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Synthesis of a novel asparagine-linked heptasaccharide structure via *p*-methoxybenzyl-assisted β-mannosylation

Yuki Ohnishi, Hiromune Ando, Tsubasa Kawai [1], Yoshiaki Nakahara[1], Yukishige Ito *

Institute of Physical and Chemical Research (RIKEN) and CREST, Japan Science and Technology Corporation (JST), Wako-shi, Saitama 351-0198, Japan

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Abstract

Synthesis of a core heptasaccharide asparagine N^4-{α-D-mannopyranosyl-(1→6)-[(α-D-mannopyranosyl)-(1→3)]-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)]-(β-D-mannopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl}-L-asparagine (**1a**) found from CHO glycosylation mutant cell LEC 14 is described. The structure of **1a** is highly novel in terms of the presence of an extra GlcNAc residue linked to the 2-position of β-linked mannose. The synthesis was performed using *p*-methoxybenzyl-assisted intramolecular aglycon delivery as the key transformation. 4,6-*O*-TIDPS-protected thiomannoside methyl 2-*O*-*p*-methoxybenzyl-4,6-*O*-(1,1,3,3-tetraisopropyl)disiloxanylidene-3-*O*-trimethylsilyl-1-thio-α-D-mannopyranoside was adopted for this particular purpose, which afforded β-mannoside *p*-methoxyphenyl 2,3-*O*-(*p*-methoxybenzylidene)-4,6-*O*-(1,1,3,3-tetraisopropyl)disiloxanylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside stereoselectively in 75% yield.

Keywords: Glycoprotein; Asparagine-linked glycans; Stereoselective; β-Manno glycoside; Intramolecular aglycon delivery

1. Introduction

Among various types of glycocojugate-related glycan chain structures those derived from complex type asparagine (Asn) linked glycoproteins are of particular interest in terms of biological significance as well as structural diversity and complexity [1]. Besides the typical structural variations that arise from branching, sialylation, fucosylation, bisecting GlcNAc addition and polylactosaminylation, novel types of terminal and non-terminal modifications have been identified. Particularly unique structures have been identified by selection of lectin-resistant mutant strains. For instance, Stanley and coworkers recently reported the presence of novel Asn-linked glycan chains that carry an additional GlcNAc attached to a Man^{III} or $GlcNAc^{II}$ residue (Fig. 1). Those structures were found in dominant Chinese hamster ovary (CHO) glycosylation mutant cells, LEC14 [2] and LEC18 [3], which in turn were selected by resistance to *Pisum sativum* agglutinin (PSA) and Lens culinaris agglutinin (LCA), respectively.

After extensive enzymatic trimming, a major part of the LEC14-derived complex type glycan chain was isolated as a nonasaccharide **1b** [2], while the LEC18 *N*-glycan core struc-

* Corresponding author. Tel.: +81-48-4679430; fax: +81-48-4624680.

E-mail address: yukito@postman.riken.go.jp (Y. Ito).

[1] Present address: Department of Industrial Chemistry, Tokai University, Hiratsuka-shi, Kanagawa 259-1292, Japan.

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ture was identified as the heptasaccharide **2b** [3]. In 1998 synthesis of the pentasaccharide portion **2a**, which is a partial structure of LEC 18-derived *N*-glycan **2b**, was reported, where β-linked Man^{III} was incorporated using conventional insoluble silver salt protocol, which proceeded in low yield (22%) and nonstereoselective (α:β = 1.4:1) manner [4].

With recent establishment of *p*-methoxybenzyl (PMB)-assisted β-mannosylation as a powerful method for the efficient and completely stereoselective synthesis of Asn-linked glycans (Scheme 1) [5–8], we decided to apply this methodology to the synthesis of the novel heptasaccharide core structure **1a** derived from the LEC 14-derived glycan chain.

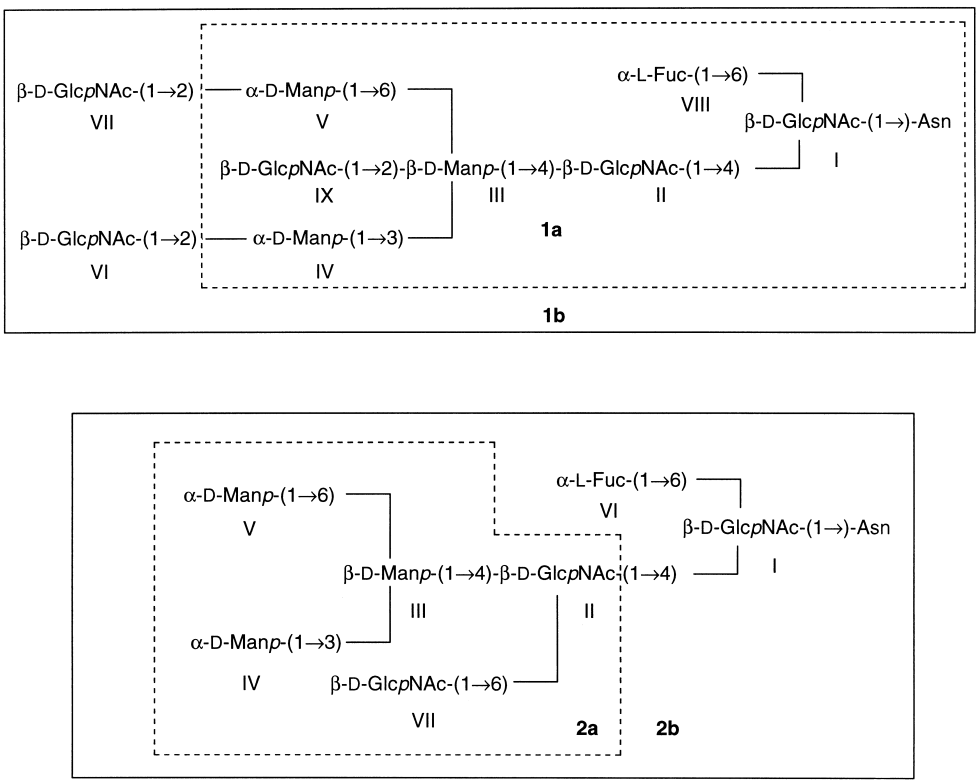
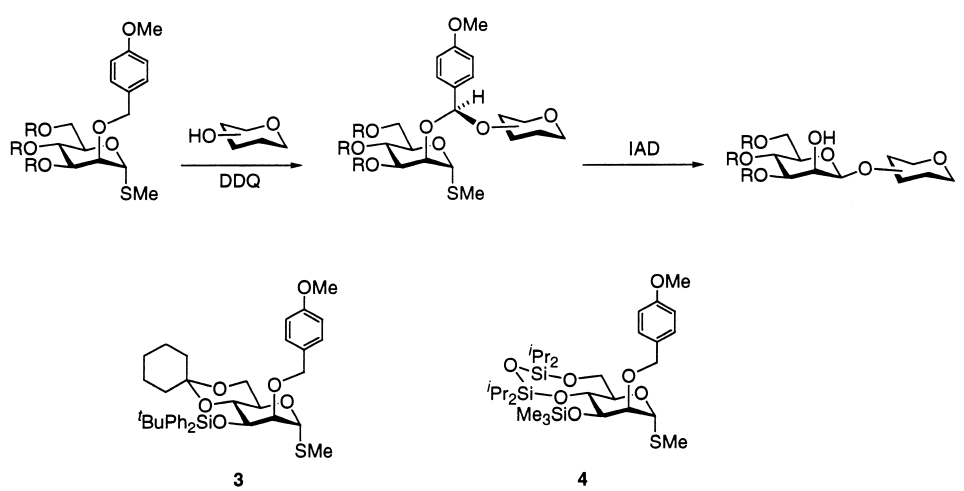
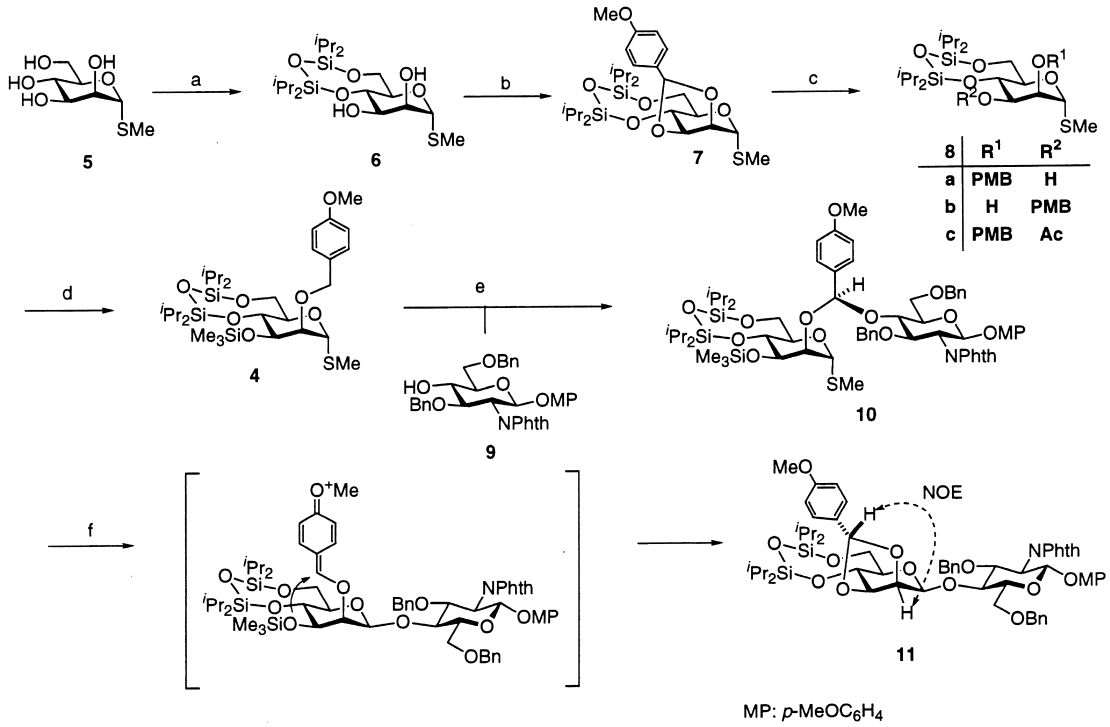


Fig. 1. Novel core structures of Asn-linked glycan chain from LEC14 (top) and LEC18 (bottom) CHO cells.



Scheme 1. PMB-assisted intramolecular aglycon delivery for stereoselective β-mannosylation.



Scheme 2. Synthesis of βMan-(1→4)-GlcNAc component. Reagents and conditions: (a) $TIPDSCl_2$, imidazole, MeCN, 90%; (b) p-$MeOC_6H_4CH(OMe)_2$, PPTS, MeCN, 85%; (c) DIBAL, toluene, −40 °C, 86%; (d) Me_3SiCl, imidazole, DMF, 94%; (e) DDQ, CH_2Cl_2, 4Å MS, 96%; (f) MeOTf, DBMP, $Cl(CH_2)_2Cl$, 4Å MS, 78%.

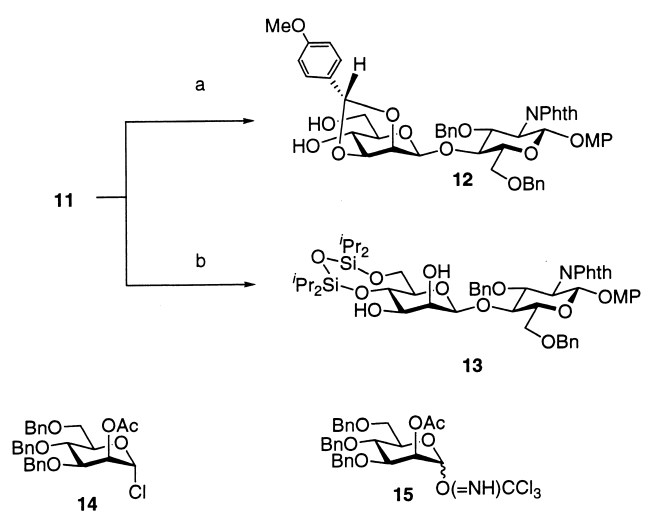
2. Results and discussion

The flexibility of our PMB-assisted β-mannosylation has been documented in the previous reports on the synthesis of typical core structures of Asn-linked glycans [6,8]. Based on this concept, we have successfully established the highly optimized protocol to achieve β-mannosylation, which affords biologically relevant βMan-(1→4)-GlcNAc building blocks in ~75–85% yield [8]. Specifically, it was found that the efficacy of this transformation is rather sensitive to the protecting group patterns and 4,6-*O*-cyclohexylidene (**3**), and tetraisopropyldisiloxanylidene (TIPDS) (**4**) protected mannosyl donors gave optimum results. Since the first chemical synthesis of prototypical complex type undecasaccharide was recently achieved by using 4,6-*O*-cyclohexylidene-carrying **3** [8], the applicability of the alternative donor **4** for complex oligosaccharide synthesis was explored in this study.

The key disaccharide building block was designed as **11** [7], which was prepared from mannosyl donor **4** as depicted in Scheme 2. Preparation of requisite **4** commenced with thiomannoside **5**, which was reacted with $TIPDSCl_2$ and imidazole in acetonitrile to afford **6** (90%). Subsequent formation of the cyclic *p*-methoxybenzylidene acetal was effected by treatment with *p*-anisaldehyde dimethylacetal and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) to afford **7** (85%). Reductive ring opening of the cyclic acetal was effected by diisobutylaluminum hydride (DIBAL-H) [9] in toluene at −40 °C, which predominantly gave 2-*O*-PMB-protected **8a** in 86% yield, together with a small amount of regioisomer **8b** (**8a**:**8b** = 14:1). The structure of **8a** was confirmed by transformation into the corresponding 3-*O*-Ac derivative **8c**. The ^1H NMR spectrum of **8c** revealed the downfield shift of the H-3 proton (δ 5.03). The selectivity of ring opening was somewhat sensitive to the temperature. Namely, the **8a**:**8b** ratio dropped down to 7:1 and 2:1 at −20 and 0 °C, respectively. Further protection of the C-3 position as a trimethylsilyl ether afforded **4**. β-Mannosylation was conducted in a standard manner. Thus, treatment of glucosamine derived acceptor **9** [10] with **4**

and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dichloromethane in the presence of 4Å molecular sieves (4Å MS) afforded the mixed acetal **10**, which was isolated in 96% yield after purification by florisil column chromatography. ^{1}H NMR (270 MHz) analysis revealed that it consisted of a single isomer, and, based on the empirical rule reported previously [11], the configuration of the acetalic carbon was assigned to be S. Low-field shifts of H-1^{Man} (δ 5.94) and single benzylic proton (δ 5.11) were diagnostic for the stereochemical assignment. Intramolecular aglycon delivery (IAD) [12] was effected by methyl trifluoromethanesulfonate (MeOTf) and 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) in 1,2-dichloroethane to afford a 78% yield of β-manno glycoside **11**. The 2,3-*O*-*p*-methoxybenzylidene cyclic acetal regenerated, presumably by the intramolecular trapping of the immediate cationic product by trimethylsilyl ether, again proved to be stereochemically homogeneous. The orientation of the *p*-methoxyphenyl substituent was assigned as endo, based on ^{1}H NMR NOE experiments.

Compound **11** was selectively deprotected to **12** and **13**, which were subjected to α-mannosylation using either glycosyl chloride **14** [13] or trichloroacetimidate **15** [14]. (Scheme 3). Rather unexpectedly, reaction of 4,6-diol **12** with mannosyl chloride **14** turned out to be non-regioselective to give a mixture of 4- and 6-O-glycosylated products. On the other hand,

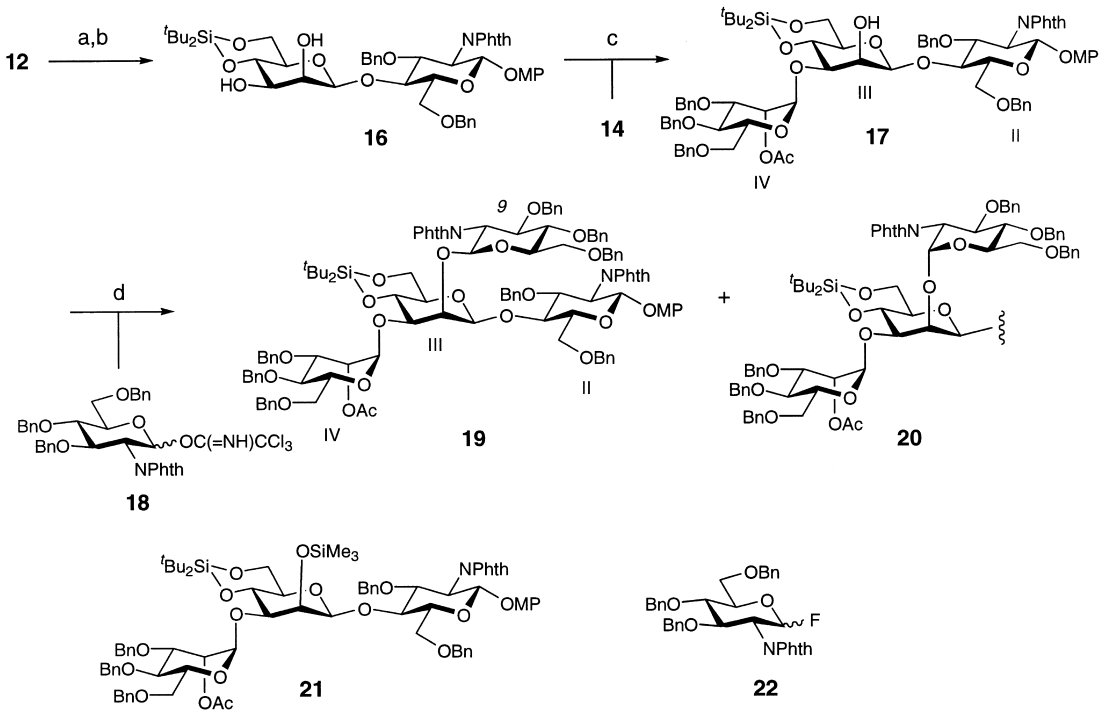


Scheme 3. Selective deprotection of **11**. Reagents and conditions: (a) PPTS, MeOH–CH_2Cl_2, 85%; (b) TBAF, THF, 99%.

attempted α-mannosylation of 2,3-diol **13** using trichloroacetimidate **15** (BF_3·OEt_2, CH_2Cl_2, −78 °C) was accompanied by the migration of the TIDPS group, and formation of the desired 3-O-glycosylated product was not observed. In order to avoid these complexities, 4,6-O-protection was exchanged to the more robust di-*tert*-butylsilanediyl (DTBS) group. Thus, treatment of 4,6-diol **12** with DTBS(OTf)$_2$ and DBMP was followed by the removal of the *p*-methoxybenzylidene group with PPTS in methanol to give **16** in 93% yield. Selective mannosylation at the C-3 position with glycosyl chloride **14** by the action of silver triflate proceeded uneventfully to give trisaccharide **17** in 83% yield (Scheme 4).

The introduction of the crucial glucosamine residue onto trisaccharide **17** was performed by using glycosyl trichloroacetimidate **18** [15]. In spite of the presence of a 1,2-trans directing group, trimethylsilyl trifluoromethansulfonate (TMSOTf) catalyzed coupling yielded an anomeric mixture of tetrasaccharides, which was contaminated with silylated trisaccharide **21**. This mixture was treated with PPTS (to remove TMS) and separated by silica gel chromatography to afford the desired tetrasaccharide **19** and corresponding α isomer **20** (ratio 5:1, combined yield 85%). Although conditions for this glycosylation was examined to some extent (Table 1), the formation of **20** could not be suppressed completely. Attempted glycosylation with fluoride **22** (AgOTf, Cp_2HfCl_2–CH_2Cl_2) [16] proved to be less satisfactory (72%, 19:20 = 2.4:1). The failure to attain complete β-stereoselectivity indicates that the combination of a highly hindered **17** and the phthalimido-carrying glycosyl donor may be a mismatched one [17].

Subsequent transformation to hexasaccharide was performed as depicted in Scheme 5. Previously reported azide **25** [6c] was employed as the fucosylated GlcNAc component. Preparation of tetrasaccharide donor **24** from **18** was performed in a standard manner. The fragment coupling reaction of these components was performed by the action of BF_3·OEt_2 in dichloromethane, and hexasaccharide **26** was obtained in 38% yield. Activation with TMSOTf was less satisfactory to afford a 24% yield of **26**.



Scheme 4. Incorporation of (1→2)-linked βGlcNAc residue. Reagents and conditions: (a) DTBS(OTf)$_2$, DBMP, DMF; (b) PPTS, MeOH–CH_2Cl_2, 93% (two steps); (c) **14**, AgOTf, DBMP, 4Å MS 83%; (d) Lewis acid, solvent, 4Å MS.

Table 1
Glycosylation of **17** with trichloroacetimidate **18**

Entry [a]	Solvent	Promoter (equiv)	Temperature (°C)	**19**/**20** [b]	Yield (%)
1	CH_2Cl_2	TMSOTf (0.2)	−20 to rt	3.3	<10
2	toluene	TMSOTf (0.2)	0	6.7	68
3	1:1 toluene–hexane	TMSOTf (0.2)	0	4.0	89
4	toluene	TMSOTf (1.0)	−20 to 0	5.0	87
5	toluene	$BF_3{\cdot}OEt_2$ (0.2)	0	1.4	26
6	toluene	TESOTf (0.2)	0	5.2	56
7	toluene	TIPSOTf (0.2)	0	5.8	44

[a] All reactions were performed in the presence molecular sieves.
[b] Determined by ^{1}H NMR spectroscopy.

In order to incorporate an α-linked mannose residue, hexasaccharide **26** was subjected to the removal of the DTBS group under desilylating conditions. For this particular purpose, tributylammonium hydrogen fluoride (TBAHF), which has been reported by Furusawa and co-workers [18], gave us a highly satisfactory result to afford **27** in an excellent yield (90%). On the other hand, attempted deprotection under more standard conditions using tetrabutylammonium fluoride–acetic acid was rather sluggish and was accompanied by the formation of several by-products.

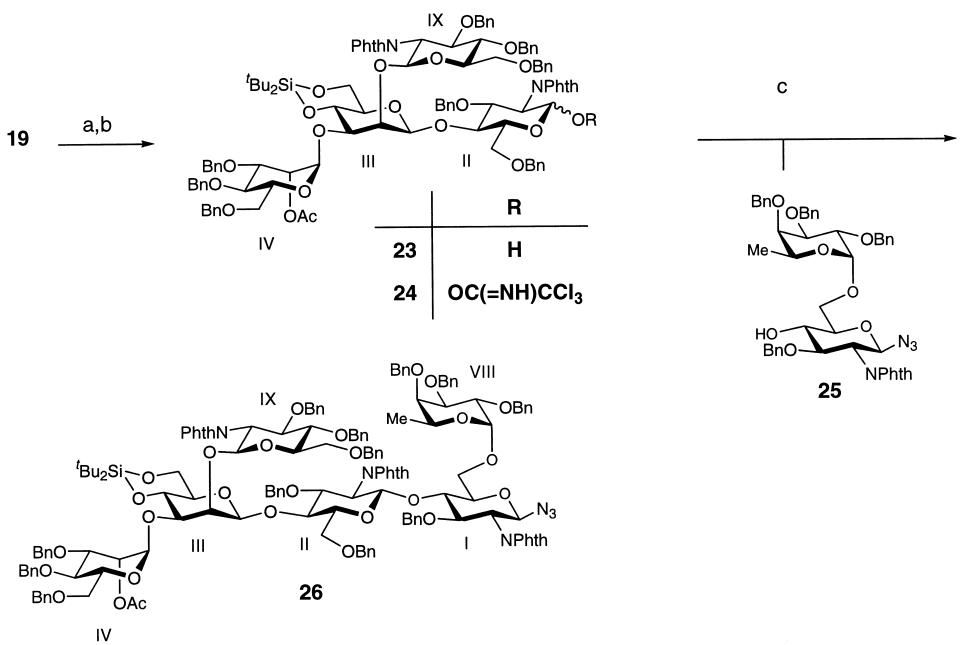
In practice, regioselective glycosylation of **27** turned out to be less straightforward than expected. Silver triflate-promoted glycosylation using glycosyl chloride **14** afforded C-6 mannosylated heptasaccharide **28** (34%), together with the regioisomeric product (20%). This result was in sharp contrast to our previous observation, where the glycosylation of pentasaccharide **29** with **14** proceeded regioselectively to afford the 6-O-glycosylated product in high yield. Presumably, the steric hindrance of the C-6 hydroxyl groups is comparable with that of C-4 due to the presence of an axially oriented glucosamine residue having bulky substituents.

Azide-carrying heptasaccharide **28** was converted into glycosyl asparagine **32** as follows. Removal of phthalimide groups with ethylenediamine in ethanol, followed by N-acetylation, afforded **30**. Chemoselective reduction of the azide group with Lindlar catalyst, in the presence of Cbz-Asp-OBn-derived anhydride **31** [19], gave the protected asparagine-linked heptasaccharide **32**. Finally, hydrogenolytic deprotection was performed with $Pd(OH)_2$–C in ethanol–THF–water to afford the target Asn-linked heptasaccharide **1a** (Scheme 6). The ^1H NMR of **1a** revealed the presence of seven anomeric protons (α-Man × 2, β-GlcNAc × 3, β-Man, and α-Fuc) with expected coupling constants.

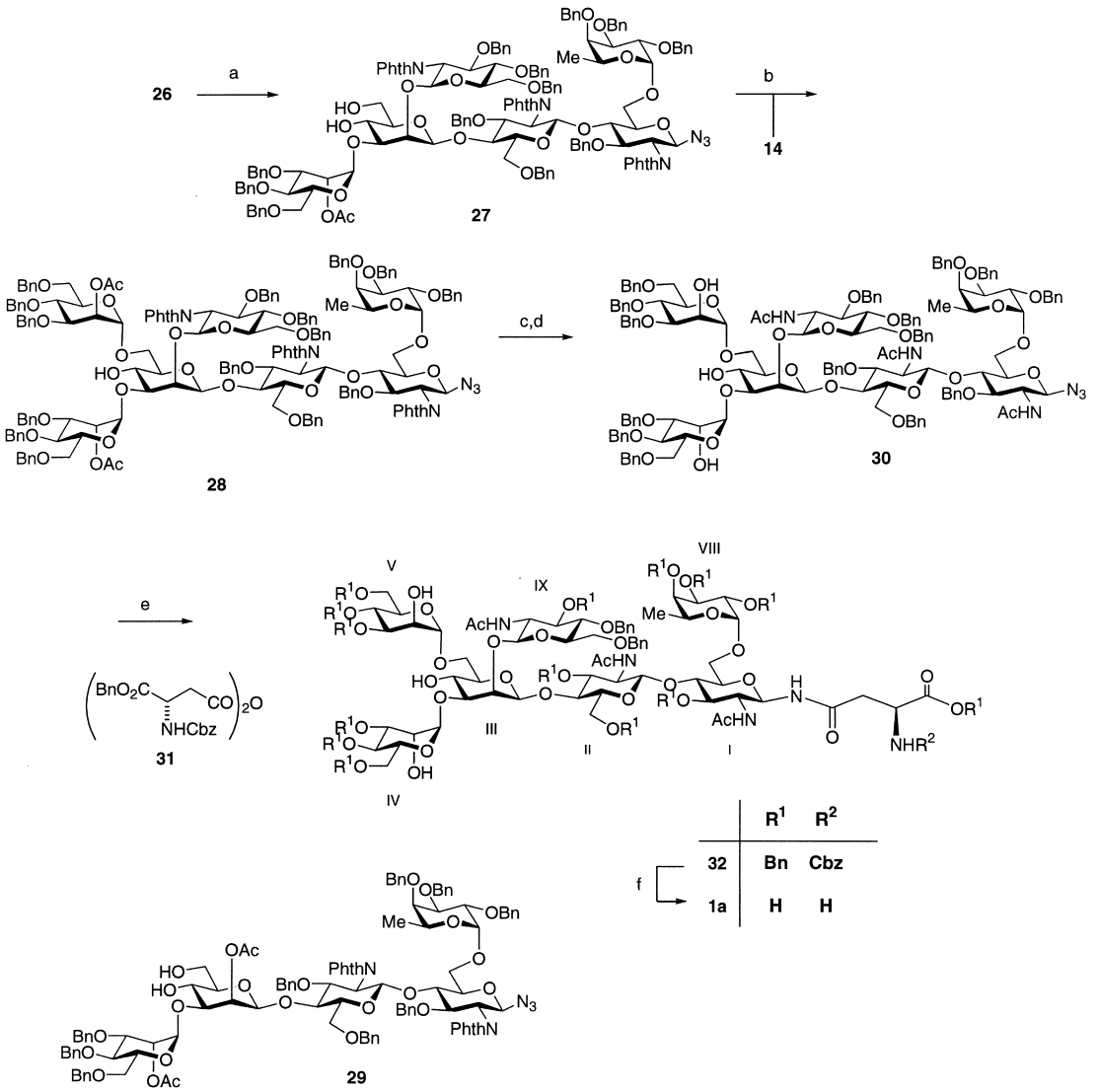
In summary, the chemical synthesis of the core heptasaccharide structure of LEC 14-derived *N*-glycan was achieved using PMB-assisted stereoselective β-mannosylation as the key transformation.

3. Experimental

General methods.—Starting materials and reagents were purchased from standard vendors and used without purification unless otherwise noted. All reactions sensitive to air or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Analytical thin-layer chromatography (TLC) was developed on E. Merck Silica Gel 60 F_{256} plates (0.25 mm thickness). Preparative TLC was performed using E. Merck Silica Gel 60 F_{256} plates (0.5 or 1.0 mm thickness). Column chromatography was performed on E. Merck Silica Gel 60 (60–230 mesh) or Florisil (60–100 mesh, Kanto Chemical Co., Inc.). Flash column chromatography was carried out using E. Merck Silica Gel 60 (230–400 mesh). NMR spectra were obtained on a JEOL EX-270, EX-400, α-500, and/or β-600 spectrometer (^1H at 270, 400, 500, and/or 600 MHz, and ^{13}C at 67.5, 100, 125, and/or 150 MHz) at ambient temperature unless otherwise noted. ^1H chemical shifts are in parts per million (δ) adjusted to Me_4Si ($CDCl_3$ and C_6D_6) at 0 ppm and *t*-BuOH (D_2O) at 1.23 ppm as an internal reference, respectively. ^{13}C chemical shifts were referenced with $CDCl_3$ at 77.0 ppm or C_6D_6 at 128.0 ppm. Optical rotations were measured on a JASCO DIP370 digital polarimeter. Mass spectra were obtained on a JEOL SX-102A mass spectrometer in fast-atom bombardment (FAB) mode with *m*-nitrobenzyl alcohol as the matrix with NaI as the additive to promote molecular ions as $[M + Na]^+$.



Scheme 5. Coupling with fucosylated GlcNAc residue. Reagents and conditions: (a) CAN, MeCN–toluene–water, 71%; (b) Cl_3CCN, DBU, toluene, 86%; (c) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 4Å MS, 38%.



Scheme 6. Synthesis of heptasaccharide asparagine. Reagents and conditions: (a) TBAHF, THF, 90%; (b) AgOTf, CH_2Cl_2, 4Å MS, 34%; (c) $H_2N(CH_2)_2NH_2$, EtOH, reflux; (d) Ac_2O, MeOH, 94%; (e) H_2, Lindlar cat., EtOAc–MeOH, 63%; (f) H_2, $Pd(OH)_2$–C, EtOH–THF–water, 61%.

*Methyl 4,6-*O*-(1,1,3,3-tetraisopropyldisiloxanylidene)-1-thio-α-*D*-mannopyranoside* (**6**). —1,3 - Dichloro - 1,1,3,3 - tetraisopropyldisiloxane (5.46 mL, 17.0 mmol) was added to an ice-cold water soln of **5** (3.00 g, 14.2 mmol) and imidazole (3.87 g, 56.5 mmol) in dry acetonitrile (60 mL). After being stirred at 0 °C for 30 min, the mixture was quenched with MeOH and filtered through Celite. The filtrate was coevaporated with toluene, and the residue was purified by silica gel column chromatography (3:17 EtOAc–hexane) to afford compound **6** (5.50 g, 90%); $[\alpha]_D$ +85.3° (*c* 1.46, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 5.26 (s, 1 H, H-1), 4.19–4.06 (m, 3 H, H-2, H-4, H-6a), 3.91–3.82 (m, 3 H, H-3, H-5, H-6b), 2.55 (d, 1 H, *J* 4.0 Hz, OH), 2.44 (d, 1 H, *J* 5.3 Hz, OH), 2.11 (s, 3 H, SMe), 1.12–1.04 (m, 28 H, *i*-Pr). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 86.0, 72.9, 72.4, 72.3, 67.4, 61.0, 17.5, 17.20, 17.16, 17.0, 13.7, 13.6, 13.2, 12.5, 12.4. Anal. Calcd for $C_{19}H_{40}O_6SSi_2$: C, 50.40; H, 8.90. Found: C, 50.66; H, 8.91.

*Methyl 2,3-*O*-p-methoxybenzylidene-4,6-*O*-(1,1,3,3-tetraisopropyl)disiloxanylidene-1-thio-α-*D*-mannopyranoside* (**7**).—Pyridinium *p*-toluenesulfonate (PPTS, 68 mg, 0.27 mmol) was added to an ice-cold water soln of compound **6** (1.19 g, 2.63 mmol) and 4-methoxybenzaldehyde dimethyl acetal (0.7 mL, 4

mmol) in dry acetonitrile (12.5 mL). After being stirred at room temperature (rt) for 15 h, the mixture was diluted with EtOAc, and quenched by aq $NaHCO_3$. The organic layer was washed successively with aq $NaHCO_3$, water, and brine, dried over Na_2SO_4, and was evaporated in vacuo. The residue was purified by silica gel column chromatography (1:99 EtOAc–toluene), followed by a column of Bio-beads S-X4, to afford compound **7** (1.27 g, 85%) as a diastereomeric mixture (endo:exo 1.3:1; assignments based on differential NOE); $[\alpha]_D$ +43.3° (*c* 1.31, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) major isomer: δ 7.41 (d, 2 H, *J* 8.6 Hz, Ar), 6.85 (d, 2 H, *J* 8.6 Hz, Ar), 5.87 (s, 1 H, C*H*Ar), 5.70 (s, 1 H, H-1), 4.24–4.07 (m, 3 H), 3.96–3.84 (m, 3 H), 3.81 (s, 3 H, OMe), 2.14 (s, 3 H, SMe), 1.12–0.84 (m, 28 H, *i*-Pr); minor isomer: δ 7.34 (d, 2 H, *J* 8.6 Hz, Ar), 6.89 (d, 2 H, *J* 8.6 Hz, Ar), 6.05 (s, 1 H, C*H*Ar), 5.58 (s, 1 H, H-1), 4.42 (dd, 1 H, *J* 7.9 and 5.3 Hz, H-3), 4.22–4.15 (m, 3 H), 3.96–3.85 (m, 2 H), 3.78 (s, 3 H, OMe), 2.10 (s, 3 H, SMe), 1.12–0.84 (m, 28 H, *i*-Pr); ^{13}C NMR (67.5 MHz, $CDCl_3$) major isomer: δ 160.2, 129.6, 127.7, 113.5, 103.5, 82.0, 79.3, 78.2, 70.5, 69.3, 60.8, 55.3; minor isomer: δ 160.2, 130.9, 127.3, 113.7, 102.5, 82.4, 79.7, 76.5, 70.2, 65.3, 60.9, 55.2; additional peaks δ 17.3, 17.2, 17.12, 17.07, 16.9, 16.8, 13.8, 13.4, 13.3, 13.13, 12.4, 12.3, 12.2. Anal. Calcd for $C_{27}H_{46}O_7SSi_2$: C, 56.81; H, 8.12. Found: C, 56.79; H, 8.18.

*Methyl 2-*O-p-*methoxybenzyl-4,6-*O-*(1,1,3,3-tetraisopropyl)disiloxanylidene-1-thio-*α*-*D*-mannopyranoside* (**8a**).—Diisobutylaluminum hydride (DIBAL-H; 0.95 M solution in hexane, 3.60 mL, 3.42 mmol) was added to a precooled (−40 °C) soln of compound **7** (607.7 mg, 1.06 mmol) in dry toluene (20 mL). The mixture was stirred at the same temperature for 1 h, quenched by 0.5 M aq potassium sodium tartarate (20 mL), and then stirred at rt for 1 h. The clear reaction mixture was diluted with EtOAc. The organic layer was washed successively with water and brine, dried over Na_2SO_4, and was evaporated in vacuo. The residue was purified by silica gel column chromatography (1:9–3:7 EtOAc–hexane) to afford compound **8a** (524.4 mg, 86%) and the corresponding regioisomer **8b** (ca. 6%). The position of the *p*-methoxybenzyl group was confirmed by acetylation of **8a** (Ac_2O–pyridine, 40 °C, 18 h) to give **8c**; ^1H NMR (400 MHz, $CDCl_3$): δ 5.29 (s, 1 H, H-1), 5.03 (dd, 1 H, *J* 9.4 and 3.2 Hz, H-3), 4.50 (t, 1 H, *J* 9.4 Hz, H-4), 2.10 and 2.09 (2 s, 6 H, SMe, Ac).

Compound **8a**: $[\alpha]_D$ +21.3° (*c* 0.85, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 7.25 (d, 2 H, *J* 8.7 Hz, Ar), 6.86 (d, 2 H, *J* 8.7 Hz, Ar), 5.41 (s, 1 H, H-1), 4.68 (d, 1 H, *J* 11.2 Hz, benzylic), 4.41 (d, 1 H, *J* 8.7 Hz), 4.17 (dd, 1 H, *J* 12.5 and 1.7 Hz, H-6a), 4.11 (dd, 1 H, *J* 9.2 and 8.9 Hz, H-4), 3.88–3.77 (m, 4 H, H-2, H-3, H-5, H-6b), 3.81 (s, 3 H, OMe), 2.23 (d, 1 H, *J* 9.2 Hz, OH), 2.10 (s, 3 H, SMe), 1.10–0.99 (m, 28 H, *i*-Pr); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 129.7, 129.3, 113.8, 82.8, 79.7, 73.1, 71.8, 67.9, 61.1, 55.3, 17.5, 17.4, 17.34, 17.25, 17.0, 13.9, 13.6, 13.3, 12.40, 12.37. Anal. Calcd for $C_{27}H_{48}O_7SSi_2$: C, 56.61; H, 8.44. Found: C, 56.50; H, 8.53.

Corresponding regioisomer **8b**: ^1H NMR (400 MHz, $CDCl_3$): δ 7.26 (d, 2 H, *J* 8.5 Hz, Ar), 6.87 (d, 2 H, *J* 8.3 Hz, Ar), 5.25 (s, 1 H, H-1), 4.63 (d, 1 H, *J* 11.7 Hz, benzylic), 4.52 (d, 1 H, *J* 11.5 Hz, benzylic), 4.22 (t, 1 H, *J* 9.2 Hz, H-4), 4.18 (dd, 1 H, *J* 12.5 and 1.5 Hz, H-6a), 3.98–3.80 (m, 6 H, H-2, H-5, H-6b, OMe), 3.67 (dd, 1 H, *J* 9.2 and 3.2 Hz, H-3), 2.53 (s, 1 H, OH), 2.09 (s, 3 H, SMe), 1.08–1.02 (m, 28 H, *i*-Pr).

*Methyl 2-*O-p-*methoxybenzyl-4,6-*O-*(1,1,3,3-tetraisopropyl)disiloxanylidene-3-*O*-trimethylsilyl-1-thio-*α*-*D*-mannopyranoside* (**4**).—Chlorotrimethylsilane (0.25 mL, 2.0 mmol) was added to an ice-cold water soln of compound **8a** (741.3 mg, 1.29 mmol) and imidazole (267.9 mg, 3.94 mmol) in dry DMF (18 mL). The mixture was stirred at rt for 2.5 h, quenched with Et_3N, and diluted with EtOAc. The organic layer was washed successively with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (1:19 EtOAc–hexane) to afford compound **4** (780.7 mg, 94%); $[\alpha]_D$ +49.3° (*c* 0.92, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 7.26 (d, 2 H, *J* 8.4 Hz, Ar), 6.83 (d, 2 H, *J* 8.4 Hz, Ar), 5.25 (d, 1 H, *J* 1.1 Hz, H-1), 4.60 (s, 2 H, benzylic), 4.38 (t, 1 H, *J* 9.2 Hz, H-4),

4.16 (dd, 1 H, *J* 12.5 and 1.7 Hz, H-6a), 3.82 (m, 1 H, H-6b), 3.80 (s, 3 H, OMe), 3.73 (brd, 1 H, *J* 9.2 Hz, H-5), 3.65 (dd, 1 H, *J* 3.3 and 1.1 Hz, H-2), 2.10 (s, 3 H, SMe), 1.16–1.02 (m, 28 H, *i*-Pr), 0.11 (s, 9 H, $SiMe_3$); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 158.9, 130.9, 128.9, 113.5, 84.7, 80.3, 74.4, 73.4, 72.4, 66.8, 61.2, 55.3, 17.9, 17.4, 17.3, 17.2, 17.11, 17.05, 13.8, 13.5, 13.4, 13.1, 12.0. Anal. Calcd for $C_{30}H_{56}O_7SSi_3$: C, 55.86; H, 8.75. Found: C, 55.79; H, 8.82.

p-*Methoxyphenyl 3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside-methyl 4′,6′*-O-*(1,1,3,3-tetraisopropyl)disiloxanylidene-3′*-O-*trimethylsilyl-1′-thio-α*-D-*mannopyranoside-2′,4*-p-*methoxybenzylidene acetal* (**10**).—DDQ (219 mg, 0.96 mmol) was added to an ice-cold water mixture of compounds **4** (504.2 mg, 0.78 mmol) and **9** (580.2 mg, 0.98 mmol) and 4Å MS in dry CH_2Cl_2 (15 mL). After being stirred at rt for 2 h, the mixture was quenched with an aq soln of ascorbic acid (0.7%)–citric acid (1.3%)–NaOH (0.9%) (10 mL). The resulting lemon-yellow mixture was diluted with Et_2O, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$ and brine, and the combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by florisil column chromatography (1:49 EtOAc–toluene) to afford mixed acetal **10** (929.4 mg, 96%); ^1H NMR (270 MHz, $CDCl_3$): δ 5.94 (s, 1 H, H-1^{Man}), 5.81 (s, 1 H, C*H*Ar), 5.55 (d, 1 H, *J* 8.2 Hz, H-1^{GlcN}), 5.11 (d, 1 H, *J* 12.5 Hz, benzylic), 3.81 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 2.21 (s, 3 H, SMe), 1.01–0.80 (m, 28 H, *i*-Pr), 0.04 (s, 9 H, $SiMe_3$); HRFABMS: Calcd for $C_{65}H_{87}NNaO_5SSi_3$ $[M+Na]^+$: 1260.5002. Found: 1260.4921.

p-*Methoxyphenyl* O-*[2,3*-O-*(*p-*methoxybenzylidene)-4,6*-O-*(1,1,3,3-tetraisopropyl)disiloxanylidene-β*-D-*mannopyranosyl]-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**11**).—To a stirred mixture of acetal **10** (929.4 mg, 0.75 mmol), DBMP (796.0 mg, 3.88 mmol), and 4Å MS (3.5 g) in dry $(CH_2Cl)_2$ (90 mL) was added to a soln of MeOTf in $(CH_2Cl)_2$ (1 M, 3.0 mL, 3.0 mmol) at 0 °C. After being stirred at rt for 0.5 h and then at 60 °C for 14 h, the reaction was quenched with Et_3N (3.0 mL), diluted with Et_2O, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$, water, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (1:19 EtOAc–toluene) to afford compound **11** (658.6 mg, 78%); $[\alpha]_D$ +35.1° (*c* 0.61, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 7.80–6.67 (m, 22 H, Ar), 5.94 (s, 1 H, C*H*Ar), 5.61 (d, 1 H, *J* 8.6 Hz, H-1^{GlcN}), 5.05 (d, 1 H, *J* 12.9 Hz, benzylic), 4.90 (brs, 1 H, H-1^{Man}), 4.78 (d, 1 H, *J* 12.0 Hz, benzylic), 4.70 (d, 1 H, *J* 12.9 Hz, benzylic), 3.72 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 2.92 (brd, 1 H, *J* 9.9 Hz, H-3^{Man}), 1.02–0.51 (m, 28 H, *i*-Pr); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 160.0, 155.3, 150.9, 138.9, 137.8, 133.6, 129.8, 128.5, 128, 4, 128.2, 128.0, 127.8, 127.7, 126.8, 123.2, 118.7, 114.3, 113.3, 104.4, 99.2, 97.6, 80.1, 78.7, 77.5, 77.2, 77.1, 76.0, 74.7, 74.6, 73.6, 68.9, 68.0, 55.8, 55.5, 55.2, 17.4, 17.3, 17.2, 17.1, 17.0, 16.8, 13.1, 13.0, 12.6, 12.2. Anal. Calcd for $C_{56}H_{53}NO_{14}$: C, 69.77; H, 5.54; N, 1.45. Found: C, 69.57; H, 5.62; N, 1.21.

p-*Methoxyphenyl 2,3*-O-p-*methoxybenzylidene-β*-D-*mannopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**12**).—TBAF (1 M solution in THF, 1.8 mL, 1.8 mmol) was added to an ice-cold water solution of **11** (642.3 mg, 0.57 mmol) in THF (15 mL), and the solution was stirred at 0 °C for 45 min. The resulting mixture was diluted with $CHCl_3$ and washed with water (×2). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (3:17–3:2 EtOAc–toluene) to afford **12** (499.0 mg, 99%); $[\alpha]_D$ +47.0° (*c* 0.45, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$): δ 7.67–6.66 (m, 22 H, Ar), 5.90 (s, 1 H, benzylidene), 5.62 (d, 1 H, *J* 8.2 Hz, H-1^{GlcN}), 4.95 (s, 1 H, H-1^{Man}), 4.92 and 4.72 (d, 1 H each, *J* 12.0 Hz, benzylic), 4.56–4.45 (m, 4 H), 4.25 (dd, 1 H, *J* 9.6 and 8.9 Hz, H-3^{GlcN}), 4.10–4.02 (m, 2 H), 3.86–3.65 (m, 2 H), 3.72 and 3.70 (s, 3 H each, OMe), 3.49 (m, 1 H), 3.21 (m, 1 H, H-5^{GlcN}); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 160.3, 155.3, 150.7, 138.1, 137.6, 133.8, 131.5, 129.1, 128.4, 128.1, 128.0, 127.93, 127.87, 127.3, 127.1, 123.3, 118.7, 114.3, 113.6, 104.9,

98.3 (C-1^{II}), 97.8 (C-1^{I}), 79.9, 78.2, 77.5, 76.8, 76.0, 75.4, 74.7, 73.8, 69.9, 67.9, 62.1, 55.6, 55.4, 55.3; HRFABMS: Calcd for $C_{49}H_{49}NNaO_{14}$ $[M+Na]^+$: 898.3051. Found: 898.3042.

p-*Methoxyphenyl 4,6*-O-*di*-tert-*butylsilanediyl*-*β*-D-*mannopyranosyl*-*(1→4)*-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-*β*-D-*glucopyranoside* (**16**).—A solution of di-*tert*-butysilanediyl ditriflate (0.14 mL, 0.37 mmol) was added to an ice-cold water soln of compound **12** (265.7 mg, 0.31 mmol) and DBMP (202 mg, 0.98 mmol) in dry DMF (10 mL), and the mixture was stirred at rt for 1.5 h. The reaction was quenched with Tris (37.5 mg), and stirred at rt for 10 min. The resulting mixture was diluted with EtOAc, and washed successively with aq Na_2CO_3, water, and brine. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was passed through a pad of silica gel (1:4 EtOAc–toluene) to afford a crude mixture of the corresponding silylene (ca. 294 mg), which was dissolved in 5:2 CH_2Cl_2–MeOH (7 mL). A solution of PPTS (0.05 M in MeOH, 1 mL, 0.05 mmol) was added, and the solution was stirred at rt for 2.5 h. The resulting mixture was diluted with EtOAc and washed with aq $NaHCO_3$ and brine, successively. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was subjected successively to a column of Bio-beads SX-4 (toluene) and silica gel column chromatography (1:4–1:1 EtOAc–toluene) to afford **16** (253.9 mg, 93%); $[\alpha]_D$ +47.5° (*c* 0.75, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.67–6.68 (18 H, Ar), 5.60 (d, 1 H, *J* 8.2 Hz, H-1^{GlcN}), 4.79 (d, 1 H, *J* 12.4 Hz, benzylic), 4.74 (d, 1 H, *J* 12.0 Hz, benzylic), 4.72 (s, 1 H, H-1^{Man}), 4.53–4.41 (m, 4 H), 4.14 (dd, 1 H, *J* 9.6 and 8.2 Hz, H-3^{GlcN}), 4.06 (dd, 1 H, *J* 10.1 and 5.1 Hz, H-6a^{GlcN}), 3.70 (s, 3 H, OMe), 3.33 (m, 1 H, H-3^{Man}), 3.14 (ddd, 1 H, *J* 9.7, 9.6, and 4.8 Hz, H-5^{GlcN}), 2.63 (d, 1 H, *J* 4.5 Hz, OH), 2.57 (d, 1 H, *J* 2.1 Hz, OH), 1.01 (s, 9 H, *t*-Bu), 0.97 (s, 9 H, *t*-Bu); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 155.3, 150.6, 138.1, 137.5, 133.7, 128.4, 127.9, 127.8, 127.6, 123.3, 118.7, 114.3, 100.6 (C-1^{Man}), 97.8 (C-1^{GlcN}), 78.6, 77.7, 77.2, 74.8, 74.7, 74.1, 73.8, 73.7, 70.9, 70.3, 68.5, 66.0, 55.6, 27.5, 27.1, 22.7, 20.0; HRFABMS: Calcd for $C_{49}H_{59}NNaO_{13}Si$ $[M+Na]^+$: 920.3653. Found: 920.3651.

p-*Methoxyphenyl 3,4,6*-*tri*-O-*benzyl*-*α*-D-*mannopyranosyl*-*(1→3)*-*4,6*-*di*-tert-*butylsilanediyl*-*β*-D-*mannopyranosyl*-*(1→4)*-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-*β*-D-*glucopyranoside* (**17**).—Compounds **16** (274.0 mg, 0.31 mmol) and **14** (229.7 mg, 0.45 mmol), DBMP (161 mg, 0.74 mmol), and 4Å MS (1.0 g) were suspended in dry CH_2Cl_2 and cooled down to −40 °C. Silver triflate (147.1 mg, 0.57 mmol) was added, and the mixture was stirred for 2 h while gradually being warmed up to −30 °C. The reaction was quenched with aq Na_2CO_3, diluted with EtOAc, and filtered through Celite. The filtrate was dried over $MgSO_4$ and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (7:93–3:17 EtOAc–toluene) to afford compound **17** (347.1 mg, 83%); $[\alpha]_D$ +27.4 (*c* 0.57, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.67–6.67 (33 H, Ar), 5.58 (d, 1 H, *J* 8.3 Hz, H-1^{II}), 5.52 (dd, 1 H, *J* 3.1 and 1.8 Hz, H-2^{IV}), 5.21 (d, 1 H, *J* 1.8 Hz, H-1^{III}), 4.89–4.29 (m, 13 H), 4.14–3.45 (m, 14 H), 3.70 (s, 3 H, OMe), 3.12 (ddd, 1 H, *J* 9.9, 5.0, and 5.0 Hz, H-5), 2.49 (s, 1 H, OH), 2.13 (s, 3 H, OAc), 1.03 (s, 9 H, *t*-Bu), 0.94 (s, 9 H, *t*-Bu); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 169.8, 155.4, 150.8, 138.3, 138.0, 137.9, 137.7, 133.8, 131.5, 128.5, 128.43, 128.36, 128.32, 128.2, 128.1, 127.9, 127.8, 127.73, 127.6, 127.60, 127.2, 123.3, 118.7, 114.3, 100.4 (C-1^{III}), 99.7 (C-1^{IV}), 97.7 (C-1^{II}), 79.6, 78.5, 78.2, 75.1, 74.7, 74.6, 74.5, 73.51, 73.46, 73.3, 71.9, 71.8, 71.0, 70.8, 69.2, 68.5, 68.4, 66.2, 55.6, 55.5, 27.5, 26.9, 22.5, 21.0, 19.8; HRFABMS: Calcd for $C_{78}H_{89}NNaO_{19}Si$ $[M+Na]^+$: 1394.5696. Found: 1394.5677.

p-*Methoxyphenyl 2*-O-*acetyl*-*3,4,6*-*tri*-O-*benzyl*-*α*-D-*mannopyranosyl*-*(1→3)*-*[3,4,6*-*tri*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-*β*-D-*glucopyranosyl*-*(1→2)]*-*4,6*-*di*-tert-*butylsilanediyl*-*β*-D-*mannopyranosyl*-*(1→4)*-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-*β*-D-*glucopyranoside* (**19**).—Trimethylsilyl trifluoromethansulfonate (29 μL, 0.16 mmol) was added to a precooled (−20 °C) mixture of compound **17** (194.6 mg, 0.14 mmol) and **18** (252.2 mg, 0.43 mmol) in dry toluene (10 mL) containing 4Å

MS (1.0 g), and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with aq $NaHCO_3$, diluted with EtOAc, and filtered through Celite. The filtrate was washed successively with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was subjected to a column of Bio-beads SX-1 (toluene) that was followed by further purification with silica gel column chromatography (1:4–1:1 EtOAc–toluene) to afford a mixture of **19**/**20** (256 mg), which contains trimethylsilylated acceptor **21**.

This mixture was treated with PPTS (18 mg, 0.072 mmol) in 3:5 MeOH–CH_2Cl_2 (8 mL). After 12 h, the mixture was diluted with EtOAc, and washed successively with aq $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (1:4–1:1 EtOAc–toluene) to afford a mixture of **19**/**20** (5:1 ratio, 233.3 mg, 85%) and trisaccharide **17** (17.3 mg, 9%). Pure **19** was finally obtained by preparative TLC (5:4:1 toluene–$CHCl_3$–EtOAc).

Compound **19**: $[\alpha]_D$ +56.3° (*c* 0.82, $CHCl_3$); ^{1}H NMR (270 MHz, C_6D_6): δ 7.91–6.56 (m, Ar), 6.13 (d, 1 H, *J* 8.6 Hz, H-1^{II}), 6.04 (dd, 1 H, *J* 3.0 and 1.8 Hz, H-2^{IV}), 5.90 (d, 1 H, *J* 11.0 Hz, benzylic), 3.21 (s, 3 H, OMe), 1.72 (s, 3 H, OAc), 1.33 (s, 9 H, *t*-Bu), 1.05 (s, 9 H, *t*-Bu); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 169.4, 156.0, 151.4, 139.6, 139.3, 139.2, 139.1, 139.0, 138.9, 133.6, 133.4, 132.8, 132.0, 128.8, 128.7, 128.6, 128.54, 128.49, 128.23, 128.17, 128.1, 127.9, 127.8, 127.3, 127.2, 127.0, 126.7, 123.8, 123.5, 119.1, 114.9, 104.6 100.9 98.6, 98.2, 82.6, 80.4, 80.2, 79.0, 78.9, 78.8, 76.5, 75.9, 75.7, 75.5, 74.9, 74.8, 74.6, 74.2, 73.9, 73.7, 73.6, 72.8, 72.0, 71.7, 70.1, 69.9, 69.2, 66.5, 60.1, 57.3, 56.7, 55.1, 28.2, 27.5, 23.0, 20.7, 20.2, 14.4; HRFABMS: Calcd for $C_{113}H_{120}O_{25}N_2SiNa$ $[M+Na]^+$: 1955.7847. Found: 1955.7817.

2-O-*Acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-[*3,4,6-tri*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(1→2)]-*4,6-di*-tert-*butylsilanediyl*-β-D-*mannopyranosyl*-(1→4)-*3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-D-*glucopyranose* (**23**).—Ceric ammonium nitrate (CAN, 378 mg, 0.69 mmol) was added to a biphasic solution of **19** (263.3 mg, 0.14 mmol) in 4:3:2 toluene–MeCN–water (3.6 mL), and the mixture was stirred at rt. After 1 h, the mixture was diluted with EtOAc, washed successively with water, aq $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was subjected to a column of Bio-beads SX-4 (toluene), followed by silica gel column chromatography (3:17–1:3 EtOAc–toluene) to afford **23** (176.9 mg, 71%); $[\alpha]_D$ +67.0° (*c* 0.44, $CHCl_3$); ^{1}H NMR (270 MHz, C_6D_6): δ 7.93–6.69 (m, Ar), 6.03 (brs, 1 H, H-2^{IV}), 5.87 (d, 1 H, *J* 8.4 Hz, H-1^{IX}), 5.62 (s, 1 H, H-1^{IV}), 5.47 (dd, 1 H, *J* 7.7 and 5.8 Hz, H-1^{II}), 5.13 (d, 1 H, *J* 11.0 Hz, benzylic), 1.72 (s, 3 H, Ac), 1.33 (s, 9 H, *t*-Bu), 1.04 (s, 9 H, *t*-Bu); ^{13}C NMR (67.5 MHz, C_6D_6): δ 104.6, 100.9, 98.6, 93.4, 82.6, 80.3, 80.0, 79.1, 78.7, 76.6, 75.9, 75.7, 75.5, 75.1, 74.7, 74.5, 74.3, 74.2, 73.9, 73.5, 72.8, 72.0, 71.6, 70.3, 70.0, 69.2, 66.5, 58.4, 57.3, 28.2, 27.5, 23.0, 20.7, 20.2; HR-FABMS: Calcd for $C_{106}H_{114}N_2NaO_{24}Si$ $[M+Na]^+$: 1849.7847. Found: 1849.7806.

2-O-*Acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-[*3,4,6-tri*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(1→2)]-*4,6-di*-O-tert-*butylsilanediyl*-β-D-*mannopyranosyl*-(1→4)-*3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-D-*glucopyranosyl trichloroacetimidate* (**24**).—DBU (1 M solution in toluene, 30 μL, 0.03 mmol) was added to an ice-cold water solution of compound **23** (109.5 mg, 0.06 mmol) and trichloroacetonitrile (1.0 mL, 10 mmol) in dry toluene (3.5 mL), and the mixture was warmed up to rt. After 4.5 h, the mixture was subjected to silica gel column chromatography (1:9 EtOAc–toluene) to afford compound **24** (102.0 mg, 86%); ^{1}H NMR (270 MHz, C_6D_6): δ 8.51 (s, 1 H, NH), 7.82–6.70 (m, Ar), 6.05 (dd, 1 H, *J* 2.8 and 2.0 Hz, H-2^{IV}), 5.88 (d, 1 H, *J* 8.4 Hz, H-1^{II} or H-1^{IX}), 5.64 (s, 1 H, H-1^{IX} or H-1^{II}), 5.13 (d, 1 H, *J* 11.0 Hz, benzylic), 1.73 (s, 3 H, OAc), 1.32 (s, 9 H, *t*-Bu), 1.05 (s, 9 H, *t*-Bu).

2-O-*Acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-[*3,4,6-tri*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(1→2)]-*4,6*-O-*di*-tert-*butylsilanediyl*-β-D-*mannopyranosyl*-(1→4)-*3,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl*-(1→4)-

[2,3,4-tri-O-*benzyl*-α-L-*fucopyranosyl*-(1→6)]-*3*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl azide* (**26**).—To a precooled (−20 °C) mixture of compounds **24** (62.9 mg, 0.03 mmol) and **25** (123.4 mg, 0.15 mmol) in dry CH_2Cl_2 (1.5 mL) containing 4Å MS (100 mg), $BF_3{\cdot}OEt_2$ (5 μL, 0.04 mmol) was added and the mixture was stirred at the same temperature. After 2 h an additional amount of $BF_3{\cdot}OEt_2$ (2.5 μL, 0.02 mmol) was added, and the mixture was stirred for one additional hour. The mixture was quenched with aq $NaHCO_3$, diluted with EtOAc, and filtered through Celite. The filtrate was washed successively with water, aq $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was subjected to a column of Bio-beads SX-1 (toluene), and hexasaccharide-containing fractions were further purified by silica gel column chromatography (5–10% EtOAc in toluene) to afford compound **26** (31.8 mg, 38%); $[\alpha]_D$ +23.4° (*c* 0.32, $CHCl_3$); ^{1}H NMR (270 MHz, C_6D_6): δ 7.82–6.61 (m, Ar), 6.01 (brs, 1 H, H-2^{IV}), 5.85 (d, 1 H, *J* 8.2 Hz, H-1), 5.81 (d, 1 H, *J* 7.3 Hz, H-1), 5.61 (s, 1 H, H-1^{IV}), 5.34–5.26 (m, 5 H), 5.14 (d, 1 H, *J* 10.9 Hz, benzylic), 1.70 (s, 3 H, OAc), 1.29 (s, 9 H, *t*-Bu), 1.25 (d, 3 H, *J* 6.4 Hz, H-6^{VIII}), 1.03 (s, 9 H, *t*-Bu); ^{13}C NMR (67.5 MHz, C_6D_6): δ 105.1, 100.5, 97.9, 97.4, 85.5, 83.2, 80.7, 80.1, 79.3, 78.8, 78.6, 78.4, 77.2, 76.6, 75.8, 75.2, 74.9, 74.8, 74.5, 74.4, 73.9, 73.7, 73.6, 73.3, 72.9, 72.7, 71.9, 71.7, 69.9, 69.3, 67.0, 63.9, 57.7, 57.5, 56.2, 28.2, 27.5, 23.0, 20.7, 20.2, 17.0; HRFABMS: Calcd for $C_{154}H_{160}N_6NaO_{33}Si$ $[M+Na]^+$: 2672.0693: Found 2672.0706.

2-O-*Acetyl*-*3,4,6*-*tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)-*[3,4,6*-*tri*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl*-(1→2)]-β-D-*mannopyranosyl*-(1→4)-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl*-(1→4)-*[2,3,4*-*tri*-O-*benzyl*-α-L-*fucopyranosyl*-(1→6)]-*3*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl azide* (**27**).—Tributylammonium hydrogen fluoride (1.6 M solution in THF, 0.1 mL, 0.16 mmol) was added to a solution of compound **26** (46.4 mg, 17.5 μmol), and the solution was stirred at rt for 1 h. The resulting mixture was quenched with water, diluted with EtOAc, and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was successively purified by silica gel column chromatography (1:1 EtOAc–toluene) and preparative TLC (1:1 EtOAc–toluene) to afford compound **27** (39.7 mg, 90%); $[\alpha]_D$ +41.1° (*c* 0.75, $CHCl_3$); ^{1}H NMR (270 MHz, C_6D_6): δ 7.93–6.62 (m, Ar), 6.24 (brs, 1 H, H-2^{IV}), 6.10 (brs, 1 H, H-1^{IV}), 5.97 (d, 1 H, *J* 8.4 Hz, H-1), 5.93 (d, 1 H, *J* 9.2 Hz, H-1), 5.45 (d, 1 H, *J* 12.9 Hz, benzylic), 5.37 (d, 1 H, *J* 13.0 Hz, benzylic), 5.29 (d, 1 H, *J* 9.2 Hz, H-1), 5.24–5.19 (m, 2 H, H-1, benzylic), 3.05 (m, 1 H), 2.78 (d, 1 H, *J* 10.0 Hz, OH), 2.16 (s, 3 H, OAc), 1.23 (d, 3 H, *J* 6.4 Hz, H-6^{VIII}); ^{13}C NMR (67.5 MHz, C_6D_6): δ 104.6, 97.76 (×2), 97.66, 97.2, 96.4; HRFABMS: Calcd for $C_{146}H_{144}N_6NaO_{33}$ $[M+Na]^+$: 2531.9672. Found: 2531.9763.

2-O-*Acetyl*-*3,4,6*-*tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→6)-*[2*-O-*acetyl*-*3,4,6*-*tri*-O-*benzyl*-α-D-*mannopyranosyl*-(1→3)]-*[3,4,6*-*tri*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl*-(1→2)]-β-D-*mannopyranosyl*-(1→4)-*3,6*-*di*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl*-(1→4)-*[2,3,4*-*tri*-O-*benzyl*-α-L-*fucopyranosyl*-(1→6)]-*3*-O-*benzyl*-*2*-*deoxy*-*2*-*phthalimido*-β-D-*glucopyranosyl azide* (**28**).—Compounds **27** (18.0 mg, 7.2 μmol) and **14** (4.6 mg, 9.0 μmol) and 4Å MS (1.0 g) were suspended in dry CH_2Cl_2 (2.0 mL). Silver triflate (3.0 mg, 11.0 mmol) was added, and the mixture was stirred for 1.5 h. The reaction was quenched with aq Na_2CO_3, diluted with EtOAc, and filtered through Celite. The filtrate was dried over $MgSO_4$ and the solvent was evaporated in vacuo. The residue was subjected to a column of Bio-beads SX-3 (3:1 toluene–$CHCl_3$) and heptasaccharide-containing fractions were further purified by preparative TLC (1:3 EtOAc–toluene) to afford compound **28** (7.2 mg, 34%), the corresponding regioisomer (4.3 mg, 20%) and recovered **27** (2.8 mg, 16%).

Compound **28**: $[\alpha]_D$ +39.7° (*c* 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6): δ 7.98–6.63 (m, 87 H, Ar), 6.23 (dd, 1 H, *J* 2.6 and 1.5 Hz, H-2^{IV} or H-2^{V}), 6.09 (brs, 1 H, H-1), 5.95 (d, 1 H, *J* 8.3 Hz, H-1), 5.90 (d, 1 H, *J* 8.3 Hz, H-1), 5.69 (d, 1 H, *J* 2.8, 1.5 Hz, H-2^{V} or H-2^{IV}), 5.43 (d, 1 H, *J* 12.9 Hz, benzylic),

5.37 (d, 1 H, *J* 12.7 Hz, benzylic), 5.31 (d, 1 H, *J* 9.3 Hz, H-1), 5.26–5.22 (m, 2 H, H-1, benzylic), 5.07–4.86 (m, 7 H), 4.79–3.73 (m, 54), 3.63 (dd, 1 H, *J* 10.0 and 7.7 Hz), 3.57–3.46 (m, 3 H), 3.39 (brs, 1 H), 3.19–3.15 (m, 2 H), 2.86 (d, 1 H, *J* 9.8 Hz, OH), 2.00 (s, 3 H, OAc), 1.72 (s, 3 H, OAc), 1.25 (d, 3 H, *J* 6.3 Hz, H-6^{VIII}); ^{13}C NMR (125 MHz, C_6D_6): δ 105.3, 97.81, 97.75, 97.4, 85.5, 84.0, 80.4, 80.24, 80.18, 79.2, 79.1, 78.5, 77.1, 76.6, 76.1, 75.9, 75.3, 75.1, 74.9, 73.6, 73.4, 73.3, 73.23, 73.17, 72.9, 72.4, 72.1, 71.8, 70.1, 69.9, 69.5, 69.4, 69.0, 66.9, 57.5, 56.9, 56.1, 30.2, 20.9, 20.7, 16.8; HRFABMS: Calcd for $C_{146}H_{144}N_6NaO_{33}$ $[M+Na]^+$: 2531.9672. Found: 2531.9763.

*3,4,6-Tri-*O*-benzyl-α-*D*-mannopyranosyl-(1→6)-[3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1→3)]-[2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl-(1→2)]-β-*D*-mannopyranosyl-(1→4)-*O*-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1→6)]-2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl azide* (**30**).—Compound **28** (9.8 mg, 3.3 mmol) was treated with ethylenediamine (1.0 mL) in EtOH (3 mL) under reflux. After 16 h an additional amount of ethylenediamine (0.2 mL) was added and the mixture was stirred at the same temperature for 5 h. The resulting mixture was evaporated in vacuo, coevaporated with toluene (×3), and was dissolved in MeOH (3 mL). The solution was treated at 0 °C with Ac_2O (0.75 mL) for 1.5 h and evaporated in vacuo. The residue was subjected to a column of Bio-beads SX-3 (3:1 toluene–$CHCl_3$) to afford compound **30** (8.1 mg, 94%); $[\alpha]_D$ −12.6° (*c* 0.54, $CHCl_3$); ^{1}H NMR (400 MHz, C_6D_6): δ 7.57–6.60 (m, Ar), 6.53 (brs, 1 H, NH), 6.34 (s, 1 H, H-1), 5.98 (brs, 1 H, NH), 5.83 (s, 1 H, H-1), 5.69 (d, 1 H, *J* 8.3 Hz, H-1), 3.16 (d, 1 H, *J* 9.0 Hz), 3.00 (m, 1 H, H-2), 2.07 (s, 3 H, NAc), 1.76 (s, 3 H, NAc), 1.70 (s, 3 H, NAc), 1.30 (d, 3 H, *J* 6.3 Hz, H-6^{VIII}); ^{13}C NMR (100 MHz, C_6D_6): δ 104.0, 99.8, 98.7, 98.1, 97.9, 88.75, 81.7, 81.0, 80.6, 79.7, 79.32, 79.26, 79.1, 79.0, 78.2, 77.2, 76.9, 76.2, 75.5, 74.9, 74.7, 73.8, 73.6, 73.4, 73.1, 73.0, 72.9, 72.5, 72.3, 72.0, 71.8, 71.1, 70.8, 70.3, 69.8, 69.6, 67.5, 67.3, 63.8, 63.5, 60.3, 55.5, 30.3, 24.1, 23.4, 23.1, 17.1; HRFABMS: Calcd for $C_{153}H_{171}N_6NaO_{34}$ $[M+Na]^+$: 2636.1836. Found: 2636.1846.

*N^2-Benzyloxycarbonyl-N^4-{3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1→6)-[3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl-(1→3)]-[2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl-(1→2)]-β-*D*-mannopyranosyl-(1→4)-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl-(1→4)-*O*-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1→6)]-2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl}-*L*-asparagine benzyl ester* (**32**).—DCC (8.8 mg, 43 μmol) was added to a solution of Z-Asp-OBn (25.8 mg, 72.2 μmol) in dry CH_2Cl_2 (1.5 mL), and the mixture was stirred at 0 °C for 30 min. The precipitate was filtered off, and the filtrate was dissolved in EtOAc (1.0 mL). A solution of compound **30** (5.0 mg, 1.9 μmol) in MeOH (1.0 mL) was added, followed by Lindlar catalyst. The mixture was stirred under a H_2 atmosphere at rt for 5 h and then filtered through a membrane filter. The filtrate was evaporated in vacuo and purified by a column of Bio-beads SX-3 (3:1 toluene–$CHCl_3$). The asparagine-linked heptasaccharide-containing fractions were further purified by preparative TLC (1:9 MeOH–$CHCl_3$) to afford compound **32** (3.5 mg, 63%); ^{1}H NMR (600 MHz, C_6D_6): δ 7.52–6.98 (m, Ar), 6.33 (brs, 1 H, NH), 5.81 (s, 1 H, H-1^{IV}), 5.72 (d, 1 H, *J* 7.8 Hz, H-1), 5.23 (brs, 1 H, H-1), 5.15 (d, 1 H, *J* 11.2 Hz, benzylic CH_2), 3.15 (brs, 1 H), 3.04 (brs, 1 H, H-2), 2.75 (brs, 1 H, CH_2^{Asn}), 2.53 (brs, 1 H, CH_2^{Asn}), 2.09 (s, 3 H, NAc), 1.75 (s, 3 H, NAc), 1.71 (s, 3 H, NAc), 1.33 (d, 3 H, *J* 5.9 Hz, H-6^{VIII}).

*N^4-{α-*D*-mannopyranosyl-(1→6)-[α-*D*-mannopyranosyl-(1→3)]-[2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→2)]-β-*D*-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1→4)-[α-*L*-fucopyranosyl-(1→6)]-2-acetamido-2-deoxy-β-*D*-glucopyranosyl}-*L*-asparagine* (**1a**).—Compound **32** (3.2 mg, 1.1 μmol) was hydrogenated over $Pd(OH)_2$–C (3.5 mg) in 4:2:1 EtOH–THF–water (1.4 mL) at rt for 62 h. The resulting mixture was filtered through a membrane filter, and the filtrate was evaporated in vacuo. The residue was passed through a C_{18} reversed-phase cartridge (water) to afford, after lyophilization, compound **1a** (1.0 mg, 61%);

^{1}H NMR (500 MHz, D_2O, 50 °C): δ 5.12 (d, 1 H, J 1.2 Hz, H-1^{IV}), 5.06 (d, 1 H, J 9.7 Hz, H-1^{I}), 4.89 (1 H, J 2.2 Hz, H-1^{IV}), 4.87 (1 H, J 3.7 Hz, H-1^{F}), 4.76 (brs, 1 H, H-1^{II}), 4.73 (d, 1 H, J 8.9 Hz, H-1^{II} or H-1^{IX}), 4.71 (d, 1 H, J 7.5 Hz, H-1^{IX} or H-1^{II}), 2.71 (dd, 1 H, J 16 and 4.9 Hz, CH_2^{Asn}), 2.45 (dd, 1 H, J 16 and 8.9 Hz, CH_2^{Asn}), 2.08 (s, 3 H, NAc), 2.03 (s, 3 H, NAc), 2.01 (s, 3 H, NAc), 1.20 (d, 3 H, J 6.8 Hz, H-6^{VIII}).

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