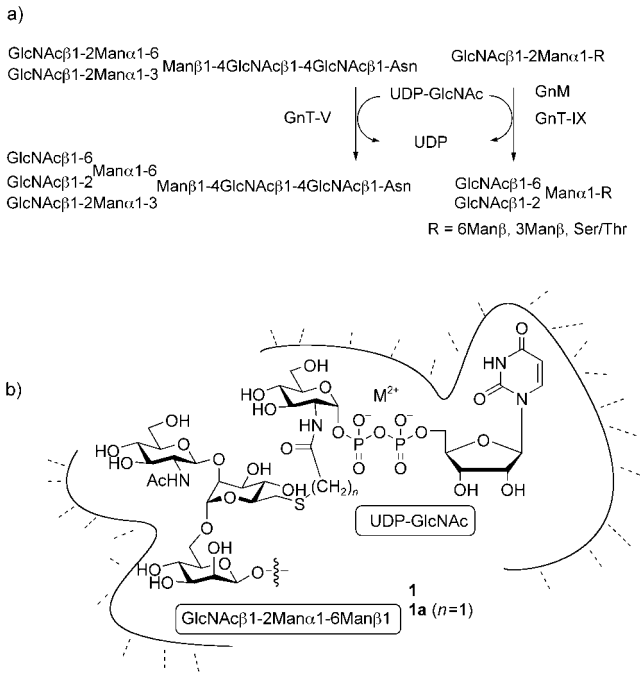

Scheme 1. a) Substrate specificities of GnT-V and GnT-IX; b) bisubstrate-type inhibitors consist of acceptor trisaccharide and UDP–GlcNAc moieties. UDP = uridine diphosphate.

Glycosyltransferase Inhibitor

Synthesis of a Bisubstrate-Type Inhibitor of *N*-Acetylglucosaminyltransferases**

*Shinya Hanashima, Shino Manabe, Kei-ichiro Inamori, Naoyuki Taniguchi, and Yukishige Ito**

Glycosyltransferases are a group of enzymes responsible for the biosynthesis of glycoconjugate oligosaccharides.[1] Among them, *N*-acetylglucosaminyltransferases (GnTs) are key enzymes in the production of highly branched complex *N*-glycan structures. GnT-V transfers an *N*-acetylglucosamine (GlcNAc) residue to the core α1-6-mannose (Man) arm to form a β1-6 linkage (Scheme 1 a).[2] The recently identified GnT-IX is a homologue of GnT-V that is exclusively expressed in the brain.[3] GnT-IX has a broader specificity and transfers GlcNAc to both α1-6- and α1-3-mannose structures, as well as to *O*-linked GlcNAcβ1-2Man.[4] The GlcNAcβ1-6-branched glycans are often extended by the polylactosamine ((Galβ1-4GlcNAc)$_n$, Gal = galactose) structure and present ligands for cell-adhesion molecules. It is well known that levels of β1-6-branched glycans are increased in tumor cells,[5] and GnT-V is involved in cancer metastasis.[6a–e] Also, relationships with T-cell activation and angiogenesis have been revealed.[6f,g] The exclusive expression of GnT-IX in the brain is in sharp contrast with the ubiquitous expression of GnT-V, and this difference suggests roles for GnT-IX in neuronal development and functions.[3]

It is expected that suppression of GnT-V may be useful for the treatment of cancer.[7] In addition to a gene-knockout strategy,[8] chemical inhibition would be an promising approach. In fact, several attempts to develop inhibitors of GnT-V that mimic acceptor substrates have been reported.[9] In this paper, we report the synthesis of compound **1a**, which is a prototype for bisubstrate-type inhibitors of type **1** (Scheme 1 b).[10] These inhibitors were designed to contain both donor (UDP–GlcNAc) and acceptor components, with the proposed mechanism of inverting GnTs[11] taken into consideration. As the acceptor component, the trisaccharide (GlcNAcβ1-2Manα1-6Manβ) was incorporated, because it was previously reported by Tahir and Hindsgaul to serve as an efficient acceptor substrate of GnT-V.[12] In order to approach our target **1a**, a convergent route was adopted; namely, the trisaccharide and donor components were constructed separately and combined together by chemoselective ligation.

Synthesis of the acceptor trisaccharide was conducted by using solution-phase polymer-support technology,[13] which utilized low-molecular-weight ($M_W \approx 750$) poly(ethylene glycol) monomethyl ether (MPEG), as shown in Scheme 2.

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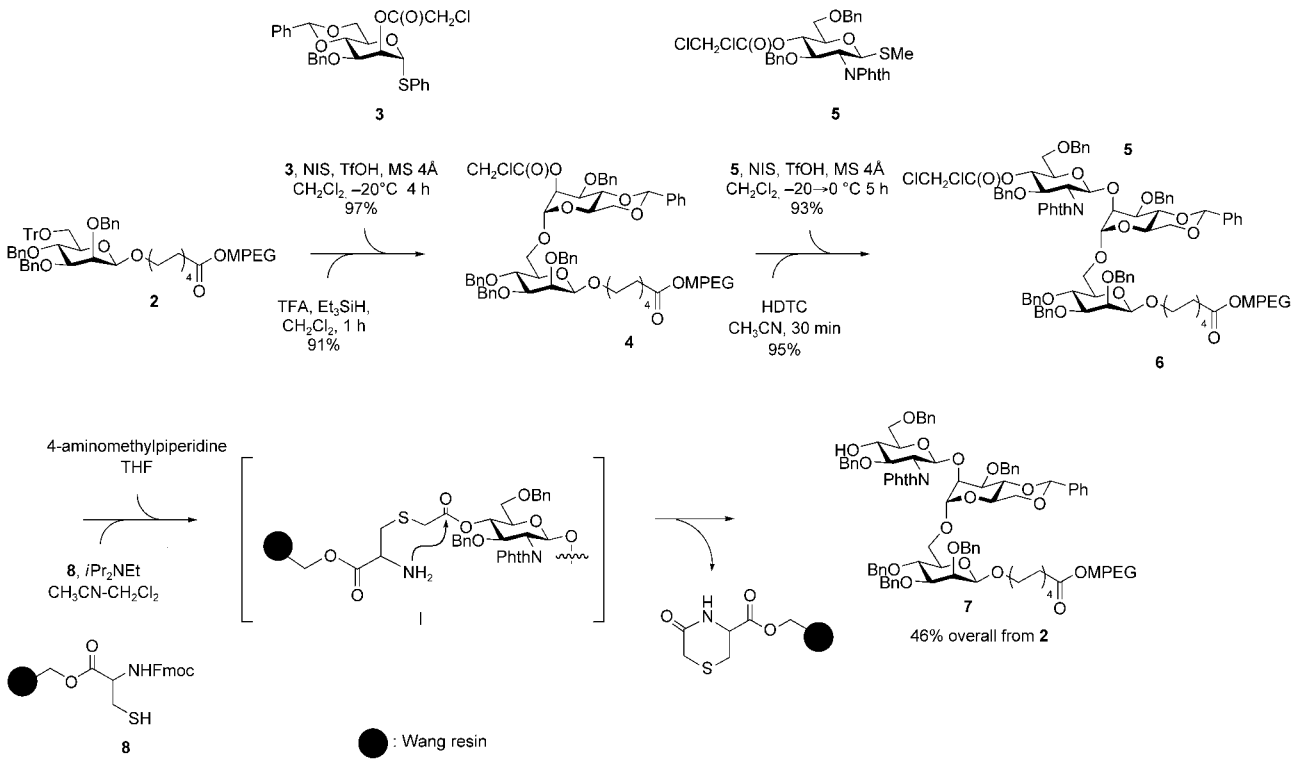
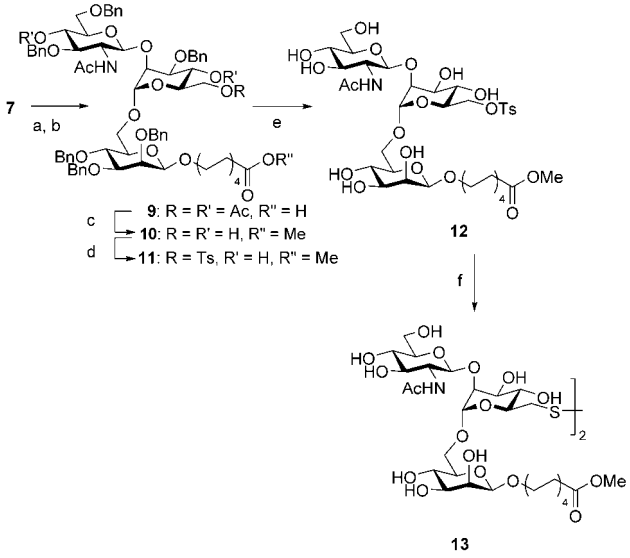


Scheme 2. Polymer-resin hybrid synthesis of trisaccharide **7**. Tr = triphenylmethyl = trityl, Bn = benzyl, TFA = trifluoroacetic acid, Phth = phthaloyl, NIS = *N*-iodosuccinimide, TfOH = trifluoromethanesulfonic acid, HDTC = hydrazinedithiocarbonate, Fmoc = 9-fluorenylmethoxycarbonyl.

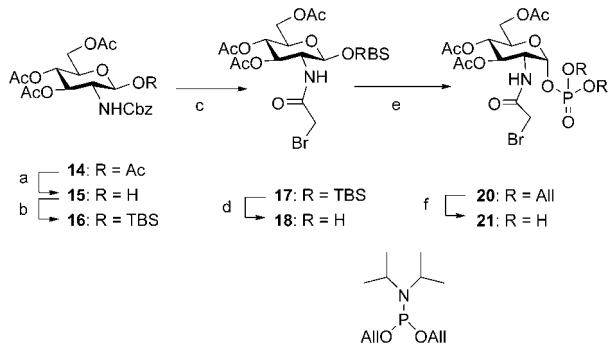
During the whole process, MPEG functioned as a polar tag and products were retrieved by chromatography on short silica gel columns.[14] In the first place, MPEG-supported **2** was prepared by using Hodosi and Kovác's β-mannosylation, as described before.[13d,15] The trityl group was removed with TFA (40 equiv) and Et_3SiH (20 equiv), and the liberated alcohol was glycosylated with phenylthiomannoside **3** with NIS and TfOH (1.1 equiv) to afford disaccharide **4**.[16,17] Cleavage of the chloroacetyl group[18] and glycosylation with **5** provided trisaccharide **6**. At this stage, the product was subjected to capture–release purification. Namely, compound **6** was captured with cysteine-conjugated Wang resin **8** through the reaction between the chloroacetyl and thiol groups. Liberation of the amino group from the Fmoc protection with 4-aminomethylpiperidine initiated cyclization and trisaccharide **7** was obtained in 46% overall yield and with high purity.

Further deprotection[13d] and introduction of the thiol group were conducted as depicted in Scheme 3. Acidic cleavage of the benzylidene acetal, ethylenediamine treatment, and acetylation afforded compound **9** in 77% yield. Subsequent deacetylation and esterification gave **10** in 85% yield. The contaminating αGlcNAc isomer (< 10%) was separated at this stage. Tosylation of the primary hydroxy group was best performed by using DMAP as a base to afford **11**, and cleavage of the benzyl ethers gave **12**. The tosyl group of **12** was substituted with SAc by using AcSK (5 equiv) in DMF at 70 °C. Deacetylation with NaOMe was accompanied by disulfide formation to provide **13**.

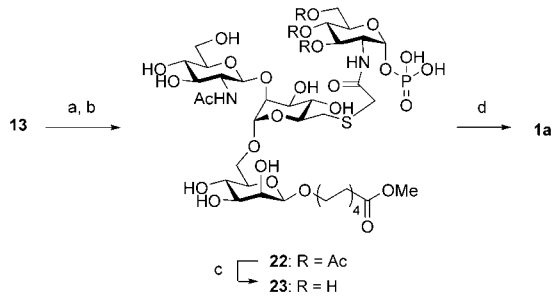


Scheme 3. Preparation of acceptor **13**: a) 60% aqueous AcOH, 60 °C, 87%; b) 1. 1 M KOH, EtOH/THF, reflux; 2. ethylenediamine, 1-BuOH, 100 °C; 3. Ac_2O, pyridine, 77%; c) 1. 0.05 M NaOMe/MeOH; 2. $TMSCHN_2$, PhH, MeOH, 85%; d) TsCl, DMAP, CH_2Cl_2, 60%; e) H_2, 20% $Pd(OH)_2$/C, AcOH, MeOH, 88%; f) 1. AcSK, DMF, 70 °C; 2. 0.05 M NaOMe/MeOH, 90%. Ts = tosyl = toluene-4-sulfonyl, TMS = trimethylsilyl, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide.

Glucosamine phosphate **21** was synthesized as shown in Scheme 4. Compound **14** was subjected to anomeric de-



Scheme 4. Preparation of phosphate **21**: a) NH_2NH_2/AcOH, THF, 94%; b) TBSCl, imidazole, DMF, 95%; c) 1. H_2, 10% Pd/C, EtOAc; 2. $(BrCH_2CO)_2O$, pyridine, CH_2Cl_2, 98%; d) 47% aqueous HF, CH_3CN, 93%; e) 1. **19**, 1*H*-tetrazole, CH_2Cl_2, −10°C; 2. TBHP, −40→0°C, 57%; f) $[Pd(PPh_3)_4]$, Et_3SiH, AcOH, toluene, 85%. Cbz = benzyloxycarbonyl, TBS = *tert*-butyldimethylsilyl, All = allyl, TBHP = *tert*-butylhydroperoxide.



Scheme 5. Chemoselective one-pot ligation and coupling with UMP: a) TCEP-HCl, MeOH, H_2O; b) **21**, *i*Pr_2NEt; c) Et_3N, 51% (three steps); d) UMP–morpholidate, 1*H*-tetrazole, pyridine, 78%. UMP = uridine monophosphate, TCEP-HCl = tris(carboxyethyl)phosphine hydrochloride.

acetylation, and the hemiacetal was masked with a TBS group to give **16**. Removal of the Cbz group was conducted by hydrogenolysis in ethyl acetate,[19] and subsequent *N*-bromoacetylation gave **17** in 98% yield.

The TBS group was removed with 47% aqueous HF in CH_3CN to afford hemiacetal **18** in 93% yield. Direct phosphorylation turned out to be problematic because of base sensitivity of the bromoacetamide moiety. However, treatment with **19** and 1*H*-tetrazole at low temperature (−10°C) readily produced a phosphite compound that was oxidized with TBHP to afford phosphate **20** in 57% yield.[20,21] Cleavage of the allyl groups by using $[Pd(PPh_3)_4]$ and Et_3SiH afforded **21** in 85% yield.

Sequential ligation and deprotection were conducted in a one-pot procedure (Scheme 5). Disulfide **13** was reduced to the thiol derivative with TCEP in MeOH/H_2O. Subsequent addition of bromoacetamide **21** and *i*Pr_2NEt afforded crude **22**, which was deacetylated with excess Et_3N to give **23** in 51% yield from **13** over 3 steps (see the Experimental Section). The modified morpholidate method reported by Wittmann and Wong[22] was used to introduce UMP; namely, monophosphate **23** was treated with UMP–morpholidate in the presence of 1*H*-tetrazole in pyridine for 4 days to afford **1a** in 78% yield. The structure of **1a** was rigorously confirmed by 2D NMR spectroscopy (DQF-COSY, HMQC) and mass spectrometry (MALDI-TOF).[23]

Inhibitory activities of **1a** toward GnT-V and GnT-IX were evaluated, and the results are summarized in Table 1.[24,25] The affinity of **1a** to GnT-V ($K_i = 103$ μM) was only modest in comparison with the acceptor substrate ($K_m = 150$ μM). However, its activity toward GnT-IX was much greater ($K_i = 7.2$ μM). In order to investigate the kinetic mechanism and physiological role of GnT-IX, compound **1a** would therefore be a useful molecular probe.

In summary, we have synthesized bisubstrate-type analogue **1a** as a GnT-V inhibitor by using a polymer-resin hybrid strategy. Ligation of bromoacetamide **21** and disulfide **13**, after coupling with UMP, gave the desired compound **1a**. Simple extension of this chemistry would provide access to various homologues differing in the structure of the acceptor component (for example, Man, GlcNAcβ1-2Man, Manα1-6Man) or the donor–acceptor distance. The latter task may well be achieved immediately simply by changing the bromoacyl group of **17** (see Scheme 4). Further studies are in progress along these lines to discover more potent and selective inhibitors of GnT-V and GnT-IX.

Table 1: Inhibitory activities of **1a** toward GnT-V and GnT-IX.

Enzyme	Acceptor	K_m [mM][a]		K_i [μM][b]
		Acceptor	UDP–GlcNAc	**1a**
GnT-V	GnGn-bi-PA[c]	0.150	4.0–6.0	103
GnT-IX	GnMSer-PAES[d]	–	–	7.2

[a] Michaelis constant. [b] Inhibition constant. [c] GnGn-bi-PA = bi-pyridylaminated Gn-Gn; see ref. [25]. [d] GnMSer-PAES = pyridylaminoethylsuccinimyl GnM; see Scheme 1a and ref. [4].

Experimental Section

Preparation of **22** by a one-pot procedure: TCEP (3.5 mg, 17 μmol) was added to a solution of disulfide **13** (4.0 mg, 2.7 μmol) in MeOH/H_2O (7:3, 1 mL). After stirring for 1 h, GlcNAc derivative **21** (9.3 mg, 18 μmol) and *i*Pr_2NEt (20 μL) were added, and the mixture was stirred for 28 h. Et_3N (0.1 mL) was then added, and the mixture was stirred for an additional day. This was followed by concentration and purification by chromatography with a Sep-Pak C18 cartridge (MeOH/H_2O 0:100→40:60) afforded **22** (2.9 mg, 0.00283 mmol, 51%).

Enzyme assay: GnT-V activity was assayed by using pyridyl aminated acceptor substrate and cell lysate from COS-1 cells transfected with the GnT-V expression vector as an enzyme source.[3,25] GnT-IX activity was assayed by using pyridyl amino ethyl succinamyl acceptor substrate and partially 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 or GnM-S-PAES) and various concentrations of UDP–GlcNAc and the inhibitor in 100 mM β-morpholinoethanesulfonic acid (pH 6.25) or 3-(*N*-morpholine)propanesulfonic acid (pH 7.5) buffer containing 200 mM GlcNAc, 0.5% Triton X-100, and 10 mM ethylenediaminetetraacetate.

The reaction was terminated by boiling for 3 min and then centrifugated at 15000 rpm for 5 min. The resulting supernatant was analyzed by HPLC.

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