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First Total Synthesis of a GPI-Anchored Peptide

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A GPI-anchored dipeptide of sperm CD52 antigen was prepared through a convergent synthesis. First, the dipeptide with its *C*-terminus free and the GPI with its nonreducing end phosphoethanolamine bearing a free amino group were synthesized separately. Then, the two building blocks were coupled with use of EDC/HOBt as the condensation reagent. Finally, the GPI-anchored peptide was deprotected to give the target molecule **1**.

Introduction

Many proteins and glycoproteins are anchored to eukaryotic cells by glycosylphosphatidyl inositols (GPIs),[1] and the GPI-anchored proteins/glycoproteins, as well as the GPIs, play a pivotal role in numerous biological functions.[2−4] To study these functions, it is essential to access homogeneous glycoforms of GPIs and GPI-anchored structures. The problem is that it is difficult, if not impossible, to prepare homogeneous glycoconjugates biochemically. Therefore, chemical synthesis becomes an important tool for obtaining homogeneous, well-defined samples for these investigations.

Figure 1 highlights the typical structure of a GPI-anchored protein or glycoprotein.[1] All GPIs share a conserved common core with the tetrasaccharide linked to the 6-*O*-position of inositol. The variable lipid chains are attached to the 1-*O*-position of inositol through a phosphate group. The GPI may have other carbohydrate residues as branches, and sometimes the inositol is modified by an acyl group. The peptides/glycopeptides are attached to the nonreducing end of the GPI glycans via the *C*-termini and a phosphoethanolamine group.

Since the first characterization of a GPI by Ferguson in 1988,[5,6] the structural complexity of GPIs has attracted significant interest in their total synthesis. Several GPIs were therefore prepared by Ferguson,[7,8] Fraser-Reid,[9,10] Ley,[11,12] Martin-Lomas,[13] Ogawa,[14−16] Schmidt,[17−21] Seeberger,[22,23] and us.[24] The *P. faclciparum* GPI was further conjugated to a carrier protein through an artificial linker to form a malaria vaccine that could induce protective immune reactions in animals.[23] However, there has been no report yet describing the synthesis of a natural GPI-peptide/glycopeptide.

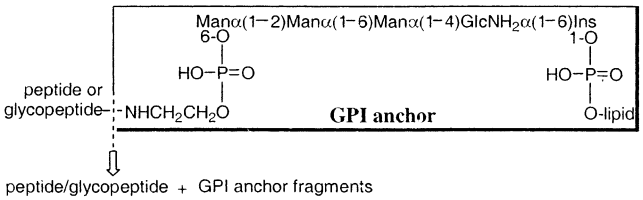

FIGURE 1. Typical structure of GPI-anchored proteins/glycoproteins.

This paper describes the first total synthesis of a naturally linked GPI-peptide conjugate **1** (Figure 2). The synthetic target is a fragment of sperm CD52. CD52 antigens play an important role in the human immune system[25−27] and the human reproduction process.[28−30]

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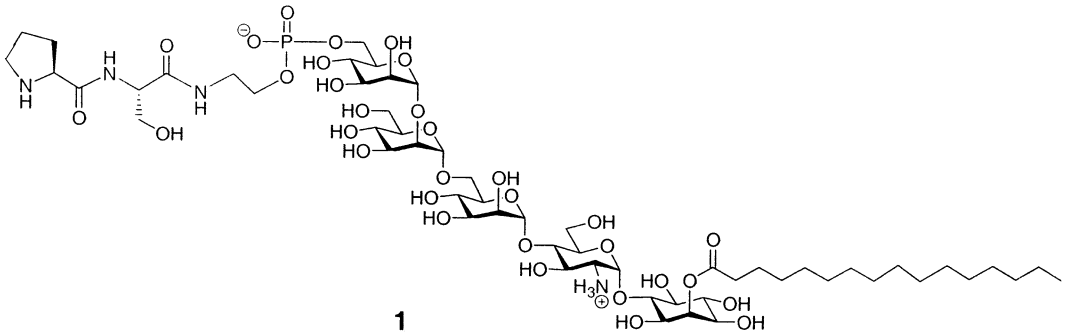

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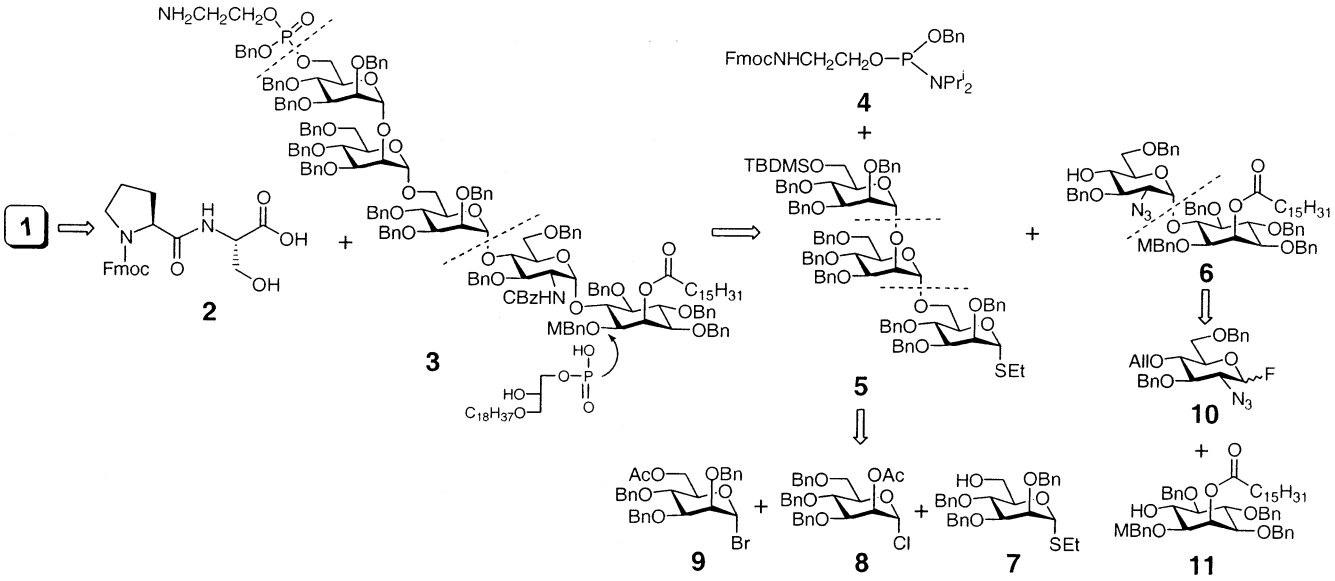
FIGURE 2. The synthetic target.

SCHEME 1. Retrosynthetic Analysis



Additionally, the unique structure of sperm CD52 also causes our attention. For example, the GPI of sperm CD52 has a long acyl chain on the inositol 2-*O*-position. Thus, the synthesis of sperm CD52 and its GPI is challenging in regard to the planning of protecting tactics and the preparation of the required inositol derivatives.

Results and Discussion

For retrosynthesis of **1** (Scheme 1), the amido bond between the peptide and GPI was disconnected first to yield two key fragments **2** and **3**. As mentioned above, this amido linkage is shared by all GPI-anchored proteins and glycoproteins (Figure 1). Then, a synthetic strategy thus developed may be of general application. Moreover, preparing the GPI and the peptide separately and coupling the two fragments at the final stage could also lead to a convergent synthetic procedure and avoid the potential chemistry compatibility problems.

In the synthesis of GPI fragment **3**, the benzyl group was chosen as the permanent protection, since it can be later deprotected globally under mild conditions. The presence of an ester bond in the final synthetic target prohibited acyl groups, such as acetyl and benzoyl, from being used.

Fragment **3** was further disconnected into three smaller pieces **4**, **5**, and **6**. The primary amino group in **4** was protected by an Fmoc that can be easily and selectively removed. Fragment **5** was designed as a thioglycoside to facilitate its direct use as a glycosyl donor, while the 6-*O*-position at its nonreducing end had to be differentiated from that of other hydroxyls. This design also enabled the use of semiorthogonal glycosylations in the preparation of **5** from monosaccharides **7**, **8**, and **9**.

Meanwhile, **10** and **11** were designed as the building blocks of **6**. The azido group in **10** was stable to most reactions involved, whereas it could be easily reduced to form D-glucosamine. The 1-, 2-, and 6-*O*-positions of **11** had to be discriminated, as the 6-*O*-position is linked to the GPI glycan and the 1-*O*- and 2-*O*-positions are respectively linked to the phospholipid and acyl group in the natural structure of sperm CD52. We planned to protect its 1-*O*-position with a *p*-methoxylbenzyl (MBn), which can be selectively removed by mild oxidants for

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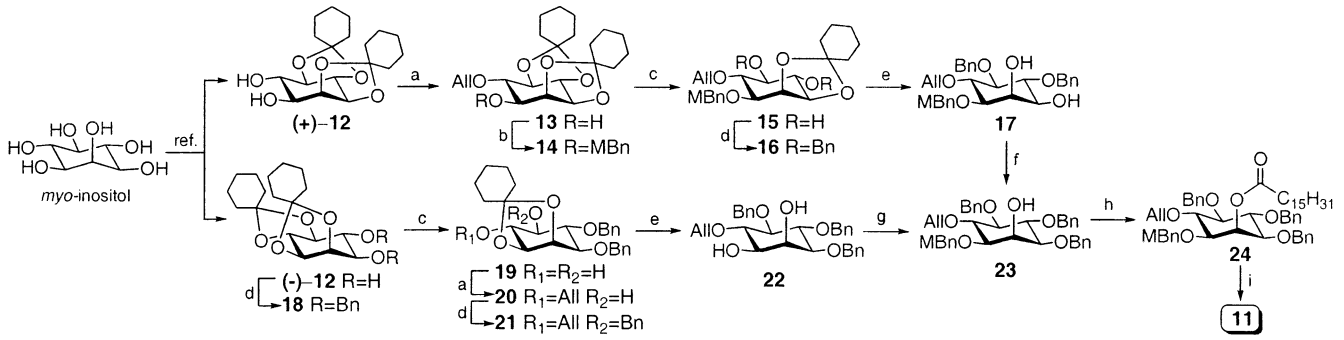
SCHEME 2[a]



[a] Reagents and conditions: (a) Bu_2SnO, toluene, reflux, 2.5 h; then AllBr, CsF, DMF, rt, 16 h; 71−80%; (b) MBnCl, NaH, DMF, rt, 3 h; 85%; (c) AcCl, MeOH−CH_2Cl_2, rt, 10 min; 60−68%; (d) BnBr, NaH, DMF, rt, 3 h; >99%; (e) AcCl, MeOH−CH_2Cl_2, rt, 2 h; 78−89%; (f) Bu_2SnO, toluene, reflux, 2.5 h; then BnBr, CsF, DMF, rt, 15 h; 98%; (g) Bu_2SnO, toluene, reflux, 2.5 h; then MBnCl, CsF, KI, DMF, rt, 16 h; 94%; (h) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2, rt, overnight; >99%; (i) $PdCl_2$, NaOAc, AcOH, H_2O, rt, 18 h; 94%.

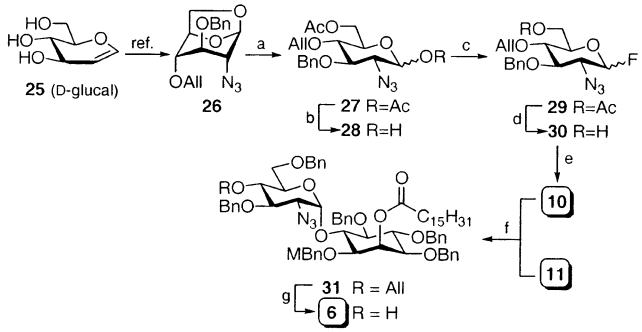
the introduction of phospholipid, while the acyl group was brought in early on.

Though many protocols for selective protection and deprotection of inositols have been developed recently,[31−38] preparing inositol derivatives that contain several uniquely modified hydroxyl groups, e.g. **11**, can be a major undertaking, especially since these derivatives are usually used as the basic starting materials and demanded in large quantities in GPI synthesis. To meet this demand, we have developed a new synthetic procedure (Scheme 2) for an inositol derivative **23**[24] having 1-*O*-, 2-*O*-, and 6-*O*-positions differently protected. These positions were also distinguished from other hydroxyl groups. This new procedure is highlighted by repeated uses of tin complex to facilitate the regioselective alkylations, as well as the use of both enantiomers of **12**, for synthesizing the same target molecules **23** and **11**. The synthetic efficiency was thus significantly improved, and **11** was prepared on multigram scales.

The optically pure intermediates (+)-**12** and (−)-**12** were prepared from *myo*-inositol according to a reported procedure, using a selective enzymatic reaction to resolve the enantiomers.[31] After a series of regioselective protections and deprotections, both (+)-**12** and (−)-**12** were transformed to **23** in 32% and 42% overall yields. Then, a palmitoyl group was introduced to the only free 2-OH in **23**, using DCC as the coupling reagent to afford **24** (>99%). The allyl group in **24** was finally removed by acetic acid and $PdCl_2$, and the resulting **11** was ready as a glycosyl acceptor to be coupled with **10**.

The glycosyl fluoride **10** as a glycosyl donor was prepared from commercial D-glucal (**25**, Scheme 3). The transformation of **25** to **26** was accomplished according

SCHEME 3[a]



[a] Reagents and conditions: (a) Ac_2O−AcOH (1:1), H_2SO_4 (cat.), 0 °C, 15 min; 72%; (b) $BnNH_2$, Et_2O, rt, 2 h; >99%; (c) DAST, CH_2Cl_2, 0 °C, 30 min; >99%; (d) NaOMe, MeOH, rt, 2 h; >99%; (e) BnBr, NaH, DMF, rt, 3 h; 75%; (f) AgOTf, $HfCp_2Cl_2$, MS 4 Å, Et_2O; 40%; (g) $PdCl_2$, NaOAc, AcOH, H_2O, rt, 18 h, 99%.

to a literature procedure.[39,40] The five-membered ring in **26** was opened via acetolysis in acetic anhydride and acetic acid (1:1), using a small amount of concentrated sulfuric acid as a catalyst, yielding **27** as an anomeric mixture (α:β 3.5:1.0). The anomeric acetyl group was then removed with benzylamine, and the resulting hemiacetal **28** was treated with DAST at low temperature to afford the corresponding glycosyl fluoride **29** (α:β 1:6).

The reaction of **10** and **11** was effected by dichlorohafnocene and silver perchlorate[41,42] in anhydrous ether to afford a mixture containing both the α-pseudodisaccharide (**31**, $GlcN_3$: H-1 δ 5.65, $J_{1,2}$ = 3.8 Hz; C-1 δ 97.5) and its β-anomer, slightly in favor of the former (4:3). This result was different from most references that report moderate yields of only α-anomer in similar reactions, which might suggest that the long acyl chain on the 2-*O*-position of inositol had a significant influence on the reaction. It was also observed that solvent choice would affect the stereochemical outcome, as the glycosylation in DCM produced a mixture slightly in favor of the

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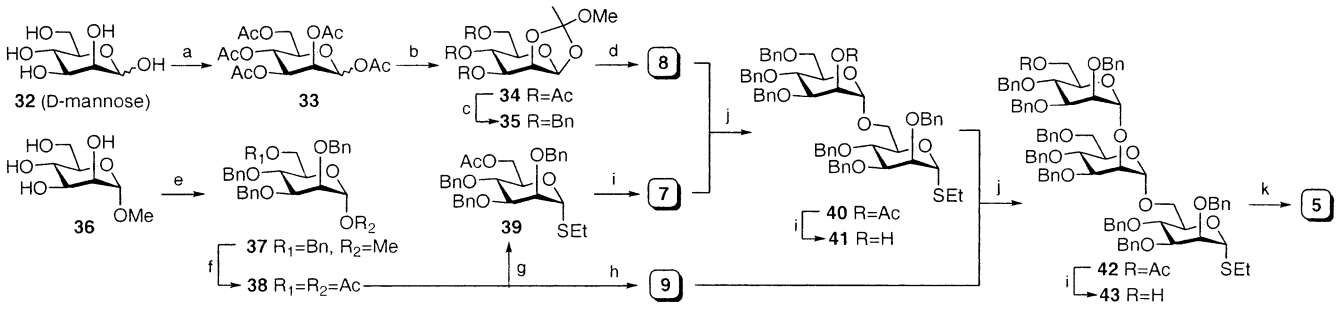
SCHEME 4[a]



[a] Reagents and conditions: (a) Ac_2O, AcOH, pyr, rt, overnight; 95%; (b) a. HBr, AcOH, rt, 6 h; b. 2,6-lutidine, MeOH−CH_2Cl_2, rt, 12 h; 87%; (c) BnCl, KOH, reflux, 4 h; 66%; (d) TMSCl, CH_2Cl_2; >99%; (e) NaH, BnBr, DMF, rt, 16 h; >99%; (f) Ac_2O, HOAc, H_2SO_4, 0 °C, 0.5 h; 70%; (g) BF_3·Et_2O, EtSH, 0 °C, 0.5 h; 65%; (h) HBr in HOAc, CH_2Cl_2; (i) NaOMe, MeOH; 91%; (j) AgOTf, MS 4 Å, CH_2Cl_2; 53−82%; (k) TBDMSCl, imidazole, DMAP, DMF; 92%.

β-anomer. Though difficult, the two anomers were separable on a silica gel column. However, we found that after the removal of the 4′-*O*-allyl group by acetic acid and $PdCl_2$, the resulting **6** was easily separated from its β-anomer via flash column chromatography. Several grams of the building block **6** was thus obtained.

Building block **5** was constructed from three monosaccharide units **7**, **8**, and **9** as shown in Scheme 4. As mentioned, we intended to have an alkylthio group linked to the reducing end of **5** so that it can be directly used as a glycosyl donor.[43] Meanwhile, the application of orthogonal glycosylations[44−46] in the construction of **5** could save several steps of anomeric deprotection and activation.

Monosaccharide **8** was readily prepared from D-mannose following a reported procedure,[47] while **7**[48] and **9** were both derived from α-methyl mannoside **36** (Scheme 4). Thus, after perbenzylation of **36**, the resulting **37** was treated with acetic acid, anhydride, and sulfuric acid to acetolyze the protecting groups on the 1-*O*- and 6-*O*-positions to afford **38**. When **38** reacted with ethanethiol using trifluoroborane etherate as the promoter, the α-thioglycoside **39** (H-1: δ 5.33, $J_{1,2}$ = 1.3 Hz) was formed in 65% yield. Deacetylation of **39** gave building block **7**. On the other hand, treating **38** with hydrogen bromide in acetic acid afforded the glycosyl bromide **9**.

The glycosylation of **7** by **8** with silver triflate as the promoter was stereospecific and gave the α-disaccharide **40** (H-1′: δ 4.94, $J_{1,2}$ = 1.2 Hz; C-1′: δ 99.6) in 82% yield. On treatment with sodium methoxide, **40** was transformed to the glycosyl acceptor **41**, which was then glycosylated by **9**, again with silver triflate as the promoter, to give the trisaccharide **42** (53%). The stereochemistry of the latter glycosyl linkage was supported by the NMR data (H-1″: δ 5.14, $J_{1,2}$ = 1.2 Hz). The 6″-acetyl group in **42** was subsequently replaced by a *tert*-butyldimethylsilyl (TBDMS) group to afford the trimannose building block **5**.

The coupling of **5** with **6** (Scheme 5) was accomplished under the promotion of *N*-iodosuccinimide (NIS) and triflic acid (TfOH) to yield the pseudopentasaccharide **44** (92%, H-1′: δ 5.21, $J_{1,2}$ < 1 Hz). Its MALDI-TOF-MS also supported the structural assignment. Then, the TBDMS group was removed with tetrabutylammonium fluoride (TBAF) to afford **45**. This reaction was clean but unexpectedly slow, probably due to the large steric bulk of the substrate.

Direct phosphorylation of **45** with 2 equiv of **4** led to a slow and incomplete reaction, yielding only 15% of **46**. The use of 7 equiv of **4** could push the reaction to completion but complications arose due to side reactions involving the azido moiety. This result was a surprise to us, as literature reported good results in similar reactions with a large excess (up to 8 equiv) of the phosphorylating reagents. The unusual reactivity of **45** might be caused by the long acyl chain on the inositol. To avoid this problem, the azide was thus reduced with triethylphosphine, and the benzyloxycarbonyl (CBz) group was used to protect the resulting free amine. Both reactions were clean, showing only one new spot on TLC, and the MALDI-TOF-MS of the product offered a single molecular peak that was consistent with **47**. However, its room temperature ^{1}H NMR spectrum showed two sets of signals (ca. 8:1). Recording the NMR at 50 °C coalesced the signals into one set that agreed well with **47**, suggesting that **47** has two rotamers at room temperature. **47** was obtained from **45** in 70% yield.

Once the azido moiety was converted to an amide, the phosphorylation reaction was rather smooth. Treating **47** with **4** and tetrazole, and then *tert*-butyl hydroperoxide, gave an inseparable diastereoisomeric mixture (4.5:1) of **48** in 84% yield. The removal of Fmoc with a dimethylamine/THF solution (1 M) yielded the GPI fragment **3** having a free amino group at its nonreducing end.

The dipeptide fragment **2** was easily prepared from the reaction of commercial perfluorophenyl (Pfp) ester of Fmoc-proline with free serine in DMF. After purification with a silica gel column, **2** (4 equiv) was coupled with **3**, using HOBt/EDC, to give **49** in 60% isolated yield (Scheme 6). The unprotected side chain of serine did not affect the coupling reaction. Fmoc was then removed, and the product **50** was carefully purified by column chro-

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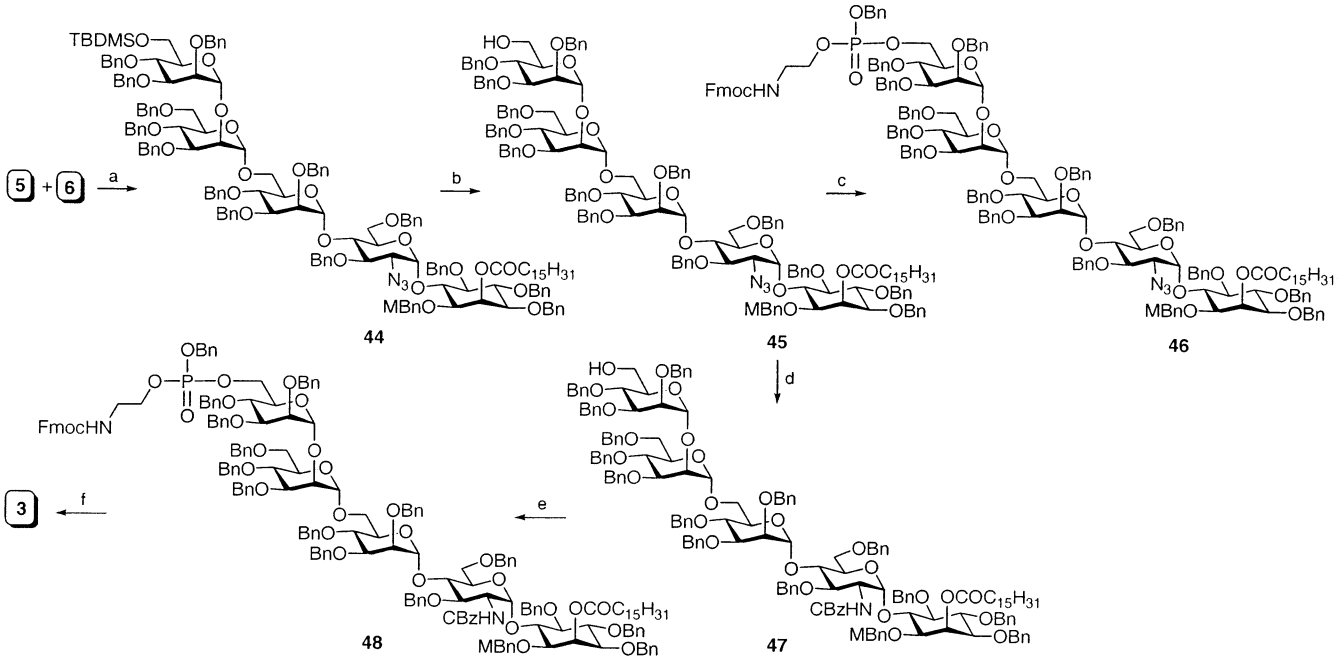
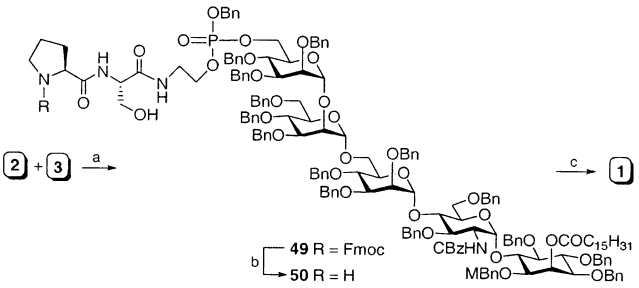
SCHEME 5[a]



[a] Reagents and conditions: (a) NIS, TfOH, DCM-ether (1:1), 0 °C, 30 min; 90%; (b) TBAF, HOAc, DCM, 35−40 °C, 3 d; 95%; (c) **4** (2 equiv), tetrazole, DCM−MeCN (3:1), rt, 6 h; then t-BuOOH; 15%; (d) PEt_3, CBz_2O, MeOH−DCM (1:2), rt, overnight; 70%; (e) **4** (7 equiv), tetrazole, DCM−MeCN (3:1), rt, 6 h; then t-BuOOH; 84%; (f) Me_2NH, THF, rt, 1 h, 95%.

SCHEME 6[a]



[a] Reagents and conditions: (a) EDC, HOBt, DCM-DMF (10:1), rt, overnight; 60%; (b) Me_2NH, THF, rt, 1 h; 85%; (c) 10% Pd/C, H_2, CH_3Cl−MeOH−H_2O (10:10:3), 1 d; 91%.

matography. Global deprotection of **50** in hydrogen atmosphere with 10% Pd/C as a catalyst offered the final synthetic target **1** as a white solid (91%).

The 1D ^1H, ^{31}P NMR, 2D $^1H-^1H$, $^1H-^{13}C$ NMR, and MS of the final product agreed well with the expected structure of **1**. For instance, the chemical shifts and ^3J coupling constants of the anomeric protons (δ 5.34, 5.20, 5.13, 4.98; all $J_{1,2} < 1$ Hz; Figure 3a,b) clearly indicated the α-glycosyl linkages of sugar units. The α-configurations were also supported by the chemical shifts of anomeric carbons (δ: 97.9, Glc; 100.0, Man; 102.8, Man; 104.2, Man) and the $^1J_{C,H}$ coupling constants between the anomeric H and C ($^1J_{C,H}$ = 174 (Glc), 174 (Man), 174 (Man), 174 Hz (Man)) derived from its heteronuclear multiple quantum coherence (HMQC) spectrum (Figure 3c). According to Bock and Petersen,[49] $^1J_{C,H}$ coupling constants greater than 170 Hz are observed in α-glycosides and those smaller than 170 Hz are observed in β-glycosides. The ^1H and ^{13}C signals of the 2-*O*-position of inositol shifted downfield to δ 5.41 and 75.6, respectively, indicating that this position is linked to the acyl group. The α-proton signals (δ 4.45 and 4.38) of the amino acid residues were also distinctive. Both were triplets (Figure 3a in D_2O) and had $^3J_{H,H}$ coupling of 5.4 and 7.2 Hz, respectively. The chemical shift (δ 1.72) of the ^{31}P signal showed the phosphate group.

In summary, this paper described the first total synthesis of a GPI-anchored peptide through a highly convergent procedure, in which a key synthetic design was to separately prepare the properly protected peptide and GPI. The two fragments were effectively coupled between the free peptide *C*-terminus and the free amino group of phosphoethanolamine at the nonreducing end of GPI, using EDC/HOBt as the condensation reagent. To facilitate the preparation of the key building blocks, e.g. the core trimannose **5** and the specifically protected inositol **23**, new synthetic routes were developed that could lead to the expected structures on large scales. The results may be applied to the synthesis of other GPI-anchored structures. On the other hand, unusual reactions were observed with the 2-*O*-palmitoyl derivatives of inositol, suggesting the significant influence of steric hindrance caused by the large acyl group.

Experimental Section

(+)-6-*O*-Allyl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (13).[33] A mixture of (+)-**12** (1.8 g, 5.3 mmol) and Bu_2SnO (1.37 g, 5.5 mmol) in 60 mL of toluene was refluxed with azeotropic removal of water for 2.5 h. After concentration to dryness under reduced pressure, to the residue were added DMF (30 mL), CsF (2.4 g, 15 mmol), and allyl bromide (0.58 mL, 6.4 mmol) at −10 °C. The mixture was stirred at room

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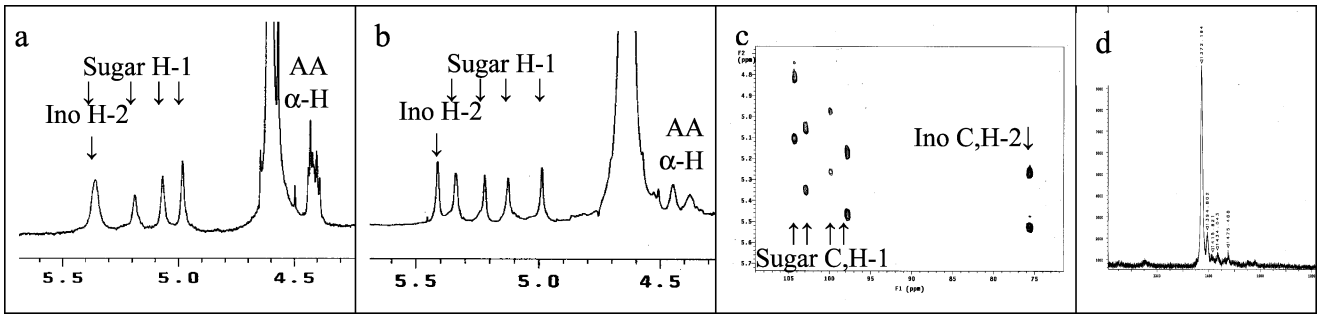

FIGURE 3. ^{1}H NMR spectra of the final synthetic target **1** in D_2O (a) and CD_3OD (b): the anomeric region of sugar units, α-Hs of amino acids, and 2-H of inositol. ^{1}H−^{13}C HQMC spectrum (coupled) of **1** in CD_3OD (c): the anomeric region. MALDI-TOF-MS spectrum of **1** (d).

temperature for 16 h, and then diluted with ethyl acetate. The organic solution was washed with brine, dried with Na_2SO_4, and then concentrated under vacuum. Column chromatography of the residue gave **13** as a colorless syrup (1.62 g, 80%). $[\alpha]_D$ +8.2 (*c* 0.8, CH_2Cl_2) (lit.[33] $[\alpha]_D$ +4.3 (*c* 2.8, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.87−6.00 (m, 1 H), 5.32 (dd, J = 17.2, 2.4 Hz, 1 H), 5.20 (d, J = 10.3 Hz, 1 H), 4.42 (dd, J = 7.4, 3.6 Hz, 1 H), 4.31 (t, J = 7.2 Hz, 1 H), 4.10−4.27 (m, 3 H), 3.98 (m, 1 H), 3.82 (dd, J = 7.9, 1.7 Hz, 1 H), 3.48 (dd, J = 10.5, 7.9 Hz, 1 H), 2.60 (d, J = 1.4 Hz, 1 H), 1.34−1.77 (m, 20 H). ^{13}C NMR ($CDCl_3$, 50 MHz): 134.4, 117.4, 113.0, 111.3, 79.8, 78.7, 76.7, 76.5, 75.3, 72.2, 70.6, 36.7, 36.6, 36.3, 33.5, 25.2, 25.1, 23.9, 23.8, 23.7, 23.4.

6-*O*-Allyl-1-*O*-(*p*-methoxybenzyl)-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (14).[33,50] To a solution of **13** (1.8 g, 4.73 mmol) and 4-methoxybenzyl chloride (0.77 mL, 5.7 mmol) in DMF (30 mL) was added NaH (284 mg, 60%, 7.1 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. The excess NaH was neutralized by MeOH, and the mixture was diluted with ethyl acetate. After workup as mentioned above, column chromatography of the residue afforded **13** as a colorless syrup (2.0 g, 85%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30 (d, J = 8.6 Hz, 2 H), 6.81 (d, J = 8.6 Hz, 2 H), 5.81−5.93 (m, 1 H), 5.27 (dd, J = 17.2, 1.7 Hz, 1 H), 5.17 (dd, J = 10.4, 1.6 Hz, 1 H), 4.68 (d, J = 11.8 Hz, 1 H), 4.62 (d, J = 11.8 Hz, 1 H), 4.26−4.34 (m, 2 H), 3.96−4.13 (m, 3 H), 3.81 (s, 3 H), 3.77 (dd, J = 8.0, 3.0 Hz, 1 H), 3.69 (t, J = 3.1 Hz, 1 H), 3.42 (dd, J = 10.6, 7.8 Hz, 1 H), 1.35−1.79 (m, 20 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 159.3, 134.6, 130.3, 129.5, 117.0, 113.8, 112.8, 111.6, 79.6, 78.8, 78.5, 77.0, 76.5, 75.1, 73.0, 70.7, 55.3, 36.7, 36.5, 34.7, 25.2, 25.1, 24.0, 23.8, 23.7.

(−)-6-*O*-Allyl-1-*O*-(*p*-methoxybenzyl)-2,3-*O*-cyclohexylidene-*myo*-inositol (15).[33] A solution of **14** (2.0 g, 4.0 mmol) in MeOH−CH_2Cl_2 (1:3, 68 mL) was stirred with acetyl chloride (87 μL) at room temperature for 10 min. After the reaction was quenched with triethylamine (0.4 mL), the mixture was concentrated. Column chromatography of the residue yielded **15** as a colorless syrup (1.0 g, 60%). $[\alpha]_D$ −8.3 (*c* 3.0, CH_2Cl_2) (lit.[33] $[\alpha]_D$ +6.5 (*c* 3, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30 (d, J = 8.8 Hz, 2 H), 6.87 (d, J = 8.8 Hz, 2 H), 5.87−6.04 (m, 1 H), 5.16−5.35 (m, 2 H), 4.70, 4.65 (2d, J = 12 Hz, 2 H), 4.44 (ddt, J = 5.6, 12.6, 1.4 Hz, 1 H), 4.16−4.25 (m, 2 H), 3.89 (dd, J = 7.5, 5.2 Hz, 1 H), 3.81 (s, 3 H), 3.71 (dt, J = 2.2, 10.0 Hz, 1 H), 3.59−3.62 (m, 2 H), 3.20−3.32 (m, 1 H), 2.88 (t, J = 2.6 Hz, 2 H), 1.33−1.77 (m, 10 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 134.9, 130.1, 129.7, 117.2, 113.9, 110.7, 80.0, 78.2, 77.4, 75.2, 73.9, 73.7, 73.1, 72.4, 55.3, 37.8, 35.2, 25.0, 23.9, 23.6.

(+)-6-*O*-Allyl-1-*O*-(*p*-methoxybenzyl)-4,5-di-*O*-benzyl-*myo*-inositol (17). To the solution of **15** (1.0 g, 2.38 mmol) and benzyl bromide (1.0 mL, 6.5 mmol) in DMF (25 mL) was added NaH (560 mg, 60%, 14.2 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. The excess NaH was neutralized by MeOH, and the solution was diluted with ethyl acetate. The organic layer was washed with water and brine, dried, and condensed to give crude (−)-6-*O*-allyl-1-*O*-(*p*-methoxybenzyl)-2,3-*O*-cyclohexylidene-*myo*-inositol (**16**) as a colorless syrup (>99%). To the solution of crude **16** in MeOH−CH_2Cl_2 (1:3, 88 mL) was added acetyl chloride (0.l9 mL), and the mixture was stirred at room temperature for 2 h. Then, triethylamine (0.73 mL) was added. Following concentration, column chromatography of the residue gave **17** (0.97 g, 78%). $[\alpha]_D$ +13.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29−7.36 (m, 12 H), 6.89 (d, J = 8.7 Hz, 2 H), 5.94−6.06 (m, 1 H), 5.30 (dd, J = 17.2, 1.8 Hz, 1 H), 5.18 (d, J = 8.5 Hz, 1 H), 4.95 (d, J = 11.2 Hz, 1 H), 4.92 (d, J = 10.7 Hz, 1 H), 4.81 (d, J = 10.7 Hz, 1 H), 4.74 (d, J = 11.2 Hz, 1 H), 4.67 (d, J = 11.4 Hz, 1 H), 4.62 (d, J = 11.4 Hz, 1 H), 4.30−4.44 (m, 2 H), 4.14 (m, 1 H), 3.73−3.84 (m, 5 H), 3.36−3.48 (m, 3 H), 2.53 (br, 1 H), 2.47 (d, J = 2.8 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 159.5, 138.6, 135.3, 130.0, 129.6, 128.6, 128.5, 128.1, 128.0, 127.9, 127.7, 116.7, 114.0, 83.3, 81.4, 81.3, 79.5, 75.8, 75.6, 74.6, 72.5, 71.8, 69.4, 55.3. HRFABMS: Calcd for $C_{31}H_{37}O_7$ 519.2364, found 519.2382.

(−)-6-*O*-Allyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (23).[51] A mixture of **17** (0.97 g, 1.86 mmol) and Bu_2SnO (0.56 g, 2.23 mmol) in toluene (60 mL) was refluxed under N_2 with azeotropic removal of water for 2.5 h and was then concentrated to dryness. To the residue were added DMF (20 mL), CsF (0.71 g, 4.7 mmol), and benzyl bromide (0.28 mL, 2.38 mmol) at −10 °C. After being stirred at −10 °C for 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction was diluted with ethyl acetate, washed with water, dried with Na_2SO_4, and finally concentrated to dryness. Column chromatography of the residue afforded **23** as a colorless syrup (1.11 g, 97.5%). $[\alpha]_D$ −8 (*c* 1.0, $CHCl_3$) (lit.[51] $[\alpha]_D$ −10 (*c* 1.2, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31−7.36 (m, 17 H), 6.92 (d, J = 8.5, Hz 2 H), 5.96−6.08 (m, 1 H), 5.32 (dd, J = 17.3, 1.6 Hz, 1 H), 5.19 (d, J = 10.4 Hz, 1 H), 4.85−4.94 (m, 4 H), 4.73 (s, 2 H), 4.68 (d, J = 11.4 Hz, 1 H), 4.64 (d, J = 11.4 Hz, 1 H), 4.42 (m, 2 H), 4.19 (br, 1 H), 3.98 (t, J = 9.5 Hz, 1 H), 3.85 (m, 1 H), 3.84 (s, 3 H), 3.42 (t, J = 9.3 Hz, 1 H), 3.39 (dd, J = 9.6, 2.5 Hz, 1 H), 3.33 (dd, J = 9.6, 2.5 Hz, 1 H), 2.48 (s, 1 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 159.4, 138.9, 138.8, 138.1, 135.4, 130.2, 129.5, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 127.6, 116.7, 114.0, 83.2, 81.2, 81.0, 79.8, 79.3, 76.0, 75.9, 74.6, 72.8, 72.5, 67.8, 55.4.

(−)-3,4-Di-*O*-benzyl-1,2-*O*-cyclohexylidene-*myo*-inositol (19).[32] The benzylation of (−)-**12** (2.27 g, 6.67 mmol) followed the same procedure described for **16**, which offered (−)-3,4-di-*O*-benzyl-1,2;5,6-di-*O*-cyclohexylidene-*myo*-inositol (**18**) as a colorless syrup in >99% yield. It was directly used in the next step. Thus, to the solution of **18** in MeOH−CH_2Cl_2

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(1:3, 112 mL) was added acetyl chloride (145 μL). The mixture was stirred at room temperature for 10 min, and then triethylamine (0.67 mL) was added. After this mixture was concentrated, column chromatography of the residue afforded **19** as a colorless syrup (1.98 g, 68%). $[\alpha]_D$ −5.8 (*c* 1.0, $CHCl_3$) (lit.[32] $[\alpha]_D$ −16.5 (*c* 1, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31−7.41 (m, 10 H), 5.00 (d, *J* = 11.3 Hz, 1 H), 4.77 (s, 2 H), 4.71 (d, *J* = 11.3 Hz, 1 H), 4.32 (dd, *J* = 5.1, 3.8 Hz, 1 H), 3.93 (dd, *J* = 7.6, 5.3 Hz, 1 H), 3.68−3.78 (m, 3 H), 3.31 (dd, *J* = 10.0, 8.5 Hz, 1 H), 2.64 (br, 2 H), 1.41−1.80 (m, 10 H).

(−)-6-*O*-Allyl-3,4-di-*O*-benzyl-1,2-*O*-cyclohexylidene-*myo*-inositol (20).[52] Regioselective allylation of **19** (1.95 g, 4.43 mmol) followed the same procedure described for **13**. The reaction afforded **20** as a colorless syrup (1.5 g, 71%). $[\alpha]_D$ −2.1 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29−7.37 (m, 10 H), 5.90−6.00 (m, 1 H), 5.30 (dd, *J* = 17.2, 1.6 Hz, 1 H), 5.18 (d, *J* = 10.3 Hz, 1 H), 4.88 (d, *J* = 11.3 Hz, 1 H), 4.71−4.78 (m, 3 H), 4.38 (dd, *J* = 12.8, 5.5 Hz, 1 H), 4.30 (dd, *J* = 5.7, 3.9 Hz, 1 H), 4.22 (dd, *J* = 12.7, 5.9 Hz, 1 H), 4.03 (t, *J* = 6.9 Hz, 1 H), 3.81 (t, *J* = 8.0 Hz, 1 H), 3.70 (dd, *J* = 8.0, 3.9 Hz, 1 H), 3.61 (dd, *J* = 9.7, 7.0 Hz, 1 H), 3.45 (dd, *J* = 9.8, 8.0 Hz, 1 H), 2.64 (br, 1 H), 1.30−1.84 (m, 10 H).

(−)-6-*O*-Allyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (22).[51] Benzylation of **20** (1.5 g, 3.13 mmol) following the same procedure described above gave **21** in >99% yield. The product was directly used in the next step. Thus, after it was dissolved in MeOH−CH_2Cl_2 (1:3, 116 mL), acetyl chloride (0.25 mL) was added. The mixture was stirred at room temperature for 2 h, and then triethylamine (0.96 mL) was added. The solution was concentrated, and column chromatography of the residue produced **22** as a colorless syrup (1.37 g, 89%). $[\alpha]_D$ −31 (*c* 1.0, $CHCl_3$) (lit.[51] $[\alpha]_D$ −35 (*c* 1.5, $CHCl_3$)). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29−7.42 (m, 15 H), 5.92−6.01 (m, 1 H), 5.28 (dd, *J* = 17.6, 1.6 Hz, 1 H), 5.18 (dd, *J* = 10.4, 1.7 Hz, 1 H), 4.80−4.93 (m, 4 H), 4.74 (d, *J* = 11.7 Hz, 1 H), 4.70 (d, *J* = 11.7 Hz, 1 H), 4.39−4.46 (m, 1 H), 4.25−4.30 (m, 1 H), 4.22 (t, *J* = 2.8 Hz, 1 H), 3.94 (t, *J* = 9.5 Hz, 1 H), 3.72 (t, *J* = 9.5 Hz, 1 H), 3.40−3.48 (m, 3 H), 2.54 (br, 2 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 138.7, 138.6, 137.8, 135.0, 128.6, 128.4, 128.0, 128.0, 128.0, 127.9, 127.7, 117.3, 83.2, 81.7, 81.0, 80.1, 76.0, 75.7, 74.4, 72.8, 71.7, 69.3.

(−)-6-*O*-Allyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (23). A solution of **22** (1.36 g, 2.78 mmol) and Bu_2SnO (0.83 g, 3.34 mmol) in toluene (100 mL) was refluxed under N_2 with azeotropic removal of water for 2.5 h. The reaction mixture was then concentrated to dryness under vacuum. To the residue was added DMF (30 mL), CsF (1.06 g, 7.05 mmol), NaI (0.7 g, 4.17 mmol), and 4-methoxbenzyl chloride (0.61 mL, 4.17 mmol) at −10 °C. After being stirred at −10 °C for 1 h, the reaction mixture was allowed to warm to room temperature and stirred for 16 h. The solution was then diluted by ethyl acetate, washed with water, dried over Na_2SO_4, and concentrated. Column chromatography of the residue afforded **23** (1.59 g, 94%). Its spectroscopic data were exactly the same as those of the product obtained from the previous synthetic route.

(−)-6-*O*-Allyl-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (24). To a solution of **23** (1.59 g, 2.6 mmol), palmitic acid (0.9 g, 3.5 mmol), and DMAP (50 mg, 0.4 mmol) in dry DCM (20 mL) was added DCC (0.72 g, 3.5 mmol) at room temperature. The mixture was stirred under argon for 1 day, and the reaction was monitored with TLC. When the reaction was over, the solution was concentrated in a vacuum. Column chromatography of the residue gave **24** as a colorless syrup (2.2 g, >99%). $[\alpha]_D$ −11.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29−7.37 (m, 17 H), 6.88 (d, *J* = 8.5 Hz, 2 H), 5.92−6.06 (m, 1 H), 5.84 (t, *J* = 2.6 Hz, 1 H), 5.32 (dd, *J* = 17.1, 1.5 Hz, 1 H), 5.17 (d, *J* = 10.4 Hz, 1 H), 4.85−4.90 (m, 3 H), 4.79 (d, *J* = 10.7 Hz, 1 H), 4.74 (d, *J* = 11.2 Hz, 1 H), 4.66 (d, *J* = 10.8 Hz, 1 H), 4.53 (d, *J* = 11.2 Hz, 1 H), 4.47 (d, *J* = 10.8 Hz, 1 H), 4.39 (dd, *J* = 12.1, 5.8 Hz, 1 H), 4.28 (dd, *J* = 12.1, 5.8 Hz, 1 H), 3.84 (t, *J* = 9.4 Hz, 1 H), 3.82 (s, 3 H), 3.72 (t, *J* = 9.4 Hz, 1 H), 3.37−3.48 (m, 3 H), 2.38 (t, *J* = 7.3 Hz, 2 H), 1.64 (m, 2 H), 1.12−1.37 (m, 24 H), 0.90 (t, *J* = 7.0 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.3, 159.3, 138.8, 138.6, 137.8, 135.4, 130.0, 129.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 116.6, 113.8, 113.7, 83.0, 81.5, 81.2, 78.4, 78.0, 76.3, 76.0, 74.7, 72.2, 71.9, 66.5, 55.3, 34.6, 32.0, 29.8, 29.6, 29.5, 29.4, 29.1, 25.4, 22.8, 14.2. FABMS: calcd for $C_{54}H_{72}O_8$ 791.5, found 791.5.

(−)-2-*O*-Hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (11). To a solution of **24** (2.2 g, 2.6 mmol) in acetic acid (12 mL) and water (13 drops) were added $PdCl_2$ (0.75 g, 4.23 mmol) and NaOAc (0.75 g) at room temperature. After 18 h of stirring, the mixture was concentrated in a vacuum. The residue was dissolved in ethyl acetate, and the solution was washed with aq $NaHCO_3$, brine, and water. The organic solution was dried over Na_2SO_4 and concentrated. Flash chromatography of the residue gave **11** as a colorless syrup (1.98 g, 94%). $[\alpha]_D$ −4.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29−7.40 (m, 17 H), 6.90 (d, *J* = 8.3 Hz, 2 H), 5.90 (bs, 1 H), 4.71−4.93 (m, 6 H), 4.57 (d, *J* = 11.3 Hz, 1 H), 4.42 (d, *J* = 10.7 Hz, 1 H), 3.98 (t, *J* = 9.5 Hz, 1 H), 3.87 (t, *J* = 9.5 Hz, 1 H), 3.82 (s, 3 H), 3.52 (dd, *J* = 9.5, 2.3 Hz, 1 H), 3.43 (t, *J* = 9.2 Hz, 1 H), 3.29 (dd, *J* = 9.8, 2.0 Hz, 1 H), 2.55 (s, 1 H), 2.40 (t, *J* = 7.3 Hz, 2 H), 1.65 (m, 2 H), 1.20−1.40 (m, 24 H), 0.91 (t, *J* = 6.0 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 173.4, 159.6, 138.8, 138.7, 137.8, 129.9, 129.5, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.7, 114.0, 82.8, 81.3, 78.5, 77.2, 76.0, 75.8, 72.9, 72.3, 71.5, 65.7, 55.3, 34.5, 32.0, 29.8, 29.6, 29.4, 29.1, 25.3, 22.8, 14.2. HRFABMS: calcd for $C_{51}H_{68}O_8Na^+$ 831.4811, found 831.4786.

1,6-Di-*O*-acetate-4-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-D-glucopyranose (27).[53] To the solution of **26** (317 mg, 1.0 mmol) in acetic anhydride (5 mL) was added a solution of H_2SO_4 in HOAc (2%, 5 mL) with stirring at 0 °C. Fifteen minutes later, $NaOAc{\cdot}3H_2O$ (500 mg) was introduced to quench the reaction. The reaction mixture was concentrated, and the residue was dispensed in ethyl acetate. The organic solution was washed with aqueous $NaHCO_3$ and, brine, and then dried and concentrated. Column chromatography of the residue gave **27** as a colorless syrup (302 mg, 72%, α:β 3.5:1.0). ^{1}H NMR ($CDCl_3$, 300 MHz) of the α-isomer: δ 7.38 (m, 5 H), 6.22 (d, *J* = 3.6 Hz, 1 H), 5.94 (m, 1 H), 5.16−5.30 (m, 2 H), 4.89 (s, 2 H), 4.27−4.36 (m, 3 H), 4.09 (m, 1 H), 3.89 (m, 2 H), 3.47−3.59 (m, 3 H), 2.17 (s, 3 H), 2.08 (s, 3 H).

6-Acetate-4-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-D-glucopyranose (28).[53] To the solution of **27** (2.25 g, 5.4 mmol) in ether (50 mL) was added benzylamine (8.8 mL, 81 mmol). The reaction mixture was stirred at room temperature for 2 h. After the reaction mixture was washed with dilute aq HCl solution and water, the organic layer was dried and concentrated. Column chromatography of the crude product afforded **28** (>99%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (m, 5 H), 5.90 (m, 1 H), 5.17−5.30 (m, 3 H), 4.89 (s, 2 H), 3.95−4.44 (m, 6 H), 3.38−3.54 (m, 3 H), 2.09 (s, 3 H).

4-*O*-Allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranosyl Fluoride (10).[38] To the solution of **28** (2.3 g, 6.1 mmol) in dry DCM (100 mL) was added DAST (1.22 mL, 8.2 mmol) under argon with stirring at 0 °C. The mixture was allowed to warm to room temperature and stirred for another 30 min. Normal workup of the reaction afforded an anomeric mixture (α:β ca. 1:6) of 6-*O*-acetyl-4-*O*-allyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-D-glucopyranosyl fluoride (**29**, >99%). This crude product was directly treated with NaOMe (0.6 mmol) in MeOH (30 mL) for deacetylation. The solution was stirred at room temperature for 2 h and neutralized with Amberlyst ion-exchange resin. The mixture was concentrated to give the

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(53) Koshida, S.; Suda, Y.; Fukui, Y.; Ormsby, J.; Sobel, M.; Kusumoto, S. *Tetrahedron Lett.* **1999**, *40*, 5725−5729.

crude 4-*O*-allyl-2-azido-3-*O*-benzyl-2-deoxy-D-glucopyranosyl fluoride (**30**). Finally, **30** was benzylated according to the procedure described above to afford **10** (1.95 g, 75%, α:*β* 1:6). This inseparable anomeric mixture was directly used in the next step of the reaction. ^{1}H NMR ($CDCl_3$, 300 MHz) of α-isomer: *δ* 5.64 (dd, $J_{1,F}$ = 52.8 Hz, 1 H). ^{1}H NMR ($CDCl_3$, 300 MHz) of *β*-isomer: *δ* 5.03 (dd, $J_{1,F}$ = 52.8 Hz, 1 H).

6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-2-propenyl-α-D-glucopyranosyl)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (31). After the mixture of silver triflate (206 mg, 0.8 mmol), hafnocene dichloride (190 mg, 0.5 mmol), and MS 4 Å (1.5 g) in diethyl ether (5 mL) was stirred at room temperature for 0.5 h and then cooled to −15 °C, a solution of **11** (180 mg, 0.22 mmol) and **10** (143 mg, 0.33 mmol) in dry ether (2 mL) was introduced. The mixture was allowed to warm to room temperature and stirred overnight. Then, DCM (30 mL) was added. The mixture was filtered through a Celite pad, and the filtrated was washed with aq $NaHCO_3$ and brine, dried with Na_2SO_4, and finally concentrated. The crude product was carefully purified by silica gel column to give **31** as a colorless syrup (106 mg, 40%), as well as the *β*-isomer (79 mg, 30%). **31**: $[\alpha]_D$ +51 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 7.21−7.44 (m, 27 H), 6.85 (d, *J* = 8.6 Hz, 2 H), 5.93 (bs, 1 H), 5.72−5.85 (m, 1 H), 5.65 (d, *J* = 3.8 Hz, 1 H), 4.49−5.15 (m, 12 H), 4.40 (d, *J* = 9.4 Hz, 1 H), 4.23 (d, *J* = 11.9 Hz, 1 H), 4.16 (dd, *J* = 12.6, 5.5 Hz, 1 H), 3.85−4.05 (m, 5 H), 3.80 (s, 3 H), 3.52−3.61 (m, 3 H), 3.47 (t, *J* = 9.4 Hz, 1 H), 3.08−3.21 (m, 3 H), 2.40 (t, *J* = 7.1 Hz, 2 H), 1.62 (m, 2 H), 1.17−1.40 (m, 24 H), 0.89 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 50 MHz): *δ* 173.4, 159.4, 138.6, 138.2, 138.0, 137.9, 137.6, 135.1, 130.3, 129.3, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.0, 127.9, 127.6, 116.0, 113.8, 97.5, 81.8, 80.8, 79.8, 79.3, 78.4, 78.2, 76.2, 75.8, 75.3, 75.2, 73.5, 73.3, 72.1, 71.5, 70.1, 67.6, 65.5, 63.0, 55.3, 34.4, 32.0, 29.8, 29.6, 29.5, 29.4, 29.1, 25.3, 22.8, 14.2. 6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-2-propenyl-*β*-D-glucopyranosyl)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (*β*-isomer): $[\alpha]_D$ −15 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 7.19−7.43 (m, 27 H), 6.81 (d, *J* = 8.7 Hz, 2 H), 5.81−5.97 (m, 1 H), 5.79 (m, 1 H), 3.76 (s, 3 H), 3.25−5.32 (m, 28 H), 2.22 (m, 2 H), 1.60 (m, 2 H), 1.14−1.40 (m, 24 H), 0.89 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 50 MHz): *δ* 173.2, 159.2, 138.6, 138.5, 138.3, 137.9, 137.7, 134.5, 130.4, 129.4, 117.9, 117.4, 113.7, 101.0, 109.7, 83.4, 83.0, 81.8, 78.4, 78.1, 77.9, 77.6, 76.2, 75.8, 75.6, 75.0, 74.5, 74.0, 73.4, 72.5, 72.1, 70.5, 68.4, 67.4, 65.4, 59.9, 55.3, 34.4, 32.0, 29.8, 29.6, 29.6, 29.4, 29.1, 25.2, 22.8, 14.2. FABMS: calcd for $C_{74}H_{93}N_3O_{12}$ 1215.6, found 1215.7.

6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (6). To the solution of **31** (a mixture of α,*β*-isomers; 1.3 g, 1.07 mmol) in acetic acid (12 mL) and water (13 drops) were added $PdCl_2$ (0.32 g, 1.8 mmol) and NaOAc (0.32 g) at room temperature. After 18 h of stirring, the reaction mixture was concentrated in a vacuum. The residue was dissolved in ethyl acetate, and the solution was washed with aq $NaHCO_3$, brine, and water. The organic solution was dried over Na_2SO_4 and then concentrated to dryness. Flash chromatography of the crude product gave **6** (0.53 g) and its *β*-isomer (0.46 g). **6**: $[\alpha]_D$ +27.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 7.19−7.47 (m, 27 H), 6.87 (d, *J* = 8.1 Hz, 2 H), 5.95 (br, 1 H), 5.67 (d, *J* = 3.6 Hz, 1 H), 3.46−4.09 (m, 8 H), 3.81 (s, 3 H), 3.30 (t, *J* = 3.7 Hz, 2 H), 3.17 (dd, *J* = 10.1, 3.4 Hz, 1 H), 2.41 (t, *J* = 7.2 Hz, 2 H), 2.11 (d, *J* = 3.5 Hz, 1 H), 1.64 (m, 2 H), 1.15−1.40 (m, 24 H), 0.91 (t, *J* = 6.6 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 50 MHz): *δ* 173.4, 159.5, 138.5, 138.3, 138.2, 138.1, 137.6, 130.4, 129.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 113.8, 97.5, 81.8, 81.0, 79.3, 79.2, 78.4, 77.7, 76.1, 75.8, 75.0, 74.8, 73.5, 72.2, 71.6, 69.6, 69.2, 65.5, 62.6, 55.3, 34.5, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 25.3, 22.8, 14.2. 6-*O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-*β*-D-glucopyranosyl)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (*β*-isomer): $[\alpha]_D$ −18 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 7.24−7.44 (m, 27 H), 6.84 (d, *J* = 8.7 Hz, 2 H), 5.81 (t, *J* = 2.7 Hz, 1 H), 3.78 (s, 3 H), 2.79 (d, *J* = 2.0 Hz, 1 H), 2.28 (dt, *J* = 7.4, 2.8 Hz, 2 H), 1.57 (m, 2 H), 0.90 (t, *J* = 6.5 Hz, 3 H). FABMS: calcd for $C_{71}H_{89}N_3O_{12}$ 1175, found 1175.

Ethyl *O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-l-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (40). To the solution of **35** (2.53 g, 5.0 mmol) in DCM (20 mL) was added 1.0 M trimethylsilyl chloride (7.5 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was concentrated in a vacuum to give the glycosyl chloride **8** as avsyrup that was directly used without further purification. After the mixture of **7** (2.1 g, 4.25 mmol), AgOTf (2.6 g, 10 mmol), and 4 Å MS (5 g) in dry DCM (30 mL) was stirred at room temperature for 1 h and then cooled to −40 °C, a solution of **8** in DCM (10 mL) was added. After the reaction mixture was warmed to room temperature and stirred overnight, it was filtered off through Celite, and the resulting solution was washed with brine, dried, and concentrated. Column chromatography of the residue gave **40** as a colorless syrup (3.46 g, 82%). $[\alpha]_D$ +66 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): *δ* 7.38 (d, *J* = 7.2 Hz, 2 H), 7.21−7.32 (m, 26 H), 7.12 (dd, *J* = 7.2, 1.8 Hz, 2 H), 5.47 (dd, *J* = 3.0, 2.4 Hz, 1 H), 5.33 (s, 1 H), 4.94 (d, *J* = 1.2 Hz, 1 H), 4.91 (d, *J* = 11.4 Hz, 1 H), 4.84 (d, *J* = 10.8 Hz, 1 H), 4.71 (d, *J* = 12.6 Hz, 1 H), 4.63−4.66 (m, 3 H), 4.55 (s, 2 H), 4.48 (d, *J* = 10.8 Hz, 1 H), 4.42−4.45 (m, 3 H), 4.08 (ddd, *J* = 9.6, 5.4, 1.8 Hz, 1 H), 3.93 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.82−3.90 (m, 5 H), 3.76 (ddd, *J* = 9.6, 3.6, 1.8 Hz, 1 H), 3.69 (dd, *J* = 9.6, 3.6 Hz, 1 H), 3.67 (dd, *J* = 10.8, 1.8 Hz, 1 H), 3.58 (dd, *J* = 10.8, 1.8 Hz, 1 H), 2.45−2.58 (m, 2 H), 2.14 (s, 3 H), 1.19 (t, *J* = 7.2 Hz, 3 H). FABMS: calcd for $C_{62}H_{76}NO_{13}S$ 1074, found 1074.

Ethyl *O*-(3,4,6-Tri-*O*-benzyl-l-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (41). A solution of sodium (46 mg, 2.0 mmol) in methanol (20 mL) was added to a stirred solution of **40** (3.46 g, 3.57 mmol) in DCM (3 mL). After being stirred at room temperature for 1 h, the reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried and concentrated. The residue was purified with a silica gel column to give **41** as a colorless syrup (3.0 g, 91%). $[\alpha]_D$ +471.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): *δ* 7.39 (d, *J* = 7.2 Hz, 2 H), 7.24−7.34 (m, 26 H), 7.16 (dd, *J* = 7.2, 1.8 Hz, 2 H), 5.34 (s, 1 H), 5.07 (d, *J* = 1.8 Hz, 1 H), 4.92 (d, *J* = 10.8 Hz, 1 H), 4.83 (d, *J* = 10.8 Hz, 1 H), 4.70 (d, *J* = 12.0 Hz, 1 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.63 (d, *J* = 12.0 Hz, 1 H), 4.62 (d, *J* = 12.0 Hz, 1 H), 4.59 (s 2 H), 4.56 (d, *J* = 12.0 Hz, 1 H), 4.52 (d, *J* = 10.8 Hz, 1 H), 4.49 (d, *J* = 10.8 Hz, 1 H), 4.47 (d, *J* = 12.0 Hz, 1 H), 4.12 (dd, *J* = 3.6, 1.2 Hz, 1 H), 4.09 (ddd, *J* = 10.2, 4.8, 1.2 Hz, 1 H), 3.83−3.96 (m, 6 H), 3.77 (ddd, *J* = 9.6, 3.6, 1.8 Hz, 1 H), 3.73 (dd, *J* = 12.0, 1.8 Hz, 1 H), 3.68 (dd, *J* = 10.8, 4.2 Hz, 1 H), 3.61 (dd, *J* = 10.8, 1.8 Hz, 1 H), 2.49−2.60 (m, 2 H), 1.22 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* 138.6, 138.5, 138.3, 138.2, 138.1, 137.9, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 99.6, 81.8, 80.4, 79.7, 76.6, 75.1, 75.0, 74.8, 74.2, 73.4, 72.2, 72.1, 71.8, 71.5, 71.1, 68.9, 68.1, 66.3, 25.4, 15.1. HRFABMS: calcd for $C_{56}H_{62}O_{10}$ 926.4064, found 926.4034; calcd for $C_{56}H_{62}O_{10}Na$ ($M + Na^+$) 949.3961, found 949.3945.

Ethyl *O*-(6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (42). To the solution of **7** (1.07 g, 2.0 mmol) in DCM (20 mL) was added 33% HBr in HOAc (1.0 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was washed with saturated $NaHCO_3$ solution and brine and then dried and concentrated in a vacuum to give the glycosyl bromide **9** as a syrup that was directly used without further purification. After the mixture of **41**(1.0 g, 1.08 mmol), AgOTf (0.52 g, 2.0 mmol), and 4 Å MS (2 g) in dry DCM (20 mL) was stirred at room

temperature for 1 h, it was cooled to −40 °C. To this mixture was added the solution of **8** in DCM (5 mL). The reaction mixture was then warmed to room temperature and stirred overnight. The solution was filtered off, washed with brine, dried, and concentrated. Column chromatography of the residue afforded **42** as a colorless syrup (0.8 g, 53%). $[\alpha]_D$ +48.6 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.15−7.40 (m, 45 H), 5.39 (s, 1 H), 5.14 (s, 1 H), 4.91−4.94 (m, 3 H), 4.84 (d, J = 12.0 Hz, 1 H), 2.56 (m, 2 H), 2.01 (s, 3 H), 1.23 (t, J = 7.2 Hz, 3 H). FABMS: calcd for $C_{85}H_{92}O_{16}S$ 1400.6, found 1400.6.

Ethyl *O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (5). A solution of sodium (2.3 mg, 0.1 mmol) in methanol (5 mL) was added to a stirred solution of **42** (0.8 g, 0.6 mmol) in DCM (10 mL). After being stirred at room temperature for 3 h, the reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried with Na_2SO_4 and concentrated to give the crude ethyl *O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**43**), which was directly used in the next step. To the solution of **43**, DMAP (22 mg, 0.2 mmol), and imidazole (82 mg, 1.2 mmol) in dry DMF (15 mL) was added TBDMSCl (121 mg, 0.8 mmol) at 0 °C. After the mixture was warmed to room temperature and stirred for 3 h, it was diluted with 50 mL of ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated in a vacuum. Finally, the residue was purified with a silica gel column to give **5** (810 mg, 92%). $[\alpha]_D$ +25.6 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz): δ 7.05−7.35 (m, 45 H), 5.29 (s, 1 H), 5.14 (d, J = 1.2 Hz, 1 H), 4.88 (d, J = 10.8 Hz, 1 H), 4.84 (d, J = 10.8 Hz, 1 H), 4.81 (d, J = 1.2 Hz, 1 H), 4.75 (d, J = 10.8 Hz, 1 H), 4.65 (d, J = 12.0 Hz, 1 H), 4.56−4.58 (m, 3 H), 4.49 (s, 2 H), 4.35−4.46 (m, 9 H), 4.08 (t, J = 2.4 Hz, 1 H), 4.04 (m, 1 H), 3.93 (t, J = 9.6 Hz, 1 H), 3.77−3.87 (m, 8 H), 3.64−3.72 (m, 4 H), 3.58 (dd, J = 11.4, 4.2 Hz, 1 H), 3.55 (dd, J = 10.8, 1.8 Hz, 1 H), 3.49 (dd, J = 11.4, 1.8 Hz, 1 H), 2.40−2.55 (m, 2 H), 1.15 (t, J = 7.2 Hz, 3 H), 0.83 (s, 9 H), 0.01 (s, 3 H), 0.00 (s, 3 H). FABMS: calcd for $C_{89}H_{104}O_{15}SSi$ 1472, found, 1472, 1495 ($M + Na^+$).

***O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2-*O*-hexadecanoyl(palmitoyl)-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (44).** After the mixture of MS 4A (3.0 g), **5** (720 mg, 0.488 mmol), and **6** (410 mg, 0.348 mmol) in DCM and ethyl ether (1:1, 20 mL) was stirred at room temperature for 1 h and then cooled to 0 °C, NIS (220 mg, 0.97 mmol) was added. The mixture was stirred for another 30 min and cooled to −10 °C, whereupon TfOH (6.1 μL, 0.035 mmol) in DCM (3 mL) was added. The reaction mixture was warmed to 0 °C and stirred for 30 min. Finally, triethylamine was added to quench the reaction. The molecular sieves were filtered off and the solution was diluted with ethyl ether. The organic layer was separated, washed, dried, and concentrated. Column chromatography of the residue afforded **44** as a colorless syrup (810 mg, 90%). $[\alpha]_D$ +44.8 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz): δ 7.03−7.36 (m, 72 H), 6.82 (d, J = 9.0 Hz, 2 H), 5.91 (t, J = 2.4 Hz, 1 H), 5.68 (d, J = 3.6 Hz, 1 H), 5.21 (s, 1 H), 5.12 (d, J = 1.2 Hz, 1 H), 4.95 (d, J = 10.8 Hz, 1 H), 4.93 (d, J = 11.4 Hz, 1 H), 4.91 (d, J = 10.2 Hz, 1 H), 4.88 (d, J = 10.2 Hz, 1 H), 4.86 (d, J = 10.8 Hz, 1 H), 4.72−4.75 (m, 4 H), 4.68 (d, J = 10.8 Hz, 1 H), 4.63 (d, J = 9.6 Hz, 1 H), 4.62 (d, J = 10.8 Hz, 1 H), 4.59 (d, J = 10.8 Hz, 1 H), 4.52 (d, J = 11.4 Hz, 1 H), 4.51 (d, J = 12.0 Hz, 1 H), 4.48 (d, J = 12.0 Hz, 1 H), 4.46 (d, J = 12.0 Hz, 1 H), 4.17 (d, J = 12.0 Hz, 1 H), 3.93 (t, J = 9.6 Hz, 1 H), 3.78 (dd, J = 9.6, 3.0 Hz, 1 H), 3.76 (s, 3 H), 3.73−3.75 (m, 2 H), 3.69 (t, J = 2.4 Hz, 1 H), 3.66 (t, J = 9.6 Hz, 1 H), 3.51 (dd, J = 9.6, 3.0 Hz, 1 H), 3.38 (m, 1 H), 3.29 (dd, J = 10.8 Hz, 1 H), 3.14 (d, J = 10.2, 3.6 Hz, 1 H), 2.34 (m, 2 H), 1.58 (m, 2 H), 1.25 (m, 24 H), 0.86 (t, J = 7.2 Hz, 1 H), 0.85 (s, 9 H), 0.02 (s, 3 H), 0.00 (s, 3 H). MALDI-TOF-MS: calcd for $C_{158}H_{187}N_3O_{27}Si$ 2586, found 2586.

***O*-(2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2-*O*-hexadecanoyl(palmitoyl)-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (45).** After **44** (600 mg, 232 μmol) was dissolved in DCM (15 mL) under nitrogen, TBAF (1 M solution in THF, 2.24 mL, 2.24 mmol) and acetic acid (130 μL, 2.28 mmol) were added to the solution at 0 °C. The mixture was warmed to room temperature and stirred at 35−40 °C for 72 h. After removal of the solvent in a vacuum, the residue was purified by flash column chromatography to give **45** (545 mg, 95%). $[\alpha]_D$ +39.0 (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz): δ 7.05−7.34 (m, 72 H), 6.84 (d, J = 8.4 Hz, 2 H), 5.93 (t, J = 2.4 Hz, 1 H), 5.69 (d, J = 3.6 Hz, 1 H), 5.24 (d, J = 1.8 Hz, 1 H), 5.03 (d, J = 1.8 Hz, 1 H), 4.97 (d, J = 10.8 Hz, 1 H), 4.93 (d, J = 12.0 Hz, 1 H), 4.91 (d, J = 12.0 Hz, 1 H), 4.90 (d, J = 10.2 Hz, 1 H), 4.88 (d, J = 1.2 Hz, 1 H), 4.87 (d, J = 11.4 Hz, 1 H), 4.78 (d, J = 11.4 Hz, 1 H), 4.75 (d, J = 11.4 Hz, 1 H), 4.73 (d, J = 12.0 Hz, 1 H), 4.70 (d, J = 10.8 Hz, 1 H), 4.64 (d, J = 10.2 Hz, 1 H), 4.62 (d, J = 11.4 Hz, 1 H), 4.59 (d, J = 11.4, 1 H), 4.56 (d, J = 12.0, 1 H), 4.54 (d, J = 12.0, 1 H), 4.52 (d, J = 12.0 Hz, 1 H), 4.49 (d, J = 12.0 Hz, 1 H), 4.48 (s, 2 H), 4.47 (d, J = 12.0 Hz, 1 H), 4.44 (d, J = 11.4 Hz, 1 H), 4.21 (d, J = 12.6 Hz, 1 H), 4.13 (d, J = 12.6 Hz, 1 H), 3.97 (t, J = 9.6 Hz, 1 H), 3.78 (s, 3 H), 3.38 (t, J = 9.0 Hz, 2 H), 3.29 (dd, J = 11.4, 3.6 Hz, 1 H), 3.17 (dd, J = 10.2, 3.6 Hz, 1 H), 2.38 (m, 2 H), 1.61 (m, 2 H), 1.26 (m, 24 H), 0.88 (t, J = 7.2 Hz, 1 H). MALDI-TOF-MS: calcd for $C_{152}H_{173}N_3O_{27}$ 2472, found 2495 ($M + Na^+$).

***O*-(2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-{2-(benzyloxycarbonyl)amino-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl}-(1→6)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (47).** To the mixture of **45** (120 mg, 48.5 μmol) and dibenzyl dicarbonate (140 mg, 0.49 mmol) in methanol and DCM (1:2, 20 mL) was added triethylphosphine (72 μL, 0.49 mmol) under nitrogen at 0 °C. The reaction mixture was then warmed to room temperature and stirred at room temperature overnight. After the removal of solvents in a vacuum, the residue was purified by flash chromatography to afford **47** (88 mg, 70%). TLC: R_f 0.50 (acetone, hexane, and DCM 4:10:2). ^1H NMR ($CDCl_3$, 600 MHz, 25 °C) major isomer: δ 6.67 (d, J = 7.8 Hz, 2 H), 5.89 (s, 1 H), 5.67 (d, J = 10.2 Hz, 1 H), 5.44 (d, J = 3.0 Hz, 1 H), 5.21 (s, 1 H), 5.09 (d, J = 11.4 Hz, 1 H), 5.05 (s, 1 H), 4.83 (s, 1 H), 3.58 (s, 3 H), 2.28 (m, 2 H), 1.61 (m, 2 H), 1.25 (m, 24 H), 0.88 (t, J = 7.2 Hz, 1 H). ^1H NMR ($CDCl_3$, 600 MHz, 25 °C) minor isomer: δ 6.72 (d, J = 7.8 Hz, 2 H), 5.92 (s, 1 H), 5.27 (d, J = 10.2 Hz, 1 H), 5.23 (s, 1 H). ^1H NMR ($CDCl_3$, 600 MHz, 50 °C): 6.69 (d, J = 6.6 Hz, 2 H), 5.87 (s, 1 H), 5.64 (d, J = 9.0 Hz, 1 H), 5.43 (d, J = 3.6 Hz, 1 H), 5.20 (s, 1 H), 5.05 (d, J = 12.0 Hz, 1 H), 5.02 (s, 1 H), 4.82 (s, 1 H), 3.59 (s, 3 H), 2.27 (m, 2 H), 1.56 (m, 2 H), 1.25 (m, 24 H), 0.88 (t, J = 7.2 Hz, 1 H). MALDI-TOF-MS: calcd for $C_{160}H_{181}NO_{29}$ 2580, found 2603 ($M + Na^+$).

***O*-{2,3,4-Tri-*O*-benzyl-6-*O*-[benzyl-[*N*-(9-fluorenylmethoxycarbonyl)]aminoethylphosphonato]-α-D-mannopyranosyl}-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-benzyloxycarbonylamino-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (48).** To a solution of **47** (70 mg, 27 μmol) and phosphitamide **4** (0.19 mmol, 7 equiv) in dry DCM and acetonitrile (3:1, 6 mL) was added 1*H*-tetrazole (0.19 mmol, 0.41 mL of 0.47 M in CH_3CN). The mixture was stirred at room temperature for 6 h, and then *tert*-butyl hydroperoxide (0.50 mmol, 0.1 mL of 5 M

in decane) was added at −20 °C. The solution was warmed to room temperature and stirred for another 1.5 h and finally concentrated. Column chromatography of the residue gave **48** (68 mg, 84%) as a mixture of two diastereoisomers. TLC: R_f 0.44 (acetone, hexane, and DCM 4:10:2). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.73 (d, J = 7.8 Hz, 2 H), 7.55 (br, 2 H), 7.35 (t, J = 7.2 Hz, 1 H), 6.66 (d, J = 5.4 Hz, 2 H), 5.89 (s, 1 H), 5.74 (t, J = 9.6 Hz, 1 H/2), 5.73 (t, J = 9.6 Hz, 1 H/2), 5.43 (br, 1 H), 5.28 (m, 1 H), 5.21 (br, 1 H), 3.55 (s, 3 H/2), 3.54 (s, 3 H/2), 2.27 (m, 2 H), 1.55 (m, 2 H), 1.25 (m, 24 H), 0.88 (t, J = 7.2 Hz, 1 H). ^{31}P NMR ($CDCl_3$): δ −0.70. MALDI-TOF-MS: calcd for $C_{184}H_{203}N_2O_{34}P$ 3015, found 3038 (M + Na^+).

***O*-{2,3,4-Tri-*O*-benzyl-6-*O*-[benzyl-[*N*-[*N*-(9-fluorenylmethoxycarbonyl)-L-prolinyl-L-serinyl]aminoethyl]phosphonato]-α-D-mannopyranosyl}-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-benzyloxycarbonylamino-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-2-*O*-hexadecanoyl-1-*O*-(*p*-methoxybenzyl)-3,4,5-tri-*O*-benzyl-*myo*-inositol (49).** Compound **48** (60 mg, 20 μmol) was dissolved in a 2 M solution of dimethylamine in THF (5 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated. The residue was dissolved and concentrated several times with chloroform as solvent to remove the excessive dimethylamine. The resulting crude product **3** (TLC: R_f 0.40; EtOAc, hexane, DCM, and methanol 3:3:3:1) was directly used in the next step. Thus, after the crude **3**, HOBt (13.5 mg, 100 μmol), and **2** (34 mg, 80 μmol) were dissolved in DCM and DMF (10 mL, 10:1), to the solution was added EDC (19.2 mg, 100 μmol) at 0 °C under nitrogen atmosphere. The reaction mixture was then warmed to room temperature and stirred overnight, which was followed by concentration in a vacuum. Column chromatography of the residue gave **49** (38.4 mg, 60%). TLC: R_f 0.54 (EtOAc, hexane, DCM, and methanol 3:3:3:1). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.73 (m, 2 H), 7.53 (m, 2 H), 7.36 (t, J = 7.2 Hz, 1 H), 6.66 (d, J = 8.4 Hz, 2 H), 5.90 (br, 1 H), 5.74 (m, 1 H), 5.43 (d, J = 3.6 Hz, 1 H), 5.20 (br, 1 H), 5.12 (br, 1 H), 5.08 (d, J = 12.0 Hz, 1 H), 4.73 (s, 1 H), 3.57 (s, 3 H), 2.28 (m, 2 H), 2.12 (m, 2 H), 1.94 (m, 1 H), 1.84 (m, 1 H), 1.56 (m, 2 H), 1.25 (m, 24 H), 0.88 (t, J = 7.2 Hz, 1 H). HMQC (^{13}C 150 MHz, ^{1}H 600 MHz): 100.6/5.20 (Man-1), 99.3/5.43 (Glu-1), 99.2/5.12 (Man-1), 99.2/4.73 (Man-1), 65.3/5.90 (Ino-2). ^{31}P NMR ($CDCl_3$): δ 3.04. MALDI-TOF-MS: calcd for $C_{193}H_{216}N_3O_{37}P$ 3198, found 3221 (M + Na^+).

***O*-{6-*O*-[*N*-(L-Prolinyl-L-serinyl)aminoethyl]phosphonato-α-D-mannopyranosyl}-(1→2)-*O*-(α-D-mannopyranosyl)-(1→6)-*O*-(α-D-mannopyranosyl)-(1→4)-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→6)-2-*O*-hexadecanoyl-*myo*-inositol (1).** After **49** (24 mg, 7.5 μmol) was dissolved in 2 M of dimethylamine in THF (4 mL), the reaction mixture was stirred at room temperature for 1 h, which was followed by concentration in a vacuum. Column chromatography of the residue gave the free amine **50** (19 mg, 6.38 μmol, 85%): R_f 0.33 (DCM and methanol 10:1). The mixture of **50** (19 mg) and Pd/C (50 mg, 10%) in $CHCl_3$, MeOH, and H_2O (4 mL, 10:10:3) was then stirred in H_2 atmosphere for 1 d. The reaction mixture was filtered off through a Celite pad with a mixture of MeOH, H_2O, and $CHCl_3$ (5:5:1) as the eluent. The filtrate was concentrated in a vacuum to afford the synthetic target **1** (8 mg, 91%) as white powder. ^{1}H NMR (D_2O, 600 MHz, 35 °C): δ 5.36 (br, 2 H), 5.19 (s, 1 H), 5.07 (s, 1 H), 4.98 (s, 1 H), 4.43 (t, J = 5.4 Hz, 1 H), 4.40 (t, J = 7.2 Hz, 1 H). ^{1}H NMR (CD_3OD, 600 MHz, 35 °C): δ 5.41 (s, 1 H), 5.34 (s, 1 H), 5.22 (s, 1 H), 5.13 (s, 1 H), 4.98 (s, 1 H), 4.45 (br, 1 H), 4.38 (br, 1 H). HMQC (CD_3OD, ^{13}C 150 MHz, ^{1}H 600 MHz, 35 °C): δ 75.6/5.41 (Ino-2), 97.9/5.34 (Glc-1), 102.8/5.22 (Man-1), 100.0/5.13 (Man-1), 104.2/4.98 (Man-1), 57.6/4.45 and 4.38 (Pro and Ser α-C/H). ^{31}P NMR (CD_3OD): δ 1.72. MALDI-TOF-MS: calcd for $C_{57}H_{102}N_3O_{32}P$ 1372, found 1372, 1395 (M + Na^+), 1434 (M + Na + K^+).

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

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