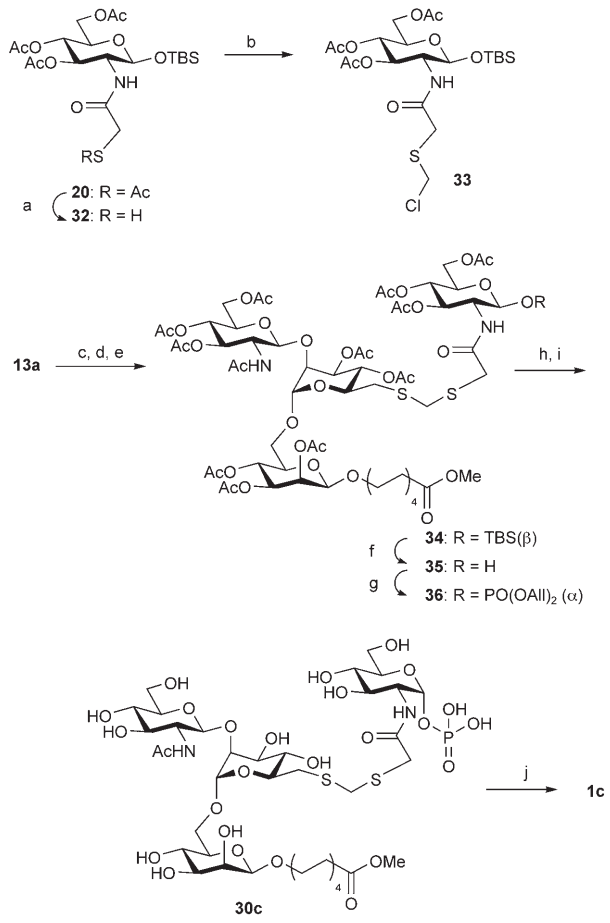
Coupling between the thiol derived from **13a** and GlcNAc derivative **33** was conducted in the presence of a stoichiometric amount of Cs_2CO_3. The coupled product was acetylated to give **34**, which was desilylated by using a HF/pyridine complex to afford the hemiacetal **35** in 31% yield from the disulfide **13a**. A phosphite group was introduced into **35** at higher temperature (45 °C), and subsequent oxidation afforded the phosphate **36**. Then, the allyl ester was cleaved using $[Pd(PPh_3)_4]$. Although NaOMe-mediated deacetylation was accompanied by a partial loss of the anomeric phosphate, the desired compound **30c** could be isolated in 45% yield from **36**. Subsequent coupling with UMP gave **1c** in 63% yield.



Scheme 5. Synthesis of **1a**, **1d**, and **1e**. a) i) TCEP-HCl, MeOH/H_2O, ii) **19**, iPr_2NEt, iii) Et_3N, **30a**; 58%; b) i) nBu_3P, THF, H_2O, ii) **28** or **29**, Cs_2CO_3, DMF, iii) Et_3N, MeOH/H_2O, **30d**; 37%, **30e**; 63%; c) UMP-morpholidate, 1*H*-tetrazole, pyridine, **1a**; 78%, **1d**; 58%, **1e**; 61%.



Scheme 6. Synthesis of **1b**. a) nBu_3P, THF, H_2O, b) pyridyl disulfide, 0.5 M HCl, MeOH/H_2O, c) **23**, MeOH/1 M NH_4OAc, 47%, d) UMP-morpholidate, 1*H*-tetrazole, pyridine, 56%.



Scheme 7. Synthesis of **1c**. a) H_2NNH_2•AcOH, THF; b) bromochloromethane, *i*Pr_2NEt, CH_3CN; c) *n*Bu_3P, THF/H_2O; d) **33**, Cs_2CO_3, DMF; e) Ac_2O, pyridine; f) HF/pyridine, DMF, 31% form 13a; g) i) **17**, 1*H*-tetrazole, $Cl(CH_2)_2Cl$, 45°C, ii) TBHP, Me_2S, 85%; h) $[Pd(PPh_3)_4]$, Et_3SiH, AcOH, toluene, 40°C, i) 0.05 M NaOMe, MeOH, 45%; j) UMP-morpholidate, 1*H*-tetrazole, pyridine, 63%.

Analysis of enzyme inhibition: The inhibitory activities of compounds **1a**–**e** against GnT-V and IX are summarized in Table 1. Enzyme assays were conducted using pyridylaminated substrates (GnGn-bi-PA and GnMSer-PAES) as described previously.[32,33] All had significant inhibitory effects on the two GnT-V and -IX.[34] In both cases, the extent of inhibition was clearly dependent upon the length of the linker. For GnT-V, compounds having a longer linker showed stronger activity, with a single exception (**1a** vs **1b**), **1e** being the most active ($K_i = 18.3$ μM). Since the K_m value of GnT-V toward acceptor and donor substrates was reported to be 0.150 mM and 4–6 mM, respectively, an enhancement in binding was evident, except for **1b**. On the other hand, GnT-IX was uniformly more sensitive to inhibition by **1a**–**e**. The strongest activity ($K_i = 4.7$ μM) against GnT-IX was exhibited by **1b**, which was least active toward GnT-V. Compounds **1c**–**e** having longer linkers all had similar levels of activity. From the results, it is suggested that these enzymes differ significantly in the relative orientations of donor- and acceptor-binding sites, in spite of their high homology.

Table 1. Inhibitory activity for GnT-V and IX.

Inhibitor	GnT-V	GnT-IX
	K_i [μM]	
1a	71.9	10.1
1b	119.3	4.7
1c	47.1	17.6
1d	26.9	21.5
1e	18.3	15.1

Conclusion

Among various types of glycoconjugates, complex-type glycoproteins are particularly important. GnTs are key enzymes to produce highly branched N-glycans having diverse structures. Therefore, potent inhibitors of GnTs are expected to be valuable tools in glycobiology. Because the three-dimensional structures of GnTs are yet to be explored, bisubstrate-type inhibitor would help clarifying the active site structure and mechanism of action of this class of enzyme.

Here we designed and synthesized bisubstrate-type inhibitors for GnTs having both an acceptor trisaccaride and a donor UDP–GlcNAc tethered by a linker of various lengths. For the construction of the trisaccharide component, we applied the oligosaccharide synthesis strategy using MPEG as a support. Coupling reactions with various GlcNAc phosphates were conducted under chemoselective ligation conditions. Finally, incorporation of the UDP component provided designed bisubstrate-type derivatives. It was clearly observed that their potencies toward GnT-V and -IX were sensitive to the linker length. Interestingly, the modes of correlation between linker length and activities were markedly different between these enzymes. These results suggest that, although they are homologous to each other, the distances of donor- and acceptor-binding sites are quite different.

Experimental Section

General procedure: ^1H and ^{13}C NMR spectra were measured by JEOL EX-400 or ECP-500 spectrometer. ^1H and ^{13}C chemical shifts in $CDCl_3$ were revealed in ppm relative to the $CHCl_3$ or TMS signal adjusted to 7.24 or 0 ppm, and the $CDCl_3$ signal adjusted to 77.0 ppm, respectively. Chemical shifts in CD_3OD were revealed relative to solvent peaks adjusted to 3.30 (^1H) and 49.5 ppm (^{13}C), respectively. Chemical shifts in D_2O are relative to DOH (4.65 ppm, ^1H) and CH_3OH (49.0 ppm, ^{13}C), respectively. Optical rotations were measured by JASCO DIP-301. MALDI-TOF MS spectra were measured by Shimadzu AXIMA-CFR using DHBA and CHCA as matrix. ESI-TOF MS spectra were measured by JEOL JMS-T100 LC spectrometer. Molecular Sieves 4 Å were purchased from Nakalai Tesque Inc (Kyoto) and dried at 170°C under vacuum immediately prior to use.

PEG-supported products were obtained in a following manner: The mixture was loaded on a pad of silica gel and was first washed with hexanes/AcOEt 1:4 to remove non-PEG supported materials. Subsequently, PEG-supported fractions were eluted by EtOAc/MeOH 1:3. The fractions were evaporated and thoroughly dried under high vacuum before used for the next reaction.

Synthetic procedures

8-(MPEG)oxycarbonyloctyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (2b): Et_3SiH (3.5 mL, 22 mmol) and TFA (3.5 mL, 45 mmol) were added dropwise at 4 °C to a solution of trityl ether **2a** (1.65 g, 1.10 mmol) in CH_2Cl_2 (50 mL). After stirring for 30 min at 4 °C under Ar atmosphere, the mixture was neutralized with sat. $NaHCO_3$ solution. The aqueous phase was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by short silica gel chromatography (hexanes/EtOAc 1:4, then EtOAc/MeOH 3:1) afforded 6-*O*-unprotected mannoside derivative **2b** (1.38 g, 1.03 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46–7.26 (m, 15H), 4.96 (d, 1H, ^2J(H,H) = 12.5 Hz; PhC*H*), 4.95 (d, 1H, ^2J(H,H) = 11.0 Hz; PhC*H*), 4.86 (d, 1H, ^2J(H,H) = 12.5 Hz; PhC*H*), 4.64 (d, 1H, ^2J(H,H) = 10.8 Hz; PhC*H*), 4.53 (d, 1H, ^2J(H,H) = 12.0 H; PhC*H*), 4.47 (d, 1H, ^2J(H,H) = 11.7 Hz; PhC*H*), 4.40 (s, 1H, H-1), 3.93–3.41 (overlapped by PEG-CH_2O signal), 3.32 (m, 1H, H-5), 2.30 (t, 2H, ^3J(H,H) = 7.3 Hz; CH_3), 1.61 (m, 4H), 1.32 (m, 8H).

8-(MPEG)oxycarbonyloctyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-chloroacetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (4): MS 4 Å (3.7 g) was added to a solution of **2b** (1.226 g, 0.916 mmol) and donor **3** (1.294 g, 2.194 mmol) in CH_2Cl_2 (40 mL), and the reaction mixture was stirred at room temperature for 10 min under Ar atmosphere. Then, the mixture was cooled to −20 °C, to which were added NIS (612 mg, 2.72 mmol) and TfOH (180 μL, 2.03 mmol). After stirring for 10 h at −20 °C, the mixture was filtered through Celite, and the filtrate was washed with 5% $Na_2S_2O_3$ solution. The aqueous phase was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by short silica gel chromatography (hexanes/EtOAc 1:4, then EtOAc/MeOH 3:1) yielded **4** (1.592 g, 0.91 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47–7.20 (m, 25H), 5.59 (s, 1H), 5.52 (m, 1H; H-$2_{\alpha Man}$), 4.99 (d, 1H, ^2J(H,H) = 12.7 Hz; PhC*H*), 4.94 (d, 1H, ^2J(H,H) = 11.2 Hz; PhC*H*), 4.87 (s, 1H, H-$1_{\alpha Man}$), 4.85 (d, 1H, ^2J(H,H) = 12.4 Hz; PhC*H*), 4.65 (d, 1H, ^2J(H,H) = 12.2 Hz; PhC*H*), 4.59 (d, 1H, ^2J(H,H) = 12.2 Hz; PhC*H*), 4.35 (s, 1H, H-$1_{\beta Man}$), 4.23 (dd, 1H, ^2J(H,H) = 10.0, ^3J(H,H) = 4.4 Hz; H-$6a_{\alpha Man}$), 4.15 (s, 2H, ClCH_2COO), 4.00–3.40 (overlapped with PEG-CH_2O signal), 3.35 (2H, H-$5_{\beta Man}$, OCH_2-alkyl).

8-(MPEG)oxycarbonyloctyl 3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-chloroacetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (6): Freshly prepared hydrazinedithiocarbonate (HDTC)[20] (0.42 M, 6.5 mL) was added to a solution of **4** (1.592 g, 0.907 mmol) in CH_3CN (50 mL), and stirred for 1 h at room temperature. The mixture was concentrated and dissolved in $CHCl_3$. The organic phase was washed with 10% citric acid solution, and the aqueous phase was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by short silica gel chromatography (hexane/EtOAc 1:4, then EtOAc/MeOH 3:1) yielded 2-*O*-unprotected disaccharide (1.474 g, 0.878 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47–7.22 (m, 25H), 5.59 (s, 1H), 5.00 (d, 1H, ^3J(H,H) = 1.5 Hz; H-$1_{\alpha Man}$), 4.97 (d, 1H, ^2J(H,H) = 12.2 Hz; PhC*H*), 4.92 (d, 1H, ^2J(H,H) = 11.0 Hz; PhC*H*), 4.84 (d, 1H, J = 12.2 Hz; PhC*H*), 4.73 (d, 1H, J = 12.0 Hz PhC*H*), 4.62 (d, 1H, ^2J(H,H) = 12.0 Hz; PhC*H*), 4.56 (d, 1H, ^2J(H,H) = 12.0 Hz; PhC*H*), 4.52 (d, 1H, ^2J(H,H) = 11.0 Hz; PhC*H*), 4.47 (d, 1H, ^2J(H,H) = 11.7 Hz; PhC*H*), 4.35 (s, 1H, H-$1_{\beta Man}$), 4.23 (dd, 1H, ^2J(H,H) = 9.3, ^3J(H,H) = 3.9 Hz; H-$6a_{\alpha Man}$), 4.13 (dd, 1H, ^3J(H,H) = 1.2, 3.2 Hz; H-$2_{\alpha Man}$), 4.08 (t, 1H, ^3J(H,H) = 9.3 Hz; H-$4_{\alpha Man}$), 3.92–3.50 (overlapped with PEG-CH_2O signal), 3.37 (m, 1H, OCH_2-b), 3.35 (m, 1H, H-$5_{\beta Man}$), 2.28 (t, 2H, J = 7.3 Hz), 1.58 (br, 4H), 1.28 (br, 8H).

4 Å MS (500 mg) was added to a solution of the disaccharide (226.3 mg, 0.135 mmol) and donor **5** (162.5 mg, 0.273 mmol) in CH_2Cl_2 (6 mL), and the reaction mixture was stirred at room temperature for 10 min under Ar atmosphere. Then, the mixture was cooled to −20 °C, to which were added NIS (73.9 mg, 0.329 mmol) and TfOH (5 μL, 0.06 mmol). After stirring for 19 h at −10 °C, the mixture was filtered through Celite, and the filtrate was washed with 5% $Na_2S_2O_3$ solution. The aqueous phase was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by short silica gel chromatography (hexane/EtOAc 1:4, then EtOAc/MeOH 3:1) yielded **6** (281.0 mg, 0.125 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88–6.96 (m, 34H), 5.42 (s, 1H), 5.28–5.23 (2H, H-1_{GN}, H-4_{GN}), 5.02 (d, 1H, ^2J(H,H) = 12.7 Hz; PhC*H*), 4.89 (d, 1H, ^2J(H,H) = 12.9 Hz; PhC*H*), 4.78 (d, 1H, ^2J(H,H) = 11.2 Hz; PhC*H*), 4.74 (d, 1H, ^2J(H,H) = 13.0 Hz; PhC*H*), 4.67 (d, 1H, ^2J(H,H) = 12.9 Hz; PhC*H*), 4.60–4.54 (m, 3H, H-$1_{\alpha Man}$, PhC*H*), 4.46–4.33 (m, 4H, PhC*H*), 4.32 (s, 1H, H-$1_{\beta Man}$), 4.29 (d, 1H, ^2J(H,H) = 11.0 Hz; PhC*H*), 4.08 (m, 1H, C*H*alkyl), 3.92–3.34 (overlapped with PEG-CH_2O signal), 3.19 (m, 1H, H-$5_{\beta Man}$), 3.02 (t, 1H, ^2J(H,H) = ^3J(H,H) = 10.2 Hz; H-$6b_{\alpha Man}$), 2.30 (t, 2H, ^3J(H,H) = 7.3 Hz), 1.62 (br, 4H), 1.30 (br, 8H).

8-(MPEG)oxycarbonyloctyl 3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (8)

Capture: Fmoc-Cys on Wang resin **7** (0.58 mmol g^{-1}, 710 mg, 0.412 mmol) and *i*Pr_2NEt (150 μL, 2.87 mmol) were added to a solution of trisaccharide **6** (281 mg, 0.125 mmol) in CH_3CN/CH_2Cl_2 (1:1, 4 mL). The mixture was shaken for 24 h at room temperature, and resin was rinsed with $CHCl_3$/MeOH, and dried under reduced pressure.

Release: The trisaccharide-loaded resin was swelled in THF (5 mL), and 4-aminomethylpiperidine (727 mg, 6.37 mmol) was added. The mixture was shaken for 24 h, and resin was rinsed with $CHCl_3$/MeOH. The organic phase was washed with 10% citric acid solution, and the aqueous phase was extracted with $CHCl_3$ (2×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by short silica gel chromatography (hexane/EtOAc 1:4, then EtOAc/MeOH 3:1) yielded **8** (171.5 mg, 0.080 mmol, 49% from **2**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88–6.95 (m, 34H), 5.40 (s, 1H), 5.23 (d, 1H, ^3J(H,H) = 8.1 Hz; H-1_{GN}), 5.02 (d, 1H, ^2J(H,H) = 12.9 Hz; PhC*H*), 4.90 (d, 1H, ^2J(H,H) = 12.7 Hz; PhC*H*), 4.81–4.75 (2H, PhC*H*), 4.72 (d, 1H, ^2J(H,H) = 12.9 Hz; PhC*H*), 4.64 (d, 1H, ^2J(H,H) = 12.9 Hz; PhC*H*), 4.58 (d, 1H, ^2J(H,H) = 12.4 Hz; PhC*H*), 4.54 (d, 1H, ^2J(H,H) = 12.4 Hz; PhC*H*), 4.50 (d, 1H, ^2J(H,H) = 11.7 Hz; PhC*H*), 4.48 (s, 1H, H-$1_{\alpha Man}$), 4.44 (d, 1H, ^2J(H,H) = 11.7 Hz; PhC*H*), 4.37–4.28 (5H, H-$1_{\beta Man}$, H-2_{GN}, H-3_{GN}, PhC*H*), 4.09 (m, 1H, C*H*alkyl), 3.92–3.34 (overlapped with PEG-CH_2O signal), 3.18 (m, 1H, H-$5_{\beta Man}$), 3.00 (t, 1H, ^2J(H,H) = ^3J(H,H) = 10.2 Hz; H-$6b_{\alpha Man}$), 2.29 (t, 2H, ^3J(H,H) = 7.3 Hz), 1.62 (br, 4H), 1.30 (br, 8H).

8-Carboxyoctyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (9): A solution of MPEG-ester **8** (47.9 mg, 0.0221 mmol) in 80% aqueous AcOH (5 mL) was stirred for 2 h at 60 °C. After cooling to room temperature, the mixture was concentrated and purified by silica gel chromatography (EtOAc/MeOH 100:0 → 3:1) to afford triol (43.7 mg, 0.0215 mmol, 97%).

A solution of crude triol (139.4 mg, 0.0671 mmol) in THF/EtOH (1:1, 10 mL) was added 1 N KOH (1 mL). The mixture was stirred for 6 h at 80 °C, and cooled to room temperature. After neutralized with Amberlyst 15E, the resin was removed by filtration, and the filtrate was concentrated, and dried under reduced pressure.

The residue was dissolved in *n*BuOH (6 mL), to which was added ethylenediamine (1.5 mL). The mixture was stirred for 10 h at 100 °C, cooled to room temperature, and evaporated in vacuo. The residue was suspended in pyridine (6 mL) and Ac_2O (3 mL) was added under ice-water cooling. After stirring for 20 h at room temperature, the mixture was concentrated, co-evaporated with toluene, and purified by preparative TLC (toluene/EtOAc/AcOH 5:5:0.1) to give **9** (64.5 mg, 0.0466 mmol, 70%).

8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (10): A solution of carboxylic acid **9** (64.5 mg, 0.0466 mmol) in 0.05 M NaOMe in MeOH (5.0 mL) was stirred at room temperature under Ar atmosphere for 20 h. The mixture was neutralized with Amberlyst 15E, filtered, and the filtrate was concentrated, and dried under reduced pressure. The residue was dissolved in MeOH/benzene (1:1, 4 mL) and a hexane solution of $TMSCHN_2$ (2.0 M) was added dropwise until the yellow color persisted for more than 5 min. After quenching with AcOH, the solution was concentrated and purified

with preparative TLC ($CHCl_3$/MeOH 25:1) to afford **10** (50.2 mg, 0.03945 mmol, 85%). $[\alpha]_D^{23} = -40$ ($c = 1.13$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.45$ (d, 2H, $^3J(H,H) = 6.3$ Hz), 7.34–7.13 (m, 28H), 5.85 (br, 1H), 4.99 (d, 1H, $^2J(H,H) = 12.7$ Hz; PhC*H*), 4.90–4.85 (m, 3H), 4.83 (d, 1H, $^3J(H,H) = 8.3$ Hz), 4.73 (d, 2H, $^2J(H,H) = 12.8$ Hz; PhC*H*), 4.66 (d, 1H, $^2J(H,H) = 11.7$ Hz; PhC*H*), 4.52–4.39 (m, 6H), 4.34 (s, 1H), 4.22 (m, 1H), 4.01 (m, 1H), 3.96–3.85 (4H), 3.71–3.56 (11H), 3.54–3.44 (m, 4H), 3.37 (m, 1H), 3.31 (m, 1H), 2.29 (t, 2H, $^3J(H,H) = 7.6$ Hz), 1.86 (s, 3H), 1.61 (m, 4H), 1.31 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.0$, 171.1, 138.2–137.4, 128.3–127.3, 101.8, 99.4, 98.3, 82.5, 80.0, 76.1, 75.1, 74.9, 74.9, 74.2, 73.7, 73.6, 73.43, 73.38, 73.0, 72.9, 72.5, 71.4, 71.2, 70.3, 69.8, 66.5, 65.5, 62.0, 56.2, 51.5, 34.1, 29.7, 29.34, 29.32, 29.2, 26.2, 25.0, 23.6; HR ESI-MS: *m/z:* calcd for $C_{72}H_{89}NO_{18}Na$: 1278.5978; found 1278.5973 $[M+Na]^+$.

8-Methoxycarbonyloctyl 2-acetamido-3,6-di-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3-*O*-benzyl-6-*O*-tosyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranoside (11): DMAP (3.5 mg, 0.029 mmol) and TsCl (4.2 mg, 0.022 mmol) were added at 4°C to a solution of triol **10** (26.0 mg, 0.021 mmol) in CH_2Cl_2. After stirring for 10 h at room temperature under Ar atmosphere, the second portions of DMAP (3.0 mg, 0.025 mmol) and TsCl (3.2 mg, 0.017 mmol) were added. After the additional stirring for 13 h, the mixture was diluted with $CHCl_3$ and washed with 0.5M HCl. The aqueous phase was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification with preparative TLC ($CHCl_3$/MeOH 20:1) produced **11** (17.6 mg, 0.0125 mmol, 60%). $[\alpha]_D^{23} = -41$ ($c = 0.69$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.71$ (d, 2H, $^3J(H,H) = 8.5$ Hz), 7.45–7.14 (m, 32H), 6.08 (d, 1H, $^3J(H,H) = 6.8$ Hz), 5.19 (d, 1H, $^3J(H,H) = 8.3$ Hz), 4.99 (d, 1H, $^2J(H,H) = 12.7$ Hz; PhC*H*), 4.89 (d, 1H, $^2J(H,H) = 11.5$ Hz; PhC*H*), 4.87 (d, 1H, $^2J(H,H) = 12.7$ Hz; PhC*H*), 4.81 (d, 1H, $^3J(H,H) = 1.7$ Hz), 4.77–4.71 (m, 3H), 4.51–4.31 (m, 9H), 4.14 (dd, 1H, $^3J(H,H) = 2.1$, 10.8 Hz), 4.06–3.82 (m, 5H), 3.73–3.51 (m, 12H), 3.50 (dd, 1H, $^3J(H,H) = 3.2$, 9.5 Hz), 3.35 (m, 1H), 3.32 (m, 1H), 3.00 (m, 1H), 2.42 (s, 3H), 2.29 (t, 2H, $^3J(H,H) = 7.6$ Hz), 1.94 (s, 3H), 1.58 (4H), 1.31 (8H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 174.0$, 171.9, 144.5, 138.6–137.4, 133.1, 129.7–127.3, 102.0, 98.4, 97.4, 82.5, 79.2, 77.2, 75.7, 74.8, 74.5, 74.2, 73.7, 73.6, 73.5, 73.2, 71.9, 71.3, 71.2, 70.3, 70.1, 69.7, 68.8, 67.2, 65.0, 58.6, 51.5, 34.2, 29.8, 29.39, 29.36, 29.2, 26.2, 25.1, 23.8, 21.7; HR ESI-MS: *m/z:* calcd for $C_{79}H_{95}NO_{20}SNa$: 1432.6066, found 1432.6059 $[M+Na]^+$.

8-Methoxycarbonyloctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-*O*-tosyl-α-D-mannopyranosyl-(1→6)-β-D-mannopyranoside (12): A solution of tosylate **11** (286.7 mg, 0.191 mmol) in MeOH (20 mL) containing AcOH (0.5 mL) was hydrogenated over $Pd(OH)_2$/C (20%, 253 mg) under vigorous stirring for 12 h. After additional stirring under an N_2 atmosphere for 30 min, the catalyst was filtered off through Celite, and the filtrate was concentrated. Purification by silica gel chromatography ($CHCl_3$/MeOH 10:1 to $CHCl_3$/MeOH/H_2O 6:2:0.2) gave **12** (147.0 mg, 0.169 mmol, 88%). $[\alpha]_D^{22} = -12$ ($c = 1.12$ in methanol); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.79$ (d, 2H, $^3J(H,H) = 8.3$ Hz), 7.43 (d, 2H, $^3J(H,H) = 8.1$ Hz), 4.86 (s, 1H, H-$1_{\alpha Man}$), 4.47 (d, 1H, $^3J(H,H) = 8.3$ Hz; H-1_{GN}), 4.46 (s, 1H, H-$1_{\beta Man}$), 4.27 (dd, 1H, $^3J(H,H) = 1.7$, 10.4 Hz), 4.07 (dd, 1H, $^3J(H,H) = 7.3$, 10.4 Hz), 4.01 (dd, 1H, $^3J(H,H) = 1.5$, 3.4 Hz), 3.89–3.27 (m, 20H), 2.45 (s, 3H), 2.30 (t, 2H, $^3J(H,H) = 7.6$ Hz), 1.92 (s, 3H), 1.58 (m, 4H), 1.31 (8H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 175.9$, 174.1, 146.3, 134.2, 131.0, 129.0, 101.8, 100.8, 98.5, 77.8, 77.6, 76.9, 75.3, 75.2, 72.4, 72.0, 71.6, 70.7, 68.4, 67.8, 62.6, 57.1, 52.0, 49.3, 34.8, 30.7, 30.4, 30.3, 30.1, 27.0, 26.0, 23.4, 21.6; HR ESI-MS: *m/z:* calcd for $C_{37}H_{59}NO_{20}SNa$: 892.3249, found 892.3289 $[M+Na]^+$.

Compound 13a: A solution of tosylate **12** (22.3 mg, 0.0256 mmol) in DMF (1.5 mL) was added AcSK (9.2 mg, 0.081 mmol), and stirred for 9 h at 65°C under N_2 atmosphere. The mixture was cooled to room temperature, to which were added MeOH/H_2O 7:3 (3 mL) and Et_3N (0.3 mL). After stirring for 15 h at room temperature, the mixture was concentrated. Purification by size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4) gave **13a** (13.4 mg, 9.2 μmol, 72%). $[\alpha]_D^{22} = +31$ ($c = 0.71$ in methanol); ^{1}H NMR (400 MHz, D_2O, 40°C): $\delta = 4.93$ (2H), 4.59–4.57 (m, 4H, overlapped with HOD), 4.11 (dd, 2H, $^3J(H,H) = 2.0$, 3.2 Hz), 4.00–3.30 (m, 44H), 2.83 (dd, 2H, $^3J(H,H) = 9.3$, $^2J(H,H) = 13.9$ Hz), 2.37 (t, 4H, $^3J(H,H) = 7.6$ Hz), 2.05 (s, 6H), 1.61–1.58 (m, 8H), 1.31 (m, 16H); ^{13}C NMR (100 MHz, D_2O, 40°C): $\delta = 174.3$, 171.7, 97.5, 96.9, 94.4, 73.8, 73.3, 72.3, 70.9, 70.7, 68.3, 68.1, 67.4, 67.34, 67.27, 67.1, 64.6, 64.1, 58.2, 52.8, 46.4, 38.9, 31.3, 26.3, 26.0–25.7, 22.7, 21.9, 20.1; HR ESI-MS: *m/z:* calcd for $C_{60}H_{104}N_2O_{34}S_2Na$: 1483.5810, found 1483.5834 $[M+Na]^+$.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-β-D-glucopyranoside (15)**: Hydrazine acetate (916 mg, 9.95 mmol) was added at 4°C to a solution of **14** (4.53 g, 9.41 mmol) in THF (120 mL). After stirring for 24 h at room temperature, ice-water was added and the mixture was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Separation by silica gel chromatography (toluene/EtOAc 5:1 → 1:1) gave hemiacetal (3.86 g, 8.79 mmol, 93%).

A solution of the hemiacetal (3.85 g, 8.76 mmol) in DMF were added TBSCl (2.00 g, 13.3 mmol) and imidazole (1.79 g, 26.3 mmol) at 4°C. After stirring for 11 h at room temperature, the mixture was quenched with 10% aqueous citric acid. Aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 15:1 → 4:1) gave **15** (4.61 g, 8.33 mmol, 95%). $[\alpha]_D^{22} = +9.9$ ($c = 1.15$ in chloroform); ^{1}H NMR (400 MHz, C_6D_6, 70°C): $\delta = 7.25$–7.00 (m, 5H), 5.28 (t, 1H, $^3J(H,H) = 10.0$ Hz; H-3), 5.07 (t, 1H, $^3J(H,H) = 9.6$ Hz; H-4), 5.04 (s, 2H), 4.57 (d, 1H, $^3J(H,H) = 7.6$ Hz; NH), 4.33 (br, 1H, H-1), 4.17 (dd, 1H, $^3J(H,H) = 5.6$, $^2J(H,H) = 12.0$ Hz; H-6a), 4.11 (dd, 1H, $^3J(H,H) = 2.8$, $^2J(H,H) = 12.0$ Hz; H-6b), 3.58 (m, 1H, H-2), 3.40 (m, 1H, H-5), 1.74–1.68 (9H), 0.96 (s, 9H), 0.16 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6, 60°C): $\delta = 170.1$, 169.7, 169.0, 155.8, 137.3, 128.6–127.8, 96.7, 72.8, 72.4, 69.9, 67.0, 62.6, 58.9, 26.0, 20.31, 20.26, 18.3, −3.9, −4.8; elemental analysis calcd (%) for $C_{26}H_{39}NO_{10}Si$: C 56.40, H 7.10, N 2.53; found: C 56.39, H 7.11, N 2.46.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-bromoacetamido-2-deoxy-β-D-glucopyranoside (16)**: 10% Pd/C (96 mg) was added to a solution of **15** (536.0 mg, 0.968 mmol) in EtOAc, and the mixture was stirred under H_2 atmosphere for 2.5 h. After additional stirring for 15 min under N_2 atmosphere, the catalyst was filtered off through Celite, and the filtrate was concentrated, and exposed to high-vacuum for 2 h. The resulting solid was dissolved in CH_2Cl_2 (10 mL), and treated with bromoacetic anhydride (336 mg, 1.29 mmol) and pyridine (100 μL, 1.24 mmol) at 4°C. After stirring for 30 min at 4°C, the mixture was neutralized with 10% aqueous citric acid. The aqueous phase was extracted with CH_2Cl_2 (2×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 15:1 → 4:1) gave **16** (513.2 mg, 0.950 mmol, 98%). $[\alpha]_D^{24} = +2.5$ ($c = 0.97$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.33$ (d, 1H, $^3J(H,H) = 8.8$ Hz; NH), 5.19 (dd, 1H, $^3J(H,H) = 9.6$, 10.8 Hz; H-3), 4.92 (t, 1H, $^3J(H,H) = 9.6$ Hz; H-4), 4.79 (d, 1H, $^3J(H,H) = 8.0$ Hz; H-1), 4.08 (dd, 1H, $^3J(H,H) = 6.0$, $^2J(H,H) = 12.4$ Hz; H-6a), 4.01 (dd, 1H, $^3J(H,H) = 2.8$, $^2J(H,H) = 12.4$ Hz; H-6b), 3.73 (m, 1H, H-2), 3.69 (s, 2H), 3.61 (m, 1H, H-5), 1.96–1.91 (9H), 0.86 (s, 9H), 0.11 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.3$, 170.2, 169.0, 165.1, 95.4, 77.2, 71.7, 70.2, 68.7, 62.3, 57.1, 28.6, 25.5, 20.67, 20.65, 20.60, 17.8, −4.2, −5.3; elemental analysis calcd (%) for $C_{20}H_{34}BrNO_9Si$: C 44.44, H 6.34, N 2.59; found: C 44.37, H 6.27, N 2.53.

3,4,6-Tri-*O*-acetyl-2-bromoacetamido-2-deoxy-α-D-glucopyranosyl phosphate diallyl ester (18): A solution of **16** (394.5 mg, 0.7299 mmol) in CH_3CN (5.0 mL) was added 47% aq. HF (750 μL), and stirred for 11 h at room temperature. The mixture was neutralized with aq. $NaHCO_3$, and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 5:1 → 1:1) gave the hemiacetal (290.3 mg, 0.6811 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.65$ (1H, $^3J(H,H) = 9.2$ Hz; NH), 5.37 (t, 1H, $^3J(H,H) = 9.6$ Hz; H-3), 5.32 (t, 1H, $^3J(H,H) = 4.0$ Hz), 5.15 (t, 1H, $^3J(H,H) = 9.6$ Hz; H-4), 4.29–4.13 (m, 4H, H-2, H-5, H-6a, H-6b), 3.81 (s, 2H), 3.19 (d, 1H, $^3J(H,H) = 2.0$ Hz; OH), 2.11–2.04 (3s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.7$, 170.3, 168.9, 165.5, 91.2, 77.2, 70.3, 67.9,

67.7, 61.8, 52.7, 28.3, 20.74, 20.70, 20.59; elemental analysis calcd (%) for $C_{14}H_{20}BrNO_9$: C 39.45, H 4.73 N 3.29; found: C 39.68, H 4.69, N3.24.

Amidite **17** (175 μL, 0.662 mmol) and tetrazole (96.3 mg, 1.37 mmol) were added at −20 °C to a solution of the hemiacetal (183.9 mg, 0.431 mmol) in CH_2Cl_2 (2.5 mL). After stirring at −10 °C for 50 min under Ar atmosphere, the mixture was cooled to −40 °C and added *tert*-butyl hydroperoxide (TBHP, 5.0–6.0 M in decane, 300 μL). After stirring for 6 h at −40 to 0 °C, the mixture was quenched with 5% aq. $Na_2S_2O_3$ and extracted with $CHCl_3$ (3×). Combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, concentrated, and purified with silica gel chromatography (toluene/EtOAc 2:1 → 1:2) to give **18** (181.2 mg, 0.358 mmol, 83%). $[\alpha]_D^{22} = +65$ ($c = 0.66$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.98$ (br, 1H, NH), 5.97 (m, 2H), 5.74 (dd, $^3J(H,H) = 2.8$, $^3J(P,H) = 5.6$ Hz; H-1), 5.44–5.29 (m, 5H), 5.20 (t, 1H, $^3J(H,H) = 9.6$ Hz; H-4), 4.61 (m, 4H), 4.38 (m, 1H, H-2), 4.29–4.22 (m, 2H, H-5, H-6a), 4.11 (d, 1H, $^2J(H,H) = 10.8$ Hz; H-6b), 3.85 (d, 1H, $^2J(H,H) = 13.2$ Hz), 3.78 (d, 1H, $^3J(H,H) = 13.2$ Hz), 2.11–2.00 (3s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.6$, 170.1, 168.9, 166.0, 131.7 (d, $^3J(P,C) = 6.6$ Hz), 131.6 (d, $^3J(P,C) = 6.6$ Hz), 118.9, 118.8, 95.3 (d, $^2J(P,C) = 6.6$ Hz), 69.6, 69.4, 68.7 ($^2J(P,C) = 5.8$ Hz), 68.6 ($^2J(P,C) = 5.8$ Hz), 67.1, 61.2, 52.5 (d, $^3J(P,C) = 7.5$ Hz), 28.0, 20.64, 20.62, 20.5.

3,4,6-Tri-*O*-acetyl-2-bromoacetamido-2-deoxy-α-D-glucopyranosyl phosphate (19): Et_3SiH (350 μL, 2.19 mmol), AcOH (130 μL, 2.27 mmol), and $[Pd(PPh_3)_4]$ (14 mg, 0.012 mmol) were added to a solution of **18** (133.0 mg, 0.2268 mmol) in toluene (4 mL), and the reaction mixture was stirred for 2.5 h at 40 °C under Ar atmosphere. Then, the mixture was cooled to room temperature, concentrated, and purified by silica gel chromatography ($CHCl_3$/MeOH/AcOH 30:1:0 → 6:2:0.1) to give **19** (97.9 mg, 0.193 mmol, 85%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.84$ (br, 1H, H-1), 5.24 (t, 1H, $^3J(H,H) = 9.2$ Hz; H-3), 5.02 (t, 1H, $^3J(H,H) = 10.0$ Hz; H-4), 4.25–4.13 (3H, H-2, H-5, H-6a), 4.04 (d, 1H, $^2J(H,H) = 11.6$ Hz; H-6b), 3.83 (d, 1H, $J(H,H) = 10.8$ Hz), 3.67 (d, 1H, $J(H,H) = 10.8$ Hz), 1.97 (s, 3H), 1.91 (s, 3H), 1.89 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 172.3$, 171.8, 171.1, 169.9, 95.5 (d, $^2J(P,C) = 5.8$ Hz), 71.7, 70.2, 69.6, 62.8, 53.5 (d, $^3J(P,C) = 9.1$ Hz), 28.4, 20.7, 20.63, 20.61; HR ESI-MS: *m/z:* calcd for $C_{14}H_{20}BrNO_{12}P$: 503.9907, found 503.9926 $[M-H]^-$.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-(acetylthio)acetamido-2-deoxy-β-D-glucopyranoside (20)**: A solution of bromoacetamide **16** (1.10 g, 2.04 mmol) in CH_3CN (10 mL) were added AcSH (180 μL, 2.52 mmol) and iPr$_2$NEt (550 μL, 3.16 mmol) at 4 °C. The mixture was stirred for 1 h at 4 °C, and quenched with aq. $NaHCO_3$. The aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 2:1) to give **20** (1.01 g, 1.88 mmol, 93%). $[\alpha]_D^{22} = +33$ ($c = 0.91$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.31$ (d, 1H, $^3J(H,H) = 9.5$ Hz; NH), 5.16 (dd, 1H, $^3J(H,H) = 9.3$, 10.7 Hz; H-4), 5.01 (t, 1H, $^3J(H,H) = 9.7$ Hz; H-3), 4.81 (d, 1H, $^3J(H,H) = 8.1$ Hz; H-1), 4.18 (dd, 1H, $J(H,H) = 5.9$, 12.2 Hz; H-6a), 4.12 (dd, 1H, $J(H,H) = 2.7$, 12.0 Hz; H-6b), 3.87 (m, 1H, H-2), 3.69 (m, 1H, H-5), 3.53 (d, 1H, $J(H,H) = 14.9$ Hz), 3.39 (d, 1H, $J(H,H) = 14.7$ Hz), 2.41 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 0.88 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 195.9$, 170.6, 170.4, 169.2, 167.9, 95.9, 72.0, 71.8, 68.9, 62.4, 56.4, 32.8, 30.1, 25.6, 20.71, 20.68, 20.61, 17.9, −4.2, −5.2; elemental analysis calcd (%) for $C_{22}H_{37}BNO_{10}SSi$: C 49.33, H 6.96, N 2.91; found: C 49.3, H 6.96, N 2.91.

3,4,6-Tri-*O*-acetyl-2-(acetylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate diallyl ester (21): HF/pyridine (100 μL) was added at 4 °C to a solution of **20** (275.3 mg, 0.514 mmol) in THF (2 mL). After stirring for 5.5 h at room temperature, the mixture was neutralized with sat $NaHCO_3$ solution. The aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography ($CHCl_3$/MeOH 100:1 → 30:1) gave the hemiacetal (168.6 mg, 0.400 mmol, 78%).

To a solution of the hemiacetal (168.6 mg, 0.400 mmol) in CH_2Cl_2 (2 mL) were added 1*H*-tetrazole (123.1 mg, 1.76 mmol) and diallylphosphoramidite **17** (190 μL, 0.719 mmol) at −20 °C. After stirring for 1 h at −10 °C under Ar atmosphere, the mixture was cooled to −40 °C, and Me_2S (600 μL) and TBHP (300 μL; 5–6 M in decane) were added. The mixture was stirred for 2.5 h at −40 to 0 °C and quenched with 5% $Na_2S_2O_3$ solution. The aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography ($CHCl_3$/EtOAc 6:1 → 2:1) gave **21** (173.6 mg, 0.307 mmol, 77%). $[\alpha]_D^{24} = +91$ ($c = 0.96$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.53$ (d, 1H, $^3J(H,H) = 9.0$ Hz; NH), 5.97 (m, 2H), 5.69 (dd, 1H, $^3J(H,H) = 3.4$, $^3J(P,H) = 5.9$ Hz; H-1), 5.45–5.15 (6H), 4.64 (m, 4H), 4.36 (m, 1H, H-2), 4.25 (dd, 1H, $J(H,H) = 4.1$, 12.4 Hz; H-6a), 4.16 (m, 1H, H-5), 4.08 (dd, 1H, $J(H,H) = 2.2$, 12.2 Hz; H-6b), 3.56 (d, 1H, $J(H,H) = 15.1$ Hz), 3.41 (d, 1H, $J(H,H) = 14.9$ Hz), 2.42 (s, 3H), 2.08 (s, 3H), 2.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 195.3$, 171.0, 170.4, 169.0, 168.2, 132.1, 132.0, 131.9 (d, $^3J(P,C) = 6.6$ Hz), 119.0, 118.8, 95.7 (d, $^2J(P,C) = 5.8$ Hz), 69.7, 69.6, 68.9 (d, $^2J(P,C) = 5.8$ Hz), 68.7 (d, $^2J(P,C) = 5.8$ Hz), 67.3, 61.4, 52.0, 51.9, 32.6, 30.2, 20.73, 20.70, 20.6; HR ESI-MS: *m/z:* calcd for $C_{22}H_{32}NO_{13}PSNa$: 604.1211, found 604.1230 $[M+Na]^+$.

3,4,6-Tri-*O*-acetyl-2-(acetylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate (22): Et_3SiH (480 μL, 3.00 mmol), AcOH (175 μL, 3.05 mmol), and $[Pd(PPh_3)_4]$ (33 mg, 0.029 mmol) were added to a solution of **21** (170.8 mg, 0.3020 mmol) in toluene (3 mL). The mixture was stirred for 14 h at 40 °C under Ar atmosphere, and concentrated. Purification by silica gel chromatography ($CHCl_3$/MeOH/AcOH 30:1:0 → 8:4:0.1) was followed by size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4) to give **22** (77.4 mg, 0.154 mmol, 51%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.46$ (dd, 1H, $^3J(H,H) = 3.4$, $^3J(P,H) = 6.3$ Hz; H-1), 5.19 (dd, 1H, $J(H,H) = 9.5$, 10.7 Hz; H-3), 5.00 (t, 1H, $^3J(H,H) = 10.0$ Hz; H-4), 4.21–4.17 (2H, H-2, H-6a), 4.12 (m, 1H, H-5), 4.02 (dd, 1H, $J(H,H) = 2.2$, 12.2 Hz; H-6b), 3.06 (d, 1H, $J(H,H) = 15.3$ Hz), 3.43 (d, 1H, $J(H,H) = 15.4$ Hz), 2.27 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 195.9$, 172.2, 171.1, 170.9, 95.8 (d, $^2J(P,C) = 5.8$ Hz), 71.4, 70.3, 69.5, 53.4 (d, $^3J(P,C) = 8.3$ Hz), 33.5, 30.1, 20.74, 20.65, 20.59; HR ESI-MS: *m/z:* calcd for $C_{16}H_{23}NO_{13}PS$: 500.0628, found 500.0663 $[M-H]^-$.

2-Deoxy-2-mercaptoacetamido-α-D-glucopyranosyl phosphate triethylammonium salt (23): Et_3N (0.75 mL) was added to a solution of **22** (32.8 mg, 0.0654 mmol) in degassed MeOH/H_2O (5 mL, 7:3), and stirred for 1 d under Ar atmosphere. The mixture was concentrated, and dried under reduced pressure. Purification was not conducted because of the instability of the material.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-(2-bromoethylthio)acetamido-2-deoxy-β-D-glucopyranoside (24)**: 2-Mercaptopropanol (190 μL, 2.709 mmol) and iPr$_2$NEt (0.9 mL, 5.17 mmol) were added to a solution of bromoacetamide **16** (1.134 g, 2.099 mmol) in CH_3CN (15 mL). After stirring for 4 h, the mixture was concentrated and dissolved in $CHCl_3$. The solution was washed with 10% citric acid solution and brine (2×), dried over Na_2SO_4, filtered, concentrated, and dried under vacuum. Then, the crude alcohol was dissolved in CH_2Cl_2 (20 mL), to which were added NBS (453 mg, 2.54 mmol) and Ph_3P (667 mg, 2.54 mmol) at 4 °C. The mixture was stirred for 1 h at 4 °C under Ar atmosphere and diluted with $CHCl_3$. The organic phase was washed with 5% $Na_2S_2O_3$ solution and brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification with silica gel chromatography (toluene/EtOAc 6:1 → 2:1) gave bromide **24** (1.206 g, 2.01 mmol, 96%). $[\alpha]_D^{24} = +5.9$ ($c = 0.95$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.71$ (d, 1H, $^3J(H,H) = 9.2$ Hz; NH), 5.30 (dd, 1H, $^3J(H,H) = 9.2$, 10.8 Hz; H-3), 5.04 (t, 1H, $^3J(H,H) = 9.2$ Hz; H-4), 4.93 (d, 1H, $^3J(H,H) = 8.0$ Hz; H-1), 4.23–4.11 (m, 2H, H-6a, H-6b), 3.84 (m, 1H, H-2), 3.73 (m, 1H, H-5), 3.49 (t, 2H, $^3J(H,H) = 7.6$ Hz), 3.19 (s, 2H), 2.94 (m, 2H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 0.87 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.7$, 170.5, 169.3, 168.0, 95.8, 72.1, 71.9, 69.0, 62.5, 57.0, 35.9, 34.9, 29.7, 25.7, 25.6, 20.9, 20.8, 20.7, −4.1, −5.0; HR ESI-MS: *m/z:* calcd for $C_{22}H_{38}BrNO_9SSiNa$: 622.1118, found 622.1143 $[M+Na]^+$; elemental analysis calcd (%) for $C_{22}H_{38}BrNO_9SSi$: C 44.00, H 6.38, N 2.33; found: C 44.11, H 6.31, N 2.32.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-(3-bromopropylthio)acetamido-2-deoxy-β-D-glucopyranoside (25)**: 3-Mercaptopropanol (210 μL,

2.43 mmol) and *i*Pr$_2$NEt (1.25 mL, 7.18 mmol) were added to a solution of the bromoacetamide **16** (1.096 g, 2.028 mmol) in CH_3CN (10 mL). After stirring for 7.5 h, the mixture was concentrated, and dissolved in $CHCl_3$. The mixture was washed with 10% citric acid solution and brine (2×), dried over Na_2SO_4, filtered, concentrated, and dried under vacuum. Then, the crude alcohol was dissolved in CH_2Cl_2 (15 mL), to which were added NBS (444 mg, 2.49 mmol) and Ph_3P (645 mg, 2.46 mmol) at 4°C. The mixture was stirred for 1 h at 4°C under Ar atmosphere, and diluted with $CHCl_3$. The organic phase was washed with 5% aqueous $Na_2S_2O_3$ solution and brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification with silica gel chromatography (toluene/EtOAc 6:1 → 2:1) gave bromide **25** (1.178 g, 1.917 mmol, 95%). $[\alpha]_D^{24} = +5.0$ ($c = 0.75$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.67$ (d, 1H, ^3J(H,H) = 8.8 Hz), 5.19 (dd, 1H, ^3J(H,H) = 9.3, 10.7 Hz; H-3), 4.91 (t, 1H, ^3J(H,H) = 10.0 Hz; H-4), 4.82 (d, 1H, ^3J(H,H) = 7.8 Hz; H-1), 4.07 (dd, 1H, ^3J(H,H) = 5.9, ^2J(H,H) = 12.2 Hz; H-6a), 4.02 (dd, 1H, ^3J(H,H) = 2.7, ^2J(H,H) = 12.2 Hz; H-6b), 3.71 (m, 1H, H-2), 3.62 (m, 1H, H-5), 3.38 (ddd, 2H, ^3J(H,H) = 1.2, 6.3, ^2J(H,H) = 12.7 Hz; CH_2Br), 3.04 (s, 2H), 2.55 (t, 2H, ^3J(H,H) = 7.1 Hz), 1.99 (t, 2H, ^3J(H,H) = 6.8 Hz), 1.95 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H), 0.75 (s, 9H), −0.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.5$, 170.2, 169.3, 168.2, 95.8, 72.1, 71.9, 69.1, 62.5, 57.0, 35.9, 35.9, 31.7, 31.5, 31.0, 25.6, 20.82, 20.76, 20.69, 18.0, −4.1, −5.0; HR ESI-MS: *m/z:* calcd for $C_{23}H_{40}BrNO_9SSi$: 636.1274, found 636.1264 $[M+H]^+$.

3,4,6-Tri-*O*-acetyl-2-(2-bromoethylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate diallyl ester (26): 47% Aqueous HF (1.0 mL) was added at 4°C to a solution of **25** (496.2 mg, 0.826 mmol) in CH_3CN (9 mL). The mixture was stirred for 10 h at room temperature, and neutralized with sat. $NaHCO_3$ solution, and the aqueous phase was extracted with $CHCl_3$ (3×). Combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 2:1 → 1:1) gave hemiacetal (281.8 mg, 0.579 mmol, 70%).

To a solution of the hemiacetal (279.2 mg, 0.574 mmol) in CH_2Cl_2 (5 mL) were added amidite **17** (310 μL, 1.17 mmol) and 1*H*-tetrazole (162.1 mg, 2.31 mmol) at −20°C. After stirring for 1 h at −10°C under Ar atmosphere, the mixture was cooled to −40°C, and Me_2S (1 mL) and TBHP (500 μL, 5–6 M in decane) were added. The mixture was stirred at −40 to 0°C for 2.5 h. The mixture was quenched with 5% $Na_2S_2O_3$ solution and extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification with silica gel chromatography (toluene/EtOAc 4:1 → 2:3) gave **26** (251.5 mg, 0.389 mmol, 68%). $[\alpha]_D^{23} = +55$ ($c = 0.95$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.89$ (d, 1H, ^3J(H,H) = 8.5 Hz; NH), 5.97 (m, 2H), 5.73 (dd, ^3J(H,H) = 3.2, ^3J(P,H) = 5.9 Hz; H-1), 5.44–5.27 (m, 5H), 5.20 (t, 1H, ^3J(H,H) = 9.8 Hz), 4.64–4.57 (m, 4H), 4.39 (m, 1H, H-2), 4.26 (dd, 1H, ^3J(H,H) = 3.8, ^2J(H,H) = 12.2, H-6a), 4.22 (m, 1H, H-5), 4.10 (dd, 1H, ^3J(H,H) = 2.0, ^2J(H,H) = 12.4 Hz; H-6b), 3.48 (t, 2H, ^3J(H,H) = 7.6 Hz), 3.21 (s, 2H), 2.95 (t, 2H, J(H,H) = 7.6 Hz), 2.09 (s, 3H), 2.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 171.0$, 170.4, 169.0, 168.7, 132.0, 131.9 (d, ^3J(P,C) = 6.6 Hz), 131.8, (d, ^3J(P,C) = 6.6 Hz), 119.1, 119.0, 95.6 (d, ^2J(P,C) = 5.8 Hz), 69.9, 69.7, 68.9 (d, ^2J(P,C) = 5.0 Hz), 68.7 (d, ^2J(P,C) = 5.0 Hz), 67.3, 61.4, 52.3 (d, ^3J(P,C) = 8.3 Hz), 35.6, 34.8, 29.6, 20.8, 20.7, 20.6; elemental analysis calcd (%) for $C_{22}H_{33}BrNO_{12}PS$: C 40.88, H 5.15, N 2.17; found: C 41.02, H 5.11, N 2.19.

3,4,6-Tri-*O*-acetyl-2-(3-bromopropylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate diallyl ester (27): 47% Aqueous HF was added at 4°C to a solution of **25** (809.3 mg, 1.317 mmol) in CH_3CN (8.5 mL) and the reaction mixture was stirred for 13 h at room temperature. Then, the mixture was neutralized with a saturated $NaHCO_3$ solution, and the aqueous phase was extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc 3:1 → 1:1) gave hemiacetal (597.3 mg, 1.19 mmol, 90%).

To a solution of the hemiacetal (431.6 mg, 0.863 mmol) in CH_2Cl_2 (5 mL) were added amidite **17** (460 μL, 1.74 mmol) and 1*H*-tetrazole (302.2 mg, 4.31 mmol) at −20°C. After stirring for 1 h at −10°C under Ar atmosphere, the mixture was cooled to −40°C, to which Me_2S (1.5 mL) and TBHP (750 μL, 5–6 M in decane) were added. The mixture was stirred at −40 to 0°C for 1.5 h. After the reaction, mixture was quenched with 5% $Na_2S_2O_3$ solution and extracted with $CHCl_3$ (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated. Purification with silica gel chromatography (toluene/EtOAc 4:1 → 2:3) gave **27** (442.2 mg, 0.669 mmol, 78%). $[\alpha]_D^{22} = +44$ ($c = 0.76$ in chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.93$ (d, 1H, ^3J(H,H) = 9.3 Hz; NH), 6.02–5.89 (m, 2H), 5.73 (dd, 1H, ^3J(H,H) = 3.4, ^2J(P,H) = 5.8 Hz; H-1), 5.44–5.28 (5H), 5.20 (t, 1H, J(H,H) = 9.8 Hz), 4.64–5.58 (m, 4H), 4.40 (m, 1H), 4.25 (dd, 1H, J(H,H) = 3.9, 12.2 Hz), 4.20 (m, 1H), 4.10 (dd, 1H, J(H,H) = 2,2, 12.4 Hz), 3.49 (t, 2H, J(H,H) = 6.3 Hz), 3.20 (d, 1H, J(H,H) = 16.3 Hz), 3.15 (d, 1H, J(H,H) = 16.4 Hz), 2.66 (t, 2H, J(H,H) = 7.1 Hz), 2.12–2.03 (3s, 9H, Ac); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.8$, 170.3, 169.0, 168.8, 131.8 (d, J(P,C) = 6.6 Hz), 131.7 (d, J(P,C) = 6.5 Hz), 119.1, 118.9, 95.6 (d, J(P,C) = 6.6 Hz), 69.9, 69.6, 68.9 (d, J(P,C) = 5.0 Hz), 68.7 (d, J(P,C) = 5.0 Hz), 67.3, 61.3, 52.1 (d, J(P,C) = 8.3 Hz), 35.6, 31.6, 31.5, 31.0, 20.71, 20.67, 20.5; HR ESI-MS: *m/z:* calcd for $C_{23}H_{35}BrNO_{12}PSNa$: 682.0699, found 682.0651 $[M+Na]^+$.

3,4,6-Tri-*O*-acetyl-2-(2-bromoethylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate (28): Et_3SiH (240 μL, 1.50 mmol), AcOH (85 μL, 1.5 mmol), and $[Pd(PPh_3)_4]$ (16.9 mg, 0.015 mmol) were added to a solution of the phosphate **26** (95.1 mg, 0.147 mmol) in toluene (2 mL). After stirring for 24 h at 40°C under Ar atmosphere, the mixture was concentrated, and purified with silica gel chromatography ($CHCl_3$/MeOH/AcOH 30:1:0 → 4:1:0.1). Final purification with size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4) yielded **28** (71.5 mg, 0.1263 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.69$ (br, 1H), 5.32 (t, 1H, J(H,H) = 10.0 Hz), 5.19 (t, 1H, J(H,H) = 9.5 Hz), 4.50–4.07 (m, 4H), 3.57 (m, 2H), 3.29 (m, 2H), 2.74 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.9$, 171.4, 170.8, 169.3, 94.1 (br), 71.7, 70.1, 71.7, 70.1, 69.2, 67.9, 61.0, 58.6, 52.0 (br), 35.5, 34.6, 31.9, 30.2, 20.9–20.7; HR ESI-MS: *m/z:* calcd for $C_{16}H_{24}BrNO_{12}PS$: 563.9940, found 563.9967 $[M-H]^-$.

3,4,6-Tri-*O*-acetyl-2-(3-bromoethylthio)acetamido-2-deoxy-α-D-glucopyranosyl phosphate (29): Et_3SiH (490 μL, 3.07 mmol), AcOH (180 μL, 3.14 mmol), and $[Pd(PPh_3)_4]$ (36.0 mg, 0.0312 mmol) were added to a solution of the allyl ester **27** (200.8 mg, 0.3040 mmol) in degassed toluene (4 mL). After stirring for 14 h at 40°C under Ar atmosphere, the mixture was concentrated and purified with silica gel chromatography ($CHCl_3$/MeOH/AcOH 30:1:0 → 6:3:0.1), following size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4) gave **29** (99.4 mg, 0.1713 mmol, 56%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.55$ (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 6.1 Hz; H-1), 5.27 (dd, 1H, ^3J(H,H) = 9.5, 11.0 Hz; H3), 5.07 (t, 1H, J(H,H) = 10.2 Hz), 4.28–4.4.15 (m, 3H, H-2, H-5, H-6a), 4.09 (dd, J(H,H) = 2.0, 12.2 Hz), 3.50 (t, 2H, J(H,H) = 6.3 Hz), 3.19 (d, 1H, J(H,H) = 14.5 Hz), 3.13 (d, 1H, J(H,H) = 14.5 Hz), 2.68 (m, 2H), 2.10–1.94 (m, 11H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 172.7$, 172.3, 171.9, 171.1, 95.9 (^2J(P,C) = 5.8 Hz), 71.5, 70.3, 69.5, 62.7, 49.5 (^3J(P,C) = 9.1 Hz), 35.6, 33.0, 32.7, 31.7, 20.8, 20.7, 20.6; HR ESI-MS: *m/z:* calcd for $C_{17}H_{26}BrNO_{12}PS$: 578.0097, found 578.0087 $[M-H]^-$.

Compound 30a: TCEP-HCl (10.8 mg, 0.0377 mmol) was added to a solution of the disulfide **13a** (10.8 mg, 0.00740 mmol) in MeOH/H_2O (7:3; 1 mL) and the mixture was stirred for 3 h at room temperature under Ar atmosphere. Then, bromoacetamide **19** (12.8 mg, 0.0254 mmol) and *i*Pr$_2$NEt (20 μL, 0.12 mmol) were added. After stirring for 3 h at room temperature under Ar atmosphere, Et_3N (150 μL) was added and stirring continued for additional 10 h, and Et_3N (150 μL) was added to complete the reaction. The mixture was further stirred for 9 h, and concentrated. Purification by SepPak C-18 cartridge (MeOH/H_2O 0:100 → 50:50), and size-exclusion chromatography (Sephadex G-15, H_2O) gave **30a** (8.8 mg, 6.5 μmol, 58%). ^{1}H NMR (400 MHz, D_2O, 40°C): $\delta = 5.42$ (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 7.1 Hz; H-1$_{GN1P}$), 4.91 (s, 1H, H-1$_{\alpha Man}$), 4.65–4.56 (2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.13 (dd, 1H, ^3J(H,H) = 1.5, 3.4 Hz; H-2$_{\alpha Man}$), 4.03–3.35 (25H), 3.68 (s, 3H, CH_3COO), 3.11 (dd, 1H, ^3J(H,H) = 2.4, ^2J(H,H) = 13.9 Hz; H-6a$_{\alpha Man}$), 2.74 (dd, 1H, ^3J(H,H) = 8.3, ^2J(H,H) = 13.9 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.3 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR

ESI-MS: *m/z:* calcd for $C_{36}H_{66}N_2O_{26}SP$: 1029.3362, found 1029.3314 $[M-H]^-$.

Compound 1a: Phosphate **30a** (2.7 mg, 2.4 μmol) and UMP-morpholidate (5.3 mg, 7.7 μmol) were co-evaporated with dry pyridine 3× and dried under vacuum for 1 h. The residue was redissolved in dry pyridine (0.5 mL), to which was added 1*H*-tetrazole (1.2 mg, 0.017 mmol), and stirred for 3 d under Ar atmosphere. Resultant mixture was quenched with H_2O and concentrated. The residue was applied to SepPak C-18 cartridge (MeOH/H_2O 0:100 → 60:40). Subsequent purification with size-exclusion chromatography (Sephadex G-15, H_2O) gave **1a** (2.5 mg, 1.9 μmol, 78%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 7.93 (d, 1H, ^3J(H,H) = 8.1 Hz; H-6$_{Uracil}$), 5.98–5.95 (2H, H-5$_{Uracil}$, H-1$_{Rib}$), 5.52 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 6.3 Hz; H-1$_{GN1P}$), 4.91 (s, 1H, H-1$_{\alpha Man}$), 4.65–4.57 (2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.36–4.20 (5H, H-2$_{Rib}$, H-3$_{Rib}$, H-4$_{Rib}$, H-5a$_{Rib}$, H-5b$_{Rib}$), 4.13 (br, 1H, H-2$_{\alpha Man}$), 4.02–3.13 (25H), 3.64 (s, 3H, CH_3COO), 3.11 (d, 1H, ^2J(H,H) = 13.2 Hz; H-6a$_{\alpha Man}$), 2.73 (dd, 1H, ^3J(H,H) = 8.3, ^2J(H,H) = 13.7 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, J = 7.6 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{47}H_{76}N_4O_{34}P_2SNa$: 1357.3435, found 1357.3417 $[M-2H+Na]^-$.

Compound 30d: nBu$_3$P (10 μL, 0.040 mmol) was added to a solution of disulfide **13a** (10.0 mg, 6.8 μmol) in degassed THF/H_2O (1 mL, 9:1). After stirring for 5 h at room temperature, the mixture was concentrated and dissolved in H_2O. The aqueous phase was washed with Et_2O (3×), concentrated, and dried under vacuum. Resultant thiol was dissolved in degassed DMF (0.7 mL), to which were added GlcNAc derivative **28** (12.2 mg, 0.0215 mmol) and Cs_2CO_3 (10.8 mg, 0.033 mmol). The mixture was stirred at 40°C under Ar atmosphere for 10 h. The mixture was directly applied to size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4). Appropriate fractions were collected, evaporated and lyophilized to give **30d** (5.5 mg, 0.0051 mmol, 37%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 5.44 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 6.8 Hz; H-1$_{GN1P}$), 4.89 (s, 1H, H-1$_{\alpha Man}$), 4.65–4.56 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.12 (br, 1H, H-2$_{\alpha Man}$), 4.02–3.35 (25H), 3.69 (s, 3H, CH_3COO), 3.08 (d, 1H, ^2J(H,H) = 12.7 Hz; H-6a$_{\alpha Man}$), 2.89 (br, 4H), 2.68 (dd, 1H, ^3J(H,H) = 9.0, ^2J(H,H) = 14.1 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.6 Hz; CH_2COO), 2.06 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{40}H_{70}N_2O_{26}PS_2$: 1089.3396, found 1089.3423 $[M-H]^-$.

Compound 1d: Compound **30d** (5.1 mg, 0.0047 mmol) was treated with UMP-morpholidate (9.8 mg, 0.014 mmol) in the presence of 1*H*-tetrazole (1.6 mg, 0.023 mmol), as described for **1a**. Successive purification with SepPak C-18 cartridge, Sephadex G-15, H_2O, preparative TLC, and SepPak C-18 cartridge afforded **1d** (3.8 mg, 0.00272 mmol, 58%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 7.93 (d, 1H, ^3J(H,H) = 8.1 Hz; H-6$_{Uracil}$), 5.98–5.95 (m, 2H, H-5$_{Uracil}$, H-1$_{Rib}$), 5.52 (dd, 1H, ^3J(H,H) = 3.4, ^3J(P,H) = 6.8 Hz; H-1$_{GN1P}$), 4.90 (s, 1H, H-1$_{\alpha Man}$), 4.65–4.56 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.38–4.17 (m, 5H, H-2$_{Rib}$, H-3$_{Rib}$, H-4$_{Rib}$, H-5a$_{Rib}$, H-5b$_{Rib}$), 4.12 (br, 1H, H-2$_{\alpha Man}$), 4.02–3.33 (m, 25H), 3.68 (s, 3H, CH_3COO), 3.08 (d, 1H, ^2J(H,H) = 13.2 Hz; H-6a$_{\alpha Man}$), 2.89 (br, 4H, SCH_2CH_2S), 2.66 (dd, 1H, ^3J(H,H) = 9.0, ^3J(H,H) = 13.4 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.3 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{49}H_{80}N_4O_{34}P_2S_2Na$ 1417.3468, found 1417.3516 $[M-2H+Na]^-$.

Compound 30e: nBu$_3$P (10 μL, 0.040 mmol) was added to a solution of disulfide **13a** (10.1 mg, 0.0069 mmol) in degassed THF/H_2O (0.7 mL, 9:1). After stirring for 5 h at room temperature, the mixture was concentrated and dissolved in H_2O. The aqueous phase was washed with Et_2O (3×) and concentrated, and dried under reduced pressure. Resultant thiol was dissolved in degassed DMF (0.7 mL), to which were added GlcNAc derivative **29** (12.4 mg, 0.0214 mmol), and Cs_2CO_3 (10.2 mg, 0.031 mmol). The mixture was stirred at 40°C under Ar atmosphere for 12 h, and applied to size-exclusion chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:4). The appropriate fraction was collected and lyophilized to give **30e** (9.7 mg, 0.088 mmol, 63%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 5.44 (dd, 1H, ^3J(H,H) = 2.9, ^3J(P,H) = 7.1 Hz; H-1$_{GN1P}$), 4.89 (s, 1H, H-1$_{\alpha Man}$), 4.64–4.59 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.11 (br, 1H, H-2$_{\alpha Man}$), 4.01–3.30 (m, 25H), 3.68 (s, 3H, CH_3COO), 3.06 (dd, 1H, ^2J(H,H) = 13.4 Hz; H-6a$_{\alpha Man}$), 2.72 (m, 4H, SCH_2), 2.63 (dd, 1H, ^3J(H,H) = 8.8, ^2J(H,H) = 13.4 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.6 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.92 (m, 2H), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{41}H_{72}N_2O_{26}PS_2$: 1103.3552, found 1103.3558 $[M-H]^-$.

Compound 1e: Compound **30e** (4.9 mg, 4.4 μmol) was treated with UMP-morpholidate (9.6 mg, 0.014 mmol) and 1*H*-tetrazole (1.6 mg, 0.023 mmol), in a manner as described for **1a**. Successive chromatographic purification afforded **1e** (3.8 mg, 0.0027 mmol, 61%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 7.93 (d, 1H, ^3J(H,H) = 8.1 Hz; H-6$_{Uracil}$), 5.98–5.95 (m, 2H, H-5$_{Uracil}$, H-1$_{Rib}$), 5.52 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 6.8 Hz; H-1$_{GN1P}$), 4.90 (s, 1H, H-1$_{\alpha Man}$), 4.64–4.54 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.37–4.15 (m, 5H, H-2$_{Rib}$, H-3$_{Rib}$, H-4$_{Rib}$, H-5a$_{Rib}$, H-5b$_{Rib}$), 4.11 (dd, 1H, ^3J(H,H) = 1.7, 3.4 Hz; H-2$_{\alpha Man}$), 4.02–3.32 (m, 25H), 3.68 (s, 3H, CH_3COO), 3.06 (dd, 1H, ^3J(H,H) = 2.0, ^2J(H,H) = 13.7 Hz; H-6a$_{\alpha Man}$), 2.71 (m, 4H, SCH_2), 2.62 (dd, 1H, ^3J(H,H) = 9.0, ^2J(H,H) = 13.9 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.3 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.90 (m, 2H), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{50}H_{82}N_4O_{34}P_2S_2Na$: 1431.3625, found 1431.3672 $[M-2H+Na]^-$.

Compound 31: nBu$_3$P (10 μL, 0.040 mmol) was added to a solution of disulfide **13a** (9.7 mg, 6.6 μmol) in degassed THF/H_2O (1 mL, 9:1). After stirring for 5 h at room temperature, the mixture was concentrated and dissolved in H_2O. The aqueous phase was washed with Et_2O (3×), concentrated, and dried under vacuum. Resultant thiol was dissolved in degassed MeOH/H_2O (6 mL, 1:1), to which were added 0.5 M HCl (0.15 mL) and pyridyl disulfide (15.1 mg, 0.069 mmol) at −20°C under Ar atmosphere under vigorous stirring. The mixture was stirred for 2 h at −20°C to room temperature and evaporated in vacuo. The residue was re-dissolved in H_2O, and the solution was washed with Et_2O (3×) to remove excess pyridyl disulfide, concentrated, and dried under vacuum. No further purification was attempted because of the instability of this compound.

Compound 30b: A solution of crude thiol **23** in degassed MeOH/1 M aq. NH_4OAc (1.5 mL, 1:1) was added crude **31** dissolved in 0.5 mL MeOH/1 M aq. NH_4OAc solution at −20°C under Ar atmosphere. After stirring for 6 h at −20°C to room temperature, the mixture was concentrated. Purification by SepPak C-18 cartridge column (MeOH/H_2O 0:100 → 50:50), and gel filtration chromatography (Sephadex G-15, H_2O) gave **30b** (6.6 mg, 6.2 μmol, 47%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 5.40 (dd, 1H, ^3J(H,H) = 2.9, ^3J(P,H) = 7.1 Hz; H-1$_{GN1P}$), 4.90 (s, 1H, H-1$_{\alpha Man}$), 4.65–4.56 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.13 (br, 1H, H-2$_{\alpha Man}$), 4.03–3.35 (m, 25H), 3.68 (s, 3H, CH_3COO), 3.37 (dd, 1H, ^3J(H,H) = 2.2, ^3J(H,H) = 13.9 Hz; H-6a$_{\alpha Man}$), 2.87 (dd, 1H, ^3J(H,H) = 8.8, ^2J(H,H) = 13.9 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.6 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.31 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{38}H_{66}N_2O_{26}PS_2$: 1061.3083, found 1061.3049 $[M-H]^-$.

Compound 1b: Disulfide linked **30b** (4.7 mg, 0.0044 mmol) and UMP-morpholidate (9.3 mg, 0.014 mmol) were co-evaporated with dry pyridine (3×) and dried under vacuum for 1 h. Then, the mixture was dissolved in dry pyridine (0.5 mL), to which was added 1*H*-tetrazole (1.7 mg, 0.024 mmol). The mixture was stirred for 3 d under Ar atmosphere, quenched with H_2O and concentrated. The residue was purified as described for **1a** to give **1b** (3.4 mg, 0.0025 mmol, 56%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 7.93 (d, 1H, ^3J(H,H) = 8.3 Hz; H-6$_{Uracil}$), 5.98–5.95 (2H, H-5$_{Uracil}$, H-1$_{Rib}$), 5.53 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 7.1 Hz; H-1$_{GN1P}$), 4.90 (d, 1H, ^3J(H,H) = 1.5 Hz; H-1$_{\alpha Man}$), 4.65–4.57 (m, 2H, H-1$_{GN}$, H-1$_{\beta Man}$, overlapped with HOD signal), 4.37–4.18 (m, 5H, H-2$_{Rib}$, H-3$_{Rib}$, H-4$_{Rib}$, H-5a$_{Rib}$, H-5b$_{Rib}$), 4.13 (dd, 1H, ^3J(H,H) = 1.7, 3.4 Hz; H-2$_{\alpha Man}$), 4.02–3.42 (m, 25H), 3.64 (s, 3H, CH_3COO), 3.37 (dd, 1H, ^3J(H,H) = 2.4, ^2J(H,H) = 14.1 Hz; H-6a$_{\alpha Man}$), 2.86 (dd, 1H, ^3J(H,H) = 8.5, ^2J(H,H) = 13.9 Hz; H-6b$_{\alpha Man}$), 2.38 (t, 2H, ^3J(H,H) = 7.6 Hz; CH_2COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{47}H_{76}N_4O_{34}P_2S_2Na$: 1389.3155, found 1389.3111 $[M-2H+Na]^-$.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-mercaptoacetamido-β-D-glucopyranoside (32)**: Hydrazine acetate (248 mg, 1.61 mmol) was added to a solution of the thioacetate **20** (744 mg, 1.39 mmol) in THF (18 mL). After stirring for 3.5 h at room temperature, the mixture was di-

luted with 10% citric acid solution. The solution was extracted with $CHCl_3$ (2×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, concentrated, and dried under vacuum to produce thiol **32** (683 mg, 1.39 mmol, quant.) This compound was not purified to avoid dimerization; HR ESI-MS: *m/z:* calcd for $C_{20}H_{35}N_2O_9SSiNa$: 516.1700, found 516.1684 $[M+Na]^+$.

***tert*-Butyldimethylsilyl 3,4,6-tri-*O*-acetyl-2-(chloromethylthio)acetamido-2-deoxy-β-D-glucopyranoside (33)**: iPr$_2$NEt (45 μL, 0.26 mmol) was added at 4°C to a solution of the thiol **32** (42.5 mg, 0.0861 mmol) in CH_3CN/$BrCH_2Cl$ (1:1, 1 mL). After stirring for 5 h at 4°C under Ar atmosphere, the mixture was concentrated, and dried under vacuum for 1 h to afford **33**, which was used for the next reaction without purification.

Compound 34: nBu$_3$P (35 μL, 0.14 mmol) was added to a solution of disulfide **13** (38.6 mg, 0.0264 mmol) in degassed THF/H_2O (9:1, 1 mL). After stirring for 5 h at room temperature under Ar atmosphere, the mixture was concentrated, and dissolved in H_2O. The aqueous phase was washed with Et_2O (3×), concentrated, and dried under reduced pressure to afford crude thiol. It was then dissolved in DMF (1 mL), to which were added crude compound **33** (0.08610 mmol) and Cs_2CO_3 (26.5 mg, 0.0813 mmol). The reaction mixture was stirred for 24 h at 40°C under Ar atmosphere, Ac_2O (1 mL) and pyridine (2 mL) were added, and the mixture was stirred further for 20 h. The mixture was concentrated, and purified with silica gel chromatography (chloroform/EtOAc 3:1 → 1:3) to give **34**.

Compound 35: A solution of crude **34** in DMF (2 mL) was added HF/pyridine complex (100 μL), and stirred for 14 h at room temperature. The mixture was neutralized with sat. $NaHCO_3$ solution under cooling. The aqueous phase was extracted with $CHCl_3$ (3×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered and concentrated. Purification by preparative TLC (toluene/acetone 3:2) gave **35** (23.8 mg, 0.016 mmol, 31% from **13**).

Compound 36: A solution of hemiacetal **35** (23.1 mg, 0.0158 mmol) in dichloroethane (1 mL) were added amidite **17** (10 μL, 0.038 mmol) and 1*H*-tetrazole (4.0 mg, 0.057 mmol). After stirring for 7 h at 45°C under Ar atmosphere, the mixture was cooled to −20°C, Me_2S (200 μL) and TBHP (100 μL, 5–6 M in decane) were added, and stirred for 13 h at −20 to 0°C, and then quenched with 5% $Na_2S_2O_3$ solution. The aqueous phase was extracted with $CHCl_3$ (3×), and the combined organic layers were washed with brine (2×), dried over Na_2SO_4, filtered and concentrated. Purification by silica gel chromatography (toluene/acetone 4:1 → 3:2 with 1% Et_3N) gave **36** (21.8 mg, 0.0135 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.90 (d, 1H, J = 8.5 Hz; N*H*), 6.65 (d, 1H, ^3J(H,H) = 7.1 Hz; N*H*), 5.95 (m, 2H), 5.73 (dd, 1H, ^3J(H,H) = 3.4, ^3J(P,H) = 5.8 Hz; H-1$_{GN1P}$), 5.67 (t, 1H, ^3J(H,H) = 6.8 Hz), 5.41–4.95 (m, 15H), 4.63 (m, 4H), 4.38–3.21 (m, 18H), 2.92 (dd, 1H, ^3J(H,H) = 3.2, ^2J(H,H) = 14.6 Hz; H-6a$_{αMan}$), 2.62 (dd, 1H, ^3J(H,H) = 5.9, ^2J(H,H) = 14.4 Hz; H-6b$_{αMan}$), 2.30 (t, 2H, J(H,H) = 7.3 Hz), 2.09–1.95 (m, 36H), 1.59 (m, 4H), 1.29 (m, 8H); HR ESI-MS: *m/z:* calcd for $C_{67}H_{99}N_2O_{37}PS_2Na$: 1641.5003, found 1641.4910 $[M+H]^+$.

Compound 30c: A solution of allyl ester **36** (10.8 mg, 6.7 μmol) in degassed toluene (0.5 mL) were added Et_3SiH (22 μL, 0.14 mmol), AcOH (8 μL, 0.14 mmol), and $[Pd(PPh_3)_4]$ (1.8 mg, 0.0016 mmol). The mixture was stirred for 14 h at 40°C under Ar atmosphere, and cooled to room temperature. After evaporation, the mixture was applied to size exclusion chromatography (Sephadex LH-20, MeOH). Appropriate fractions were concentrated, and dried under reduced pressure. Then, the deallylated product was dissolved in 0.05 M NaOMe in MeOH (3 mL), and stirred for 10 h at room temperature under Ar atmosphere. The mixture was separated by size exclusion chromatography (Sephadex G-15, H_2O). Further purification by SepPak C-18 cartridge (MeOH/H_2O 0:100 → 60:40) provided **30c** (3.2 mg, 3.0 μmol, 45%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 5.38 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 7.3 Hz; H-1$_{GN1P}$), 4.89 (d, 1H, ^3J(H,H) = 1.5 Hz; H-1$_{αMan}$), 4.65–4.56 (2H, H-1$_{GN}$, H-1$_{βMan}$, overlapped with HOD signal), 4.12 (dd, 1H, ^3J(H,H) = 1.5, 3.4 Hz; H-2$_{αMan}$), 4.02–3.42 (m, 25H), 3.68 (s, 3H, C*H*$_3$COO), 3.08 (dd, 1H, ^3J(H,H) = 2.4, ^2J(H,H) = 13.9 Hz; H-6a$_{αMan}$), 2.74 (dd, 1H, ^3J(H,H) = 8.8, ^2J(H,H) = 14.1 Hz; H-6b$_{αMan}$), 2.38 (t, 2H, ^3J(H,H) = 7.3 Hz; C*H*$_2$COO), 2.06 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{39}H_{68}N_2O_{26}PS_2$: 1075.3239, found 1075.3211 $[M-H]^-$.

Compound 1c: Compound **30c** (2.7 mg, 2.5 μmol) and UMP-morpholidate (5.2 mg, 7.6 μmol) were co-evaporated with dry pyridine (3×) and dried under reduced pressure for 1 h. Then, the mixture was dissolved in dry pyridine (0.5 mL) and added 1*H*-tetrazole (1.2 mg, 0.019 mmol), and stirred for 3 d under Ar atmosphere. The mixture was quenched with H_2O and concentrated. The residue was purified as described for **1a** to give **1c** (2.2 mg, 0.0016 mmol, 63%). ^{1}H NMR (400 MHz, D_2O, 40°C): δ = 7.93 (d, 1H, ^3J(H,H) = 8.3 Hz; H-6$_{Uracil}$), 5.98–5.95 (m, 2H, H-5$_{Uracil}$, H-1$_{Rib}$), 5.52 (dd, 1H, ^3J(H,H) = 3.2, ^3J(P,H) = 7.1 Hz; H-1$_{GN1P}$), 4.89 (d, 1H, ^3J(H,H) = 1.4 Hz; H-1$_{αMan}$), 4.65–4.53 (m, 2H, H-1$_{GN}$, H-1$_{βMan}$, overlapped with HOD signal), 4.37–4.15 (m, 5H, H-2$_{Rib}$, H-3$_{Rib}$, H-4$_{Rib}$, H-5a$_{Rib}$, H-5b$_{Rib}$), 4.12 (dd, 1H, ^3J(H,H) = 1.5, 3.4 Hz; H-2$_{αMan}$), 4.01–3.40 (m, 27H), 3.68 (s, 3H, C*H*$_3$COO), 3.08 (dd, 1H, ^3J(H,H) = 2.2, ^2J(H,H) = 13.7 Hz; H-6a$_{αMan}$), 2.72 (dd, 1H, ^3J(H,H) = 8.8, ^2J(H,H) = 14.1 Hz; H-6b$_{αMan}$), 2.38 (t, 2H, ^3J(H,H) = 7.3 Hz; C*H*$_2$COO), 2.05 (s, 3H, AcN), 1.59 (br, 4H), 1.30 (br, 8H); HR ESI-MS: *m/z:* calcd for $C_{48}H_{78}N_4O_{34}P_2S_2Na$: 1403.3312, found 1403.3271 $[M-2H+Na]^-$.

Inhibitor assays: GnT-V activity was assayed using the pyridylaminated acceptor substrate and purified recombinant soluble GnT-V.[32] GnT-IX activity was assayed using the pyridylaminoethylsuccinamyl acceptor substrate and partial purified recombinant soluble GnT-IX.[4] For kinetic analyses, these enzyme sources were incubated at 37°C for 2 h with 20 μM acceptor substrate (GnGn-bi-PA[33] or GnM-S-PAES[4]) and various concentrations of UDP-GlcNAc and the inhibitor in 100 mM MES (pH 6.25) or MOPS (pH 7.5) buffer containing 200 mM GlcNAc, 0.5% Triton X-100, and 10 mM EDTA. The reaction was terminated by boiling for 3 min and then centrifuged at 15000 rpm for 5 min. The resulting supernatant was analyzed by HPLC.

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