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Synthesis of the Core Tetrasaccharide of *Trypanosoma cruzi* Glycoinositolphospholipids: Man*p*(α1→6)-Man*p*(α1→4)-6-(2-aminoethylphosphonic acid)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4

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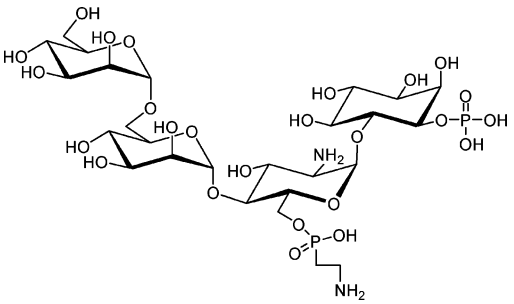

Synthesis of the core tetrasaccharide Man*p*(α1→6)-Man*p*(α1→4)-6-(2-aminoethylphosphonic acid)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4, found in glycoinositolphospholipids of *Trypanosoma cruzi* parasites, is described. The key building block, 6-*O*-(2-azido-3-*O*-benzyl-6-*O*-((2-benzyloxycarbonylaminoethyl)-phosphonic acid benzyl ester)-2-deoxy-α-D-glucopyranosyl)-1-di-*O*-benzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2,2,1]bicycloheptan-6-ylidene)-D-*myo*-inositol, was synthesized using a partially protected glucosyl D-camphorinositolphosphate and a (2-benzyloxycarbonylaminoethyl)-phosphonic acid derivative in a regioselective phosphonate esterfication. Elongation with ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-α-D-thiomannopyranoside using dimethyl(methylthio)sulfonium trifluoromethanesulfonate gave a fully protected tetrasaccharide which was successfully deprotected subsequently with sodium methoxide, sodium in liquid ammonia, and aq hydrochloric acid to give title compound.

Introduction

The cell surface of *Trypanosoma cruzi* (*T. cruzi*), the etiological agent of Chagas' disease, is covered by specific glycoinositolphospholipids (GIPLs) during the complex life cycle of the parasite.[1] Characterization and synthesis of such glycoconjugates are of great interest to an increased understanding of their virulence and pathogenesis related to the infection. GIPLs from *T. cruzi* are related to glycosylphosphatidylinositol (GPI) anchors as they contain Man*p*(α1→4)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4, the structural motif found in all GPI anchors characterized so far.[2]

In 1991, the glycosyl portion of the most abundant GIPL species in *T. cruzi* epimastigotes (Y-strain) was completely characterized.[3] Later, a major GPI membrane-anchored glycoprotein from the same morphological form of *T. cruzi* (Y-strain) was concluded to share the same core phosphoinositol oligosaccharide, Man*p*(α1→2)-Man*p*(α1→2)-Man*p*(α1→6)-Man*p*(α1→4)-6-(2-aminoethyl phosphonic acid)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4.[4] The difference between the two glycoconjugates is the substitution of two galactofuranoside (Gal*f*) residues in the GIPL species linked (β1→3) to the terminal Man*p*(α1→2)-

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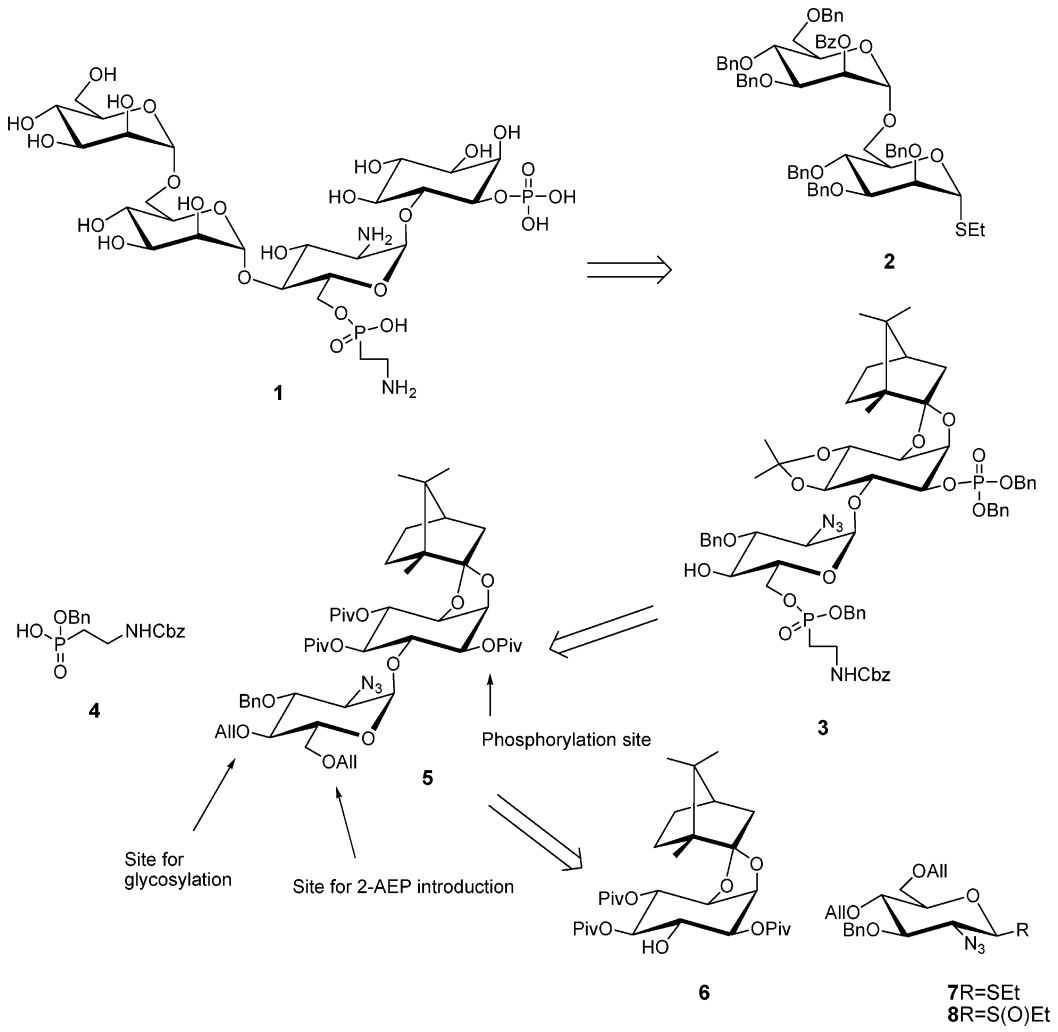

FIGURE 1. Synthetic strategy to tetrasaccharide **1**.

Man*p* disaccharide, whereas the third mannose residue distal to GlcN*p* in the mucin-like GPI-anchor contains ethanolamine phosphate (EtNP) substituted at C-6.

Further studies of *T. cruzi* epimastigote GIPLs from different strains have revealed structural diversity and the classification of two series which differ in the substitution of the third Man*p* residue distal from GlcN*p*. Series 1 has the distinguished feature for EtNP found on C-6 similar to the mucin-like GPI-anchor, whereas in series 2, C-3 is substituted with a (β1→3) Gal*f*. The inner part of *T. cruzi* GIPL glycans contain a nonacylated GlcN*p* residue and the unusual 2-aminoethyl phosphonic acid (2-AEP) motif substituted at C-6, a fragment not found in any characterized GPI-anchor. Finally, phosphatidyl-*myo*-inositol anchors the glycan domain of the GIPLs in the membrane via ceramides or 1-*O*-alkyl-2-*O*-acyl-*sn*-glycerols depending on *T. cruzi* strain.[5]

As the most abundant cell surface glycoconjugates with structural motifs unique to different strains, the conserved structures of GIPLs from *T. cruzi* most likely play an important role for the survival of the parasite. Still the precise role of the GIPLs in host−parasite interactions remains unknown, but several studies have shown the GIPLs to have host immune regulatory properties.[6] For example, glycans of *T. cruzi* GIPLs were found to stimulate B cells and immunoglobulin secretion in vitro.[7] On the other hand, the ceramide lipid of *T. cruzi* GIPLs exhibit down-regulating properties on T cell activation both in vitro and in vivo.[8] More recently, the ceramide moiety was also shown to have a down-regulating effect on host macrophages and dendritic cells.[9] Thus, *T. cruzi* GIPLs seem to have beneficial properties for the parasite to subvert the host immune response.

Synthesis of oligosaccharides correlated to *T. cruzi* GIPLs could constitute powerful tools to elucidate not only the structural motifs necessary for bioactivity and *T. cruzi* survival mechanisms but also an increased knowledge about the biosynthetic pathways of these cell surface molecules. Recently, synthesis of terminal oli-

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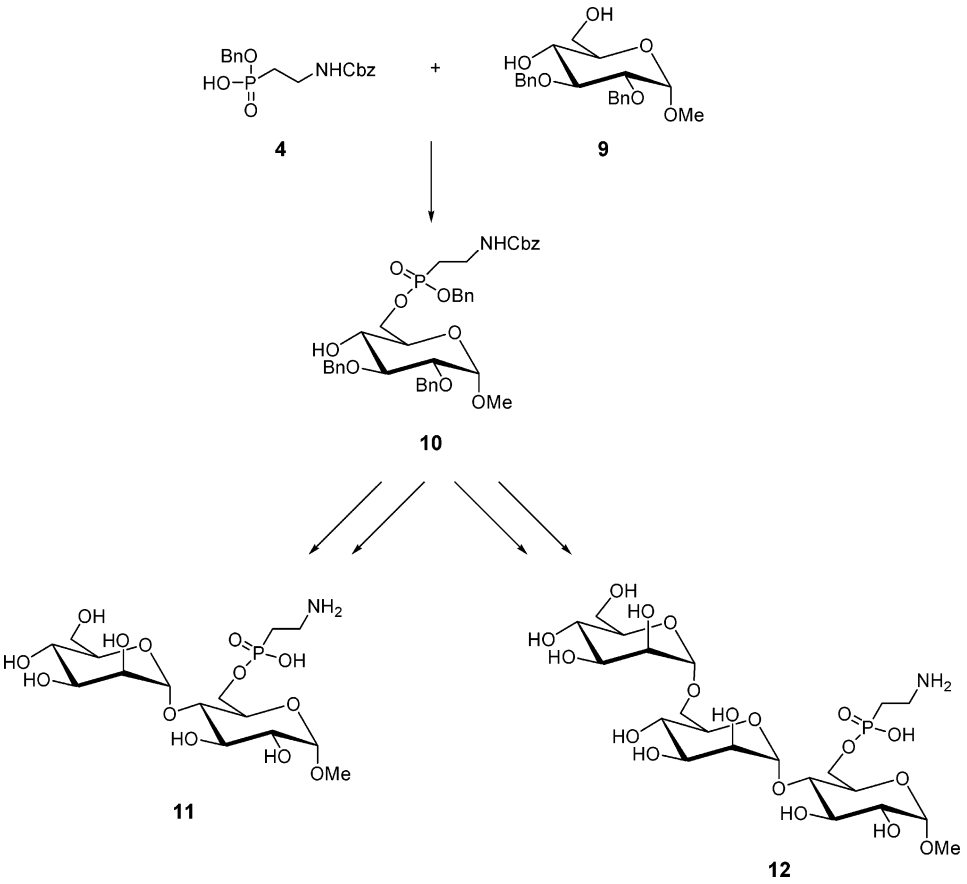
SCHEME 1. Synthesis of 2-Aminoethylphosphonic Acid Substituted Oligosaccharides 11 and 12



gosaccharide fragments of *T. cruzi* GIPLs was reported.[10] Herein, we present the first synthesis of the core tetrasaccharide Man*p*(α1→6)-Man*p*(α1→4)-6-(2-AEP)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4 (**1**) corresponding to GIPLs and GPI membrane-anchored mucins of *T. cruzi* Y-strain (Figure 1). Compound **3** was obtained after esterfication of (2-benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester (**4**) with a partially protected GlcN-*myo*-Ins-1-PO_4 compound derived from disaccharide **5**. Compound **5** was synthesized in a glycosylation with *myo*-inositol acceptor **6** and donors **7** or **8** respectively. To acquire experience in C-phosphonate chemistry (e.g., regioselective introduction of the 2-AEP motif, stability during glycosylation and deprotections) synthetic routes to two 2-AEP containing carbohydrates, oligosaccharides **11** and **12**, were developed (Scheme 1). The key step in this model study was the synthesis of compound **10** using methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**9**)[11] and monobenzyl ester **4** in a regioselective phosphonate esterfication. Taken together, the results presented will be useful in the ongoing project to synthesize the complete glycophosphatidyl inositol portion of the dominating GIPL species of *T. cruzi* epimastigotes.

Results and Discussion

Recently the complete synthesis of the *Leishmania* LPPG core heptasaccharyl *myo*-inositol was presented by our group.[12,13] The successful synthesis of this complex molecule highlighted the careful choice of protecting groups and the urgent need of flexibility in the final deprotections. We wanted to investigate whether similar synthetic strategy was possible to apply in this project to reach target molecule **1**. Hence, disconnection of molecule **1** between the Man*p*(1→4)GlcN*p* residues gave Man*p*(α1→6)-Man*p* derivative **2** and the partially protected 6-(2-AEP)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4 derivative **3** as promising building blocks. Compound **3** was planned to be synthesized from disaccharide **5**, which after removal of the pivaloylic groups, introduction of the isopropylidene acetal, phosphodiester formation, and cleavage of the allylic groups would give an appropriate GlcN-*myo*-Ins-1-PO_4 derivative to be used in the crucial regioselective condensation with the 2-AEP bearing compound **4** (Figure 1).

The (α1→6) disaccharide **2** was synthesized in 83% yield by converting ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**13**)[14] to corresponding bromosugar, followed by condensation with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**14**)[15] using silver triflate as promoter (Scheme 2). The 2-*O*-benzoyl protecting group was deliberately placed because of the possibility to be selectively removed without interfering with

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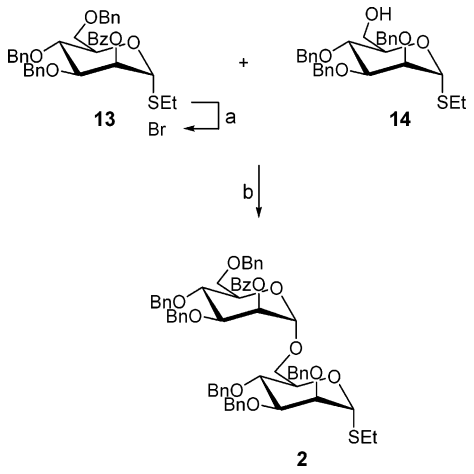
SCHEME 2[a]



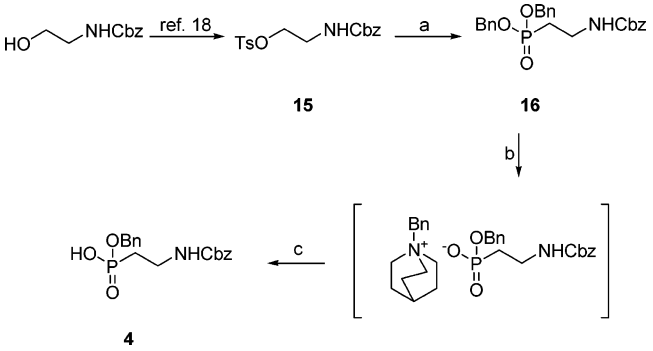
[a] Key: (a) Br_2, CH_2Cl_2; (b) AgOTf, CH_2Cl_2/ toluene (5:1), 4 Å molecular sieves, −30 °C, 83%.

the other protecting groups and thereby keeping in view future synthesis of larger structures related to GIPLs of *T. cruzi*.

Synthesis of (2-benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester (**4**) has previously been reported using 2-AEP as starting compound.[16] Without 2-AEP accessible, the synthetic route to compound **4** was based on a report by Lintunen et al. describing the synthesis of 2-AEP substituted monosaccharides with the aim to mimic transition-state of a transesterfication reaction.[17] We experienced difficulties in reproducing both the substitution of *N*-benzyloxycarbonyl-*O*-tosylethanolamine (**15**)[18] with sodium dibenzyl phosphinate, and the one-pot synthesis of benzyl (2-benzyloxycarbonylaminoethyl)-phosphonochloridate as described in the original procedure. Instead our objective became to produce crystalline **4** as a suitable compound which can be activated by various reagents aiming at the formation of a phosphonate ester with a carbohydrate. Treating **15** with a preformed mixture of dibenzyl phosphite and sodium hydride in DMF at 65 °C produced compound **16** in acceptable yield (62%). Monodebenzylation using quinuclidine[19] in toluene at 80 °C and subsequent hydrolysis of the obtained benzyl quinuclidine salt with aq 5% hydrochloric acid resulted in derivative **4** in 90% yield (Scheme 3).

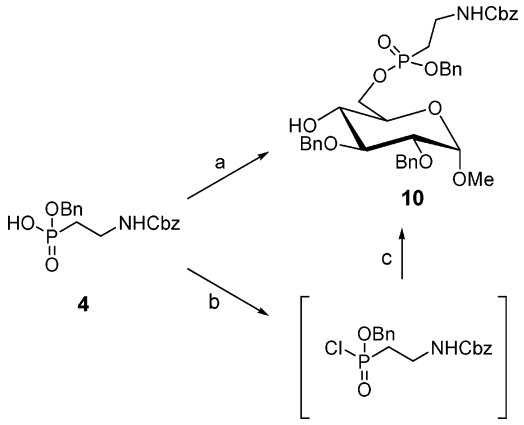
The introduction of the benzyloxycarbonyl-protected 2-AEP motif was elucidated on model compound methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**9**).[11] The first method involved phosphonate ester formation using Mitzunobu reagents.[20] Treating compounds **9** and **4** with triphenylphosphine and diisopropyl azodicarboxylate in THF at 40 °C gave compound **10** in 91% yield as a diastereomeric mixture. The regioselectivity was con-

SCHEME 3[a]



[a] Key: (a) dibenzyl phosphite, NaH, DMF, 65°C, 62%; (b) quinuclidine, toluene, 80 °C; (c) 5% aq HCl, 90%.

SCHEME 4[a]



[a] Key: (a) PPh_3, DIAD, **9**, THF, 40 °C, 91%; (b) $(COCl)_2$, cat. DMF, CH_2Cl_2, 0 °C; (c) **9**, Et_3N, CH_2Cl_2, −10 °C, 75%.

firmed by treating alcohol **10** with benzoyl chloride in pyridine to produce two double doublets in ^{1}H NMR at 5.33 (J = 9.6 9.6 Hz) and 5.26 (J = 9.8 9.8 Hz) ppm from the diastereomeric H-4 protons. Evidently, a partially carbohydrate was successfully used in a regioselective Mitzunobu phosphonate ester reaction. This is in contrary to what Saady et al. reported in their course to synthesis of 5′-phosphorylated adenosine derivatives using 2′- and 3′-nonprotected nucleosides.[21]

Now the attention was focused whether the phosphonochloridate described above could be obtained from compound **4** and used to prepare compound **10**. Treating monobenzylphosphonic acid **4** with oxalylic chloride and a catalytic amount of DMF in CH_2Cl_2 gave the phosphonochloridate (^{31}P NMR 42.6 ppm) which was found to be extremely sensitive to moisture and could therefore not be fully characterized. However, when a solution of diol **9** and triethylamine in CH_2Cl_2 was added to a solution of phosphonochloridate at −10 °C prepared in situ, derivative **10** was obtained in 75% yield (Scheme 4).

To validate the stability of the 2-AEP-substituted acceptor **10** during glycosylation, ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranoside (**17**)[22] was linked via dimethyl(methylthio)sulfonium trifluoromethanesulfonate

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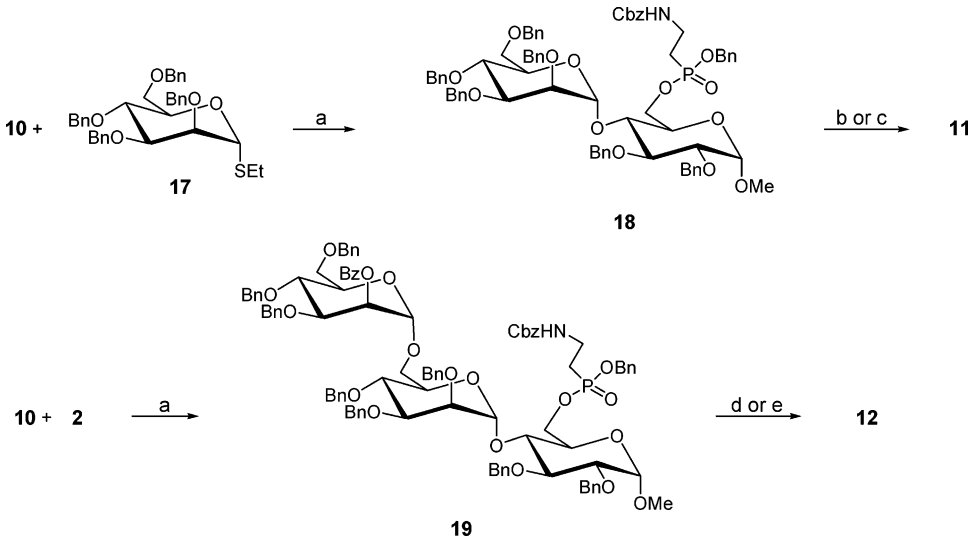
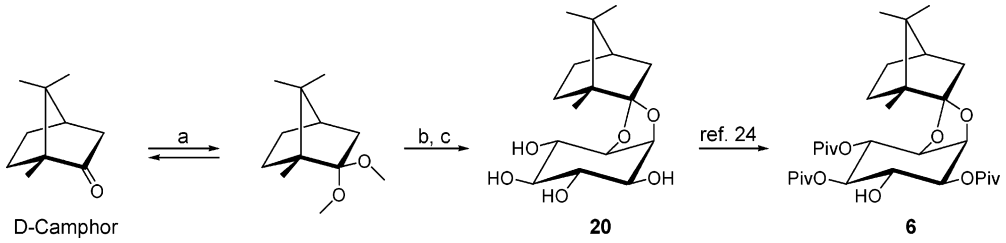
SCHEME 5[a]



[a] Key: (a) DMTST, diethyl ether, 4 Å molecular sieves, 82% for **10** + **17**; 79% for **10** + **2**; (b) 20% $Pd(OH)_2/C$, H_2, EtOAc/ EtOH/ H_2O (4:2:1), 94%; (c) Na (s), NH_3 (l), 95%; (d) (1) NaOMe, CH_2Cl_2/MeOH (2:1), (2) 20% $Pd(OH)_2/C$, H_2, EtOAc/EtOH/H_2O (4:2:1), 80%; (e) (1) NaOMe, CH_2Cl_2/MeOH (2:1); (2) Na (s), NH_3 (l), 82%.

SCHEME 6[a]



[a] Key: (a) MeOH, trimethyl orthoformate H_2SO_4; (b) *myo*-inositol, H_2SO_4, DMSO; (c) $CHCl_3$/MeOH/H_2O (50:16:1), *p*-TsOH, 37%.

(DMTST) as promoter in diethyl ether to give (α1→4) disaccharide **18** in 82% yield. The two diastereomers of **18** could be separated using conventional silica gel chromatography. Debenzylation and deprotection of the benzyloxycarbonyl group of diastereomeric **18** using palladium hydroxide on carbon ($Pd(OH)_2/C$) and H_2 (g) produced target compound **11** in 94% yield as single product with the characteristic resonances in ^{31}P NMR at 22.5 ppm and ^{13}C NMR at 24.6 (d, J = 135 Hz) and 35.5 ppm from the 2-AEP motif. Solid sodium in liquid ammonia (Na (s)/NH_3 (l)) was found as a most convenient alternative in the deprotection to target compound (Scheme 5c). Coupling of the 2-*O*-benzoylated (α1→6) disaccharide **2** with **10** using the same conditions as for **10** + **17** produced trisaccharide **19** in 79% yield. Debenzoylation with sodium methoxide in a mixture of CH_2Cl_2 and methanol followed by final deprotection using either $Pd(OH)_2/C$ and H_2 (g) or Na (s)/NH_3 (l) gave target molecule **12** in 80- and 82% yield respectively (Scheme 5). Without drawing too many conclusions from model experiments, the successful regioselective introduction of the 2-AEP motif, glycosylations, and deprotections leading to compounds **11** and **12** encouraged us to continue the proposed strategy in the synthesis of tetrasaccharide **1**.

We have recently published a procedure of the in situ preparation of dimethyl L-camphor acetal and subsequent reaction with D-*myo*-inositol to give 1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol.[23] This method was applied to D-camphor, and the resulting dimethyl D-camphor acetal was allowed to react with D-*myo*-inositol providing the chiral D-camphor *myo*-inositol acetal compound **20** in 37% yield. The partially protected *myo*-inositol derivative **6** was synthesized according to the procedure presented by Pietrusiewicz et al.[24] with excess of pivaloyl chloride in pyridine. This short synthetic route offered a chiral D-campher *myo*-inositol acceptor with a remaining C-6 hydroxylic group as the site for elongation with a suitable glucosamine donor (Scheme 6).

Synthesis of glucosamine donor **7** (91%) with a protecting group pattern in line with the synthetic strategy was performed by hydrolysis of the benzylidene acetal in ethyl 4,6-*O*-benzylidene-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (**21**)[25] and introduction of the two allylic groups. Glycosylations with acceptor **6** and different 4-*O*-allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranosyl donors, promoters, and solvents were nicely investigated by Lindberg et al. in a recent publication.[23] The most favorable yield of the corresponding GlcN*p*-Ins

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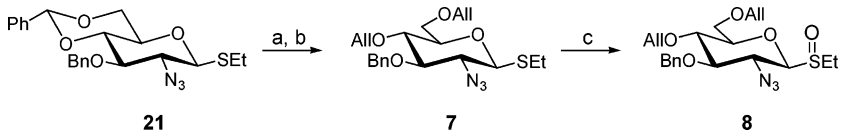
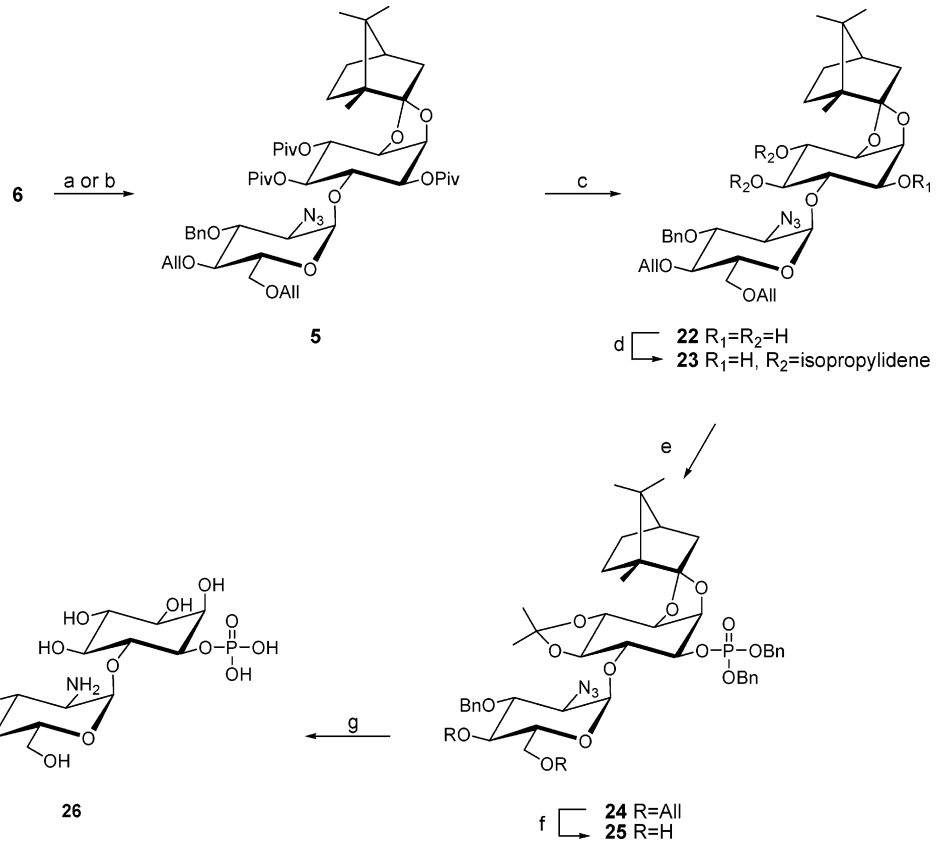
SCHEME 7[a]



[a] Key: (a) AcOH/ H_2O (6:4), 80°C; (b) AllBr, NaH, DMF, 91%; (c) *m*-CPBA, CH_2Cl_2, −60→ −20 °C, 65%.

SCHEME 8[a]



[a] Key: (a) **7**, MeOTf, Et_2O, 4 Å molecular sieves, 51%; (b) **8**, Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, toluene, −50 → 0 °C, 61%; (c) NaOH, MeOH, reflux, 83%; (d) 2,2-dimethoxypropane, pyridinium *p*-toluenesulfonate, DMF, 65%; (e) (1) dibenzyl diisopropyl phosphoramidite, 1*H*-tetrazole, CH_2Cl_2, (2) *m*-CPBA, 0 °C, 85%; (f) (1) H_2 (g), [bis(methyldiphenylphosphine)](1,5-cyclooctadiene) Ir(I)PF_6, THF, (2) NIS, H_2O, 86%; (g) (1) Na (s), NH_3 (l), (2) 0.1 M HCl, 90%.

disaccharide was obtained using an ethyl *S*-oxide donor and activation following the original procedure described by Kahne et al.[26] With these results in mind, derivative **7** was oxidized using *m*-CPBA in CH_2Cl_2 to give ethyl *S*-oxide **8** in 65% yield. Overoxidation to the corresponding ethyl sulfonylglycoside attributed to the moderate yield of compound **8** (Scheme 7).

Glycosylation to disaccharide **5** was performed using both donors. Condensation of **6** and **7** using methyl trifluoromethanesulfonate (MeOTf) as promoter in diethyl ether gave disaccharide **5** in moderate yield (51%). In comparison, activation of donor **8** with trifluoromethanesulfonic anhydride in toluene at −50 °C, and subsequent addition of acceptor **6** and 2,6-di-*tert*-butyl-4-methylpyridine produced compound **5** in 61% yield (Scheme 8).

Protecting group manipulations via cleavage of the pivaloylic groups (**5** → **22**, 83%) and introduction of the isopropylidene acetal (**22** → **23**, 65%) gave a derivative with the inositol-1-OH phosphorylation site. Compound **23** was treated with dibenzyl diisopropyl phosphoramidite[27] and 1*H*-tetrazole in CH_2Cl_2 followed by oxidation with *m*-CPBA at 0 °C to give compound **24** in 85% yield. Deallylation (**24** → **25**) was accomplished using a two-step sequence involving (a) isomerization with [bis-(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium-(I)PF_6[28] and (b) hydrolysis of the vinylic ethers under neutral conditions using *N*-iodosuccinimide and H_2O[29] to give compound **25** in 86% yield. The synthetic route was verified by deprotection of **25** using Na (s)/NH_3 (l) and aq 0.1 M hydrochloric acid to give the known derivative **26**[30] (Scheme 8).

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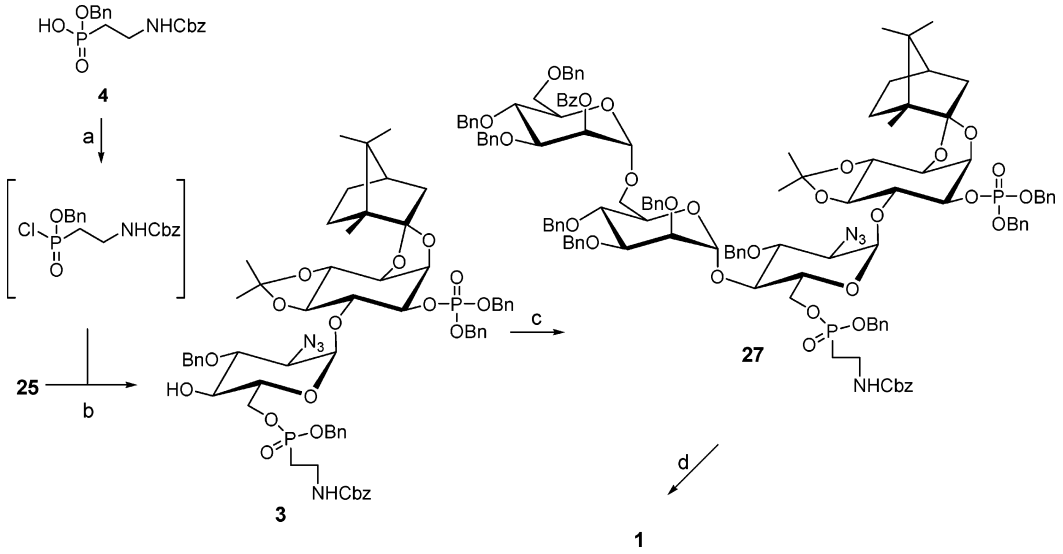
SCHEME 9[a]



[a] Key: (a) $(COCl)_2$, cat. DMF, CH_2Cl_2; (b) TEA, CH_2Cl_2, 0 °C, 82%; (c) **2**, DMTST, Et_2O, 74%; (d) (1) NaOMe, CH_2Cl_2/MeOH (2:1), (2) Na (s), NH_3 (l), (3) 0.1 M HCl, 71%.

Substitution of diol **25** with 2-AEP derivative **4** was performed using both methods described in the preparation of **10**. The Mitzunobu phosphonate esterfication of **25** → **3** was not satisfactory; compound **3** was obtained in only 40% yield. On the other hand, disaccharide **3** was isolated in 82% yield using the more reactive phosphonochloridate produced from derivative **4** (Scheme 9). Treating a diastereomeric mixture of **3** in pyridine with benzoyl chloride as described for compound **10** gave two double doublets at 5.40 (J = 9.9 9.9 Hz) and 5.47 (J = 9.9 9.9 Hz) ppm related to the diastereomeric GlcN*p* H-4 protons. Coupling of **3** with **2** using DMTST in diethyl ether gave tetrasaccharide **27** in 74% yield as a diastereomeric mixture. The strategy in the crucial removal of all protecting groups was a result from earlier observations in the synthesis of the *Leishmania* LPPG core heptasaccharyl *myo*-inositol[12,13] where acidic deacetalization performed before basic deacetylation induced phosphate migration on the inositol residue. In that particular case, these problems were solved by changing the order of deprotection to (1) basic deacetylation with sodium methoxide, (2) debenzylation with sodium in liquid ammonia, and finally (3) acidic hydrolysis of the acetals. Using the same deprotection sequence on derivative **27** was our first strategy to target compound. Thus, removal of the 2-*O*-benzoyl group was accomplished using sodium methoxide in CH_2Cl_2 and methanol. Further deprotection of this derivative using Na (s)/NH_3 (l) and subsequent acidic deacetalization produced target molecule **1** in 71% yield (Scheme 9). Deprotection of the benzyl ethers and the benzyloxycarbonyl group using Pd-$(OH)_2$/C and H_2 (g) instead of Na (s)/NH_3 (l) was not successful, the reaction did not proceed to completion. ^{31}P and ^{13}C NMR spectra of target compound **1** were in line with data reported from characterization of phosphoinositol-oligosaccharides from natural sources.[4]

Conclusions

In conclusion, we report the synthesis of the core tetrasaccharide Man*p*(α1→6)-Man*p*(α1→4)-6-(2-AEP)-GlcN*p*(α1→6)-*myo*-Ins-1-PO_4^- corresponding to GIPLs and GPI-related mucins of *T. cruzi* Y-strain. The new chemistry of the 2-AEP motif was supported through the development of synthetic routes to oligosaccharides **11** and **12**. This work will be followed up by attempts to synthesize the complete glycophosphatidyl inositol portion of the dominating GIPL species of *T. cruzi* epimastigotes by glycosylation between compound **3** and a Gal*f*(β1→3)-Man*p*(α1→2)−(Gal*f*(β1→3))-Man*p*(α1→2)-Man*p*(α1→6)-Man*p* donor, which is under construction in our laboratory.

Experimental Section

Organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo at 40 °C. NMR spectra were recorded at 25 °C unless otherwise stated. ^1H and ^{13}C chemical shifts are given in ppm relative to TMS in $CDCl_3$ (δ = 0.00), acetone in MeOH-d_4 (^{13}C: δ = 30.7, ^1H: δ = 2.15), and D_2O (^{13}C: δ = 31.00, ^1H: δ = 2.22); ^{31}P, 85% H_3PO_4 (δ = 0.00) as external reference. pH* values in D_2O were calibrated against H_2O−buffer solutions. TLC was performed on silica gel F_{254} plates with detection by UV light (254 nm) and/or by charring with AMC [ammonium molybdate 10 g, cerium(IV) sulfate 2 g, dissolved in 10% H_2SO_4 (200 mL)] followed by heating at ∼250 °C. Silica gel (0.040−0.063 mm) was used for flash chromatography (FC). MALDI-TOF mass spectra were recorded in positive mode with α-cyano-4-hydroxycinnamic acid matrix and a calibration mixture using angiotensin I (*m/z* 1297.51) as internal standard. IR spectra were recorded as KBr pellets (solids) or films on CaF_2 crystals (syrups). Gel filtrations were performed using deoxygenated H_2O containing 1% *n*-butanol as eluent.

Ethyl 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-α-D-thiomannopyranoside (2). To a solution of 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**13**)[14] (735 mg, 1.23 mmol) in CH_2Cl_2 (15 mL) was added Br_2 (67 μL, 1.30 mmol). After 30 min, the solution was co-concentrated with toluene. A mixture of the obtained crude bromosugar, ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-

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D-mannopyranoside (**14**)[15] (608 mg, 1.23 mmol), and 4 Å molecular sieves in CH_2Cl_2 (20 mL) was stirred under inert atmosphere for 15 min when the temperature was lowered to −30 °C. AgOTf (791 mg, 3.08 mmol) in toluene (4 mL) was added dropwise to the reaction mixture, and the temperature was maintained at −30 °C for 20 min followed by the addition of Et_3N (1.5 mL). The mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated. FC (light petroleum (45−65)/EtOAc 9:1 → 4:1) gave **2** (1.052 g, 1.02 mmol, 83%) as a colorless oil: R_f 0.18 (light petroleum (45−65)/EtOAc 9:1); $[\alpha]_D$ = +39 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3090, 3064, 3030, 2930, 2868, 1723, 1602, 1584, 1495, 1453, 1383, 1364, 1320, 1268, 1207, 1098, 1027, 737, 710, 697; ^1H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, 3H, J = 7.4 Hz), 2.43−2.64 (m, 2H), 3.71−3.79 (m, 2H), 3.83−3.93 (m, 4H), 3.97−4.03 (m, 2H), 4.10−4.20 (m, 3H), 4.45−4.52 (m, 4H), 4.56 (s, 2H), 4.70−4.82 (m, 4H) 4.87 (d, 1H, J = 11.0 Hz), 4.92 (d, 1H, J = 11.3 Hz), 5.07 (d, 1H, J = 1.9 Hz), 5.34 (d, 1H, J = 0.8 Hz), 5.73 (dd, 1H, J = 0.8 2.5 Hz), 7.13−7.41 (m, 32H), 7.53 (t, 1H, J = 8.0 Hz), 8.07 (d, 2H, J = 8.2 Hz); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 15.0, 25.3, 66.6, 68.7, 69.0, 71.1, 71.4, 71.6, 72.0, 72.1, 73.3, 74.2, 74.8, 75.0, 75.1, 76.4, 77.7, 80.4, 81.6, 98.0 (J_{CH} = 172 Hz), 127.4−128.4 (several peaks), 129.9, 130.0, 133.0, 137.9, 138.1 138.2, 138.5 (2C), 138.6 165.5; MALDI-TOF calcd for $C_{63}H_{66}O_{11}S$ $[M + Na]^+$ 1053, found $[M + Na]^+$ 1053. Anal. Calcd for $C_{63}H_{66}O_{11}S$: C, 73.37; H, 6.45. Found: C, 73.14; H, 6.22.

(2-Benzyloxycarbonylaminoethyl)phosphonic Acid Dibenzyl Ester (16). To a stirred solution of dibenzyl phosphite (12.06 g, 46.0 mmol) in DMF (50 mL) at 0 °C was added NaH (1.906 g, 43.7 mmol, 55% dispersion in oil). When the gas evolution had ceased, the mixture was added to a solution of *N*-benzyloxycarbonyl-*O*-tosylethanolamine (**15**)[18] (4.00 g, 11.5 mmol) in DMF (25 mL) at 65 °C. The solution was heated for 2 h, diluted with toluene, and poured into a separatory funnel with ice and water. The organic phase was washed with water, dried, filtered, and concentrated. FC (toluene/EtOAc 1:1 → 1:3) and reqrystallization from hexane gave **16** (3.12 g, 7.1 mmol, 62%) as white crystals: R_f 0.29 (toluene/EtOAc 1:3); mp 73−74 °C (from hexane); IR ν_{max} cm^{-1} 3292, 3091, 3066, 3034, 2948, 1715, 1587, 1526, 1498, 1453, 1444, 1379, 1264, 1245, 1218, 1138, 1079, 1052, 1025, 987, 873, 852, 760, 742, 729, 694, 663, 604, 519, 459; ^1H NMR (300 MHz, $CDCl_3$) δ 1.99 (dt, 2H, J = 17.9 6.6 Hz), 3.37−3.50 (m, 2H), 5.04 (dd, 2H, J = 8.6 11.6 Hz), 4.95 (dd, 2H, J = 8.6 11.6 Hz), 5.06 (s, 2H), 5.24−5.38 (bs, 1H), 7.29−7.38 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7 (d, J = 139.0 Hz), 35.1 (d, J = 4 Hz), 66.7, 67.4 (d, J = 6.5 Hz, 2C), 128.0, 128.1, 128.4, 128.5, 128.6, 136.0, 136.1, 156.1; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ 31.7; MALDI-TOF calcd for $C_{24}H_{26}NO_5P$ $[M + Na]^+$ 462.1, found $[M + Na]^+$ 462.1. Anal. Calcd for $C_{24}H_{26}NO_5P$: C, 65.60; H, 5.96. Found: C, 65.56; H, 6.04.

(2-Benzyloxycarbonylaminoethyl)phosphonic Acid Benzyl Ester (4). Compound **16** (1.50 g, 3.41 mmol) and quinuclidine (394 mg, 3.55 mmol) in dry toluene (10 mL) were heated at 80 °C for 12 h and concentrated to give the *N*-benzylquinuclidine salt of **16** [^{13}C NMR (75.4 MHz, $CDCl_3$) δ 19.9, 23.8 (3 C), 27.1 (J = 130 Hz), 36.8 (d, J = 7 Hz), 54.2 (3 C), 65.9 (d, J (CH_2P) = 5 Hz), 66.1, 67.6, 125.0, 127.1−129.0, 130.2, 137.1, 139.3, 139.4, 156.4; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) 22.2]. The obtained compound was treated with aq HCl (25 mL, 5%, v/v) for 30 min and extracted with EtOAc (3 × 50 mL). The organic phase was dried, filtered, and concentrated to give compound **4** (1.07 g, 3.07 mmol, 90%) as a white solid: mp 130−132 °C (acetone) [lit.[16] mp 131−133 °C (acetone)]; IR ν_{max} cm^{-1} 3300, 3061, 3029, 2950, 2894, 2582, 2281, 1957, 1688, 1549, 1500, 1467, 1455, 1445, 1413, 1381, 1374, 1322, 1282, 1245, 1218, 1186, 1082, 1031, 999, 864, 791, 781, 745, 699, 676, 605, 572, 516, 499, 466, 423; ^1H NMR (300 MHz, CD_3OD) δ 2.02 (dt, 2H, J = 17.9 7.8 Hz), 3.37 (dt, 2H, J = 11.8 7.7 Hz), 5.02 (d, 2H, J = 7.7 Hz), 5.04 (s, 2H), 7.27−7.40 (m, 10H); ^{13}C NMR (75.4 MHz, CD_3OD) δ 27.9 (d, J = 136 Hz), 36.4, 67.5, 67.8 (d, J = 5.9 Hz), 128.7−129.6, 138.1 (2 C) 158.5; ^{31}P NMR (decoupled, 121 MHz, CD_3OD) δ 28.9; MALDI-TOF calcd for $C_{17}H_{20}NO_5P$ $[M + Na]^+$ 372.1, found $[M + Na]^+$ 372.1.

Methyl 6-*O*-((2-Benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester)-2,3-di-*O*-benzyl-α-D-glucopyranoside (10). Method A: Phosphonate Ester Formation Using Mitzunobu Conditions. To a solution of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (**9**)[11] (400 mg, 1.07 mmol) and phosphonic acid **4** (562 mg, 1.61 mmol) in dry THF (4 mL) under argon atmosphere was added dropwise a preformed solution of triphenylphosphine (407 mg, 1.55 mmol) and diisopropylazadicarboxylate (305 μL, 1.55 mmol) in dry THF (4 mL). After 15 min, the temperature was raised to 40 °C, and stirring was maintained for an additional 3 h when MeOH (1 mL) was added. Concentration and purification by FC twice (EtOAc/toluene 4:1 and $CHCl_3$/acetone 4:1) gave compound **10** (687 mg, 0.97 mmol, 91%) as a colorless syrup.

Method B: Phosphonate Ester Formation via Phosphonochloridate. To a solution of phosphonic acid **4** (150 mg, 0.43 mmol) and a catalytic amount of dry DMF in CH_2Cl_2 (3 mL) at 0 °C and argon atmosphere was added oxalylic chloride (38 μL, 0.45 mmol) via syringe. After 30 min, the solution of crude phosponochloridate (^{31}P (decoupled, 121 MHz, $CHCl_3$) δ 42.6) was added dropwise to a solution of **9** (107 mg, 0.29 mmol) and NEt_3 (121 μL, 0.87 mmol) in CH_2Cl_2 (2 mL) at −10 °C. The reaction was quenched after 1 h with water and diluted with EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (EtOAc/toluene 4:1) gave compound **10** (153 mg, 0.22 mmol, 75%).

10: R_f 0.18 (EtOAc/toluene 3:1); $[\alpha]_D$ = +15 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3355, 3090, 3063, 3031, 2910, 1720, 1527, 1497, 1454, 1404, 1368, 1255, 1217, 1135, 1058, 1028, 914, 866, 737, 697, 460; ^1H NMR (300 MHz, $CHCl_3$, diastereomeric mixture), δ 1.92−2.10 (m, 4H), 3.32 (s, 3H), 3.33 (s, 3H), 3.38−3.68 (m, 8H), 3.78 (t, 2H, J = 9.2 Hz), 3.80 (t, 2H, J = 9.1 Hz), 4.05 (ddd, 1H, J = 1.9 7.1 11.5 Hz), 4.21 (dd, 2H, J = 2.7 7.4 Hz), 4.31 (ddd, 1H, J = 3.8 8.8 11.5 Hz), 4.57−4.84 (m, 8H), 4.94 (d, 1H, J = 11.1 Hz), 4.96 (d, 1H, J = 11.1 Hz), 5.04−5.07 (m, 8H), 5.45−5.55 (m, 2H), 7.29−7.40 (m, 40H); ^{13}C NMR (75.4 MHz, $CHCl_3$, diastereomeric mixture), δ 26.3 (d, J = 140 Hz, 2 C), 35.1 (2 C), 55.3 (2 C), 64.3 (d, J = 6.6 Hz), 64.6 (d, J = 6.6 Hz), 66.6 (2C), 67.4 (d, J = 6.6 Hz), 67.7 (d, J = 6.6 Hz), 69.4, 69.5, 69.8 (d, J = 6.0 Hz), 70.0 (d, J = 6.0 Hz), 73.1, 73.2, 75.4, 75.5, 79.5, 79.6, 81.1 (2 C), 98.2, 98.4, 127.7−128.6 (several carbons), 135.9, 136.0 136.4 (2 C), 138.0, 138.1, 138.8 (2 C), 156.2 (2 C); ^{31}P NMR (decoupled, 121 MHz, $CHCl_3$, diastereomeric mixture), δ 32.5, 32.8; MALDI-TOF calcd for $C_{38}H_{44}NO_{10}P$ $[M + Na]^+$ 728.3, found $[M + Na]^+$ 728.3. Anal. Calcd for $C_{38}H_{44}NO_{10}P$: C, 64.67; H, 6.28. Found: C, 64.41; H, 6.46.

Methyl (2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-6-*O*-((2-benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester)-2,3-di-*O*-benzyl-α-D-glucopyranoside (18). DMTST (169 mg, 0.656 mmol) was added to a mixture of ethyl 2,3,4,6-tetra-*O*-benzyl-α-D-1-thiomannopyranoside (**17**)[22] (200 mg, 0.342 mmol), derivative **10** (201 mg, 0.285 mmol), and 4 Å molecular sieves (0.3 g) in Et_2O (8 mL) at 0°C. The reaction mixture was allowed to reach rt during 1 h and stirred for an additional 5 h when Et_3N (0.5 mL) was added. After 15 min, the mixture was diluted with CH_2Cl_2, filtered through Celite, and concentrated. FC (toluene/EtOAc 1:1) gave **18** (298 mg, 0.242 mmol, 85%) as a diastereomeric mixture: IR ν_{max} cm^{-1} 3089, 3062, 3030, 2929, 1721, 1521, 1497, 1454, 1399, 1361, 1254, 1160, 1098, 1051, 1028, 911, 736, 697, 603, 458. Diastereomer A: R_f 0.34 (toluene/EtOAc 1:1); $[\alpha]_D$ = +26 (*c* 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.90−1.98 (m, 2H), 3.32 (s, 3H), 3.33−3.51 (m, 3H), 3.62−3.70 (m, 5H), 3.78−3.86 (m, 3H), 4.00 (t, 1H, J = 9.1 Hz), 4.21 (d, 1H, J = 12.1 Hz), 4.26−4.62 (m, 12H), 4.81 (d, 1H, J = 10.7 Hz), 4.94−5.09 (m, 5H), 5.24 (d, 1H, J = 2.1 Hz), 5.57 (m, 1H), 7.14−7.29 (m, 40H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 26.3 (d, J = 140 Hz), 35.1 (d, J = Hz), 55.4, 64.9 (d, J = 6.3 Hz), 66.5,

66.9 (d, J = 6.6 Hz), 69.1, 69.2 (d, J = 6.3 Hz), 72.1,72.4, 73.1, 73.2, 73.3, 74.7, 74.9, 75.1, 76.4, 77.5, 79.5, 79.9, 81.1, 97.6, 100.7 (J_{CH} = 172 Hz), 126.8−128.6, 136.2−138.6, 156.2; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ 31.9. Diastereomer B: R_f 0.29 (toluene/EtOAc 1:1); $[\alpha]_D$ = +14 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.84−2.08 (m, 2H), 3.35 (s, 3H), 3.36−3.45 (3H), 3.61 (dd, 1H, J = 8.2 9.9 Hz), 3.67−3.87 (m, 7H), 3.98 (t, 1H, J = 9.1 Hz), 4.22 (d, 1H, J = 12.1 Hz), 4.23−4.31 (m, 2H), 4.34 (d, 1H, J = 12.1 Hz), 4.44−4.64 (m, 9H), 4.80 (d, 1H, J = 10.7 Hz), 4.94−5.09 (m, 5H), 5.26 (d, 1H, J = 2.2 Hz), 5.57 (m, 1H), 7.14−7.32 (m, 40H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 26.3 (d, J = 140 Hz), 35.1, 55.4, 64.9 (d, J = 6.0 Hz), 66.5, 67.0 (d, J = 6.6 Hz), 69.1, 69.2 (d, J = 6.0 Hz), 72.1, 72.4, 73.1, 73.2, 73.3, 74.7, 74.9, 75.1, 76.4, 77.5, 79.5, 79.9, 81.1, 97.6, 100.7 (J_{CH} = 172 Hz), 126.8−128.6, 136.3−138.6, 156.2; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ 32.0; MALDI-TOF calcd for $C_{72}H_{78}NO_{15}P$ $[M + Na]^+$ 1250, found $[M + Na]^+$ 1250. Anal. Calcd for $C_{72}H_{78}NO_{15}P$: C, 70.40; H, 6.40. Found: C, 70.27; H, 6.54.

Methyl (α-D-Mannopyranosyl)-(1→4)-6-*O*-((2-aminoethyl)phosphonic acid)-α-D-glucopyranoside (11). Method A. A diastereomeric mixture of **18** (50 mg, 0.041 mmol) and 20% $Pd(OH)_2$/C (30 mg) in EtOAc/EtOH/H_2O (4 mL, 4:2:1) was treated with H_2 (g) at 1 atm for 12 h, filtered, and concentrated. Gel filtration on the residue on a Sephadex G-15 column eluted with H_2O containing 1% n-butanol gave **11** (18 mg, 0.039 mmol, 94%).

Method B. To ∼20 mL of NH_3 (l) at −33 °C was added a small amount of Na (s) which turned the mixture dark blue. A solution of **18** (45 mg, 0.037 mmol) in dry THF (2 mL) was added, and the mixture was stirred vigorously for 1 min when NH_4Cl (s) was added until the blue color disappeared. Concentration of the solution under a stream of nitrogen gas, dilution with H_2O (20 mL) and washing with Et_2O (20 mL) was followed by concentration of the aq. phase. Gel filtration of the residue on a Sephadex G-15 column eluted with H_2O containing 1% n-butanol gave **11** (16 mg, 0.035 mmol, 95%) as a white solid.

11: IR ν_{max} cm^{-1} 3404, 2938, 1635, 1456, 1384, 1192, 1143, 1059, 1029, 976, 816; $[\alpha]_D$= +116 (c 0.5, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.90−2.01 (m, 2H), 3.11−3.21 (m, 2H), 3.36 (s, 3H), 3.51−4.10 (m, 12H), 4.75 (s, 1H), 5.26 (s, 1H); ^{13}C NMR (75.4 MHz, D_2O) δ 24.6 (d, J = 135 Hz), 35.5, 55.4, 61.4, 63.4 (d, J = 5 Hz), 67.1, 69.5 (d, J = 7 Hz), 70.5 (2C), 71.5, 73.7, 74.0, 76.0, 99.3, 101.3; ^{31}P NMR (decoupled, 121 MHz, D_2O, pH* = 5.8) δ 22.5; HRMS calcd for $C_{15}H_{30}NO_{13}P$ $[M + H]^+$ = 464.1533, found 464.1538.

Methyl (2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl-(1→4)-6-*O*-((2-benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester)-2,3-di-*O*-benzyl-α-D-glucopyranoside (19). Compound **19** was synthesized following the procedure described in the preparation of **18** using **10** (100 mg, 0.142 mmol), **2** (209 mg, 0.170 mmol), and DMTST (92 mg, 0.355 mmol). FC (toluene: EtOAc 2:1) gave **19** (190 mg, 0.112 mmol, 79%) as a diastereomeric mixture: IR ν_{max} cm^{-1} 3087, 3062, 3030, 2898, 1722, 1602, 1585, 1520, 1497, 1453, 1384, 1362, 1320, 1266, 1213, 1100, 1046, 1027, 911, 736, 712, 697, 458. Diastereomer A: R_f 0.50 (toluene/EtOAc 1:1); $[\alpha]_D$ = +32 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97−2.05 (m, 2H), 3.35 (s, 3H), 3.37−3.45 (m, 3H), 3.58−4.67 (m, 34H), 4.85 (d, 1H, J = 11.3 Hz), 4.87 (d, 1H, J = 11.3 Hz), 4.95−5.11 (m, 5H), 5.13 (d, 1H, J = 1.9 Hz), 5.29 (d, 1H, J = 1.9 Hz), 5.64−5.70 (m, 1H), 5.72 (m, 1H), 7.08−7.34 (m, 52H), 7.50−7.55 (m, 1H), 8.05−8.07 (m, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 26.6 (d, J = 139 Hz), 35.1, 55.4, 64.8 (d, J = 6.3 Hz), 66.3, 66.4, 67.1 (d, J = 6.3 Hz), 68.8, 68.9, 69.2 (d, J = 5.7 Hz), 71.0, 71.8, 72.0, 72.4, 72.6, 73.1, 73.3, 74.2 (2C), 74.9, 75.1 (2C), 76.2, 77.3, 77.7, 79.6, 80.0, 81.1, 97.6 (J_{CH} = 168 Hz), 98.3 (J_{CH} = 173 Hz), 100.5 (J_{CH} = 171 Hz), 125.3−130.1, 132.9, 136.3−138.6, 156.2, 165.5; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ 31.8. Diastereomer B: R_f 0.40 (toluene/EtOAc 1:1); $[\alpha]_D$= +15 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97−2.05 (m, 2H), 3.33 (s, 3H), 3.37−4.64 (m, 37H), 4.84 (d, 1H, J = 11.0 Hz), 4.88 (d, 1H, J = 11.0 Hz), 4.98−5.11 (m, 6H), 5.28 (d, 1H, J = 1.9 Hz), 5.58−5.64 (m, 1H), 5.67 (m, 1H), 7.10−8.07 (m, 55H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 26.7 (d, J = 140 Hz), 35.1, 55.5, 64.6 (d, J = 5.7 Hz), 66.4, 66.5, 67.3 (d, J = 6.0 Hz), 68.7, 68.9, 69.0 (d, J = 6.0 Hz), 70.9, 71.8, 72.0, 72.3, 72.5, 73.0, 73.3, 74.1, 74.2, 74.9, 75.1 (2C), 76.1, 77.0, 77.7, 79.9, 80.0, 81.1, 97.6 (J_{CH} = 168 Hz), 98.3 (J_{CH} = 173 Hz), 100.4 (J_{CH} = 171 Hz), 125.3−130.1, 132.9, 136.0−138.7, 156.2, 165.3; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ 32.2; MALDI-TOF calcd for $C_{100}H_{108}NO_{21}P$ $[M + Na]^+$ 1713, found $[M + Na]^+$ 1713. Anal. Calcd for $C_{100}H_{108}NO_{21}P$: C, 71.03; H, 6.44. Found: C, 70.79; H, 6.59.

Methyl (α-D-Mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-6-*O*-((2-aminoethyl)phosphonic acid)-α-D-glucopyranoside (12). NaOMe (14 mg, 0.264 mmol) was added each to two separate diastereomeric solutions of **19** (75 mg, 0.044 mmol) in CH_2Cl_2/ MeOH (3 mL, 2:1). The solutions were stirred for 12 h, neutralized with Dowex-H^+, filtered, and concentrated separately. The obtained residues (R_f 0.46 (toluene/EtOAc 1:3) were further deprotected either by method A or B as described in the preparation of compound **11** to give **12** (method A: 22 mg, 0.035 mmol, 80%; method B: 23 mg, 0.036 mmol, 82%): $[\alpha]_D$ = +116 (c 1.0, H_2O); IR ν_{max} cm^{-1} 3406, 2939, 1635, 1458, 1399, 1384, 1191, 1135, 1061, 1030, 977, 815; ^{1}H NMR (300 MHz, D_2O) δ 1.89−2.00 (m, 2H), 3.11−3.20 (m, 2H), 3.35 (s, 3H), 3.50−3.99 (m, 17H), 4.08−4.11 (m, 1H), 4.75 (bs, 1H), 4.86 (s, 1H), 5.23 (s, 1H); ^{13}C NMR (75.4 MHz, D_2O) δ 24.7 (d, J = 134 Hz), 35.6, 55.4, 61.4, 63.5 (d, J = 5.4 Hz), 65.9, 66.6, 67.0, 69.5 (d, J = 6.6 Hz), 70.2, 70.5, 70.7, 70.8, 71.4, 72.2, 73.0, 73.6, 76.2, 99.3, 99.9, 101.3; ^{31}P NMR (decoupled, 121 MHz, D_2O, pH* = 5.2) δ 22.2; HRMS calcd for $C_{21}H_{40}NO_{18}P$ $[M + H]^+$ = 626.2061, found 626.2073.

2,3-*O*-(D-1,7,7-Trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (20). To a solution of D-camphor (32.6 g, 0.21 mol) in trimethyl orthoformate (165 mL) and methanol (33 mL) was added concentrated H_2SO_4 (320 μL). The solution was stirred for 48 h, neutralized with NaOMe (685 mg), and concentrated. The resulting residue was dissolved in toluene, the precipitated Na_2SO_4 was filtered off, and the filtrate concentrated to give crude D-camphor dimethyl acetal. To this compound and *myo*-inositol (16.86 g, 93.6 mmol) in DMSO (184 mL) was added H_2SO_4 (950 μL). The resulting mixture was stirred for 3 h at 75 °C, neutralized with NEt_3 (6.3 mL), and concentrated under vacuum at 80 °C. To the obtained residue was added DMSO to a total weight of 82 g, and $CHCl_3$ (285 mL), MeOH (17.9 mL), H_2O (5.7 mL), and p-TsOH·H_2O (63 mg) were added. The mixture was stirred overnight and neutralized with NEt_3 (2.1 mL), and the precipitate was filtered off and washed with $CHCl_3$ (200 mL × 2) to give crude product. Recrystallization from MeOH (0.1% NEt_3) twice gave pure **20** (10.9 g, 34.7 mmol, 37%): mp 233−234 °C (MeOH (0.1% NEt_3)/H_2O) [lit.[31] (MeOH) mp 231−232°C]; $[\alpha]_D$ = +44.0 (c 1.9, pyridine) [lit.[31] $[\alpha]_D$ = +44.3 (c 1.9, pyridine)]; ^{1}H and ^{13}C NMR spectra were in accordance with those previously published;[24] MALDI-TOF calcd for $C_{16}H_{26}O_6$ $[M + Na]^+$ 337.2, found $[M + Na]^+$ 337.2.

Ethyl 4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (7). Thioglucoside **21**[25] (5.00 g, 9.43 mmol) in AcOH/H_2O (50 mL, 6:4) was heated at 80 °C for 8 h and then co-concentrated with toluene several times. The obtained solid was dissolved in dry DMF (20 mL), and NaH (1.028 g, 23.58 mmol, 55% dispersion in oil) was added at 0 °C under inert atmosphere. When the gas evolution had ceased, the water bath was removed and allyl bromide (2.85 mL, 23.58 mmol) was added. The reaction mixture was stirred for 3 h, quenched with MeOH (5 mL), diluted with toluene, and washed with H_2O. The organic phase was dried, filtered, and concentrated followed by FC (toluene/EtOAc 19:1) and

(31) Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361−6374.

recrystallization in hexane to give **7** (3.60 g, 8.58 mmol, 91%): R_f 0.52 (toluene/EtOAc 9:1); $[\alpha]_D$= −46 (*c* 1.2, $CHCl_3$); mp 39−40 °C (from hexane); IR ν_{max} cm^{-1} 3085, 3030, 2980, 2923, 2870, 2215, 2110, 1647, 1498, 1465, 1453, 1428, 1389, 1387, 1355, 1280, 1264, 1147, 1114, 1092, 1055, 1000, 924, 752, 698. 680, 617, 566, 550, 478; ^{1}H NMR (300 MHz, $CDCl_3$), δ 1.31 (t, 3H, J = 7.4 Hz), 2.65−2.83 (m, 2H), 3.33−3.51 (m, 4H), 3.63 (dd, 1H, J = 4.4 11.0 Hz), 3.70 (dd, 1H, J = 1.9 11.0 Hz), 3.97−4.32 (m, 4H), 4.25 (d, 1H, J = 9.9 Hz), 4.86 (s, 2H), 5.14−5.30 (m, 4H), 5.83−5.96 (m, 2H), 7.27−7.41(m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ 14.9, 24.5, 66.0, 68.7, 72.3, 73.6, 75.5, 77.5, 79.3, 84.1, 84.9, 116.8, 116.9, 127.8, 128.0 (2C), 128.3 (2C), 134.4, 134.5, 137.7; MALDI-TOF Calcd for $C_{21}H_{29}N_3O_4S$ [M + Na]$^+$ 442.2, found [M + Na]$^+$ 442.2. Anal. Calcd for $C_{21}H_{29}N_3O_4S$: C, 60.12; H, 6.97. Found: C, 59.97; H, 6.75.

Ethyl 4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside *S*-Oxide (8). To a stirred solution of **7** (1.00 g, 2.38 mmol) in CH_2Cl_2 (20 mL) at −60 °C was added *m*-CPBA (431 mg, 2.49 mmol). The mixture was allowed to reach −20 °C during 30 min, diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (toluene/EtOAc 1:1) and reqrystallization from hexane gave **8** (674 mg, 1.55 mmol, 65%) as a white solid: R_f 0.36 (toluene/EtOAc 1:1); $[\alpha]_D$ = −26 (*c* 1.0, $CHCl_3$); mp 64−65 °C (from hexane); IR ν_{max} cm^{-1} 3071, 3033, 2981, 2967, 2930, 2873, 2856, 2219, 2116, 1652, 1499, 1454, 1420, 1356, 1346, 1273, 1120, 1081, 1061, 1020, 994, 941, 916, 777, 752, 702, 677, 611, 552; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (t, 3H, J = 7.7 Hz), 2.84 (dq, 1H, J = 15.4 7.7 Hz), 3.04 (dq, 1H, J = 15.4 7.7 Hz), 3.41−3.45 (m, 1H), 3.50 (t, 1H, J = 9.1 Hz), 3.61 (t, 1H, J = 9.1 Hz), 3.64 (dd, 1H, J = 4.1 11.0 Hz), 3.70 (dd, 1H, 1.9 11.3 Hz), 3.82 (t, 1H, J = 9.1 Hz), 3.99 (d, 1H, J = 9.1 Hz), 4.02 (m, 2H), 4.13 (dddd, 1H, J = 12.4 5.8 1.4 1.4 Hz), 4.28 (dddd, 1H, J = 12.4 5.8 1.4 1.4 Hz), 4.88 (s, 2H), 5.16−5.30 (m, 4H), 5.81−5.96 (m, 2H), 7.31−7.42 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ 7.2, 43.1, 60.9, 68.3, 72.4, 73.7, 75.7, 76.9, 80.0, 84.7, 90.6, 117.1, 117.2, 128.0, 128.1 (2C), 128.4 (2C), 134.2, 134.3, 137.5; MALDI-TOF calcd for $C_{21}H_{29}N_3O_5S$ [M + Na]$^+$ 458.2, found [M + Na]$^+$ 458.2. Anal. Calcd for $C_{21}H_{29}N_3O_5S$: C, 57.91; H, 6.71. Found: C, 57.75; H, 6.87.

6-*O*-(4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1,4,5-tri-*O*-pivaloyl-2,3-*O*-(D-1,7,7-trimethyl-[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (5). Method A. Glycosylation Using Thioglucoside 7. To a solution of thioglycoside **7** (1.00 g, 2.42 mmol), 1,4,5-tri-*O*-pivaloyl-2,3-*O*-(D-1,7,7-trimethyl[2,2,1]bicyclohept-6-ylidene)-D-*myo*-inositol (**6**)[24] (1.64 g, 2.90 mmol), and 4 Å molecular sieves (0.5 g) in dry diethyl ether (45 mL) was added MeOTf (1.10 mL, 9.72 mmol). The mixture was stirred for 16 h, Et_3N (1 mL) was added, and the mixture was diluted with DCM, filtered through Celite, and concentrated. FC (light petroleum (45−65)/EtOAc 9:1) gave **5** (1.14 g, 1.23 mmol, 51%) as a colorless syrup.

Method B. Glycosylation Using Sulfoxide 8. To a solution of *S*-oxide **8** (500 mg, 1.15 mmol) in toluene (20 mL) at −50 °C was added Tf_2O (203 μL, 1.21 mmol). After 5 min, a solution of alcohol **6** (1.04 g, 1.84 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (283 mg, 1.38 mmol) in toluene (10 mL) was added at −50 °C, and the reaction mixture was allowed to reach 0 °C during 30 min, diluted with toluene, washed with saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (light petroleum (45−65)/EtOAc 9:1) gave **5** (647 mg, 0.70 mmol, 61%) with traces of the β-anomer.

5: R_f 0.34 (light petroleum (45−65)/EtOAc 9:1); $[\alpha]_D$ = +49 (*c* 0.5, $CHCl_3$); IR ν_{max} cm^{-1} 3078, 2967, 2936, 2874, 2108, 1740, 1480, 1459, 1397, 1369, 1279, 1205, 1140, 1114, 1038, 991, 970, 924, 748, 697; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.18−1.42 (m, 30H), 1.66−1.76 (m, 2H), 1.83−2.04 (m, 2H), 3.26 (dd, 1H, J = 3.6 10.4 Hz), 3.55 (dd, 1H, J = 1.9 11.0 Hz), 3.60−3.68 (m, 2H), 3.80−3.83 (m, 1H), 3.88 (dd, 1H, J = 9.1 10.4 Hz), 3.92−3.99 (m, 1H), 4.02−4.17 (m, 3H), 4.22−4.31 (m, 2H), 4.55 (dd, 1H, J = 4.4 7.4 Hz), 4.81 (d, 1H, J = 10.4 Hz), 4.88 (d, 1H, J = 10.4 Hz), 5.00 (t, 1H, J = 5.0 Hz), 5.12−5.30 (m, 6H), 5.33 (dd, 1H, J = 4.4 8.2 Hz), 5.83−5.96 (m, 2H), 7.28−7.41 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 10.0, 20.1, 20.3, 26.9, 27.0 (3C), 27.1 (3C), 27.2 (3C), 29.6, 38.7, 38.8, 39.0, 43.0, 45.1, 47.8, 51.5, 63.0, 67.8, 69.6, 71.2, 71.3, 71.5, 71.7, 72.5, 73.7 74.7, 75.3, 75.5, 77.7, 79.4, 98.0, 116.7, 117.4, 118.0, 127.8, 128.1, 128.4, 134.4, 134.7, 138.0, 176.6, 177.2, 177.6; MALDI-TOF calcd for $C_{50}H_{73}N_3O_{13}$ [M + Na]$^+$ 946.5, found [M + Na]$^+$ 946.5. Anal. Calcd for $C_{50}H_{73}N_3O_{13}$: C, 64.98; H, 7.96. Found: C, 65.09; H, 8.02.

6-*O*-(4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (22). To a solution of compound **5** (2.50 g, 2.71 mmol) in MeOH (20 mL) was added NaOH (4.36 g, 108.9 mmol). The solution was refluxed for 30 min, diluted with toluene, and washed sequently with saturated aqueous $NaHCO_3$ and water. The organic phase was dried, filtered, and concentrated. FC (toluene/EtOAc 3:1 → 1:1) gave **22** (1.51 g, 2.25 mmol, 83%) as a colorless glue: R_f 0.50 (toluene/EtOAc 1:1); $[\alpha]_D$ = +41 (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3455, 3081, 2934, 2876, 2110, 1453, 1384, 1321, 1262, 1206, 1146, 1113, 1083, 1046, 1029, 967, 929, 700, 619; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H), 0.87 (s, 3H), 1.03 (s, 3H), 1.20−1.28 (m, 1H), 1.36−1.45 (m, 1H), 1.52 (d, 1H, J = 13.2 Hz), 1.68−1.76 (m, 2H), 2.00−2.06 (m, 2H), 3.29−3.41 (m, 3H), 3.52 (dd, 1H, J = 3.9 10.2 Hz), 3.55 (dd, 1H, J = 6.6 10.2 Hz), 3.64−3.80 (m, 4H), 3.85−4.14 (m, 7H), 4.29 (dddd, 1H, J = 1.2 1.2 5.7 12.6 Hz), 4.37 (dd, 1H, J = 3.9 6.0 Hz), 4.86 (ABq, 2H, J = 10.5 Hz), 5.07 (d, 1H, J = 3.9 Hz), 5.17−5.32 (m, 4H), 5.83−5.96 (m, 2H), 7.37−7.41 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 9.8, 20.2, 20.6, 26.9, 29.7, 44.5, 45.1, 48.0, 51.7, 64.0, 68.5, 70.1, 71.4, 72.5, 72.9, 73.8, 74.6, 74.7, 75.5, 75.6, 78.3, 80.6, 84.5, 98.6, 117.3, 117.7, 118.2, 127.9, 128.0, 128.1, 128.3,128.4, 134.0, 134.2, 137.6; MALDI-TOF calcd for $C_{35}H_{49}N_3O_{10}$ [M + Na]$^+$ 694.3, found [M + Na]$^+$ 694.3. Anal. Calcd for $C_{35}H_{49}N_3O_{10}$: C, 62.58; H, 7.35. Found: C, 62.44; H, 7.28.

6-*O*-(4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (23). Compound **22** (900 mg, 1.34 mmol) was treated with 2,2-dimethoxypropane (4.12 mL, 33.5 mmol) and pyridinium *p*-toluene-4-sulfonate (50 mg, 0.20 mmol) in DMF (8 mL). After 8 h, solid $NaHCO_3$ (0.4 g) was added, and the solution was diluted with toluene and washed with water. The organic phase was dried, filtered, and concentrated followed by FC (toluene/EtOAc 15:1 → 9:1) to give **23** (620 mg, 0.87 mmol, 65%) as a colorless syrup: R_f 0.54 (toluene/EtOAc 9:1); $[\alpha]_D$ = +95 (*c*, $CHCl_3$); IR ν_{max} cm^{-1} 3074, 2986, 2935, 2872, 2107, 1497, 1477, 1454, 1383, 1372, 1320, 1235, 1117, 1080, 1054, 1027, 1005, 925, 874, 852, 838, 795, 747, 698; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H), 0.89 (s, 3H), 1.02 (s, 3H), 1.16−1.24 (m, 1H), 1.36−1.41 (m, 1H), 1.42 (s, 3H), 1.43 (s, 3H), 1.47 (d, 1H, J = 12.9 Hz), 1.67−1.76 (m, 1H), 1.79 (t, 1H, J = 4.5 Hz), 1.99−2.09 (m, 2H), 2.72 (d, 1H, J = 1.5 Hz), 3.35 (dd, 1H, J = 3.6 10.3 Hz), 3.53−3.66 (m, 3H), 3.72 (dd, 1H, J = 3.0 11.0 Hz), 3.88−3.95 (m, 2H), 3.98 (ddd, 1H, J = 1.4 1.4 5.8 12.9 Hz), 4.04−4.21 (m, 6H), 4.30 (ddd, 1H, J = 1.4 1.4 5.8 12.1 Hz), 4.42 (dd, 1H, J = 3.3 6.9 Hz), 4.84 (d, 1H, J = 10.7 Hz), 4.89 (d, 1H, J = 10.7 Hz), 5.14 (d, 1H, J = 3.6 Hz), 5.15−5.32 (m, 4H), 5.86−6.00 (m, 2H), 7.25−7.42 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 9.7, 20.1, 20.4, 26.8, 27.1, 27.2, 29.8, 42.9, 45.1, 48.0, 51.5, 63.1, 67.9, 70.7, 72.3, 72.6, 73.9, 74.8, 74.9, 75.3, 76.4, 77.7, 78.0, 78.2, 80.0, 96.8, 112.2, 117.1, 117.2, 118.5, 127.8, 128.0, 128.4, 134.5, 134.6, 138.0; MALDI-TOF calcd for $C_{38}H_{53}N_3O_{10}$ [M + Na]$^+$ 734.4, found [M + Na]$^+$ 734.4. Anal. Calcd for $C_{38}H_{53}N_3O_{10}$: C, 64.12; H, 7.50. Found: C, 64.28; H, 7.39.

6-*O*-(4,6-Di-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-di-*O*-benzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (24). To a solution of **23** (500 mg, 0.70 mmol) and dibenzyl diisopropyl phosphoramidite (411 mg, 1.19 mmol) in CH_2Cl_2 (10 mL) was added 1*H*-tetrazole (172

mg, 2.45 mmol). After 45 min, the reaction mixture was cooled to 0 °C, and *m*-CPBA (242 mg, 1.40 mmol) was added. The mixture was stirred for 1 h, diluted with CH_2Cl_2, washed with 20% aq $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, dried, filtered, and concentrated. FC (toluene/EtOAc 6:1) gave **24** (578 mg, 0.60 mmol, 85%) as a syrup: R_f 0.47 (toluene/EtOAc 4:1); $[\alpha]_D = +43$ (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3065, 3032, 2984, 2935, 2108, 1498, 1455, 1390, 1372, 1264, 1215, 1147, 1117, 1050, 1006, 919, 849, 737, 697, 602; ^1H NMR (300 MHz, $CDCl_3$) δ 0.78 (s, 3H), 0.84 (s, 3H), 0.92 (s, 3H), 1.08−1.19 (m, 1H), 1.20−1.39 (m, 2H), 1.32 (s, 3H), 1.36 (s, 3H), 1.58−1.62 (m, 2H), 1.84−1.99 (m, 2H), 3.17 (dd, 1H, J = 3.8 10.2 Hz), 3.39 (dd, 1H, J = 8.8 10.4 Hz), 3.50−3.56 (m, 2H), 3.64 (dd, 1H, J = 2.7 11.0 Hz), 3.79−3.93 (m, 4H), 3.99−4.06 (m, 2H), 4.08−4.27 (m, 3H), 4.41−4.46 (m, 1H), 4.52−4.58 (m, 1H), 4.76 (d, 1H, J = 10.4 Hz), 4.82 (d, 1H, J = 10.4 Hz), 4.97 (d, 2H, J = 7.7 Hz), 5.03 (d, 2H, J = 8.5 Hz), 5.07 (d, 1H, J = 3.8 Hz), 5.09−5.25 (m, 4H), 5.78−5.95 (m, 2H), 7.21−7.36 (m, 15H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 9.9, 20.2, 20.4, 26.9 (2C), 27.1, 29.8, 43.9, 45.1, 47.9, 51.5, 63.1, 67.9, 69.4 (d, J = 4.9 Hz), 69.5 (d, J = 5.4 Hz), 70.7, 72.3, 73.6, 73.8, 74.2 (d, J = 4.0 Hz), 75.3, 76.7, 76.8 (d, J = 4.7 Hz), 77.6, 77.9 (d, J = 6.6 Hz), 78.0, 79.9, 97.4, 112.2, 116.9, 117.1, 119.0, 127.8−128.5, 134.6, 134.7, 135.6 (2 C), 135.7 (2 C), 138.0; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ −0.5; MALDI-TOF calcd for $C_{52}H_{66}N_3O_{13}P$ $[M + Na]^+$ 994.4, found $[M + Na]^+$ 994.4. Anal. Calcd for $C_{52}H_{66}N_3O_{13}P$: C, 64.25; H, 6.84. Found: C, 63.97; H, 6.78.

6-*O*-(2-Azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-1-di-*O*-benzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (25). A solution of compound **24** (300 mg, 0.309 mmol) and [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium(I)PF_6 (26 mg, 0.031 mmol) in THF (5 mL) was degassed and activated by treatment with H_2 (*g*) for 5 min. Stirring was continued under N_2 atmosphere for 20 min when *N*-iodosuccinimide (695 mg, 3.09 mmol) and H_2O (1 mL) were added. After 1 h, the solution was diluted with EtOAc and washed with satd aq $Na_2S_2O_3$ and satd aq $NaHCO_3$, dried, filtered, and concentrated. FC (EtOAc/toluene 2:1) gave **25** (237 mg, 0.266 mmol, 86%) as a colorless glue: R_f 0.21 (toluene/EtOAc 1:1); $[\alpha]_D = +23$ (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3065, 3033, 2984, 2936, 2109, 1497, 1455, 1390, 1373, 1262, 1111, 1085, 1053, 1010, 736, 698, 602; ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 3H), 0.89 (s, 3H), 0.99 (s, 3H), 1.13−1.21 (m, 1H), 1.32−1.41 (m, 2H), 1.37 (s, 3H), 1.42 (s, 3H), 1.65−1.70 (m, 2H), 1.91−2.01 (m, 2H), 3.19 (dd, 1H, J = 3.6 10.2 Hz), 3.49 (dd, 1H, J = 8.5 10.4 Hz), 3.61 (dd, 1H, J = 8.8 9.6 Hz), 3.70−3.87 (m, 4H), 3.92 (dd, 1H, J = 8.1 10.4 Hz), 4.09 (dd, 1H, J = 7.1 8.0 Hz), 4.22 (dd, 1H, J = 3.6 8.5 Hz 4.42−4.47 (m, 1H), 4.57−4.62 (m, 1H), 4.77 (d, 1H, J = 11.1 Hz), 4.89 (d, 1H, J = 11.1 Hz), 5.03−5.09 (m, 5H), 7.28−7.39 (m, 15H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 9.9, 14.1, 20.2, 20.4, 26.9, 27.0, 29.8, 43.7, 45.1, 47.9, 51.5, 61.8, 62.8, 69.9 (2C, d, J = 5.4 Hz), 70.9, 71.7, 73.7 (d, J = 6.0 Hz), 73.8, 75.0, 77.0 (2C), 77.5 (d, J = 6.3 Hz), 77.7 (d, J = 3.7 Hz), 80.0, 97.3, 112.5, 119.1, 127.9−128.6, 135.4−135.6, 138.0; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$) δ −0.5; MALDI-TOF calcd for $C_{46}H_{58}N_3O_{13}P$ $[M + Na]^+$ 914.4, found $[M + Na]^+$ 914.4. Anal. Calcd for $C_{46}H_{58}N_3O_{13}P$: C, 61.94; H, 6.55. Found: C, 61.77.; H, 6.49.

6-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-D-*myo*-inositol 1-Phosphate (26). Compound **25** (75 mg, 0.084 mmol) was dissolved in THF (2 mL) and added to ∼20 mL of NH_3 (l) at −33 °C containing a small amount of Na (s). The mixture was stirred vigorously for 1 min when NH_4Cl (s) was added until the blue color disappeared followed by concentration under a stream of nitrogen. The residue was dissolved in 0.1 M aq HCl (10 mL) and stirred for 5 h before neutralization with 25% NH_3 (0.1 mL). The aqueous phase was washed with Et_2O (20 mL) and concentrated, and the obtained residue was gelfiltrated on a Sephadex G-15 column eluted with H_2O containing 1% *n*-butanol to give **26** (32 mg, 0.076 mmol, 90%). ^1H, ^{13}C, and ^{31}P NMR spectra were in accordance with those previously reported.[30]

6-*O*-(2-Azido-3-*O*-benzyl-6-*O*-((2-benzyloxycarbonylaminoethyl)phosphonic acid benzyl ester)-2-deoxy-α-D-glucopyranosyl)-1-di-*O*-benzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (3). Compound **3** was synthesized according to the procedure described in the preparation of compound **10** (method B), using **4** (117 mg, 0.336 mmol), oxalylic chloride (28 μL, 0.331 mmol), DMF (5 μL), **25** (150 mg, 0.168 mmol), and Et_3N (92 μL, 0.662 mmol). FC (EtOAc/toluene 3:1 → 6:1) gave **3** (168 mg, 0.137 mmol, 82%) as a diastereomeric mixture: R_f 0.40 (EtOAc/ toluene 3:1); $[\alpha]_D = +24$ (*c* 1.0, $CHCl_3$); IR ν_{max} cm^{-1} 3090, 3067, 3035, 2984, 2954, 2935, 2110, 1719, 1636, 1498, 1456, 1384, 1260, 1119, 1049, 1011, 739, 697; ^1H NMR (300 MHz, $CDCl_3$, diastereomeric mixture) δ 0.88 (s, 6H), 0.93 (s, 6H), 1.02 (s, 6H), 1.19−1.21 (m, 2H), 1.23−1.48 (m, 16H), 1.69−1.73 (m, 4H), 1.94−2.15 (m, 8H), 3.17−3.25 (m, 2H), 3.42−3.52 (m, 6H), 3.66−3.77 (m, 2H), 3.83−4.26 (m, 12H), 4.36−4.45 (m, 2H), 4.51−4.54 (m, 2H), 4.58−4.63 (m, 2H), 4.82−4.89 (m, 3H), 4.96−5.14 (m, 19H), 5.58 (m, 1H), 5.70 (m, 1H), 7.20−7.48 (m, 50H); ^{13}C NMR (75.4 MHz, $CDCl_3$, diastereomeric mixture) δ 9.9 (2C), 20.1 (2C), 20.3 (2C), 26.0 (d, J = 139 Hz), 26.2 (d, J = 139 Hz), 26.8−27.0 (6 C), 29.7 (2C), 34.9 (2C), 43.7 (2C), 44.9 (2C), 47.8 (2C), 51.2 (2C), 62.4 (2C), 63.3 (d, J = 5.7 Hz), 64.0 (d, J = 4.9 Hz), 66.6, 66.7, 67.4 (d, J = 6.6 Hz), 67.9 (d, J = 6.3 Hz), 69.3 (2C, d, J = 5.7 Hz), 69.4 (2C, d, J = 5.7 Hz), 69.9 (2C), 70.4 (d, J = 4.3 Hz), 70.5 (d, J = 4.3 Hz), 73.4, 73.5, 73.8 (d, J = 4.6 Hz), 73.9 (d, J = 4.3 Hz), 75.1, 75.3, 76.5 (2C), 76.7 (d, J = 3.4 Hz), 76.8 (d, J = 3.4 Hz), 77.3, 77.4, 77.5 (d, J = 6.3 Hz), 77.6 (d, J = 6.3 Hz), 78.9, 79.1, 97.1 (J_{CH} = 174 Hz), 97.4 (J_{CH} = 174 Hz), 112.1, 112.3, 118.9 (2C), 127.7−128.6, 135.5−136.2, 156.1 (2C); ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$, diastereomeric mixture) δ 33.7, 33.3, −0.33, −0.39; MALDI-TOF calcd for $C_{63}H_{76}N_4O_{17}P_2$ $[M + Na]^+$ 1245, found $[M + Na]^+$ 1245. Anal. Calcd for $C_{63}H_{76}N_4O_{17}P_2$: C, 61.86; H, 6.26. Found: C, 61.67; H, 6.16.

[2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-α-D-mannopyranoside]-(1→4)-[2-azido-3-*O*-benzyl-6-*O*-(2-benzyloxycarbonylaminoethyl)-phosphonic acid benzyl ester-2-deoxy-α-D-glucopyranosyl]-(1→6)-1-di-*O*-benzylphosphoryl-4,5-*O*-isopropylidene-2,3-*O*-(D-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (27). To a stirred solution of **2** (115 mg, 0.112 mmol), **3** (114 mg, 0.093 mmol), and 4 Å molecular sieves (0.4 g) in Et_2O (10 mL) was added DMTST (53 mg, 0.205 mmol). After 7 h, NEt_3 (0.3 mL) was added to the mixture and stirring continued for 15 min followed by filtering through Celite and concentration. FC (toluene/EtOAc 3:1 → 1:1) gave **27** (151 mg, 0.069 mmol, 74%) as a diastereomeric mixture: R_f 0.29 (toluene/EtOAc 2:1); $[\alpha]_D = +40$ (*c* 1.2, $CHCl_3$); IR ν_{max} cm^{-1} 3090, 3064, 3033, 2984, 2937, 2909, 1723, 1498, 1455, 1384, 1267, 1115, 1046, 1027, 1005, 737, 679; ^1H NMR (300 MHz, $CDCl_3$, diastereomeric mixture) δ 0.85 (s, 6H), 0.91 (s, 6H), 0.99 (s, 3H), 1.00 (s, 3H), 1.15−1.46 (m, 18H), 1.64−1.74 (m, 4H), 1.91−2.12 (m, 8H), 2.98 (dd, 2H, J = 3.3 9.6 Hz), 3.26−4.67 (m, 68H), 4.83−5.10 (m, 26H), 5.17−5.20 (m, 2H), 5.31 (s, 1H), 5.33 (s, 1H), 5.68 (s, 1H), 5.72 (s, 1H), 5.98−6.04 (m, 2H), 7.08−7.36 (m, 114H), 7.50−7.56 (m, 2H), 8.02−8.08 (m, 4H); ^{13}C NMR (Varian Inova 600 Instrument, 150.8 M Hz, $CDCl_3$, diastereomeric mixture) δ 10.0 (2C), 20.2 (2C), 20.4 (2C), 26.5−27.1 (8 C), 29.7 (2C), 35.1 (2C), 43.8 (2C), 45.0 (2C), 47.9 (2C), 51.4 (2C), 62.6 (2C), 63.6, 63.9, 65.9,66.1, 66.3, 66.4, 67.0 (d, J = 5.4 Hz), 67.1 (d, J = 5.9 Hz), 68.4, 68.6, 68.7, 68.8, 69.4−69.6 (6C), 70.7, 70.8, 71.8 (2C), 71.9 (2C), 72.1 (2C), 72.7, 72.9, 73.2 (2C), 73.6, 73.7, 73.8−73.9 (4C), 74.1, 75.0 (2C), 75.1, 75.7, 75.8, 75.9, 76.4, 76.6, 76.7, 77.0−77.3 (11C), 77.6, 79.4, 79.6 (2C), 79.7, 96.6 (J_{CH} = 173 Hz), 97.0 (J_{CH} = 173 Hz), 98.4 (2C, J_{CH} = 174 Hz), 100.2 (J_{CH} = 172 Hz), 100.3 (J_{CH} = 172 Hz), 112.5, 112.6, 119.0, 119.1, 126.8−128.6, 129.9, 132.9, 135.4−136.6, 137.7, 137.7, 138.2−138.5, 156.2 (2C), 165.2,

165.4; ^{31}P NMR (decoupled, 121 MHz, $CDCl_3$, diastereomeric mixture) δ 32.2, 31.3, −0.2, −0.3; MALDI-TOF calcd for $C_{124}H_{136}N_4O_{28}P_2$ $[M + Na]^+$ 2214, found $[M + Na]^+$ 2214. Anal. Calcd for $C_{124}H_{136}N_4O_{28}P_2$: C, 67.93; H, 6.25. Found: C, 68.12; H, 6.32.

[α-D-Mannopyranosyl-(1→6)-α-D-mannopyranosyl]-(1→4)-[2-amino-6-*O*-((2-aminoethyl)phosphonic acid)-2-deoxy-α-D-glucopyranosyl]-(1→6)-1-*O*-hydrogenphosphate-D-*myo*-inositol, Ammonium Salt (1). To a stirred solution of **27** (78 mg, 0.0356 mmol) in CH_2Cl_2/MeOH (2:1, 3 mL) was added NaOMe (10 mg, 0.1852 mmol). After 16 h, the solution was diluted with CH_2Cl_2, washed with water, dried, filtered, and concentrated. The obtained residue (R_f = 0.38 (toluene/EtOAc 1:1) was dissolved in dry THF (2 mL) and added to NH_3 (l) (~20 mL) at −33 °C. To the stirred mixture was added a minimum amount of sodium for the mixture to turn deep blue. After 1 min, NH_4Cl (s) was added until the color disappeared and the mixture was concentrated under a stream of nitrogen gas. The residue was dissolved in 0.1 M HCl (10 mL), and 5 h later the mixture was neutralized with NH_3 (25%, 0.9 mL), washed with ether (2 × 15 mL), and concentrated. Gel filtration of the residue on a Sephadex G-15 column eluted with H_2O containing 1% *n*-butanol gave **1** (22 mg, 0.0253 mmol, 71%): $[\alpha]_D$ = +73 (*c* 0.6, H_2O); IR ν_{max} cm^{-1} 3415, 2926, 1630, 1383, 1189, 1095, 1066, 974, 820; ^{1}H NMR (300 MHz, D_2O) δ 1.92−2.13 (m, 2H), 3.17−3.28 (m, 2H), 3.36−3.44 (m, 2H), 3.56 (dd, 1H, *J* = 2.7 10.2 Hz), 3.64−3.69 (m, 3H), 3.72−3.73 (m, 1H), 3.77−3.94 (m, 8H), 4.00−4.20 (m, 8H), 4.25−4.28 (m, 1H), 4.93 (d, 1H, *J* = 1.5 Hz), 5.30 (d, 1H, *J* = 1.5 Hz), 5.68 (d, 1H, *J* = 3.9 Hz); ^{13}C NMR (75.4 MHz, D_2O) δ 25.1 (d, *J* = 134 Hz), 36.0, 54.5, 61.7, 63.8, 66.3, 67.0, 67.5, 70.5 (d, *J* = 6.6 Hz), 70.7, 71.0, 71.2 (4C), 72.5, 72.8, 73.1, 73.5 (2C), 76.0, 76.3 (d, *J* = 5.5 Hz), 78.0 (d, *J* = 5.4 Hz), 95.8, 100.4, 102.1; ^{31}P NMR (decoupled, 121 MHz, D_2O, pH* = 5.5) δ 22.4, 2.6; HRMS calcd for $C_{26}H_{50}N_2O_{25}P_2$ $[M + H]^+$ 853.2256, found 853.2299.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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