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Convergent Synthesis of a GPI Containing an Acylated Inositol

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Abstract: A GPI of sperm CD52 was synthesized by a highly convergent procedure, representing the first chemical synthesis of a complex GPI having an acylated inositol. The presence of a large acyl group resulted in unusual properties and reactions of the relevant intermediates, which gave rise to a number of problems. To overcome the problems and achieve the target molecule, a new synthetic strategy was developed. First, the pseudodisaccharide of 2-*O*-palmitoylinositol was phospholipidated, and then the trimannose segment and the phosphoethanolamine group were sequentially attached. Global deprotection eventually afforded the sperm CD52 GPI. The method may be useful for the synthesis of other GPIs having an acylated inositol.

Introduction

Anchoring proteins and glycoproteins to cell membranes through glycosylphosphatidylinositols (GPIs) is ubiquitous in the eukaryotic world,[1] and GPIs play a pivotal role in many biological events.[2−4] For instance, CD52, a GPI-anchored glycopeptide antigen expressed by virtually all human lymphocyte and sperm cells,[5,6] is involved both in the recognition process of human immune system and in the process of human reproduction.[7−9] Monoclonal antibodies of lymphocyte CD52 have been used to treat several immune system-related diseases,[10−16] while the identification of antibodies specific to sperm CD52 in infertile women foresees the opportunities of new contraceptive developments.[17,18]

Extensive investigations prove that the structure of GPIs is rather conserved with the phospholipid attached to the inositol 1-*O*-position. However, there are a number of GPIs having a large acyl group directly linked to the inositol 2-*O*-position.[1] It is known that the inositol 2-*O*-acylation is important for GPI biosynthesis,[19,20] but the acyl group is often deleted after the biosynthesis is accomplished.[21] The presence of a 2-*O*-acyl group in mature GPIs causes their resistance to bacterial PI-phospholipase C,[19,20] while the actual biological functions of the 2-*O*-acyl group are not clear yet. Thus, chemical synthesis of GPIs having an acylated inositol is important for their structural, metabolic, and other biological studies.

Chemical synthesis of GPIs has been a very attractive area, and several GPIs have been prepared in recent years.[22−38]

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However, to the best of our knowledge no chemical synthesis of GPIs containing a 2-*O*-acyl inositol, except for an incomplete structure,[39] has been reported, although these GPIs are of special interest to both biologists and chemists. Evidently, the presence of a huge substituent at the inositol 2-*O*-position will engender more synthetic challenges. For example, the increased steric hindrance around the inositol residue may make its phospholipidation and other modifications difficult. The potential migration of the 2-*O*-acyl group to other positions of the inositol residue may pose another problem.[40]

To study these questions and establish a potentially useful method for preparing 2-*O*-acylated GPIs, this work investigated the synthesis of a GPI of sperm CD52.

Results and Discussion

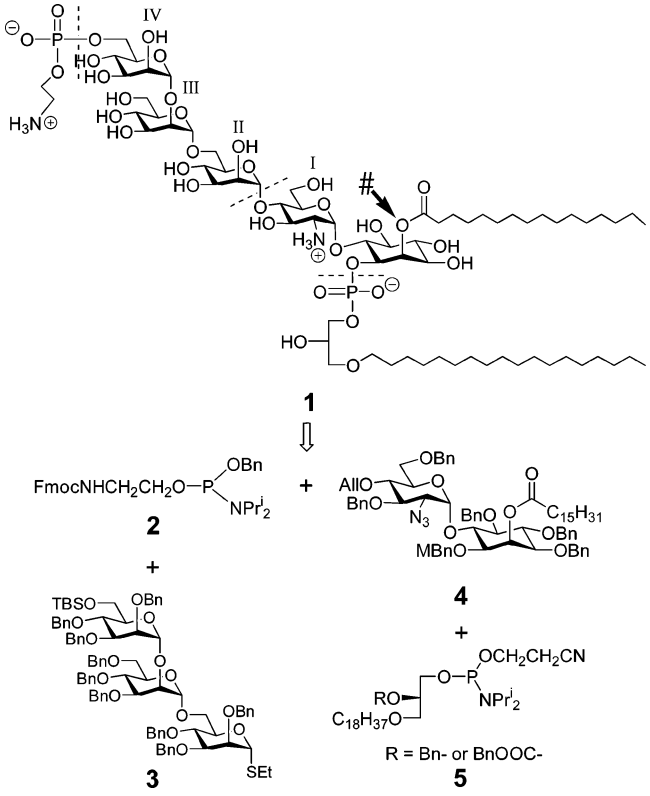
Sperm CD52 has a rather typical GPI, with the phospholipid and the conserved anchor glycan linked to the inositol 1-*O*- and 6-*O*-positions, respectively, and a phosphoethanolamine to the 6-*O*-position of Man^{IV} at the nonreducing end. Another phosphoethanolamine is attached to the 2-*O*-position of Man^{II}, which is a species-specific modification.[32] An intriguing structural feature of sperm CD52 GPI is that it has a long acyl chain, namely, a palmitoyl group, at the inositol 2-*O*-position.[5,6]

Our synthetic target is **1**, a GPI of sperm CD52 without the optional phosphoethanolamine on Man^{II}. Its retrosynthetic plan is outlined in Scheme 1. Because the phosphate and α-mannose linkages are more easily formed, they were cleaved first to yield the logical building blocks **2**, **3**, **4**, and **5**. The benzyl group was chosen as a permanent protection for hydroxyl groups, as it can be removed under mild conditions later on. Moreover, the presence of a palmitoyl in the synthetic target prevented acyl groups from being used for this purpose. An azido sugar was designed as a glucosamine derivative to facilitate the α-glycosylation. In regard to the assembly of building blocks, all reported syntheses[22−38] followed a procedure of constructing the backbone (**3** + **4**) first and attaching the phosphates at later stages.

We considered a similar strategy in our initial synthetic attempts. However, one of our concerns was whether the phospholipid could be successfully introduced to the inositol 1-*O*-position, because in **1** there are huge substituents linked to both the 2-*O*- and 6-*O*-positions adjacent to the 1-*O*-position. To address this problem, a model reaction was conducted with the pseudodisaccharide **4**.[41] It turned out that the reaction between **4**, after the selective exposure of its inositol 1-*O*-position, and **5** gave the phospholipidation product in a good yield (80%), and the azido group was stable.

Encouraged by these results, segment **4** was thus transformed to **6** and coupled with **3**. Following the selective removal of the *p*-methoxybenzyl (MBn) group, the pseudopentasaccharide **8** was obtained (Scheme 2). However, when **8** was treated with

Scheme 1



5 under the established conditions,[41] a cyclic phosphitamide (**9**) was formed as a diastereoisomeric mixture in 1.6:1.0 ratio, indicating the involvement of the azido group. The structure of **9** was supported by its NMR and MS results.

To further characterize product **9**, it was treated with sodium methoxide in methanol to remove the 2-*O*-acyl group, affording two products easily separable by flash column chromatography. The MS and NMR spectra showed that they were diastereoisomers **10a** and **10b**.

To the best of our knowledge, this is a new reaction to form cyclophosphitamides. Moreover, since the azido group was not affected during the phospholipidation of the pseudopentasaccharide and other complex structures without the 2-*O*-acyl group,[22−38] nor was it affected during the phospholipidation of the acylated pseudodisaccharide without the trimannose,[41] it seems that the coexistence of the 2-*O*-acyl group and the trimannose was critical to result in this unique reaction.

We propose that the steric interactions between the trimannose segment and the long acyl chain, even though they appear to be well separated, force **8** to adopt a conformation that has the azido group close to the free hydroxyl group. This conformational arrangement facilitates the intramolecular reaction between the phosphite group and the azido group of intermediate **13a** (Scheme 3).

Trying to circumvent this problem, the azido group of **8** was transformed to a benzyloxycarbonyl amino (CBzHN) group to offer **11** (Scheme 2). To our disappointment, there was no reaction between **11** and **5**, proved by the complete recovery of **11** from the reaction mixture. This result might indicate the dramatic increase of steric hindrance around the free hydroxyl group of **11**. In contrast, the reaction between **11** and a less bulky phosphorylating reagent dibenzyl *N*,*N*-diisopropylphosphoramidite under the same condition gave **12** in an almost

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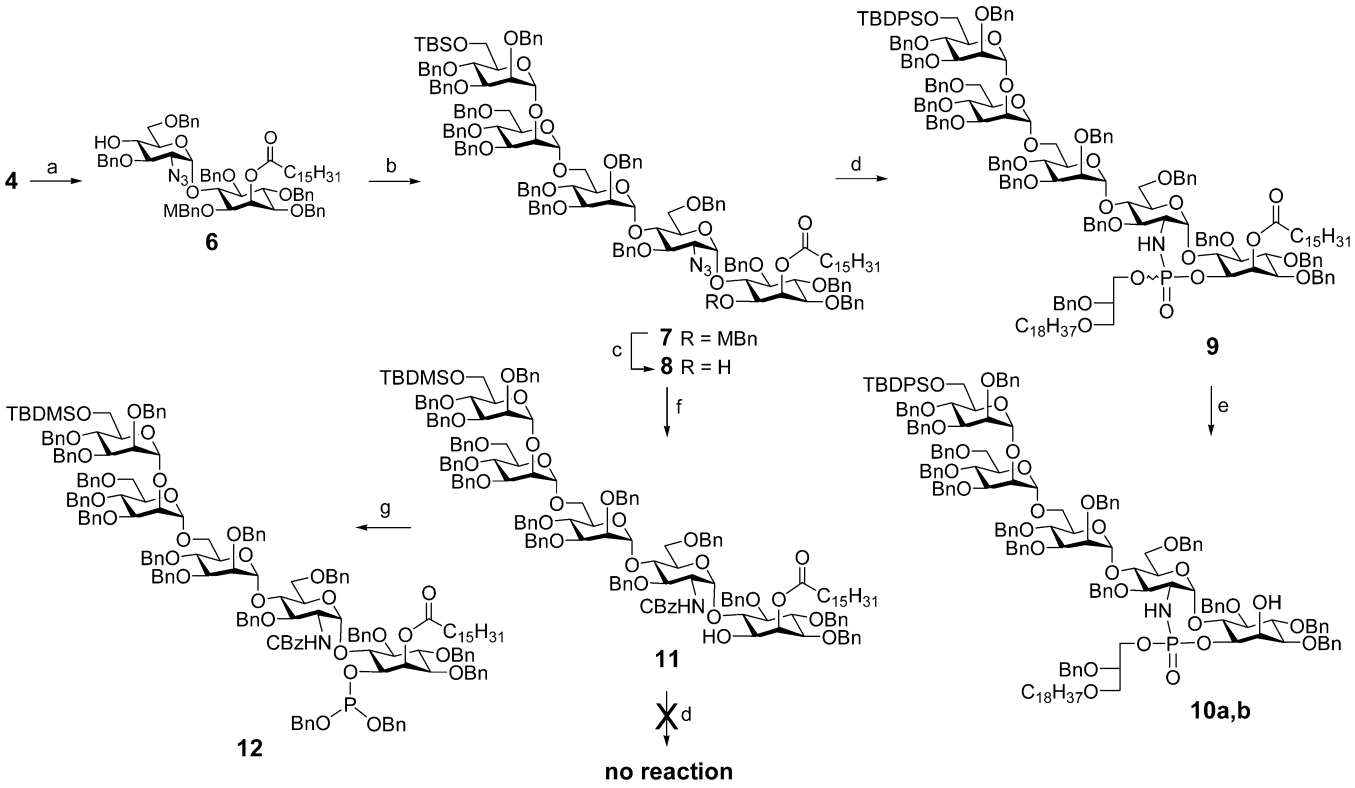
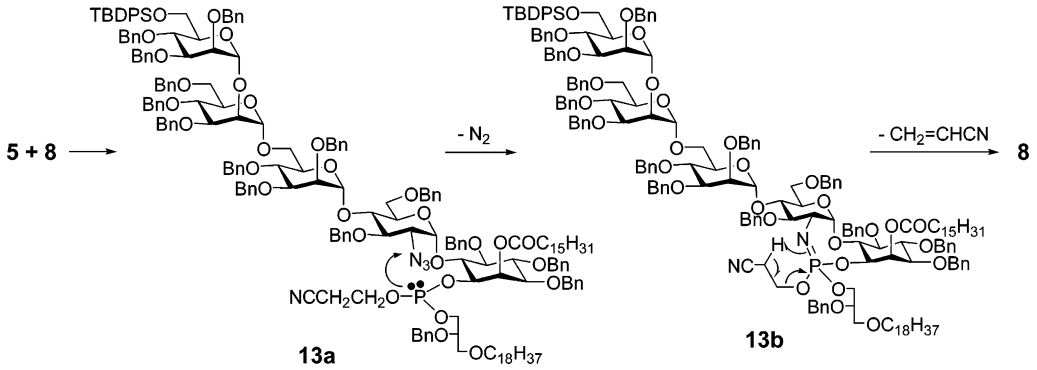
Scheme 2 [a]



[a] Reagents and conditions: (a) $PdCl_2$, 84%; (b) **3**, NIS, TfOH, 51%; (c) CAN, MeCN−H_2O, 52%; (d) **5**, tetrazole; then tBuO$_2$H, 83%; (e) NaOMe, MeOH, rt, >99%; (f) PEt_3, CBz_2O, MeOH-DCM (1:2), 65%; (g) tetrazole, $(BnO)_2PN(iPr)_2$, >99%.

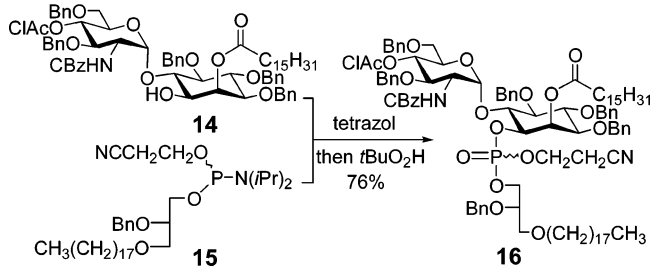
Scheme 3



quantitative yield, which further suggested that the steric effects might play a significant role. More interestingly, the reaction between **15** and **14**, which also possessed the 2-*O*-acyl and CBz groups but not the trimannose segment, was clean and afforded **16** in a good yield (Scheme 4). All these results agree well with the hypothesis that the trimannose and the long acyl chain in **7** and **8** may somehow interact with each other and cause the unexpected properties.

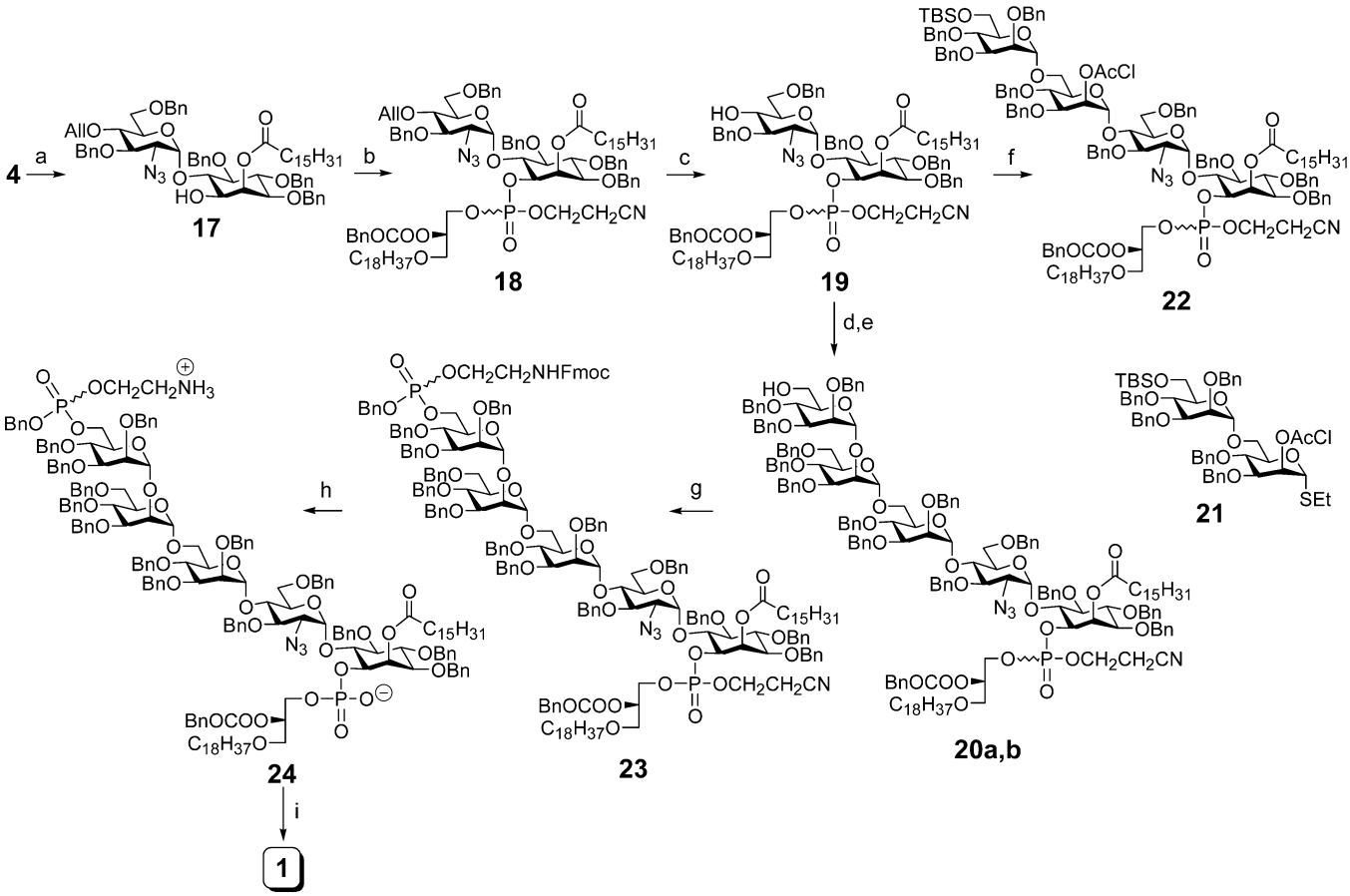
Trying to introduce the phospholipid to **8** and **11**, different reactions and conditions were tested, but to no avail. At this point it was clear that to achieve the synthetic target alternative strategies had to be considered. Since the pseudodisaccharide was phospholipidated without problem,[41] it was natural to conceive a design of coupling **4** and **5** first and introducing the trisaccharide segment **3** and the phosphoethanolamine **2** later. For such a strategy to work, the phospholipid had to be stable to the conditions employed in the glycosylation.

Scheme 4



As shown in Scheme 5, after MBn of **4** was removed with trifluoroacetic acid (TFA), the product **17** was phospholipidated by **5** in a two-step one-pot procedure to give an inseparable diastereoisomeric mixture (1:3) of **18**, which was directly applied to the deallylation with $PdCl_2$. The azido group was not affected by the phospholipidation, but the reaction could be complex, probably due to the involvement of the azido group, if the

Scheme 5 [a]



[a] Reagents and conditions: (a) 5% TFA/DCM, 93%; (b) **5**, tetrazole; then tBuO$_2$H, 68%; (c) $PdCl_2$, 61%; (d) **3**, NIS, TfOH; (e) Et_2O-BF_3, 52% (two steps); (f) **21**, NIS, TfOH, 54% (plus an ortho ester 23%); (g) **2**, tetrazole; then tBuO$_2$H, 74%; (h) DBU, 87%; (i) 10% Pd/C, H_2, 84%.

phosphite intermediate formed from the reaction of **17** and **5** was kept for an extended period before its transformation to the desirable phosphate. Nevertheless, no acyl migration was observed during the deprotection of **4** or phospholipidation of **17**. The glycosylation of **19** with **3** followed by selective deprotection of the resulting product afforded **20**. Its two diastereoisomers, **20a** and **20b**, could be separated by careful chromatography and were individually identified by NMR and MS. The yield and stereoselectivity of the glycosylation of **19** by **3** turned out to be similar to that of the reaction between **3** and **6**, showing that the phospholipid had no obvious influence on the glycosylation. To further probe this synthetic strategy, the glycosylation of **19** by a disaccharide **21** under the same conditions was performed. The reaction gave the phospholipidated pseudotetrasaccharide **22** in 54% yield, as well as an ortho ester (23%), produced from a side reaction involving the neighboring acyl group that is frequently observed in the glycosylations using 2-*O*-acylated sugars as donors. This result indicates that the strategy may be useful for other syntheses as well. The introduction of a phosphoethanolamine group to **20** by the traditional method yielded a diastereoisomeric mixture (1:1) of **23**. The removal of its Fmoc and cyanoethyl groups was achieved by treating it with DBU for a short period (2 min), whereas secondary amines, such as piperidine and dimethylamine, gave quite complex results. The HMQC NMR spectra of **24** showed two diastereoisomers with well-differentiated two sets of reporter signals of the mannose residues, which are close to the phosphorus chiral center, while the signals of $GlcN_3$ and Ino were overlapping. Global deprotection of **24** was accomplished by hydrogenolysis using 10% Pd/C as the catalyst to finally afford the synthetic target **1** that was soluble in DMSO but barely soluble in water, MeOH, or $CHCl_3$ or their mixtures. The ^{1}H NMR spectra of **1** in DMSO-d_6 did not show any aromatic proton, suggesting the complete removal of benzyl groups, which was also supported by its MS having the highest m/z peak of M + Na^+. The inositol 2-H and the carbohydrate anomeric protons of **1** were characteristic and assignable. The homogeneity of **1** was also supported by its phosphorus NMR.

In summary, this paper described the synthesis of a GPI anchor of sperm CD52 through a highly convergent procedure. It represents the first chemical synthesis of a GPI with an acylated inositol. The acyl group had a significant influence on the structures and properties of relevant intermediates. As a result, unusual and interesting behaviors and reactions, such as the novel cyclophosphitamidation reaction, were observed with **8** and **11**. Moreover, it seems that the coexistence of the 2-*O*-acyl group and the trimannose segment was decisive to cause these unexpected results. A new synthetic strategy was thus designed to overcome the problems and achieve the target. The strategy may be useful for the syntheses of other GPIs having an acyl group linked to the inositol residue. In addition, the potential interactions between the anchor glycan and the inositol 2-*O*-acyl group may be of biological relevance, even though the structures discussed herein are in the protected form. It is also worthy mentioning that no migration of the 2-*O*-palmitoyl group was observed with either the pseudodisaccharides **14** and

17 or the pseudopentasaccharides **8** and **11**. Neither was it observed during the phospholipidation reactions. These results suggest that the migration of 2-*O*-acyl group may not be a major concern in the synthesis of GPIs having 2-*O*-acylated inositols.

Experimental Section

6-*O*-[2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2-propenyl)-α-D-glucopyranosyl]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (17). After the solution of 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 (**4**, 540 mg, 0.45 mmol) in 5% TFA/DCM (10 mL) was stirred at room temperature for 2 h, the reaction mixture was diluted with DCM and washed with aqueous $NaHCO_3$ and water. The organic solution was dried with Na_2SO_4 and then concentrated to dryness in a vacuum. Flash chromatography of the crude product gave **17** (460 mg, 0.42 mmol, 93%). TLC (acetone/hexane 1:3): $R_f = 0.38$. $[\alpha]^{25}_D = +50$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.17−7.40 (m, 25 H), 5.73−5.80 (m, 2 H), 5.22 (d, $J = 4.2$ Hz, 1 H), 5.13 (dd, $J = 17.4$, 1.2 Hz, 1 H), 5.09 (d, $J = 8.4$ Hz, 1 H), 5.07 (d, $J = 12.0$ Hz, 1 H), 4.92 (d, $J = 10.2$ Hz, 1 H), 4.89 (d, $J = 10.8$ Hz, 1 H), 4.82 (d, $J = 10.2$ Hz, 1 H), 4.76 (d, $J = 12.0$ Hz, 1 H), 4.74 (d, $J = 11.4$ Hz, 1 H), 4.63 (d, $J = 10.8$ Hz, 1 H), 4.50 (d, $J = 10.8$ Hz, 1 H), 4.43 (d, $J = 12.0$ Hz, 1 H), 4.15 (dd, $J = 12.0$, 6.0 Hz, 1 H), 4.12 (d, $J = 12.0$ Hz, 1 H), 3.80−3.91 (m, 6 H), 3.72 (ddd, $J = 9.6$, 2.4, 2.4 Hz, 1 H), 3.52−3.60 (m, 3 H), 3.40 (t, $J = 9.6$ Hz, 1 H), 3.17 (dd, $J = 10.8$, 2.4 Hz, 1 H), 2.93 (dd, $J = 10.8$, 1.8 Hz, 1 H), 2.41 (t, $J = 7.2$ Hz, 2 H), 1.66 (m, 2 H), 1.34 (m, 2 H), 1.23−1.29 (m, 22 H), 0.88 (t, $J = 6.6$ Hz, 3 H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.4, 138.4, 138.4, 137.8, 134.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 116.6, 99.3, 82.3, 82.0, 80.9, 80.8, 78.6, 78.5, 77.9, 77.6, 77.6, 77.0, 76.0, 75.6, 75.5, 73.6, 73.4, 72.1, 71.4, 71.3, 68.9, 67.3, 64.4, 34.5, 32.0, 30.0, 29.9, 29.8, 29.6, 29.5, 29.4, 29.1, 25.3, 22.8, 14.2. FABMS: calcd for $C_{66}H_{85}N_3O_{11}$ 1095.6, found 1095.6.

6-*O*-[2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2-propenyl)-α-D-glucopyranosyl]-1-*O*-[(2-cyanoethoxy)-(2-*O*-benzyloxycarbonyl-3-*O*-octadecyl-sn-glycerol)-phosphono]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (18). To the solution of **17** (80 mg, 0.073 mmol) and freshly prepared **5** (200 mg, 0.295 mmol) in dry CH_2Cl_2 (5 mL) was added 1*H*-tetrazole (0.295 mmol, 0.47 mmol/L in CH_3CN). The mixture was stirred at room temperature and monitored with TLC. After **17** was completely transformed to a new product (acetone/hexane 1:3, $R_f = 0.40$) in 3 h, *t*-BuO_2H (1 mmol, 0.2 mL of 5 M solution in decane) was added at −20 °C. The reaction mixture was warmed to room temperature and stirred for another 1 h and finally concentrated. Column chromatography of the residue gave **18** (90 mg, 0.050 mmol, 68%) as an inseparable mixture of two diastereoisomers (3:1). TLC (acetone/hexane 1:3): $R_f = 0.34$. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (the major isomer) 5.88 (t, *J* 2.4 Hz, 1 H), 5.76 (m, 1 H), 5.41 (d, $J = 3.6$ Hz, 1 H), 2.69 (t, $J = 6.0$ Hz, 2 H), 2.38 (t, $J = 7.2$ Hz, 2 H), 1.45−1.62 (m, 4 H), 0.88 (t, $J = 7.2$ Hz, 6 H); δ (the minor isomer) 5.91 (t, $J = 2.4$ Hz, 1 H), 5.76 (m, 1 H), 5.44 (d, $J = 3.6$ Hz, 1 H), 2.64 (m, 2 H), 2.37 (t, $J = 7.2$ Hz, 2 H), 1.45−1.62 (m, 4 H), 0.88 (t, $J = 7.2$ Hz, 6 H). ^{31}P NMR ($CDCl_3$): δ 1.51. MALDI-TOF-MS: calcd for $C_{98}H_{137}N_4O_{18}P$ 1688, found 1710 ($M + Na^+$).

6-*O*-[2-Azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-1-*O*-[(2-cyanoethoxy)-(2-*O*-benzyloxycarbonyl-3-*O*-octadecyl-sn-glycerol)-phosphono]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (19). To the solution of **18** (60 mg, 0.036 mmol) in acetic acid (4 mL) and water (3 drops) were added $PdCl_2$ (12.5 mg, 0.070 mmol) and NaOAc (12.5 mg) at room temperature. After 30 h of stirring, the reaction mixture was diluted with ethyl acetate, and the solution was washed with aqueous $NaHCO_3$, brine, and water. The organic layer was dried with Na_2SO_4 and then concentrated to dryness. Flash chromatography of the residue gave **19** (30 mg, 0.018 mmol, 61%) as a diastereoisomeric mixture (3:1). TLC (acetone/hexane/DCM 2:5:1): $R_f = 0.51$. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (the major isomer) 5.88 (t, $J = 2.4$ Hz, 1 H), 5.42 (d, $J = 3.6$ Hz, 1 H), 2.69 (t, $J = 6.0$ Hz, 2 H), 2.38 (t, $J = 7.2$ Hz, 2 H), 1.50−1.62 (m, 4 H), 0.88 (t, $J = 7.2$ Hz, 6 H); δ (the minor isomer) 5.91 (t, $J = 2.4$ Hz, 1 H), 5.42 (d, $J = 3.6$ Hz, 1 H), 2.64 (m, 2 H), 2.36 (t, $J = 7.2$ Hz, 2 H), 1.50−1.62 (m, 4 H), 0.88 (t, *J* 7.2 Hz, 6 H). HMQC NMR ($CDCl_3$, ^{13}C 150 MHz/^{1}H 600 MHz): δ (the major isomer) 97.5/5.42 (Glc-1), 68.3/5.88 (Ino-2); δ (the minor isomer) 97.5/5.42 (Glc-1), 68.5/5.91 (Ino-2). ^{31}P NMR ($CDCl_3$): δ −1.71, −1.92. MALDI-TOF-MS: calcd for $C_{95}H_{133}N_4O_{18}P$ 1648.7, found 1671.5 ($M + Na^+$).

6-*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-*O*-[(2-cyanoethoxy)-(2-*O*-benzyloxycarbonyl-3-*O*-octadecyl-sn-glycerol)-phosphono]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (20). After the mixture of MS 4A (0.3 g), **19** (38 mg, 0.023 mmol), and **3** (74 mg, 0.046 mmol) in DCM and ethyl ether (1:1, 5 mL) was stirred at room temperature for 1 h and then cooled to 0 °C, NIS (14 mg, 0.063 mmol) was added. The mixture was stirred for another 30 min and then cooled to −10 °C, whereupon TfOH (0.6 μL, 0.0035 mmol) in DCM (0.5 mL) was added. The mixture was warmed to 0 °C and stirred for 15 min. Then, triethylamine was added to quench the reaction. The molecular sieves were filtered off, and the filtrate was diluted with ethyl ether and washed, dried, and concentrated. Column chromatography of the residue gave the product as a color less syrup. It was then dissolved in 3% of $BF_3{\cdot}Et_2O$/DCM (5 mL) at 0 °C, and the mixture was allowed to warm to room temperature and stirred for 1.5 h and finally diluted with DCM. The organic layer was washed with water, dried, and concentrated. Column chromatography of the residue gave two diastereoisomers **20a** (24 mg, 0.008 mmol, 35%) and **20b** (12 mg, 0.004 mmol, 17%). **20a**: TLC (acetone/hexane/DCM 2:5:1): $R_f = 0.45$. $[\alpha]_D$ +46.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.03−7.36 (m, 75 H), 5.88 (t, $J = 2.4$ Hz, 1 H), 5.46 (d, $J = 3.6$ Hz, 1 H), 5.18 (d, $J = 1.8$ Hz, 1 H), 5.13 (d, $J = 12.0$ Hz, 1 H), 5.09 (d, $J = 12.0$ Hz, 1 H), 5.03 (d, $J = 1.8$ Hz, 1 H), 5.01 (dd, 1 H), 4.90 (br s, 1 H), 3.30 (dd, $J = 10.2$, 3.6 Hz, 1 H), 2.69 (t, $J = 6.0$ Hz, 2 H), 2.36 (d, $J = 7.2$ Hz, 2 H), 1.59 (m, 2 H), 1.49 (m, 2 H), 1.22−1.30 (m, 54 H), 0.87 (t, $J = 7.2$ Hz, 6 H). HMQC NMR ($CDCl_3$, ^{13}C 150 MHz/^{1}H 600 MHz): 101.0/5.18 (Man-1), 99.8/5.03 (Man-1), 99.7/4.90 (Man-1), 97.6/5.46 (Glc-1), 68.4/5.88 (Ino-2). ^{31}P NMR ($CDCl_3$): δ 1.18. MALDI-TOF-MS: cacld for $C_{176}H_{217}N_4O_{33}P$, 2946; found, 2984 ($M + K^+$). **20b**: TLC (acetone/hexane/DCM 2:5:1): $R_f = 0.43$. $[\alpha]_D$ +25.4 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.03−7.36 (m, 75 H), 5.90 (t, $J = 2.4$ Hz, 1 H), 5.46 (d, $J = 3.6$ Hz, 1 H), 5.23 (d, $J = 1.2$ Hz, 1 H), 5.16 (d, $J = 12.0$ Hz, 1 H), 5.10 (d, $J = 12.0$ Hz, 1 H), 5.04 (dd, 1 H), 5.02 (d, $J = 1.8$ Hz, 1 H), 4.89 (br s, 1 H), 3.29 (dd, $J = 10.2$, 3.6 Hz, 1 H), 2.60 (m, 2 H), 2.34 (d, $J = 7.2$ Hz, 2 H), 1.48−1.60 (m, 4 H), 1.22−1.30 (m, 54 H), 0.87 (t, $J = 7.2$ Hz, 6 H). ^{31}P NMR ($CDCl_3$): δ −1.83. MALDI-TOF-MS: cacld for $C_{176}H_{217}N_4O_{33}P$ 2946, found, 2969 ($M + Na^+$).

6-*O*-{[2,3,4-Tri-*O*-benzyl-6-*O*-[benzyl-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-phosphono]-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-*O*-[(2-cyanoethoxy)-(2-*O*-benzyloxycarbonyl-3-*O*-octadecyl-sn-glycerol)-phosphono]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (23). To a solution of **20** (30 mg, 0.010 mmol) and **2** (52 mg, 0.10 mmol) in dry DCM (3 mL) was added 1*H*-tetrazole (0.10 mmol, 0.47 mmol/L in CH_3CN). After the mixture was stirred at room temperature for 1.5 h, *t*-BuO_2H (0.50 mmol, 5 mmol/L in decane) was added at −20 °C. The reaction mixture was warmed to room temperature and stirred for another 0.5 h and finally concentrated. Column chromatography of the residue gave **23** (25 mg, 0.0074 mmol, 74%) as an inseparable diastereoisomeric mixture. TLC (acetone/hexane/DCM 2:5:1): $R_f = 0.39$. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.72

(d, $J = 7.2$ Hz, 2 H), 7.54 (m, 2 H), 7.35 (t, $J = 7.2$ Hz, 2 H), 5.90 (br, 1/2 H), 5.88 (br, 1/2 H), 5.46 (br, 1/2 H), 5.25 (br, 1/2 H), 5.20 (br, 1/2 H), 3.20 (m, 1 H), 2.68 (d, $J = 6.0$ Hz, 2/2 H), 2.58 (m, 2/2 H), 2.35 (d, $J = 7.2$ Hz, 3/2 H), 2.33 (d, $J = 7.2$ Hz, 3/2 H), 1.45−1.62 (m, 4 H), 1.18−1.27 (m, 54 H), 0.87 (t, $J = 7.2$ Hz, 6 H). MALDI-TOF-MS: calcd for $C_{200}H_{239}N_5O_{38}P_2$ 3381.5, found 3404.7 ($M + Na^+$), 3420.4 ($M + K^+$).

6-*O*-{[2,3,4-Tri-*O*-benzyl-6-*O*-[benzyl-(2-aminoethyl)-phosphono]-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-*O*-[(2-*O*-benzyloxycarbonyl-3-*O*-octadecyl-sn-glycerol)-phosphono]-2-*O*-hexadecanoyl-3,4,5-tri-*O*-benzyl-*myo*-inositol (24). To the solution of **23** (10 mg, 0.003 mmol) in anhydrous DCM (3 mL) was added DBU (3 drops) at room temperature. After 2 min of stirring, the reaction was quenched by 3 drops of acetic acid. The solution was concentrated to dryness in a vacuum. Flash chromatography of the residue gave the partially deprotected product **24** (8 mg, 0.0026 mmol, 87%) as a mixture of two diastereoisomers (1:1). TLC (methanol/DCM 1:10): $R_f = 0.35$. ^{1}H NMR ($CDCl_3/CD_3OD$ 1:1, 600 MHz): δ 5.92 (br, 1 H), 5.85 (d, $J = 3.0$ Hz, 1 H), 5.32 (br, 1 H), 4.96 (d, $J = 1.2$ Hz, 1/2 H), 4.90 (br, 1/2 H), 4.87 (br, 1/2 H), 4.74 (br, 1/2 H), 3.19 (m, 1 H), 2.23 (dt, $J = 3.0$, 7.2 Hz, 2/2 H), 2.17 (dt, $J = 4.2$, 7.2 Hz, 2/2 H), 1.45−1.54 (m, 4 H), 0.83 (t, $J = 7.2$ Hz, 6 H). HMQC NMR ($CDCl_3$, ^{13}C 150 MHz/^{1}H 600 MHz): 100.9/5.32 (1/2 Man-1), 100.5/4.96 (1/2 Man-1), 100.4/4.90 (1/2 Man-1), 100.3/4.87 (1/2 Man-1), 100.2/4.74 (1/2 Man-1), 100.2/5.32 (1/2 Man-1), 95.8/5.85 (Glc-1), 69.9/5.92 (Ino-2). ^{31}P NMR ($CDCl_3$): δ −0.09, −0.38, −1.37. MALDI-TOF-MS: calcd for $C_{182}H_{226}N_4O_{36}P_2$ 3106, found 3130 ($M + Na^+$).

6-*O*-{[6-*O*-[(2-Aminoethyl)-phosphono]-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}-2-*O*-hexadecanoyl-1-*O*-[(3-*O*-octadecyl-sn-glycerol)-phosphono]-*myo*-inositol (1). The mixture of **24** (8 mg, 0.0026 mmol) and 10% Pd/C (20 mg) in $CHCl_3$, MeOH, and H_2O (10:10:3, 3 mL) was stirred in an H_2 atmosphere for 1 d. The reaction mixture was filtered off through a pad of Celite with the mixture of MeOH, H_2O, and $CHCl_3$ (3:3:1) as the eluent. The filtrate was concentrated in a vacuum to afford the synthetic target **1** (3.5 mg, 84%) as a white powder. ^{1}H NMR (DMSO-d_6, 600 MHz, 35 °C): 5.32 (br, 2 H), 5.10 (s, 1 H), 4.90 (s, 1 H), 4.75 (s, 1 H), 2.26 (br, 2 H, CH_2), 1.51 (br, 2 H, CH_2), 1.48 (br, 2 H, CH_2), 0.90 (2 t, $J = 6.0$ Hz, 6 H, 2 Me). MALDI-TOF-MS: calcd for $C_{69}H_{132}N_2O_{34}P_2$ 1595, found 1596 ($M + H^+$), 1619 ($M + Na^+$).

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Supporting Information Available: Experimental details of additional syntheses, NMR and MS data of all intermediates and the final product. This material is available free of charge via the Internet at http://pubs.acs.org.

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