Synthetic Studies on Glycosphingolipids from Protostomia Phyla: Synthesis of Amphoteric Glycolipid Analogues from the Porcine Nematode, *Ascaris suum*

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A novel amphoteric glycosphingolipid, cholinephosphoryl-(→6)-β-D-Glc*p*NAc-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→)-Cer, isolated from the porcine parasitic nematode, *Ascaris suum*, may be expected to be involved in host-parasite interactions. This glycosphingolipid analogue containing octyl residue in place of ceramide was synthesized as follows: The key reaction of this synthetic procedure is the formation of a intramolecular aglycon delivery (IAD) approach for β-selective mannosylation. Then, a coupling of phosphocholine group at the position C-6″ of 16 was attempted using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by reaction of the resulting cyclic phosphate intermediate with anhydrous trimethylamine to give 17. Subsequent debenzylation and debenzylidenation afforded target compound (2).

Key words amphoteric glycosphingolipid; *Ascaris suum*; chemical synthesis; β-mannosidic linkage; phosphocholine

Glycosphingolipids are components of cell membranes and are thought to play important roles in a variety of biological events.[1] Especially sialic acid-containing glycosphingolipids, such as gangliosides have been recognized life phenomenon, for example extracellular recognition, cell–cell interaction, differentiation, oncogenesis and immunity. A number of sialyloligosaccharides and mimetics have been synthesized, researched systematic understanding of structure-function at molecular level.[2—4] On the other hand, the structure of glycosphingolipids from invertebrate animal species differ greatly from these of mammals.[5—8] We have been interested in the relationships between the structure and biological function of glycolipid derived from them and have so far synthesized oligosaccharides were isolated from various Protostomia phyla.[9—16] In the previous paper,[17] we reported the synthesis of neutral glycosphingolipid, of which core structure was a β-D-Glc*p*NAc-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→)-Cer, in larvae of the green-bottle fly, *Lucilia caesar*.[18,19] Sugita and co-workers.[20] isolated novel amphoteric glycosphingolipid, cholinephosphoryl-(→6)-β-D-Glc*p*NAc-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→)-Cer (**1**), from the porcine parasite nematode, *Ascaris suum*. This structure has a β-D-mannopyranosidic linkage and a phosphocholine at the C-6 position of the glucosamine residue (Fig 1). Furthermore, Lochnit *et al.*[21] have been published the structure of the major component of amphoteric glycolipid, α-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-[cholinephosphoryl-(→6)]-β-D-Glc*p*NAc-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→)-Cer from *A. suum*, having many biological activities, namely stimulation-associated cytokines, tumor necrosis factor α (TNF-α), interleukin 1 (IL-1) and interleukin 6 (IL-6). We applied intramolecular aglycon delivery (IAD) approach for β-selective mannosylation[22] to the synthesis of the glycolipid analogue. The glycolipid was the target for the synthetic studies described herein as part of our investigation into synthetic oligosaccharides of structural and biological interest.

Results and Discussion

Synthesis of Monosaccharide Derivatives Syntheses of the glucopyranosyl and 2-deoxy-2-phthalimido-glucopyranosyl building blocks **4** and **10** were carried out as depicted in Chart 1. Reductive ring-opening of the benzylidene acetal in **3** with sodium cyanoborohydride-hydrogen chloride in dry diethylether afforded compound octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**).[23] On the other hand, imidate donor 3,4-di-*O*-benzyl-6-*O*-pivaloyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**10**) was obtained from 2-(trimethylsilyl)ethyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**5**)[24] by the following four step procedures. Regioselective tritylation of the starting material with trityl chlo-

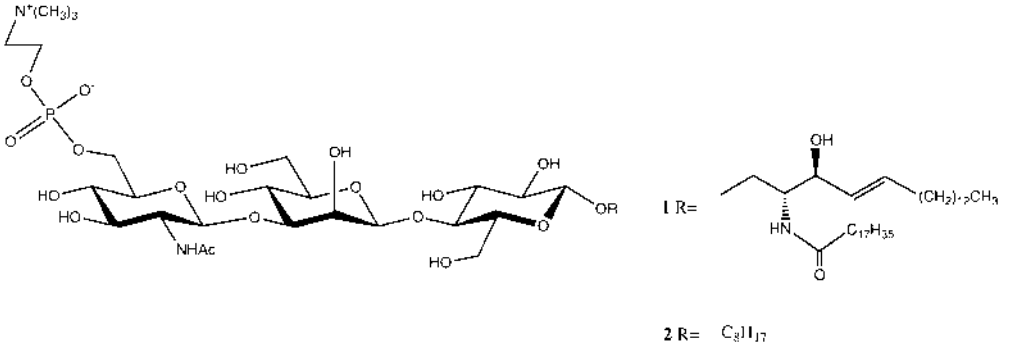

Fig. 1. Structure of an Amphoteric Glycosphingolipid from *Ascaris suum* (**1**) and Analogue (**2**)

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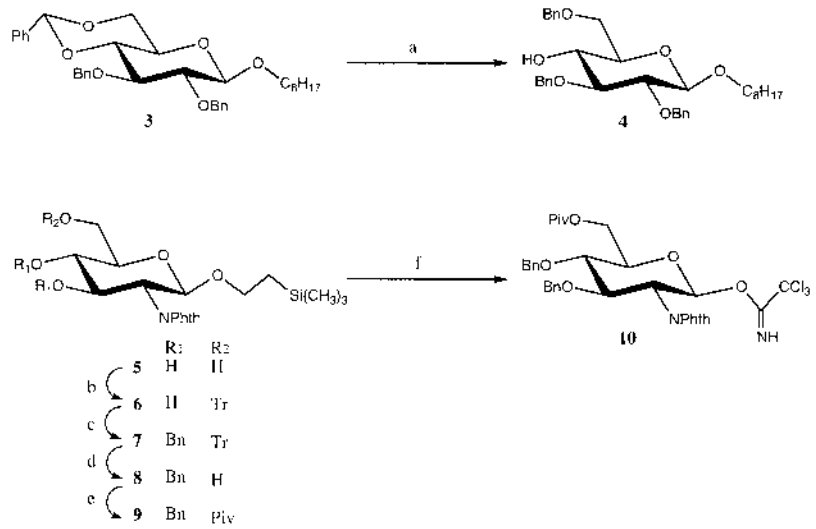
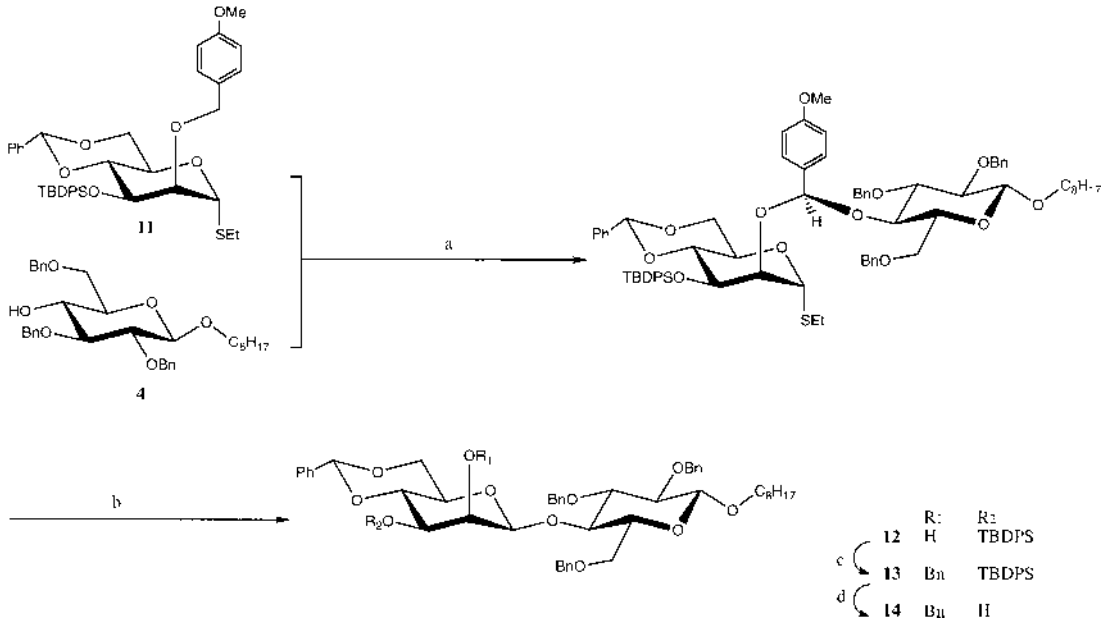


Reagents: (a) HCl–Et_2O, $NaBH_3CN$, THF, 65%; (b) trityl chloride, pyr., 77%; (c) BnBr, NaH, *n*-Bu_4NBr, DMF, 88%; (d) TsOH, CH_2Cl_2–MeOH, quant.; (e) PivCl, pyr., quant.; (f) (1) TFA, CH_2Cl_2, (2) DBU, CCl_3CN, CH_2Cl_2, quant.

Chart 1



Reagents: (a) DDQ, CH_2Cl_2; (b) MeOTf, DTBMP, CH_2Cl_2, 35% (2 steps); (c) BnBr, NaH, DMF, 72%; (d) TBAF, THF, 83%.

Chart 2

ride (**6**), followed by benzylation (**7**) and subsequent acid hydrolysis of the trityl group (**8**) and then pivaloylation, gave compound **9**. For selective removal of the 2-(trimethylsilyl)ethyl (SE) group, compound **9** was treated[24,25] with trifluoroacetic acid in dichloromethane for 1 h at 0 °C to give the 1-hydroxy compound, which, on further treatment[26] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane for 30 min at 0 °C, gave only the corresponding β-trichloroacetimidate **10** (Chart 1).

Synthesis of Oligosaccharide Derivatives β-Mannosylation was attempted in the intramolecular aglycon delivery approach. Thus, treatment of octyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**4**) with ethyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyl diphenylsilyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (**11**)[18,19] and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in dichloromethane in the presence of 4A molecular sieves (4A MS) afforded the mixed acetal in an essentially quantitative yield. A stereochemical assignment of this intermediate was mentioned by Ito and co-workers.[27] Subsequent IAD was effected by methyl trifluoromethanesulfonate (MeOTf) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in dichloromethane to afford the desired β-mannoside **12** in 35% yield and the α-mannoside was not detected. The anomeric hydrogen atom of the mannose unit appeared as a signal at δ 4.41 (s, H-1′) by the ^{1}H-NMR spectrum. The J_{CH} coupling constant of C-1 (159.3 Hz) confirmed the configuration of the β-mannoside linkage in the ^{13}C-

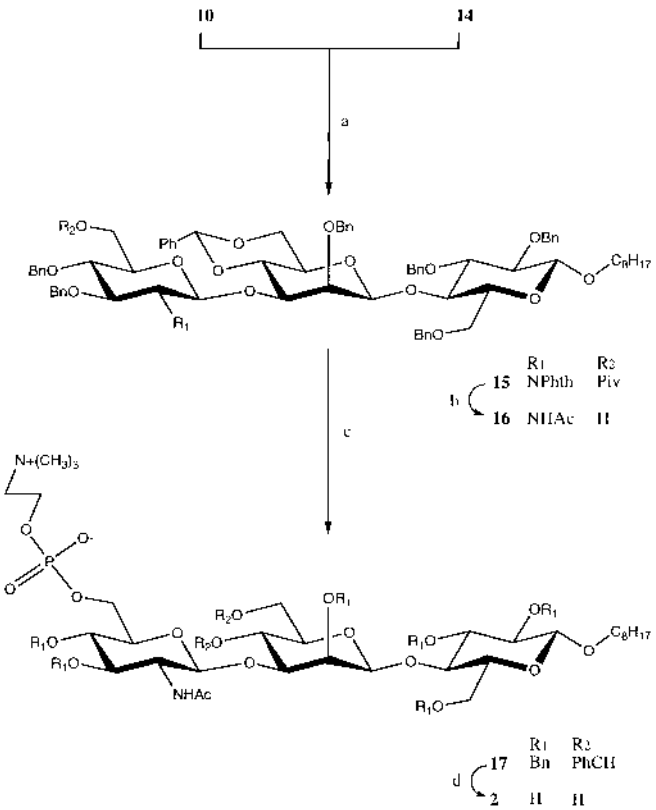
NMR spectrum.[28] Compound **12** was subjected to *O*-benzylation, followed by removal of *tert*-butyldiphenylsilyl group with tetrabutylammonium fluoride (TBAF), to give the disaccharide acceptor **14** (83%) (Chart 2). Glycosylation of **14** with the glycosyl donor **10** in the presence of trimethylsilyl trifluoromethane sulfonate (TMSOTf) and 4A MS in dichloromethane for 2 h at −25 °C gave the desired trisaccharide **15** in 94% high yield.[29] The ^{13}C-NMR spectrum showed three anomeric carbon atom signals at δ 103.42 (C-1), 102.12(C-1′) and 97.22 (C-1″). The ^{1}H-NMR spectrum of **15** showed an anomeric proton doublet for a β-glucosamine unit at δ 5.41 (d, $J_{1,2}$=7.9 Hz, H-1″) indicating the newly formed glycosidic linkage to be β-D configuration (Table 1). Removal of the pivaroyl and phthalimido group from **15** by NH_2NH_2–H_2O, then only *N*-acetylation gave **16** in 81% yield.

Synthesis of Trisaccharide Containing Phosphocholine Coupling of a phosphocholine group at the position C-6″ of **16** was attempted using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by reaction of the resulting cyclic phosphate intermediate with anhydrous trimethylamine at 65 °C in a sealed vessel to give **17** in 69% yield for two steps.[30] Compound **17** revealed an $[M+H]^+$ ion peak at *m*/*z* 1451 in the time of flight mass spectrometer (TOF-MS) spectrum. Removal of the benzyl groups from **17** by catalytic hydrogenolysis over 10% Pd–C gave the target compound **2** (Chart 3).

In conclusion, the analogue synthesis of novel amphoteric glycosphingolipid **1** was achieved by employing highly stereocontrolled glycosylation methods.

Table 1. ^{13}C-NMR Data (δ) for Selected Compounds

Carbon atom	Compound			
	12	**13**	**14**	**15**
C-1 (Glc)	103.7	103.5	103.6	103.4
($J_{C,H}$)	(157.2)	(153.1)	(155.7)	(159.3)
2	81.8	82.3	82.3	82.3
3	82.8	84.7	84.7	84.8
4	78.2	78.1	78.2	78.0
5	74.5	74.5	74.6	74.7
6	68.5	68.9	69.3	68.9
C-1 (Man)	100.1	101.7	101.9	102.1
($J_{C,H}$)	(159.3)	(163.5)	(159.2)	(159.3)
2	71.4	79.5	79.2	79.7
3	72.6	73.4	70.7	79.5
4	78.2	78.5	78.0	78.1
5	66.6	67.3	67.0	67.8
6	68.5	68.5	68.5	68.4
C-1 (GlcNAc)				97.2
($J_{C,H}$)				(167.5)
2				55.8
3				73.3
4				77.4
5				76.7
6				62.7
PhCH=	101.1	102.3	102.7	101.3
$PhCH_2$–	75.4	75.8	75.7	74.4
	74.9	75.4	75.2	74.9
	74.7	74.9	74.8	75.0
		74.4	74.7	75.1
				75.7
				76.5
OCH_2–	70.2	69.9	70.1	69.9



Reagents: (a) TMSOTf, CH_2Cl_2, 94%; (b) (1) NH_2NH_2–H_2O, EtOH, (2) Ac_2O, pyr., 81%; (c) (1) 2-chloro-2-oxo-1,3,2,-dioxaphospholane, Et_3N, benzene, (2) Me_3N, CH_3CN, 69%; (d) Pd–C, AcOH, MeOH, 90%.

Chart 3

Experimental

Optical rotations were determined with a JASCO digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM A 500 FT NMR spectrometer in $CDCl_3$ with Me_4Si as the internal standard. Matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF-MS) was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on Silica gel 60 F_{254} (E. Merck) with detection by quenching of UV fluorescence and by spraying with 10% H_2SO_4. Column chromatography was carried out on silica gel 60 (E. Merck). Octyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-*β*-D-glucopyranoside (**3**), 2-(trimethylsilyl)ethyl 2-deoxy-2-phthalimido-*β*-D-glucopyranoside (**5**) and ethyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyl diphenylsilyl-2-*O*-*p*-methoxybenzyl-1-thio-*α*-D-mannopyranoside (**11**) were prepared by literature method.[25—27]

Octyl 2,3,6-Tri-*O*-benzyl-*β*-D-glucopyranoside (4) To a solution of **3** (1.82 g, 3 mmol) and $NaBH_3CN$ (1.64 g, 24 mmol) in dry tetrahydrofuran (THF) (10 ml) was added 3A MS, and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. HCl–Et_2O was added until the solution was acidic (pH paper, gas evolution). After 1 h, the reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 6 : 1 hexane–ethyl acetate as eluent to give **4** (1.1 g, 66%).

2-(Trimethylsilyl)ethyl 6-*O*-Trityl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (6) To a solution of **5** (500 mg, 1.2 mmol) in pyridine (5 ml) was added trityl chloride (375 mg, 1.3 mmol) for 4 h at 50 °C, then concentrated. The product was purified by silica gel column chromatography using 20 : 1 $CHCl_3$–MeOH as eluent to give **6** (600 mg, 77%); $[\alpha]_D^{24}$ −16.5° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.85—7.24 (19H, m, Ph×4), 5.24 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.30 (1H, t, H-3), 4.13 (1H, t, H-2), 3.94 (1H, q, –OCH_2–), 3.62 (1H, t, H-4), 3.61 (1H, t, H-6a), 3.53—3.48 (2H, m, H-5, –CH_2–), 3.44 (1H, dd, H-6b), −0.08 (9H, s, $Si(CH_3)_3$). MALDI-TOF-MS: Calcd for $C_{38}H_{41}NO_7Si$: *m/z* 651. Found: *m/z* 674 $[M+Na]^+$.

2-(Trimethylsilyl)ethyl 3,4-Di-*O*-benzyl-6-*O*-trityl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (7) To a solution of **6** (580 mg, 0.9 mmol), tetrabutyl ammonium bromide (88 mg, 0.27 mmol) and BnBr (0.44 ml, 3.6 mmol) in *N*,*N*-dimethylformamide (DMF) (10 ml) was added NaH (66 mg, 0.27 mmol) for 2 h at 0 °C. The mixture was diluted with ethyl acetate (10 ml), then washed with water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 10 : 1 hexane–ethyl acetate as eluent to give **7** (666 mg, 88%); $[\alpha]_D^{24}$ +5.50° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.81—6.84 (34H, m, Ph×6), 5.20 (1H, d, $J_{1,2}$=7.9 Hz), 4.80, 4.69, 4.44, 4.42 (4H, each d, J_{gem}=11.2 Hz, benzyl methylene×2), 4.28 (1H, t, H-3), 4.26 (1H, t, H-2), 4.05—3.98 (2H, m, H-6a, –OCH_2–), 3.63 (1H, t, H-4), 3.60—3.54 (2H, m, H-5, H-6b), 3.28 (1H, q, –OCH_2–), −0.07 (9H, s, $Si(CH_3)_3$). MALDI-TOF-MS: Calcd for $C_{52}H_{53}NO_7Si$: *m/z* 831. Found: *m/z* 854 $[M+Na]^+$.

2-(Trimethylsilyl)ethyl 3,4-Di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (8) To a solution of **7** (315 mg, 0.34 mmol) in CH_2Cl_2 (3 ml) and MeOH (1.5 ml) was added *p*-toluene sulfonic acid (146 mg, 0.68 mmol) for 20 h at room temperature. The mixture was added Et_3N, and concentrated. The product was purified by silica gel column chromatography using 20 : 1 benzene–acetone as eluent to give **8** (293 mg, quant.); $[\alpha]_D^{24}$ +1.40° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.67—6.86 (14H, m, Ph×3), 5.19 (1H, d, $J_{1,2}$=7.9 Hz), 4.38, 4.80, 4.73, 4.45 (4H, each d, J_{gem}=11.0 Hz, benzyl methylene×2), 4.35 (1H, t, $J_{3,4}$=8.8 Hz, H-3), 4.20 (1H, t, H-2), 3.93 (1H, dd, H-6a), 3.87 (1H, q, –OCH_2–), 3.80—3.72 (2H, m, H-4, H-6b), 3.56 (1H, q, H-5), 3.43 (1H, q, –OCH_2–), −0.08 (9H, s, $Si(CH_3)_3$). MALDI-TOF-MS: Calcd for $C_{33}H_{59}NO_7Si$: *m/z* 589. Found: *m/z* 612 $[M+Na]^+$.

2-(Trimethylsilyl)ethyl 3,4-Di-*O*-benzyl-6-*O*-pivaloyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (9) To a solution of **8** (293 mg, 0.34 mmol) in pyridine (4 ml) was added pivaloyl chloride (2 ml) for 5 h at 0 °C. The mixture was diluted with MeOH (5 ml), then extracted with $CHCl_3$, washed with 5% HCl, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 4 : 1 hexane–ethyl acetate as eluent to give **9** (336 mg, quant.); $[\alpha]_D^{24}$ +0.58° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.67—6.87 (14H, m, Ph×3), 5.15 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.89, 4.80, 4.64, 4.67 (4H, each d, J_{gem}=11.2 Hz, benzyl methylene×2), 4.45 (1H, d, H-6a), 4.35 (1H, t, $J_{3,4}$=8.5 Hz, H-3), 4.22 (1H, dd, H-6b), 4.15 (1H, t, H-2), 3.86 (1H, q, –OCH_2–), 3.71 (1H, q, H-5), 3.64 (1H, t, $J_{4,5}$=9.8 Hz, H-4), 3.45 (1H, q, –OCH_2–), 1.26 (9H, t, $C(CH_3)_3$), −0.08 (9H, s, $Si(CH_3)_3$). MALDI-TOF-MS: Calcd for $C_{38}H_{47}NO_8Si$: *m/z* 673. Found: *m/z* 696 $[M+Na]^+$.

3,4-Di-*O*-benzyl-6-*O*-pivaloyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl Trichloroacetimidate (10) To a solution of **9** (26 mg, 0.04 mmol) in CH_2Cl_2 (1 ml), cooled to 0 °C, was added CF_3COOH (1 ml), and the mixture was stirred for 1 h at room temperature and concentrated. Ethyl acetate and toluene (1 : 2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (2 ml) cooled at 0 °C were added trichloroacetonitlile (161 *μ*l, 1.2 mmol) and DBU (6.9 *μ*l, 0.05 mmol). The mixture was stirred for 30 min at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (3 : 1 hexane–ethyl acetate) gave **10** (28 mg, quant.); $[\alpha]_D^{24}$ +10.3° (c=0.7, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 8.54 (1H, s, NH), 7.67—6.85 (14H, m, Ph×3), 6.41 (1H, d, $J_{1,2}$=8.5 Hz, H-1), 4.90, 4.83, 4.67, 4.50 (4H, each d, J_{gem}=10.9 Hz, benzyl methylene×2), 4.45—4.43 (3H, m, H-2, H-3, H-6a), 4.32 (1H, dd, H-6b), 3.89 (1H, q, H-5), 3.77 (1H, t, H-4), 1.24 (9H, t, $C(CH_3)_3$).

Octyl 4,6-*O*-Benzylidene-3-*O*-*tert*-butyldiphenylsilyl-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (12) To a stirred mixture of DDQ (248 mg, 1.1 mmol) and 4A MS in dry CH_2Cl_2 (3 ml) were added **11** (550 mg, 0.82 mmol), **4** (340 mg, 0.6 mmol) and 4A MS in dry CH_2Cl_2 (11 ml) at 0 °C. The mixture was stirred for 10 min at 0 °C and for 3 h at room temperature. The mixture was quenched with a solution of ascorbic acid (0.7%) and NaOH (0.9%) in water (5 ml), diluted with ethyl acetate and filtered off. The filtrate was successively washed with water, saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated to give mixed acetal. To a solution of this acetal, 2,6-di-*tert*-butyl-4-methyl-pyridine (DTBMP: 683 mg, 3.3 mmol) and 4A MS in dry CH_2Cl_2 (20 ml) were stirred for 2 h at room temperature, then cooled to 0 °C. Methyl trifluoromethanesulfonate (MeOTf: 340 ml, 3.1 mmol) was added to the mixture, which was stirred for 10 min at 0 °C and for 24 h at room temperature, then neutralized with Et_3N. The mixture was filtered off and washed with water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 8 : 1 hexane–ethyl acetate as eluent to give **12** (222 mg, 35%); $[\alpha]_D^{24}$ +11.2° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.66—7.14 (30H, m, Ph×6), 5.34 (1H, s, benzylidene methyne), 4.95, 4.87, 4.75, 4.68, 4.57, 4.30 (6H, each d, J_{gem}=10.4 Hz, benzyl methylene×3), 4.41 (1H, s, H-1′), 4.33 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.04 (1H, dd, $J_{5,6a}$=10.4 Hz, $J_{6a,6b}$=4.9 Hz, H-6a′), 3.92 (1H, q, –OCH_2–), 3.90 (1H, t, $J_{4,5}$=9.2 Hz, H-4′), 3.85 (1H, t, $J_{3,4}$=9.2 Hz, H-3), 3.72 (1H, dd, $J_{2,3}$=3.7 Hz, $J_{3,4}$=9.8 Hz, H-3′), 3.60—3.59 (2H, m, H-4, H-6b), 3.58 (1H, d, $J_{2,3}$=3.7 Hz, H-2′), 3.56 (1H, t, $J_{6a,6b}$=10.4 Hz, H-6b′), 3.48—3.45 (2H, m, H-5, –OCH_2–), 3.38—3.35 (2H, m, H-2, H-6b), 2.89 (1H, q, H-5′), 2.69 (1H, s, OH), 1.64 (2H, q, –$OCH_2\underline{CH_2}$–), 1.25 (10H, s, –CH_2–×5), 1.03 (9H, s, CH_3×3), 0.88 (3H, t, –$CH_2\underline{CH_3}$). ^{13}C-NMR ($CDCl_3$): δ 103.7 (J_{CH}=157.2 Hz, C-1), 101.1 (benzylidene methyne), 100.1 (J_{CH}=159.3 Hz, C-1′), 82.8 (C-3), 81.8 (C-2), 78.1 (C-4′), 78.1 (C-4), 75.4, 74.9, 74.7 (benzyl methylene), 74.3 (C-5), 72.6 (C-3′), 71.4 (C-2′), 70.2 (–OCH_2–), 68.6 (C-6), 68.5 (C-6′), 66.6 (C-5′), 31.8 (–$OCH_2\underline{CH_2}$–), 29.8, 29.7, 29.4, 29.3 (–$\underline{CH_2}$–), 26.8 (CH_3×3), 26.2 (–$\underline{CH_2}CH_3$), 14.1 (–$CH_2\underline{CH_3}$). MALDI-TOF-MS: Calcd for $C_{64}H_{78}O_{11}Si$: *m/z* 1050. Found: *m/z* 1073 $[M+Na]^+$.

Octyl 2-*O*-Benzyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldiphenylsilyl-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (13) To a solution of **12** (437 mg, 0.41 mmol) and BnBr (0.10 ml, 0.8 mmol) in DMF (5 ml) was added NaH (14.9 mg, 0.62 mmol) for 2 h at 0 °C. The mixture was diluted with ethyl acetate (10 ml), washed with water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 10 : 1 hexane–ethyl acetate as eluent to give **13** (341 mg, 72%); $[\alpha]_D^{24}$ −8.71° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.65—7.12 (35H, m, Ph×7), 5.31 (1H, s, benzylidene methyne), 4.95, 4.93, 4.92, 4.77, 4.76, 4.71, 4.68, 4.43 (8H, each d, J_{gem}=11.6 Hz, benzyl methylene×4), 4.30 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.22 (1H, s, H-1′), 4.19 (1H, dd, $J_{5,6a}$=10.4 Hz, $J_{6a,6b}$=4.88 Hz, H-6a′), 4.10 (1H, t, H-6a), 4.05 (1H, t, $J_{4,5}$=9.9 Hz, H-4′), 3.86 (1H, dd, $J_{2,3}$=3.3 Hz, $J_{3,4}$=12.8 Hz, H-3′), 3.83—3.78 (2H, m, H-6b, –OCH_2–), 3.51 (1H, d, H-2′), 3.46—3.45 (2H, m, H-5, –OCH_2–), 3.39—3.31 (3H, m, H-2, H-4, H-6b), 3.01 (1H, q, H-5′), 1.55 (2H, q, –$OCH_2\underline{CH_2}$–), 1.25 (10H, s, –CH_2–×5), 1.00 (9H, s, CH_3×3), 0.88 (3H, t, –$CH_2\underline{CH_3}$). ^{13}C-NMR ($CDCl_3$): δ 103.5 (C-1), 102.3 (benzylidene methyne), 101.7 (C-1′), 84.7 (C-3), 82.3 (C-2), 79.5 (C-2′), 78.5 (C-4′), 78.1 (C-4), 75.7, 75.4, 74.9, 74.4 (benzyl methylene×4), 74.5 (C-5), 73.4 (C-2), 69.9 (–OCH_2–), 68.9 (C-6), 68.5 (C-6′), 67.3 (C-5), 31.6 (–$OCH_2\underline{CH_2}$–), 29.9, 29.7, 29.4, 29.3 (–$\underline{CH_2}$–), 26.8 (CH_3×3), 26.0 (–$\underline{CH_2}CH_3$), 14.3 (–$CH_2\underline{CH_3}$). MALDI-TOF-MS: Calcd for $C_{71}H_{84}O_{11}Si$: *m/z* 1140. Found: *m/z* 1063 $[M+Na]^+$.

Octyl 2-*O*-Benzyl-4,6-*O*-benzylidene-*β*-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-*β*-D-glucopyranoside (14) To a solution of **13** (180 mg, 0.15 mmol) in THF (3 ml) was added tetrabutylammoniumfluoride, 1.0 M solution in THF (TBAF: 4 ml). The reaction mixture was stirred for 2 h at room temperature, then extracted with $CHCl_3$, washed with 5% HCl, dried (Na_2SO_4)

and concentrated. The product was purified by silica gel column chromatography using 3 : 1 hexane–ethyl acetate as eluent to give **14** (115 mg, 83%); $[\alpha]_D^{24}$ −12.7° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.48—7.25 (25H, m, Ph×5), 5.53 (1H, s, benzylidene methyne), 5.06, 4.96, 4.94, 4.86, 4.78, 4.71, 4.66, 4.54 (8H, each d, J_{gem}=11.0 Hz, benzyl methylene×4), 4.49 (1H, s, H-1′), 4.38 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.30 (1H, dd, $J_{5,6a}$=10.4 Hz, $J_{6a,6b}$=4.9 Hz, H-6a′), 4.19 (1H, d, H-6a), 3.88—3.78 (3H, m, H-2′, H-4′, H-6b′, H-3, –OCH_2–), 3.69—3.66 (2H, m, H-4, H-3′), 3.58—3.54 (2H, m, H-6b, –OCH_2–), 3.45—3.35 (2H, m, H-2, H-5), 3.28 (1H, q, H-5′), 2.38 (1H, s, OH), 1.59 (2H, s, –$OCH_2C\underline{H}_2$–), 1.25 (10H, s, –CH_2–×5), 0.87 (3H, t, –CH_2CH_3). ^{13}C-NMR ($CDCl_3$): δ 103.6 (C-1), 102.7 (benzylidene methyne), 101.9 (C-2′), 84.7 (C-3), 82.3 (C-2), 79.2 (C-2′), 78.2 (C-4), 78.0 (C-4), 75.7, 75.6, 74.8, 74.7 (benzyl methylene×4), 74.6 (C-5), 70.1 (–$O\underline{C}H_2$–), 69.3 (C-6), 68.5 (C-6′), 67.0 (C-5′), 31.6 (–$OCH_2\underline{C}H_2$–), 29.9, 29.7, 29.4, 29.3 (–$\underline{C}H_2$–), 26.0 (–$\underline{C}H_2CH_3$), 14.3 (–$CH_2\underline{C}H_3$). MALDI-TOF-MS: Calcd for $C_{55}H_{66}O_{11}$: m/z 903. Found: m/z 926 $[M+Na]^+$.

Octyl 3,4-Di-*O*-benzyl-6-*O*-pivaloyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15) To a solution of **14** (30 mg, 0.03 mmol), **10** (28 mg, 0.04 mmol) and 4A MS in dry CH_2Cl_2 (2 ml) was stirred for 2 h at room temperature and then cooled to −25 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf: 1.4 ml, 7.9 mmol) was added and the mixture was stirred for 1 h at −25 °C and for 24 h at room temperature. The mixture was filtered off and extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 40 : 1 benzene–acetone as eluent to give **15** (41 mg, 94%); $[\alpha]_D^{24}$ −7.60° (c=1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 5.41 (1H, d, H-1″), 4.31 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.24 (1H, s, H-1′). ^{13}C-NMR ($CDCl_3$): δ 103.42 (C-1), 102.12 (C-1′), 97.22 (C-1″). MALDI-TOF-MS: Calcd for $C_{88}H_{99}NO_{18}$: m/z 1458. Found: m/z 1481 $[M+Na]^+$.

Octyl 3,4-Di-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16) To a solution of **15** (40 mg, 27.4 mmol) in EtOH (5 ml) was added hydrazine monohydrate (3 ml). The reaction mixture was refluxed for 3 h, then concentrated. The residue was *N*-acetylated with Ac_2O (2 ml) and triethylamine (0.1 ml) in MeOH (4 ml). The mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The product was purified by silica gel column chromatography using 5 : 1 benzene–acetone as eluent to give **16** (28.5 mg, 81%); $[\alpha]_D^{24}$ −0.91° (c=0.7, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 5.10 (1H, d, H-1″), 4.38 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.30 (1H, s, H-1′). MALDI-TOF-MS: Calcd for $C_{77}H_{91}NO_{16}$: m/z 1285. Found: m/z 1308 $[M+Na]^+$.

Octyl 3,4-Di-*O*-benzyl-6-*O*-phosphocoline-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (17) To a cooled solution of **16** (60 mg, 46.7 μmol) and Et_3N (51 ml, 0.37 mmol) in dry benzene (1 ml) was added dropwise 2-chloro-2-oxo-1,3,2-dioxaphospholane (34 μl, 0.37 mmol). The mixture was stirred for 1 h at room temperature. The crystalline Et_3N–HCl was removed by filtration, and solvent was concentrated. The residue was dissolved saturated Me_3N in CH_3CN (3 ml), then heated in an oil bath for 48 h at 65 °C. The reaction mixture was cooled, the white solid was dissolved in chloroform and concentrated. The product was purified by iatrobeads chromatography using 1 : 1 $CHCl_3$–MeOH then 1 : 1 H_2O–MeOH as eluent to give **17** (47.8 mg, 69%); $[\alpha]_D^{24}$ −14.9° (c=1.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 5.11 (1H, d, H-1″), 4.30 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.28 (1H, s, H-1′), 4.11 (2H, m, $OC\underline{H}_2CH_2$), 3.39 (2H, m, $CH_2C\underline{H}_2N$), 2.97 (9H, s, $N(CH_3)_3$). MALDI-TOF-MS: Calcd for $C_{82}H_{103}N_2O_{19}P$: m/z 1450. Found: m/z 1451 $[M+H]^+$.

Octyl 6-*O*-Phosphocholine-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside (2) A solution of **17** (47.8 mg, 0.33 mmol) in MeOH (3 ml) and AcOH (1 ml) was hydrogenated over 10% Pd–C (50 mg) for 24 h at room temperature, then filtered through Celite and the residue was washed with methanol and concentrated. Column chromatography (1 : 1 H_2O : MeOH) of the residue on Sephadex LH-20 gave **2** (24 mg, 90%); $[\alpha]_D^{24}$ −11.9° (c=0.6, H_2O); ^{1}H-NMR (1 : 1 CD_3OD : $CDCl_3$) δ: 4.58 (1H, d, $J_{1,2}$=8.5 Hz, H-1″), 4.48 (1H, s, H-1′), 4.18 (1H, d, $J_{1,2}$=7.9 Hz, H-1), 4.08 (2H, m, $OC\underline{H}_2CH_2$), 3.39 (2H, m, $CH_2C\underline{H}_2N$), 3.15 (9H, s, $N(CH_3)_3$). HR-FAB-MS: Calcd for $C_{33}H_{64}N_2O_{19}P$ $[M+H]^+$: m/z 823.3841. Found: m/z 823.3878.

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