Synthesis of inositol glycan cyclic phosphates

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Abstract

An efficient synthesis of tri-, tetra-, and pentasaccharide cyclic phosphates 1–5, structurally related to natural inositol phosphate glycans, is reported. The title compounds were assembled by PhSeOTf-promoted glycosylation of the known glucosamine precursor, t-butyldimethylsilyl 2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (8) with protected 1-methylthio mono-, di-, and trimannosides 7a–c, and, after conversion into glycosyl fluorides, Cp₂ZrCl₂–AgOTf-promoted glycosylation of differentially protected optically pure 1D-myo-inositol 11. The syntheses were completed by installing the cyclic phosphate moieties with methylpyridinium dichlorophosphate and finally, removal of all protecting groups by dissolving-metal reduction. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the US, approximately 16 million people suffer from diabetes mellitus. Ninety to ninety-five percent of these are afflicted by non-insulin dependent diabetes mellitus (NIDDM), a condition characterized by insulin resistance. Diminished binding of insulin to its receptor usually does not adequately account for the decreased responsiveness on the cellular level. Therefore, NIDDM may be considered a disease of insulin signal transduction.1

During the past 15 years, small inositol-containing oligosaccharides have been implicated as signaling molecules in the insulin signal transduction pathway.2 These oligosaccharides are believed to be structurally similar to the glycosylphosphatidyl-inositol (GPI) membrane anchors. This idea is supported by the observation that the compound obtained by PI-PLC and Pronase release of the variant surface glycoprotein (VSG) GPI anchor from variant clone 118 of Trypanosoma brucei strain 427, presumed to have structure 6, is insulin mimetic.3,14

However, due to the miniscule amounts of biological material available and the heterogeneity of many isolates, the precise chemical structures of these putative insulin mediators are not known. At least two general classes of compounds have been identified. One class, referred to by Larner’s group4 as the pH 1.3 mediator because of the pH of an eluting solvent during purification, inhibits cAMP-dependent protein kinase and contains myo-inositol and glucosamine.5 The other, so-called pH 2 mediator, stimulates pyruvate dehydrogenase phosphatase and contains chiro-inositol and galactosamine.6

The lack of complete structural characterization has led to intense synthetic efforts by several groups7–11 in the hope of determining the structural features necessary for mimicking insulin action. Herein, we report the syn-
Fig. 1. Synthetic compounds 1–5, and natural VSG membrane anchor fragment 6.

2. Results and discussion

Synthetic strategy.—The syntheses of compounds 1–5 were designed to be modular so that assembly of a variety of oligosaccharides could be achieved rapidly (Scheme 1). The two terminal portions of each target compound, an oligomannose unit and an inositol unit, would be attached to a suitably protected linking glucosamine unit. To minimize the number of manipulations, all protecting groups remaining after final assembly of the oligosaccharides were chosen to be removable in a single deprotection step.

The known glucosamine precursor t-butyldimethylsilyl 2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (8) satisfied these criteria. We envisioned glycosylating the 4-position of 8 with mono-, di-, and trimannoses 7a, 7b, or 7c to provide the corresponding di-, tri-, and tetrasaccharides 9a–c. The methyl 1-thio-α-glycosides 7a–c were selected to utilize Ogawa’s α-selective phenylselenyl triflate-promoted glycosylation procedure. After desilylation and conversion into the glycosyl fluorides 10a–c, zirconocene dichloride-promoted coupling to myo-inositol 11 would produce fully protected tri-, tetra-, and pentasaccharides 12a–c, respectively. This glycosylation method was chosen since both the α and β anomers of the oligosaccharides were desired. Recent work by Müller’s group has
demonstrated that some IPGs with β-D inositol linkages are insulin mimetic.

Inositol 11 was chosen to allow selective removal of the carbonate moiety of 12a–c, thereby freeing the hydroxyl groups to be esterified in the cyclic phosphate moiety of the targets. Cyclic phosphorylation could then be accomplished in one step by addition of methylpyridinium dichloro-phosphate (31). Finally, removal of the benzyl protecting groups and reduction of the azido functionality in a single reductive deprotection step would produce targets 1–5.

**Synthesis of mannose methyl 1-thioglycosides 7a–c.**—Selective dimethoxytritylation of the C-6 hydroxyl of the known mannose glycoside 1319,20 (Scheme 2) with 4,4'-dimethoxytrityl tetrafluoroborate21 yielded triol 14. Exhaustive benzylaition under standard conditions ((i) NaH, DMF, 0 °C; (ii) BnBr) yielded fully protected 15. Removal of the DMT protecting group with 50% HOAc provided alcohol 16 in 64% overall yield from 13.

![Scheme 1. Overview of the syntheses of compounds 1–5.](image)

![Scheme 2.](image)
Coupling of 16 to the known methyl 1-thiomannoside 17\textsuperscript{16} (Scheme 3) using Ogawa’s protocol\textsuperscript{22} yielded the differentially protected mannose disaccharide 18. The \( \alpha \) configuration of the glycosidic linkage in the product was confirmed by the \( ^{13}\text{C} - ^{1}\text{H} \) coupling constant (171 Hz) at the anomeric position according to the observation of Bock and Pedersen\textsuperscript{23} that \( \alpha \)-mannnosides exhibit anomeric \( J_{\text{C,H}} \sim 170 \text{ Hz} \) while \( \beta \)-mannnosides exhibit anomeric \( J_{\text{C,H}} \sim 160 \text{ Hz} \). In every case in our work where a glycosidic bond was formed from mannose, the anomeric configuration of the product(s) was confirmed by this method.

Deacetylation of 18 with methanolic ammonia produced alcohol 19, which was a common intermediate in the synthesis of methyl 1-thioglycoside donors 7\textsubscript{b} and 7\textsubscript{c} (Schemes 3 and 4).

The synthesis of 7\textsubscript{b} was easily realized as shown in Scheme 3. Compound 19 was benzylationed to produce 20 and this was subjected to electrochemical oxidation in aqueous acetonitrile\textsuperscript{24} to remove the \( p \)-methoxyphenyl (PMP) protecting group providing an anomic mixture of alcohols 21. This mixture was readily converted into a mixture of anomic fluorides 22 by treatment with DAST.\textsuperscript{25} Finally, SnCl\textsubscript{4}-promoted thiomethylation with Bu\textsubscript{3}SnSMe\textsuperscript{16,26} produced the glycosyl donor 7\textsubscript{b}, ready to couple with acceptor 8.

For the synthesis of trisaccharide 7\textsubscript{c} (Scheme 4), the common intermediate 19 was glycosylated with methyl 1-thiomannoside 17 to produce trisaccharide 23. Elaboration of 23 to 7\textsubscript{c} proceeded in direct analogy to the synthesis of 7\textsubscript{b}, producing 7\textsubscript{c} in 24\% overall yield from the common intermediate 19.

Synthesis of myo-inositol 11.—The differentially protected inositol 11 (Scheme 5), ready for coupling, was prepared from known, optically pure diol 28\textsuperscript{27} by protection of the two free hydroxyl groups as the cyclic carbonate (\( N,N' \)-carbonyldimidazole, benzene) followed by removal of the triisopropylsilyl (TIPS) protecting group. Use of acetyl groups to protect the 1- and 2-positions of the inositol ring proved unsuitable because of acetyl group migrations upon fluoride-induced removal of the TIPS group at position 6. The cyclic carbonate did not suffer migration, presumably because the more rigid structure prevents intramolecular attack of the intermediate C-6 alkoxy on the carbonyl group.

Synthesis of oligosaccharides 1–5.—With the necessary precursors in hand, each final oligosaccharide could be synthesized in seven steps (Scheme 6). The mannose mono-, di-,
and trisaccharides 7a–c were coupled with glucosamine derivative 8 utilizing Ogawa’s method\textsuperscript{16} resulting in $\alpha$-linked disaccharide 9a, trisaccharide 9b, and tetrasaccharide 9c in 56, 36, and 37% yield, respectively. Fluoride-promoted desilylation of each, followed by conversion into the anomeric fluorides with DAST yielded glycosyl donors suitable for coupling with the inositol. Silver triflate and Cp$_2$ZrCl$_2$-promoted coupling of 11 with 10a–c yielded the corresponding trisaccharide 12a, tetrasaccharide 12b, and pentasaccharide 12c. In each case, a mixture of $\alpha$ and $\beta$ anomers was obtained. These were separated chromatographically and each was carried forward independently.

Removal of the cyclic carbonate moiety by alkaline hydrolysis yielded diols ready for installation of the cyclic phosphate moiety. Addition of methylpyridinium dichlorophosphate (31), prepared according to the literature procedure\textsuperscript{18} from methyl phosphorodichloridate and pyridine, to diols 30az\textsubscript{x}, 30az\textsubscript{β}, 30bz\textsubscript{z}, 30bz\textsubscript{β}, and 30cz\textsubscript{α} resulted in the formation of the protected oligosaccharide cyclic phosphates. Finally, dissolving-metal reduction (Na (s), NH$_3$ (l), $-78 ^\circ$C, 15 min) followed by careful quenching at $-78 ^\circ$C with NH$_4$Cl (s), then MeOH, resulted in removal of all benzyl protecting groups and reduction of the azido group to provide compounds 1–5 contaminated with NaCl. Pure products were obtained by desalting with Sephadex G-10 (56–85% yield).

The biological activity of these compounds is currently being evaluated and will be reported elsewhere.
3. Experimental

**General methods.**—All nonaqueous reactions were performed under an Ar atmosphere. Organic extracts were dried with anhyd MgSO₄ unless otherwise noted. Solvents were removed in vacuo on a Büchi rotary evaporator. Solvents and reagents obtained from commercial sources were used without further purification with the following exceptions. Tetrahydrofuran (THF) and benzene were distilled from Na and benzophenone. Acetonitrile, CH₂Cl₂, pyridine, and toluene were distilled from CaH₂. Benzyl bromide was fractionally distilled. N,N'-Dimethylformamide (DMF) was dried with MgSO₄, filtered, and then distilled. 1,2-Dichloroethane was dried with MgSO₄, filtered, and distilled from P₂O₅. Silver triflate was dried in vacuo (0.1 mmHg) for 24 h. Reactions were monitored by thin-layer chromatography (TLC) on Baker glass-backed silica gel plates (0.25 μm thickness) with a 254 nm fluorescent indicator. Chromatograms were visualized by one or more of the following techniques: (a) ultraviolet illumination; (b) dipping in an ethanolic solution of 2.5% p-anisaldehyde, 3.5% H₂SO₄ and 1% AcOH followed by heating; (c) dipping in an ethanolic solution of Hanes–Isherwood stain (ammonium molybdate–HCl–perchloric acid–acetone); (d) dipping in a 2-propanol solution of ninhydrin–AcOH–pyridine. Purifications were performed by flash chromatography on Baker silica gel (40 μm), by preparative TLC, or by gel-filtration utilizing Sephadex G-10. Nuclear magnetic resonance (NMR) data were obtained on a Bruker AM-300 FT NMR spectrometer operating at 300 MHz for ¹H. Tetramethylsilane (Me₄Si, 0.03%) was used as the internal standard for most ¹H and ¹³C NMR spectra. CFCl₃ and H₃PO₄ (85% in D₂O) were used as the external standards for the ¹⁹F and ³¹P NMR spectra, respectively. High-resolution and low-resolution mass spectrometry data were obtained on a JEOL AX-
was added and the mixture was extracted with ether (4 × 1 mL). The ether layer was washed with NaHCO₃ (1 M, 4 × 1 mL), dried, and concentrated. The crude sample was used directly in the next step. A small portion of the crude product was purified for analysis using flash silica gel chromatography with step gradient elution. To prevent hydrolysis, the column was first treated with 1% triethylamine in hexane. Compound 15 was purified via flash-column chromatography utilizing step gradient elution: hexane to remove excess BnBr followed by 2:1 hexane–ether to obtain a pure sample of 15; R, 15 0.40 (2:1 hexanes–ether); ¹H NMR (CDCl₃): δ 3.24 (dd, 1 H, J 5.0 Hz), 3.45 (d, 1 H, J 10.0 Hz), 3.76 (s, 9 H, OCH₃), 3.8–4.1 (m, 4 H), 4.3 (d, 1 H, J 11.0 Hz), 4.68–4.73 (m, 4 H), 4.88 (d, 1 H, J 11.0 Hz), 5.5 (s, 1 H, H-1), 6.74–6.82 (m, 5 H), 6.9 (d, 2 H, J 9.0 Hz), 7.02 (d, 2 H, J 9.0 Hz), 7.17–7.48 (m, aryl H’s, 21 H).

p-Methoxyphenyl 2,3,4-tri-O-benzyl-α-D-mannopyranoside (16).—To a solution of 15 (1.0 g, 1.0 mmol) in CHCl₃ (8 mL) was added a solution of 4 mL of 1:1 AcOH–water and the mixture was stirred for 2 h at 80 °C. When the reaction was complete, ether (25 mL) was added and the solution was neutralized with NaHCO₃ (1 M, 20 mL). The layers were separated and the aqueous layer was extracted with ether (3 × 50 mL). The combined organic extracts were dried, filtered, and concentrated. Compound 16 was purified via flash-column chromatography utilizing step gradient elution: 2:1 hexane–ether was first used to remove nonpolar side-products followed by 1:1 hexane–ether to obtain pure 16 (454 mg, 70% yield); R, 16 0.20 (1:1 hexanes–ether); ¹H NMR (CDCl₃): δ 1.9 (bs, 1 H, OH), 3.75 (s, 3 H, OCH₃), 3.77 (m, 2 H), 3.92 (m, 1 H, J 4.0 Hz), 3.98 (d, 1 H, J 10.0 Hz), 4.05–4.15 (m, 2 H), 4.69–4.77 (m, 5 H, H-2, 4 H, CH₂Ph), 4.82 (d, 1 H, J 10.0 Hz, CH₂Ph), 4.93 (d, 1 H, J 10.0 Hz, CH₂Ph), 5.40 (s, 1 H, H-1), 6.78 (d, 2 H, J 9.0 Hz, PMP), 6.88 (d, 2 H, J 9.0 Hz, PMP), 7.35–7.45 (m, 15 H, phenyl H’s); HRMS: Calcd for C₃₃H₃₆O₇Na⁺: 579.2359; Found: 579.2352.

p-Methoxyphenyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (18).—To a flame-dried flask was added 4 A molecular sieves (~100 mg), phenylselenium chloride
(0.258 g, 1.349 mmol) and toluene (2 mL). The mixture was cooled to −30 °C [CH3CN–CO2(s)] and silver trifluoromethanesulfonate (462 mg, 1.798 mmol) was added. Slowly a solution of 16 (500 mg, 0.899 mmol) and 1716 (704 mg, 1.349 mmol) in toluene (2 mL) was added to the vigorously stirred mixture. After ~30 min, NaHCO3 (1 M, ~5 mL) and bleach (~5 mL) were added to the reaction mixture. The mixture was stirred until the solution was no longer orange (pale yellow) and extractions were done with ether (3 × 5 mL). The combined organic layers were dried, filtered and concentrated. Compound 18 was purified via flash-column chromatography utilizing step gradient elution: hexane was first used to remove nonpolar side-products followed by 3:2 hexane–ether to obtain pure 18 (56–71% yield); Rf 17 0.63, Rf 18 0.49, Rf 16 0.14 (1:1 hexane–ether); 1H NMR (CDCl3): δ 2.14 (s, 3 H, CH3CO), 3.58 (s, 3 H, CH3O), 3.62 (2pseudo-t, 2 H, H-4 mannose-1, H-4 mannose-2), 3.73 (dd, 1 H, J 3.7, J 10 Hz, H-3 mannose-1), 3.82–3.89 (m, 6 H), 3.96 (dd, 1 H, H-2 mannose-1), 4.12 (dd, 1 H, J 8.9, J 3 Hz, H-3 mannose-2), 4.37 (d, 1 H, J 11 Hz, CH2Ph), 4.42 (d, 1 H, J 11 Hz, CH2Ph), 4.45 (d, 1 H, J 11 Hz, CH2Ph), 4.51 (d, 1 H, J 11 Hz, CH2Ph), 4.59 (d, 1 H, J 11 Hz, CH2Ph), 4.66 (d, 1 H, J 11 Hz, CH2Ph), 4.77 (s, 2 H, CH2Ph), 4.86 (d, 1 H, J 11 Hz, CH2Ph), 4.90 (d, 1 H, J 1.8 Hz, H-2 mannose-2), 4.93 (d, 1 H, J 11 Hz, CH2Ph), 5.40 (s, 1 H, H-1 mannose-1), 5.42 (d, J 1.8 Hz, H-1 mannose-2), 6.78 (d, 2 H, J 9 Hz, PMP), 6.98 (d, 2 H, J 9 Hz, PMP), 7.15–7.42 (m, 30 H, phenyl H); 13C NMR (CDCl3): δ 21 (H2CCO), 55 (CH2O), 66.4 (CH2), 68.4 (CH2), 68.6 (CH), 71.2 (CH), 71.4 (CH2), 71.6 (CH), 72.2 (CH2), 72.7 (CH2), 73.2 (CH2), 74.1 (CH), 74.5 (CH), 75.0 (CH), 77.0 (t, CDCl3), 77.8 (CH), 80.0 (CH), 96.6 (d, J 171 Hz, C-1), 97.8 (d, J 171 Hz, C-1), 114.5 (CH, PMP), 117.4 (CH, PMP), 127.6–128.3 (phenyl Cs), 137.9–138.6 (phenyl Cs), 150.1 (C-O, PMP), 154.8 (C-O, PMP), 170.1 (C-O).

p-Methoxycinnamyl 3,4,6-Tri-O-benzyl-α-D-mannopyranosyl-(1 →6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (19).—Compound 18 (550 mg, 0.534 mmol) was dissolved in CH2Cl2 (5 mL) and transferred to a pressure vessel. Methanol (5 mL) was added and the mixture was cooled to 0 °C. Ammonia (g) was bubbled through the solution for 5 min then the flask was tightly stoppered. The solution was stirred for 12 h at 20 °C, cooled again to 0 °C and additional NH3 (g) was added. The reaction was then stirred for 24 h at 20 °C. The solution was then transferred to a round-bottom flask and concentrated. Purification via silica gel-flash chromatography (1:1 hexane–ether) produced 19 (412 mg, 78% yield). Crude 19 was not usually purified and was used directly in the production of 20; Rf 18 0.97, Rf 19 0.40 (1:2 hexane–ether); 1H NMR (CDCl3): δ 2.28 (s, 1 H, OH), 3.48 (s, 3 H, CH2O), 3.50 (s, 1 H), 3.54–3.57 (m, 2 H), 3.63–3.76 (m, 5 H), 3.81–3.82 (m, 2 H), 3.85–3.86 (m, 1 H), 3.95 (dd, 1 H, J 3, J 11 Hz), 4.30 (d, 2 H, J 11 Hz), 4.36 (d, 2 H, J 11 Hz), 4.37 (s, 1 H), 4.46 (d, 1 H, J 11 Hz), 4.54 (s, 2 H), 4.58 (d, 2 H), 4.64 (d, 1 H, J 11 Hz), 4.75 (d, 1 H, J 11 Hz), 4.81 (d, 1 H, J 11 Hz), 5.22 (s, 1 H, H-1), 5.63 (d, 2 H, J 9 Hz, PMP), 6.68 (d, 2 H, J 9 Hz, PMP), 6.91–7.15 (m, 30 H, phenyl H’s).

p-Methoxycinnamyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1 →6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside (20).—Compound 19 (400 mg, 0.405 mmol) was dried by coevaporation with toluene, then dissolved in DMF (0.4 mL). The solution was cooled to 0 °C and NaH (40 mg of a 50% dispersion in mineral oil, 0.81 mmol) was added. The mixture was stirred for 30 min, and then BnBr (136 mg, 0.906 mL, 0.81 mmol) was added. The mixture was stirred in the dark at 20 °C and monitored by TLC. When the reaction was complete (~1 h), 5.0 mL of ether was added and the mixture was washed with NaHCO3 to remove the DMF. The ether layer was dried with MgSO4, filtered, and concentrated. Compound 20 was purified via flash-column chromatography. The crude evaporate was applied to a hexane-equilibrated column as a solution in hexane with a few drops of CH2Cl2 added to enhance solubility. The column was eluted with a step gradient: hexane was first used to remove excess BnBr followed by 1.5:1 hexane–ether to obtain pure 20 (370 mg, 85% yield); Rf 20 0.80, Rf 19 0.30 (1:1 hexane–ether); 1H NMR (CDCl3): δ 3.60 (s, 3 H, CH2O), 3.65–4.15 (m, 12 H, mannose), 4.45–4.55 (m, 4 H), 4.65–4.82 (m, 8 H), 4.88–4.95
A mixture was extracted with ether (3 mL), water (30 mL) was added, and the reaction was complete, the solution was transferred to 20 °C and cooled to 0 °C. When the reaction was complete, NaF (1.3 mL stock solution) was added. The yellowish solution was stirred at 0 °C for 15 min. When the reaction was complete, the solution was stirred for 72 h at 25 °C as a voltage of +1.55 V was applied. When the reaction was complete, the solution was transferred to a round-bottom flask and concentrated; water (30 mL) was added, and the mixture was extracted with ether (3 × 30 mL). The white precipitate, Bu₄NPF₆, was collected by filtration for future reuse. The combined ether layers were dried, filtered, and concentrated. Compounds 21 were purified via flash-column chromatography utilizing step gradient elution: 2.5:1 hexane–ether was first used to remove nonpolar side-products followed by 1:2.5 hexane–ether to obtain 21 (100 mg, 74% yield); R, 21 0.20 (1:1 hexane–ether); ¹H NMR (CDCl₃): δ 1.65 (bs, 1 H, OH), 3.28–3.40, 3.55–4.0 (2m, 12 H, mannose), 4.20–4.75, 4.82–4.90 (2m, 14 H, CH₂Ph), 5.05 (s, 1 H, H-1 mannose), 5.15 (s, 1 H, H-1 mannose), 7.10–7.40 (m, 35 H, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4,6-tri-O-benzyl-α,β-D-mannopyranosyl fluoride (22).—Compounds 21 (87 mg, 0.089 mmol) were azeotropically dried with toluene then dissolved in THF (0.25 mL) and cooled to −30 °C. Diethylaminosulfur trifluoride (DAST, 18 µL, 0.135 mmol) was added and the reaction mixture was slowly brought to 20 °C over 30 min. When the reaction was complete, the mixture was cooled to −30 °C and MeOH was added to quench any unreacted DAST. After warming the mixture to 20 °C, NaHCO₃ (1 M, ~1 mL) was added and the mixture was extracted with ether (3 × 5 mL). The combined ether layers were dried, filtered, and concentrated. Flash silica gel-column chromatography (3:1 hexane–ether) was used to obtain 22 (75 mg, 86% yield), R, 22 0.63 (1:1 hexanes–ether); ¹H NMR (CDCl₃): δ 3.60–3.78, 3.80–4.00 (2m, 12 H, mannose), 4.45–4.75, 4.80–5.20 (2m, 14 H, CH₂Ph), 5.52 (d, 1 H, J 50 Hz, H-1), 7.10–7.40 (m, 35 H, phenyl H’s).

Methyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (7b).—To pre-dried 22 (140 mg, 0.144 mmol) cooled to 0 °C was added Bu₄SnSMee in CH₂ClCH₂Cl (97 mg, 0.288 mmol, 1.3 mL of a 74 mg/mL stock solution). After stirring for 5 min, SnCl₄ in CH₂ClCH₂Cl (75 mg, 0.29 mmol, 1.3 mL of a 57 mg/mL stock solution) was added. The yellowish solution was stirred at 0 °C for 15 min. When the reaction was complete, NaF (1 M, 15 mL) and EtOAc were added and the reaction was stirred for 1 h. The resulting white precipitate was removed by filtration through Celite. The organic layer was separated, washed with NaHCO₃ (1 M, 3 × 15 mL), dried, filtered and concentrated. To obtain pure product, two flash chromatography separations were required. After the first separation (8:1 toluene–EtOAc, R, α,β RSMe ~ 0.80), the mixture of anomers was collected and applied to a second column (1:1 hexanes–ether) to produce pure 7b (122 mg, 85% yield). For 7b: R, 7b 0.56 (1:1 hexanes–ether); ¹H NMR (CDCl₃): δ 2.05 (s, 3 H, CH₃S), 3.62–4.05 (m, 12 H, mannose), 4.55–4.70 (m, 13 H, CH₂Ph), 4.88 (d, J 12 Hz, CH₃Ph), 5.10 (s, 1 H, H-1), 5.20 (s, 1 H, H-1), 7.10–7.38 (m, 35 H, phenyl H’s).

For the β anomer: R, 0.33; ¹H NMR (CDCl₃): δ 2.18 (s, 3 H, CH₃S), 3.35, 3.58–4.05 (2m, mannose), 4.39–4.98 (m, CH₂Ph and mannose), 5.15 (d, 1 H, J 1.5 Hz, H-1 mannose-2), 7.15–7.48 (m, phenyl H’s).

p-Methoxyphenyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4,6-tri-O-benzyl-α-D-mannopyranoside (23).—Compound 23 was synthesized in a manner analogous to the synthesis of compound 18 except that 19 was used as the glycosyl acceptor in place of 16. The trisaccharide product 23 was purified via flash chromato-
side-products followed by 3:1 hexane–ether to obtain pure 23 (88% yield); \( R, 23 \) 0.47 (1:1 hexanes–ether); \( ^1 \)H NMR (CDCl₃): \( \delta \) 2.12 (s, 3 H, CH₃CO), 3.58 (s, 3 H, CH₃O), 3.50–4.20 (m, 17 H, mannose), 4.10 (dd, 1 H, J 3, J 11 Hz), 4.34 (d, 1 H, J 12 Hz, CH₂Ph), 4.43 (d, 1 H, J 12 Hz, CH₂Ph), 4.48 (d, 1 H, J 12 Hz, CH₂Ph), 4.52 (s, 2 H, CH₂Ph), 4.56 (d, 1 H, J 12 Hz, CH₂Ph), 4.62 (d, 1 H, J 12 Hz, CH₂Ph), 4.63 (d, 1 H, J 12 Hz, CH₂Ph), 4.67 (s, 2 H, CH₂Ph), 4.75 (s, 2 H, CH₂Ph), 4.82 (d, 1 H, J 12 Hz, CH₂Ph), 4.86 (d, 1 H, J 12 Hz, CH₂Ph), 4.91 (d, 1 H, J 12 Hz, CH₂Ph), 4.93 (pseudo-t, 1 H, J 1 H, H-1 mannose-2), 5.03 (d, 1 H, J 1 H, H-1 mannose-3), 5.41 (d, 1 H, J 1 H, H-1 mannose-1), 5.52 (pseudo-t, 1 H, J 1 H, H-2 mannose-3), 6.70 (d, 2 H, J 9 Hz, PMP), 6.93 (d, 2 H, J 9 Hz, PMP), 7.10–7.42 (m, 45 H, phenyl H’s); \( ^{13} \)C NMR (CDCl₃): \( \delta \) 21 (CH₂CO), 53.45, 55.44 (CH₂O), 66.49, 68.83, 71.88, 72.30, 72.34, 73.30, 74.62, 75.02, 77.04 (t, CDCl₃) 78.18, 79.32, 80.15, 96.94 (d, J 170 Hz, C–H, C-1), 98.81 (d, J 171 Hz, C–H, C-1), 99.57 (d, J 173 Hz, C–H, C-1), 114.64 (CH, PMP), 117.52 (CH, PMP), 127.55–133.58 (phenyl Cs), 137.9–140.0 (aromatic), 151 (C–O, PMP), 156 (C–O, PMP), 171 (C=O); HRMS: Calculated for C₉₀H₉₄O₁₈ Na+: 1485.6337; Found: 1485.6456.

\( p\)-Methoxyphenyl 3,4,6-tri-O-benzyl-\( \alpha\)-D-mannopyranosyl-(1 \( \rightarrow \) 2)-3,4,6-tri-O-benzyl-\( \alpha\)-d-mannopyranosyl-(1 \( \rightarrow \) 6)-2,3,4-tri-O-benzyl-\( \alpha\),\( \beta\)-d-mannopyranoside (24).—Compound 24 was synthesized in a manner analogous to that utilized for the synthesis of compound 21 except that trisaccharide 25 was oxidized instead of disaccharide 20. After work-up, product 26 was purified via flash-column chromatography utilizing step gradient elution: hexane was used first to remove nonpolar side-products followed by 2:1 hexane–ether to obtain pure 25 (81% yield); \( R, 25 \) 0.49 (1:1 hexanes–ether); \( ^1 \)H NMR (CDCl₃): \( \delta \) 3.55 (s, 3 H, CH₃O), 3.60–4.12 (m, 18 H, mannose), 4.42–4.90 (m, 20 H, CH₂Ph), 4.93 (m, 1 H, J 1 H, H-1), 5.13 (d, 1 H, J 1 H, H-1), 5.42 (d, 1 H, J 2 Hz, H-1), 6.75 (d, 2 H, J 9 Hz, PMP), 6.95 (d, 2 H, J 9 Hz, PMP), 7.15–7.45 (m, 50 H, aromatic).

\( 2,3,4,6\)-Tetra-O-benzyl-\( \alpha\)-D-mannopyranosyl-(1 \( \rightarrow \) 2)-3,4,6-tri-O-benzyl-\( \alpha\)-D-mannopyranosyl-(1 \( \rightarrow \) 6)-2,3,4-tri-O-benzyl-\( \alpha\),\( \beta\)-D-mannopyranoside (26).—Compound 26 were synthesized by a procedure analogous to that utilized for the synthesis of compounds 21 except that trisaccharide 25 was oxidized instead of disaccharide 20. After work-up, product 26 was purified via flash-column chromatography utilizing step gradient elution: 2:1 hexane–ether was used first to remove nonpolar side-products followed by 1:2 hexane–ether to obtain 26 (78% yield); \( R, 26 \) 0.18 (1:1 hexanes–ether); \( ^1 \)H NMR (CDCl₃): \( \delta \) 3.60–4.00 (m, 17 H, mannose), 4.18 (m, 1 H), 4.45–4.90 (m, 20 H, CH₂Ph), 4.92 (m, 1 H, J 1 H, H-1), 4.96 (d, 1 H, J 2 Hz, H-1), 5.16 (d, 1 H, J 1 Hz, H-1), 7.15–7.40 (m, 50 H, aromatic).

\( 2,3,4,6\)-Tetra-O-benzyl-\( \alpha\)-D-mannopyranosyl-(1 \( \rightarrow \) 2)-3,4,6-tri-O-benzyl-\( \alpha\)-D-mannopyranosyl-(1 \( \rightarrow \) 6)-2,3,4-tri-O-benzyl-\( \alpha\),\( \beta\)-D-mannopyranosyl fluoride (27).—Compound 27 were synthesized in a manner analogous to the synthesis of compound 22 except that tri- saccharide 26 were fluorinated instead of di-
saccharide 21. After work-up, flash silica gel-column chromatography (2:1 hexanes–ether) was used to obtain pure 27 (as an anomic mixture), 90% yield; \( R_f \) 0.62 (1:1 hexanes–ether); \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 3.55–4.03 (m, 17 H, mannose), 4.15 (pseudo-t, 1 H, J 2 Hz), 4.40–4.89 (m, 20 H, CH\(_2\)Ph), 4.96 (d, 1 H, J 1 Hz, H-1), 5.15 (d, 1 H, J 1 Hz, H-1), 5.56 (d, 1 H, J 51 Hz, H-1 mannose-1), 7.10–7.36 (m, 50 H, phenyl H’s); \(^{19}\)F NMR (CDCl\(_3\)) \( \delta \) –138.5 (d, \( J_{FH} \) 51 Hz).

Methyl 2,3,4,6-tetra-O-benzyl-\( \alpha \)-d-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-\( \alpha \)-d-mannopyranosyl-(1→6)-2,3,4,6-tetra-O-benzyl-1-thio-\( \alpha \),\( \beta \)-D-mannopyranoside (7c).—Compound 7c was synthesized in a manner analogous to the synthesis of compound 7b except that trisaccharides were thiomethylated instead of disaccharides 22. To obtain the pure \( \alpha \) anomer, two flash chromatography column separations were required. After the first column (8:1 toluene–EtOAc, \( R_f \) \( \alpha \),\( \beta \) RSMe \( \sim \) 0.80), the mixture of anomers was collected and applied to a second column (3:2 hexanes–ether) providing 80 mg (52% yield) of 7c (\( \alpha \) anomer) and 7 mg (5% yield) of the corresponding \( \beta \) anomer; \( R_f \) 7c 0.50 (1:1 hexanes–ether); \(^1\)H NMR 7c (CDCl\(_3\)): \( \delta \) 2.05 (s, 3 H, CH\(_3\)S), 3.55–3.95, 4.05–4.10 (2m, mannose), 4.15 (pseudo-t, 1 H, J 2 Hz), 4.45–4.95 (m, 20 H, CH\(_2\)Ph), 4.95 (d, 1 H, J 1.5 Hz, H-1), 5.18 (d, 1 H, J 1.5 Hz, H-1), 7.15–7.42 (m, 50 H, phenyl H’s); \( R_f \) (\( \beta \) anomer) 0.25 (1:1 hexanes–ether); \(^1\)H NMR (CDCl\(_3\)) \( \beta \) anomer, \( \delta \) 3.40, 3.55–4.05 (2m, mannose), 4.10 (pseudo-t, 1 H, J 1 Hz), 4.40–4.95 (m, CH\(_2\)Ph and mannose), 5.20 (d, 1 H, J 1 Hz, H-1), 5.30 (d, 1 H, J 1 Hz, H-1), 5.30 (CH\(_2\)Cl\(_2\)), 7.10–7.40 (m, 50 H, phenyl H’s).

3,4,5-Tri-O-benzyl-1D-myo-inositol 1,2-cyclic carbonate (11).—To 335 mg of diol 28\(^{27}\) (0.55 mmol) in 27.5 mL of benzene was added 275 mg of 1,1’-carbonyldimimidazole (1.7 mmol). The reaction was stirred at 20 °C for 17 h and quenched by the addition of water (20 mL). The layers were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (4 × 20 mL). The combined organic extracts were washed with satd NaCl (20 mL), dried, concentrated by co-evaporation with toluene, and dissolved in 24 mL of dry THF. The reaction was cooled to 0 °C and 5.5 mL of 1 M \( t \)-butylammonium fluoride (TBAF) in THF was added. The reaction was stirred at 0 °C for 3 min and quenched by addition of 12 mL of water at 0 °C. The mixture was extracted with CH\(_2\)Cl\(_2\) (4 × 12 mL). The combined organic extracts were washed with water (2 × 10 mL) and then satd NaCl (10 mL), dried, and concentrated. Purification via flash silica-column chromatography yielded 262 mg of 11 (84%). [\( \alpha \])\(_D\) +22.7° (c 0.0075, CH\(_2\)Cl\(_2\)); \( R_f \) 11 0.29 (7:3 hexanes–EtOAc); \(^1\)H NMR (CDCl\(_3\)): \( \delta \) 2.7 (bs, 1 H, OH), 3.38 (dd, 1 H, J 4.5, 10.7 Hz), 3.79 (dd, 1 H, J 2.5, 4.5 Hz), 3.90 (pseudo-t, 1 H, J 2.8 Hz), 4.31 (d, 1 H, J 11.5 Hz, HCHPh), 4.38 (d, 1 H, J 11.5 Hz, HCHPh), 4.45 (dd, 1 H, J 7.0, 10.7 Hz), 4.56 (d, 1 H, J 11.4 Hz, HCHPh), 4.58 (d, 1 H, J 11.9 Hz, HCHPh), 4.62–2.67 (m, 2 H), 4.71 (d, 1 H, J 11.9 Hz, HCHPh), 4.86 (dd, 1 H, J 3.3, 8.7 Hz), 7–7.5 (m, 15 H, phenyl H’s); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 70.57 (C–O), 72.02 (C–O), 73.34 (C–O), 73.43 (C–O), 73.72 (C–O), 74.34 (C–O), 79.26 (C–O), 80.07 (C–O), 80.92 (C–O), 127.89 (phenyl C), 128.15 (phenyl C), 128.52 (phenyl C), 136.76 (phenyl C), 137.46 (phenyl C), 154.46 (C–O). HRMS-FAB\(^{+}\): Calcd for C\(_{38}\)H\(_{30}\)O\(_{7}\) + Na 549.1733; Found 549.1744.

t-Butyldimethylsilaryl 2,3,4,6-tetra-O-benzyl-\( \alpha \)-d-mannopyranosyl-(1→2)-2-azido-2-deoxy-3,6-di-O-benzyl-\( \beta \)-d-glucopyranoside (9a).—To a flame-dried flask containing a stir bar was added PhSeCl (60 mg, 0.313 mmol), 4 Å molecular sieves, and 2.4 mL of toluene. The reaction mixture was cooled to 0 °C and AgOTf (80 mg, 0.311 mmol) was added. After stirring at 0 °C for 10 min, the mixture was cooled to –42 °C and a solution containing 7a (120 mg, 0.206 mmol) and 8\(^{13}\) (86 mg, 0.171 mmol), pre-dried by coevaporation with toluene, in 7.5 mL of toluene was added. The reaction was stirred at –42 °C for 30 min, then quenched by addition of 1 M NaHCO\(_3\) (5 mL) followed by filtration through Celite. The layers were separated, the filtrate was diluted with CHCl\(_3\) (5 mL) and the organic layer was washed with water (3 × 5 mL), dried, and concentrated. Purification via silica gel chromatography with 4:1 hexane–ether provided 99 mg of 9a as an oil (56% yield); \( R_f \) 9a 0.26 (4:1 hexane–ether); \(^1\)H NMR
(CDCl₃): δ 0.19 (s, 3 H, CH₃), 0.20 (s, 3 H, CH₃), 0.94 (s, 9 H, t-butyl), 3.25 (dd, 1 H, J 8.6, 9.9 Hz, H-3 glucosamine), 3.36–3.42 (m, 2 H, H-5 and H-2 of glucosamine), 3.58 (dd, 1 H, J 1.5, 9.1 Hz, H-3 mannose), 3.64–3.83 (m, 7 H), 3.98 (pseudo-t, 1 H, J 9.3 Hz, H-4 mannose), 4.25 (d, 1 H, J 12.2 Hz, HCHPh), 4.35 (d, 1 H, J 12.2 Hz, HCHPh), 4.43–4.63 (m, 9 H), 4.82 (d, 1 H, J 10.8 Hz, HCHPh), 4.94 (d, 1 H, J 11.4 Hz, HCHPh), 5.30 (d, 1 H, J 2.0 Hz, H-1 mannose), 7.14–7.40 (m, phenyl H’s); ¹³C NMR (CDCl₃): δ 100.15 (d, J 10.8 Hz), 105.15 (d, J 10.9 Hz), 108.3 (d, J 10.9 Hz, HCHPh), 4.29 (d, 1 H, J 1.6 Hz, H-1 mannose), 5.14 (d, 1 H, J 1.6 Hz, H-1 mannose), 5.28 (d, 1 H, J 1.9 Hz, H-1 mannose), 7.12–7.36 (m, phenyl H’s); ¹³C NMR (CDCl₃): δ 97.12 (d, J 155 Hz, C-1 glucosamine), 99.27 (d, J 168.3 Hz, 2 × C-1 mannose), 99.83 (d, J 169.7 Hz, C-1 mannose).

2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranoside (29a).—To 360 mg (0.348 mmol) of 9a in 11 mL of THF was added 700 μL of glacial AcOH followed by 5.3 mL of t-butylammonium fluoride (TBAF, 5.32 mmol in THF). The reaction was stirred at 20 °C for 1.5 h. The mixture was quenched with 1 mL of 1 M NaHCO₃ and extracted with CHCl₃ (2 × 1 mL). The organic phase was washed sequentially with water (2 × 5 mL), satd NaCl (5 mL), dried, and concentrated. Purification via silica gel chromatography yielded 270 mg of 29a (84% yield); δ 0.29 (1:1 hexanes–ether); ¹¹B NMR (CDCl₃): δ 2.36–3.40 (m, 4 H), 3.48–4.05 (m, 22 H), 4.21 (d, 1 H, J 12.1 Hz), 4.24 (d, 1 H, J 12.1 Hz), 4.35 (d, 2 H, J 12.0 Hz), 4.41–4.46 (m, 17 H), 4.80 (d, 1 H, J 10.8 Hz), 4.82 (d, 1 H, J 10.8 Hz), 4.90 (d, 1 H, J 10.8 Hz), 4.93 (d, 1 H, J 10.8 Hz), 5.25 (d, 1 H, J 2.4 Hz, H-1 mannose of β anomer), 5.27 (d, 1 H, J 1.8 Hz, H-1 mannose of α anomer), 5.33 (d, 1 H, J 3.4 Hz, H-1 glucosamine of α anomer), 7.16–7.39 (m, phenyl H’s).
procedure outlined for compound 29a except that trisaccharide 9b was desilylated instead of disaccharide 9a. After work-up, purification via preparative TLC (1:1 hexanes–ether) yielded 1:1 anomeric mixture of alcohols (85% yield); $R_f$ 29b 0.34 (1:1 hexanes–ether); $^1$H NMR (CDCl$_3$): $\delta$ 2.43 (bs, 1 H, OH), 3.28–3.56 (m, 4 H), 3.61–4.02 (m, 14 H), 4.16 (d, 1 H, J 12.1 Hz, HCHPh), 4.31 (d, 1 H, J 12 Hz, HCHPh), 4.32 (d, 1 H, J 12 Hz, HCHPh), 4.39–4.63 (m, 13.5 H), 4.80–4.91 (m, 3 H), 5.04 (pseudo-s, 1 H, H-1 mannose 2), 5.21 (d, 0.5 H, J 1.92 Hz, H-1 mannose 1), 5.23 (d, 0.5 H, J 2.0 Hz, H-1 mannose 1), 5.26 (d, 0.5 H, J 3.32 Hz, H-1 glucosamine of $\alpha$ anomer), 7.2–7.5 (m, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 4)-2-azido-2-deoxy-3,6-di-O-benzyl-$\alpha,\beta$-d-glucopyranosyl fluoride (10a).—Compounds 10a were synthesized according to the procedure outlined for compound 22 except that 29a was fluorinated instead of 21. After work-up, the crude products were chromatographed on silica gel with 3:1 hexane–ether yielding a 1:2 $\alpha,\beta$ mixture of anomeric fluorides (84% yield); $R_f$ 10a 0.18 (3:1 hexanes–ether); $^1$H NMR (CDCl$_3$): $\delta$ 3.31–3.41(m), 3.42–3.88 (m), 3.8–4.03 (m), 4.27 (d, 1 H, J 10.2 Hz), 4.31 (d, 1 H, J 10.1 Hz), 4.39–4.66 (m), 4.81 (d, 1 H, J 10.8 Hz), 4.83 (d, 1 H, J 10.9 Hz), 4.87 (d, 1 H, J 11.2 Hz), 4.88 (d, 1 H, J 11.1 Hz), 5.09 (dd, 1 H, J 7.1, J 52.7 Hz, H-1 glucosamine of $\beta$ anomer), 5.26 (d, 1 H, J 2.2 Hz, H-1 mannose), 5.30 (d, 1 H, J 2.3 Hz, H-1 mannose), 5.66 (dd, 1 H, J 2.6, J 52.9 Hz, H-1 glucosamine of $\alpha$ anomer), 7.14–7.39 (m, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 6)-2,3,4-tri-O-benzyl-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 4)-2-azido-2-deoxy-3,6-di-O-benzyl-$\alpha,\beta$-d-glucopyranosyl fluoride (10b).—Compounds 10b were synthesized according to the procedure outlined for compound 22 except that 29b was fluorinated instead of 21. After work-up, purification via preparative TLC (1:1 hexanes–ether) yielded a 1:1 ($\alpha,\beta$) mixture of fluorides (86% yield); $R_f$ 10b 0.42 (1:1 hexanes–ether); $^1$H NMR (CDCl$_3$): $\delta$ 3.31–4.02 (m, 18 H), 4.24 (d, 1 H, J 12.4 Hz, HCHPh), 4.25 (d, 1 H, J 12.1 Hz, HCHPh), 4.35–4.67 (m, 13 H, CH$_2$Ph), 4.81–4.89 (m, 3 H, CH$_2$Ph), 5.03 (pseudo-s, 1 H, H-1 mannose 2), 5.07 (dd, 0.5 H, J 7.1, J 52.7 Hz, H-1 glucosamine of $\beta$ anomer), 5.22 (d, 0.5 H, J 1.92 Hz, H-1 mannose 1), 5.27 (d, 0.5 H, J 2.1 Hz, H-1 mannose 1), 5.60 (dd, 0.5 H, J 2.4, J 53 Hz, H-1 glucosamine of $\alpha$ anomer), 7.1–7.5 (m, 45 H, phenyl H’s).
O-benzyl-1-d-myo-inositol 1,2 cyclic carbonate (12b,\(\alpha,\beta\)).—Compounds 12b,\(\alpha,\beta\) were synthesized in a manner analogous to that for compounds 12a,\(\alpha,\beta\) except that trisaccharides 10b were used as glycosyl donor instead of disaccharides 10a. After work-up, purification via preparative TLC (7:3 hexanes–EtOAc) yielded 17.5 mg of 12b,\(\alpha\) and 6.6 mg of slightly impure 12b,\(\beta\) (57% combined yield); \(R_e\) 12b,\(\alpha\) 0.38, \(R_e\) 12b,\(\beta\) 0.46 (7:3 hexanes–EtOAc); \(^1H\) NMR (CDCl\(_3\)) 12b,\(\alpha\): \(\delta\) 3.37 (dd, 1 H, J 11.0 Hz, H-2 glucosamine), 3.49–3.7 (m, 11 H), 3.75–3.88 (m, 8 H), 3.9–4.0 (m, 3 H), 4.16 (d, 1 H, J 12.0 Hz, HCHPh), 4.27–4.65 (m, 19 H), 4.80–4.91 (m, 5 H), 5.04 (pseudo-t, 1 H, H-1 mannose), 5.23 (pseudo-s, 1 H, H-1 mannose), 5.32 (d, 1 H, J 3.6 Hz, H-1 glucosamine), 7.0–7.5 (m, 60 H, phenyl H’s); \(^13C\) NMR (CDCl\(_3\)) 12b,\(\alpha\): \(\delta\) 29.61 (anomeric C), 98.10 (anomeric C), 100.40 (anomeric C), 153.97 (carbonate carbon); LRMS: Calcd for C\(_{109}\)H\(_{111}\)N\(_3\)O\(_{21}\) (anomeric C), 153.97 (carbonate carbon); \(m/z\): 1816. 

was stirred at 20 °C for 17 h and quenched by addition of 1 M \(\text{NH}_2\text{Cl}\) (3 mL). The mixture was extracted with CH\(_2\)Cl\(_2\) (4 × 3 mL). The combined organic extracts were washed with satd NaCl (3 mL), dried, and concentrated.

Purification via preparative TLC (1:1 CHCl\(_3\)–ether) yielded 13.8 mg of diol 30a,\(\alpha\) (80% yield); \(R_e\) 30a,\(\alpha\) 0.57 (1:1 CHCl\(_3\)–ether); \(^1H\) NMR (CDCl\(_3\)): \(\delta\) 2.51 (bs, OH), 3.29–3.56 (m), 3.58–3.63 (m), 3.65–4.06 (m), 4.16 (pseudo-t, 1 H, J 2.7 Hz, H-2 inositol), 4.23 (d, 1 H, J 12.2 Hz, HCHPh), 4.26–4.38 (m), 4.44–4.68 (m), 4.72–4.94 (m), 5.23 (d, 1 H, J 2.1 Hz, H-1 mannose), 5.45 (d, 1 H, J 3.6 Hz, H-1 glucosamine), 7.10–7.39 (m, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-\(\alpha\)-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-di-O-benzyl-\(\beta\)-D-glucopyranosyl-(1→6)-3,4,5-tri-O-benzyl-1-D-myo-inositol (30a,\(\beta\)).—Compound 30a,\(\beta\) was synthesized in a manner analogous to that for compound 30a,\(\alpha\) except that 12a,\(\beta\) was hydrolyzed instead of 12a,\(\alpha\). After work-up, purification via preparative TLC (1:1 CHCl\(_3\)–ether) yielded 12.3 mg of diol 30a,\(\beta\) (75% yield); \(R_e\) 30a,\(\beta\) 0.80 (1:1 CHCl\(_3\)–ether); \(^1H\) NMR (CDCl\(_3\)): \(\delta\) 3.26 (pseudo-t, 1 H, J 10 Hz, H-2 glucosamine), 3.4–4.1 (m, 15 H), 4.2 (pseudo-t, 1 H, J 2.7, H-2 inositol), 4.25–5.1 (m, 20 H), 5.26 (pseudo-s, 1 H, H-1 mannose), 7.0–7.5 (m, 45 H, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-\(\alpha\)-D-mannopyranosyl-(1→6)-2,3,4,6-tri-O-benzyl-\(\alpha\)-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-di-O-benzyl-\(\beta\)-D-glucopyranosyl-(1→6)-3,4,5-tri-O-benzyl-1-D-myo-inositol (30b,\(\alpha\)).—Compound 30b,\(\alpha\) was synthesized in a manner analogous to that for compound 30a,\(\alpha\) except that 12b,\(\alpha\) was hydrolyzed instead of 12a,\(\alpha\). After work-up, purification via preparative TLC (1:1 CHCl\(_3\)–ether) yielded 14 mg of diol (86% yield); \(R_e\) 30b,\(\alpha\) 0.61 (1:1 CHCl\(_3\)–ether); \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 2.6 (s, 1 H, OH), 3.25–3.66 (m, 11 H), 3.71 (pseudo-t, 1 H, J 2.3 Hz), 3.79–3.85 (m, 6 H), 3.87–4.02 (m, 5 H), 4.14–4.18 (m, 2 H), 4.21–4.50 (m, 12 H), 4.54 (d, 1 H, J 11.9 Hz, HCHPh), 4.58 (d, 1 H, J 12.0 Hz, HCHPh), 4.64 (d, 1 H, J 11.3 Hz, HCHPh), 4.70–4.76 (m, 4 H), 4.82–4.90 (m, 4 H), 4.94 (d, 1 H, J 11.0 Hz, HCHPh), 5.03 (pseudo-s, 1 H, H-1 mannose), 5.22 (d, 1 H, J 1.7 Hz, H-1 mannose), 5.44 (d, 1 H, J 3.6 Hz,
H-1 glucosamine), 7.2–7.5 (m, 60 H, phenyl H’s).

2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl-
(1→6)–2,3,4-tri-O-benzyl-α-D-mannopyranosyl-
(1→4)–2-azido-2-deoxy-3,6-di-O-benzyl-β-D-glucopyranosyl-
(1→6)–3,4,5-tri-O-benzyl-1-D-myo-inositol (30b,β).—Compound 30b,β was synthesized in a manner analogous to that for compound 30a,α except that 12b,β was hydrolyzed instead of 12a,α. After work-up, purification via preparative TLC (1:1 CHCl₃–ether) yielded 4.3 mg of diol (66% yield); ¹H NMR (CDCl₃): δ 2.50 (bs, 1 H, OH), 3.28 (pseudo-t, 1 H, J 9.3 Hz), 3.35–4.07 (m, 26 H), 4.17–4.22 (m, 2 H), 4.32–4.62 (m, 13 H), 4.68–5.03 (m, 9 H), 5.24 (pseudo-s, 1 H, H-1 mannose), 7.0–7.4 (m, 60 H, phenyl Hs).

2,3,4,6-Tetra-O-benzyl-α-D-mannopyranosyl-
(1→2)–3,4,6-tri-O-benzyl-α-D-mannopyranosyl-
(1→6)–2,3,4-tri-O-benzyl-α-D-mannopyranosyl-
(1→4)–2-azido-2-deoxy-3,6-di-O-benzyl-α-D-glucopyranosyl-
(1→6)–3,4,5-tri-O-benzyl-1-D-myo-inositol (30c,α).—Compound 30c,α was synthesized in a manner analogous to that for compound 30a,α except that 12c,α was hydrolyzed instead of 12a,α. After work-up, purification via preparative TLC (1:1 CHCl₃–ether) yielded 5.1 mg of diol 30c,α (97% yield); ¹H NMR (CDCl₃): δ 2.50 (bs, 1 H, OH), 3.28–4.95 (m, 60 H), 5.02 (pseudo-s, 1 H, H-1 mannose), 5.14 (pseudo-s, 1 H, H-1 mannose), 5.25 (pseudo-s, 1 H, H-1 mannose), 5.40 (pseudo-s, 1 H, H-1 glucosamine) 7.0–7.4 (m, 75 H, phenyl H’s).

Preparation of phosphorylating reagent 31.¹⁸.—Methyl dichlorophosphoridate (PCl₂-O₂Me, 500 µL) was added slowly to 5.0 mL of freshly-distilled pyridine. The reaction was stirred at 20 °C for 30 min prior to use.

α-D-Mannopyranosyl-(1→4)–2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)–1-D-myo-inositol 1:2-(cyclic) phosphate (1).—To a solution of 30a,α (13.0 mg, 9.70 µmol) in pyridine (230 µL) was added freshly prepared phosphorylating reagent 31 (518 µL). The reaction was stirred at 20 °C until judged complete (30 min) by TLC analysis (1:1,1, CHCl₃–ether–MeOH, Rf diol 1.0, Rf cyclic phosphate 0.73, Rf acyclic 0; 1:1, CHCl₃–ether, Rf diol 0.57, Rf cyclic and acyclic phosphate 0). The reaction was quenched by addition of 1 mL of satd NaHCO₃ and concentrated by evaporation with 2 M HCl to pH 1 (~20 drops). The suspension was extracted with EtOAc (5 × 3 mL) and evaporated to dryness, dissolved in dry THF (2.5 mL). The reaction mixture was stirred at −78 °C for 15 min (blue color persisted) and then carefully quenched by addition of 167 mg of Na₂SO₄, concentrated, dried by co-evaporation with toluene, and dissolved in dry THF (6 mL). The resulting blue powder was dissolved in water (4 mL) and filtered through Celite. The filtrate was evaporated to dryness, dissolved in water (1.0 mL), and desalted by passing over 12 g of Sephadex G-10, eluting with water (2.0 mL). Evaporation of the eluate yielded 3.1 mg of 1 (56% yield over two steps); ¹H NMR (D₂O): δ 3–4 (m, 16 H), 4.35 (dd, 1 H, H-1 inositol), 4.5 (pseudo-t, 1 H, H-2 inositol), 5.1 (pseudo-s, 1 H, H-1 mannose), 5.3 (pseudo-s, 1 H, H-1 glucosamine); ³¹P NMR (D₂O): δ 3.62 (trace of acyclic phosphate), 17.14 (cyclic phosphate); HRMS (FAB, negative ion mode): Calcd for C₁₉H₃₁NO₁₇P: 564.1329; Found: 564.1354.

α-D-Mannopyranosyl-(1→4)–2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)–1-D-myo-inositol 1:2-(cyclic) phosphate (2).—Compound 2 was synthesized in a manner analogous to that for compound 1 except that β anomer 30a,β was phosphorylated and deprotected instead of the α anomer 30a,α. After work-up, desalting with Sephadex G-10 yielded 8.6 mg of 2 (84% yield over two steps); ¹H NMR (D₂O): δ 2.9 (m, 1 H), 3.3–4 (m, 15 H), 4.4 (dd, 1 H, H-1 inositol), 4.55 (pseudo-s, 1 H, H-2 inositol), 4.85 (pseudo-s, 1 H, H-1
glucosamine), 5.1 (pseudo-s, 1 H, H-1 mannose); $^{31}$P NMR (D$_2$O): $\delta$ 16.79.

$\alpha$-D-Mannopyranosyl-(1 $\rightarrow$ 6)-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 4)-2-amino-2-deoxy-$\beta$-D-glucopyranosyl-(1 $\rightarrow$ 6)-1-D-myo-inositol 1:2-(cyclic) phosphate (3).—Compound 3 was synthesized in a manner analogous to that for compound 1 except that tetrascarhide 30b,$\alpha$ was phosphorylated and deprotected instead of trisaccharide 30a.$\alpha$. After work-up, desalting with Sephadex G-10 yielded 3.1 mg of 3 (75% yield over two steps). $^1$H NMR (D$_2$O): $\delta$ 3.1–4.0 (m, 22 H), 4.35 (ddd, 1 H, J 4, 12, 20 Hz, H-1 inositol), 4.5 (pseudo-t, 1 H, J 4 Hz, H-2 inositol), 4.7 (pseudo-s, 1 H, H-1 mannose), 5.05 (pseudo-s, 1 H, H-1 mannose), 5.13 (d, 1 H, J 3.1 Hz, H-1 glucosamine); $^{31}$P NMR (D$_2$O): $\delta$ 17.12; HRMS (electrospray): Calcd for [C$_{24}$H$_{42}$NO$_{22}$P $+$ H]$^+$ 728.2014; Found: 728.2014.

$\alpha$-D-Mannopyranosyl-(1 $\rightarrow$ 6)-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 4)-2-amino-2-deoxy-$\beta$-D-glucopyranosyl-(1 $\rightarrow$ 6)-1-D-myo-inositol 1:2-(cyclic) phosphate (4).—Compound 4 was synthesized in a manner analogous to that for compound 1 except that $\beta$-tetrascarhide 30b,$\beta$ was phosphorylated and deprotected instead of $\alpha$-trisaccharide 30a.$\alpha$. After work-up, desalting with Sephadex G-10 yielded 3.1 mg of 4 (83% yield over two steps). $^1$H NMR (D$_2$O): $\delta$ 2.97 (m, 1 H), 3.33 (pseudo-t, 1 H), 3.44–4.14 (m, 20 H), 4.29 (ddd, 1 H, J 4.7, 8.1, 20 Hz, H-1 inositol), 4.5 (pseudo-t, 1 H, H-2 inositol), 4.73 (pseudo-s, 1 H, H-1 mannose), 4.81 (d, 1 H, J 7.4 Hz, H-1 glucosamine), 5.03 (pseudo-s, 1 H, H-1 mannose); $^{31}$P NMR (D$_2$O): $\delta$ 16.89.

$\alpha$-D-Mannopyranosyl-(1 $\rightarrow$ 2)-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 6)-$\alpha$-D-mannopyranosyl-(1 $\rightarrow$ 4)-2-amino-2-deoxy-$\alpha$-D-glucopyranosyl-(1 $\rightarrow$ 6)-1-D-myo-inositol 1:2-(cyclic) phosphate (5).—Compound 5 was synthesized in a manner analogous to that for compound 1 except that $\alpha$-pentascarhide 30c,$\alpha$ was phosphorylated and deprotected instead of $\alpha$-trisaccharide 30a.$\alpha$. After work-up, desalting with Sephadex G-10 yielded 1.1 mg of 5 (75% yield over two steps). $^1$H NMR (D$_2$O): $\delta$ 2.62 (pseudo-d, 1 H, J 7.7 Hz, H-2 glucosamine), 3.20 (pseudo-t, 1 H, J 9.5 Hz), 3.39–3.88 (m, 26 H), 4.36 (ddd, 1 H, H-1 inositol), 4.5 (pseudo-t, 1 H, H-2 inositol), 4.84 (pseudo-s, 1 H, H-1 mannose), 4.95 (pseudo-s, 1 H, H-1 mannose), 5.04 (pseudo-s, 1 H, H-1 mannose), 5.13 (d, 1 H, J 3.1 Hz, H-1 glucosamine); $^{31}$P NMR (D$_2$O): $\delta$ 17.12; HRMS (FAB, negative ion mode): Calcd for C$_{30}$H$_{51}$NO$_{22}$P: 888.2385; Found: 888.2415.

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References

12. See Refs. 10 and 11.