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Total Synthesis of a Glycosylphosphatidylinositol Anchor of the Human Lymphocyte CD52 Antigen

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Abstract: The first total synthesis of a glycosylphosphatidylinositol (GPI) anchor bearing a polyunsaturated arachidonoyl fatty acid is reported. This lipid is found in mammalian GPIs that do not undergo lipid remodeling, a process that has important implications in the localization and function of GPI-anchored proteins. Incorporation of the oxidation- and reduction-sensitive arachidonoyl lipid in the target GPI was accomplished by using the *para*-methoxybenzyl (PMB) group for permanent hydroxyl group protection, which featured a selective, rapid, and efficient global deprotection protocol. The flexibility of this synthetic strategy was further highlighted by the inclusion of two additional GPI core structural modifications present in the GPI anchor of the human lymphocyte CD52 antigen.

Keywords: antigens · glycolipids · GPI anchor · lipid remodeling · total synthesis

Introduction

Numerous eukaryotic cell surface proteins are anchored to the plasma membrane by glycosylphosphatidylinositols (GPIs), a family of glycolipids that are post-translationally attached to proteins at their C termini.[1] GPI-anchored proteins (GPI-APs) play important roles in many biological and pathological events, such as cell recognition and adhesion,[2] signal transduction,[3] host defense,[1c,4] and acting as receptors for viruses and toxins.[4] In the 1980s, Ferguson and coworkers elucidated the GPI anchor core structure conserved among all species: H_2NEt-(PO_4)-6-Manα(1→2)Manα(1→6)Manα(1→4)Glc$NH_2\alpha$(1→6)*myo*-inositol-1-(PO_4)-glycerolipid.[5] Modifications of the core structure are frequent and primarily include additional glycans and phosphoethanolamine groups linked to Man residues, while the phosphatidylinositol (PI) fatty acids can differ in chain length and unsaturation.[1c,d]

The structural diversity of GPI lipids is generated by the "lipid remodeling" process of GPI-APs.[6] GPI biosynthesis begins from cellular PI, which in mammalian cells typically contains a polyunsaturated arachidonoyl lipid (20:4) at the *sn*-2 position. The arachidonoyl group can be replaced by other lipids through a series of transformations in the Golgi apparatus, and as a result most mature mammalian GPI-APs contain only saturated fatty acids. However, several mature GPI-APs bearing 2-arachidonoyl PI have been identified, including a major form of the human lymphocyte CD52 antigen.[7] The effects of unremodeled lipids on the function of lymphocyte CD52 and other GPI-APs have not been thoroughly explored, though in general it is believed that the structure of GPI lipids has important implications in the localization and function of GPI-APs. For example, a recent study demonstrated that saturated fatty acids resulting from lipid remodeling are required for association of mammalian GPI-APs with lipid rafts.[8] On the other hand, unsaturated lipids found in GPIs from the protozoan parasite *Trypanosoma cruzi* are thought to be responsible for potent proinflammatory activity.[9]

The CD52 antigen is a GPI-anchored glycopeptide expressed by human lymphocyte and sperm cells.[7b,10] Although lymphocyte and sperm CD52 share an identical dodecapeptide sequence, structural variations in their *N*-glycan and GPI anchor lead to distinct biological activities.[11] Whereas lymphocyte CD52 is a target for monoclonal antibody-based therapy of immune system related diseases, such as leukemia,[12] antibodies specific to sperm CD52 have been identified in infertile women, suggesting potential for immunocontraceptive development.[13] Intriguingly, there are two major forms of lymphocyte CD52, which exhibit two extremes of GPI lipid remodeling, one carrying exclusively distearoyl-PI and the other carrying inositol-palmitoylated stearoyl-arachidonoyl PI.[7b] Evaluating the consequences of lipid structural variations on GPI-AP trafficking, surface localization, and antibody binding can be aided by chemical synthesis, which is an invaluable approach to obtaining structurally defined GPI anchors for biological study.[14]

To develop a strategy for accessing GPIs relevant to studying the effects of lipid remodeling and to continue our synthetic studies of GPI anchors[15] and the human CD52 antigen,[16] we targeted lymphocyte CD52 GPI anchor **1** for total synthesis. The target molecule contained modifications of the GPI core glycan present in lymphocyte CD52, includ-

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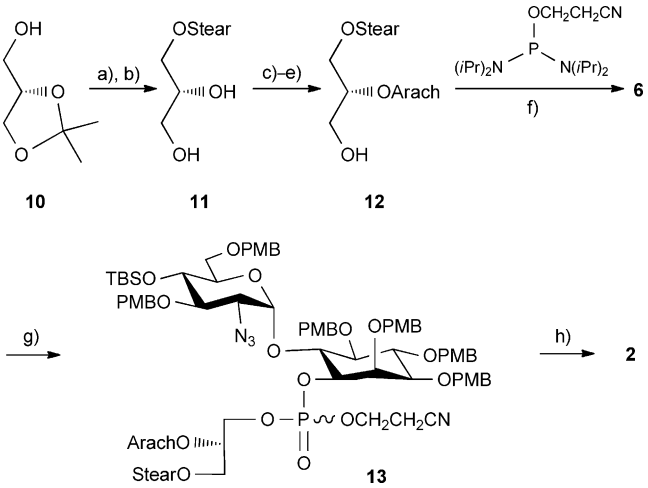
ing additional phosphoethanolamine and mannose groups linked to the 2-*O*-positions of Man-I and Man-III, respectively. Most importantly, compound **1** contained a stearoyl-arachidonoyl PI moiety, which is present in lymphocyte CD52 and other GPI-APs that do not undergo lipid remodeling. To achieve the synthetically challenging incorporation of a polyunsaturated arachidonoyl lipid in GPI **1**, we employed a strategy based on *para*-methoxybenzyl (PMB) hydroxyl protection, which was recently developed in our laboratory to enable the synthesis of GPIs carrying sensitive functionalities that are not compatible with traditional carbohydrate protection chemistries.[15d,e]

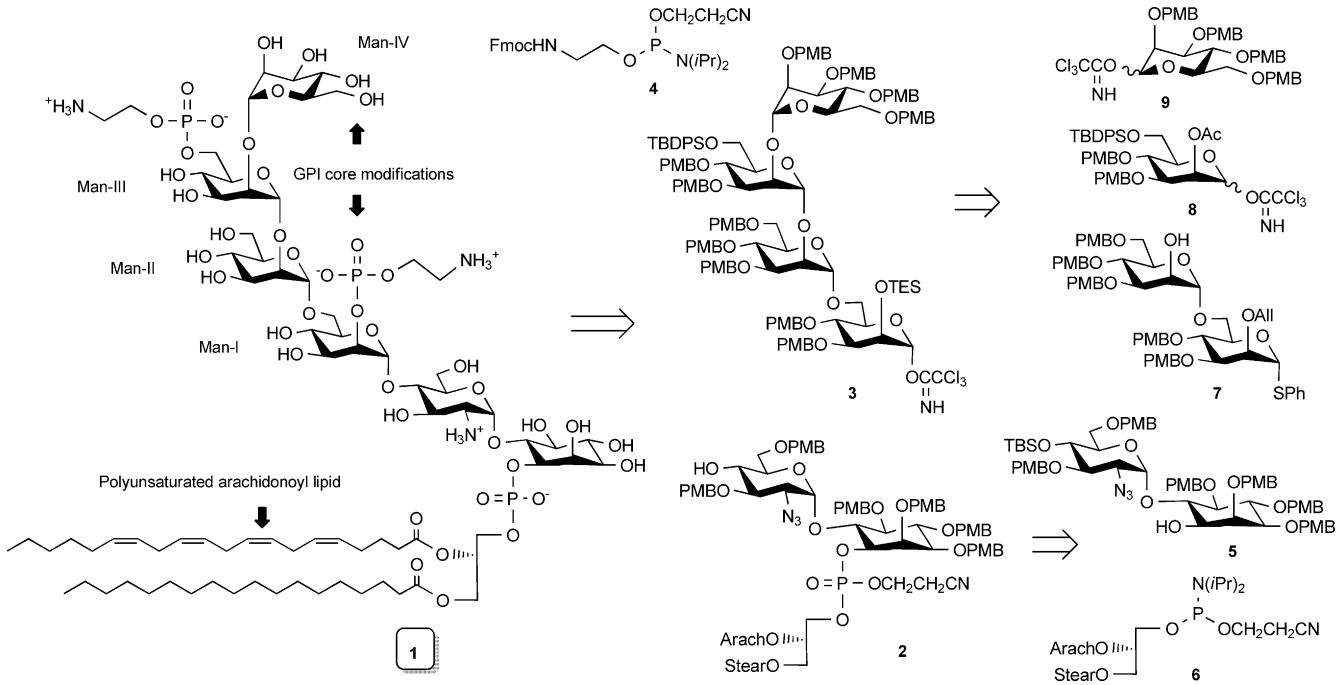
Results and Discussion

A convergent approach was used for the construction of GPI **1** that relied on a key glycosylation between PMB-protected coupling partners pseudodisaccharide acceptor **2** and tetramannosyl donor **3** (Scheme 1). Silyl ether protecting groups at the Man-I 2-*O*- and Man-III 6-*O*-positions would be selectively removed at a late stage to enable double phosphorylation of these sites with phosphoramidite **4**. Acceptor **2** could be accessed through phospholipidation of **5** with phosphoramidite **6**, which would attach the arachidonoyl-containing glycerolipid. Glycosyl donor **3** would arise from sequential α-mannosylations of disaccharide **7**. Guided by previous success, we used the Schmidt method[17] to stereoselectively couple all PMB-protected glycosylation partners.

The preparation of pseudodisaccharide **2** is shown in Scheme 2. 2-Arachidonoyl glycerides are known to be sensitive to: 1) heat, base, and acid, which promote rearrangement to the more stable 1-arachidonoyl isomer, and 2) air and light, which result in oxidation of the lipid alkenes.[18] Care was taken in the synthesis to minimize exposure to these conditions. The preparation of phosphoramidite **6** commenced with stearoylation of optically pure glycerol derivative **10**, which was carried out with EDCI and DMAP



Scheme 2. Synthesis of pseudodisaccharide **2**. All reactions involving arachidonoyl lipid were carried out with exclusion of light and air. Reagents and conditions: a) stearic acid, EDCI, DMAP, CH_2Cl_2/DMF (4:1), 70%; b) AcOH, CH_2Cl_2, H_2O, 69%; c) TBSCl, imidazole, DMF, 77%; d) arachidonic acid, EDCI, DMAP, CH_2Cl_2/DMF (9:1), 99%; e) $Et_3N{\cdot}3HF$, THF, CH_3CN, 98%; f) diisopropylammonium tetrazolide, CH_2Cl_2, CH_3CN, 88%; g) **5**, 1*H*-tetrazole, CH_2Cl_2; then *t*BuOOH, −40 °C; h) $Et_3N{\cdot}3HF$, THF, CH_3CN, 55% (two steps). DMAP: 4-dimethylamino pyridine, DMF: *N,N*-dimethylformamide, EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, TBS: *tert*-butyldimethylsilyl.

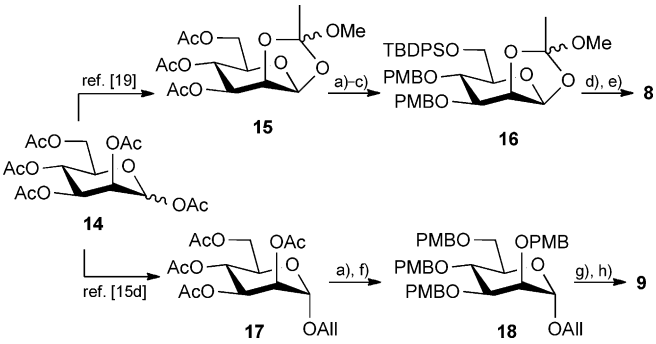


Scheme 1. Retrosynthesis of lymphocyte CD52 GPI anchor **1**. Ac: acetyl, All: allyl, Arach: arachidonoyl, Fmoc: 9-fluorenylmethoxycarbonyl, Stear: stearoyl, TBDPS: *tert*-butyldiphenylsilyl, TES: triethylsilyl.

(70%). After acid-catalyzed acetonide hydrolysis, the resulting diol **11** was treated with TBSCl and imidazole to selectively protect its primary hydroxyl group. Next, with rigorous exclusion of air, light, and moisture, the secondary alcohol was esterified with arachidonic acid in the presence of EDCI and DMAP. The reaction proceeded in 99% yield, and NMR spectroscopic analysis of the product confirmed that the polyunsaturated lipid chain was intact. Previous reports describing arachidonoyl migration during deprotection of the adjacent hydroxyl group[18] were echoed in our work, as desilylation with TBAF/AcOH was accompanied by unwanted transesterification. Fortunately, triethylamine trihydrofluoride proved mild enough to effect migration-free removal of the TBS group, and gave stearoyl-arachidonoyl glyceride **12** in 98% yield. Compound **12** was converted to its corresponding phospholipid precursor **6** by coupling with commercially available 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyl phosphoramidite in 88% yield.

Phospholipidation of the PMB-protected pseudodisaccharide **5**[15d] was realized by treatment with freshly prepared phosphoramidite **6** in the presence of 1*H*-tetrazole. Chemoselective oxidation of the resulting intermediate phosphite to phosphate **13** with *t*BuOOH was performed in situ at −40°C without affecting the sensitive lipid alkenes. After partial purification on silica gel, compound **13** was treated with triethylamine trihydrofluoride to remove the sterically hindered glucosamine 4-*O*-TBS group to afford pseudodisaccharide **2** in 55% yield over two steps. At this stage, the approximately 1:1 diastereomeric mixture was separated by semipreparative HPLC in order to simplify characterization of downstream GPI intermediates. At each step in the synthesis of **2**, the structural integrity of the arachidonoyl lipid was confirmed by NMR spectroscopy and mass spectrometry.

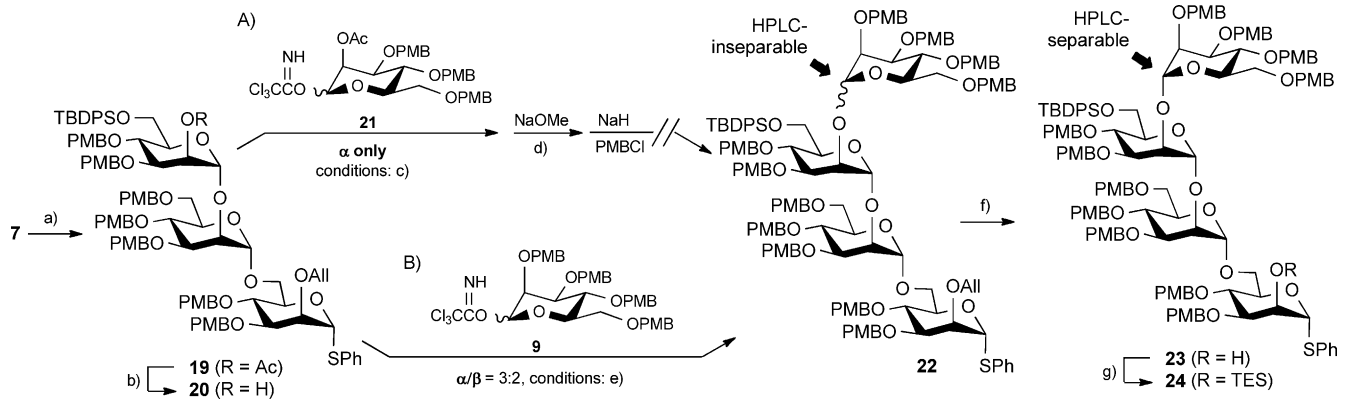
With pseudodisaccharide **2** in hand, we turned our attention to the synthesis of tetramannosyl donor **3**. Previously, we synthesized PMB-protected dimannose thioglycoside **7**,[15d] which possessed the appropriate features to access **3** en route to the synthetic target **1**: 1) orthogonal protection at the Man-I 2-*O*-position to enable later phosphorylation; 2) anomeric thioether for facile conversion to a trichloroacetimidate glycosyl donor; 3) free Man-II 2-*O*-position for extension of the glycan chain. For the latter purpose, PMB-protected mannosyl donors **8** and **9** were prepared from mannose penta-acetate **14** (Scheme 3). To obtain Man-III donor **8**, known orthoester **15**[19] was first converted to **16** by sequential deacetylation with NaOMe/MeOH, 6-*O*-silylation with TBDPSCl/imidazole (68% over two steps), and *para*-methoxybenzylation of the 3-*O*- and 4-*O*-positions (71%). After acid-catalyzed regioselective opening of the orthoester ring (78%), the resulting hemiacetal was converted to trichloroacetimidate **8** by Cl_3CCN and DBU. Man-IV donor **9** was prepared from allyl glycoside **17**. Replacement of the acetates by PMB groups gave **18** (85% over two steps), which underwent palladium-catalyzed anomeric deallylation followed by treatment with Cl_3CCN and DBU to give trichloroacetimidate **9**.



Scheme 3. Synthesis of mannosyl donors **8** and **9**. a) NaOMe, MeOH; b) TBDPSCl, imidazole, DMF, 68% (two steps); c) NaH, PMBCl, DMF, 71%; d) AcOH, CH_2Cl_2, H_2O, 78%; e) Cl_3CCN, DBU, CH_2Cl_2, 94%; f) NaH, PMBCl, DMF, 85% (two steps); g) $PdCl_2$, NaOAc, AcOH, H_2O, 70%; h) Cl_3CCN, DBU, CH_2Cl_2, 92%. DBU: 1,8-diazabicycloundec-7-ene.

Assembly of the tetramannose fragment from building blocks **7**–**9** is depicted in Scheme 4. Coupling of dimannose alcohol **7** and Man-III donor **8** was performed under standard Schmidt conditions, that is, catalytic TMSOTf and MS 4 Å in CH_2Cl_2 at 0°C. Trimannose **19** was produced in 72% yield with complete α stereoselectivity imparted by neighboring group participation from the donor 2-*O*-acetyl group, which was subsequently removed by using NaOMe/MeOH to afford **20** in 97% yield. To install the Man-IV unit, we pursued a route initiated by glycosylation of **20** with known mannosyl trichloroacetimidate **21**,[15d] which carried a temporary stereodirecting acetyl group at its 2-*O*-position that would be converted to a PMB group following α stereoselective glycosylation (Scheme 4A). Coupling of **20** and **21** under standard Schmidt conditions furnished the desired tetramannoside in a moderate 44% yield with excellent stereoselectivity. However, subsequent attempts to replace the Man-IV 2-*O*-acetate with a PMB group failed due to base-promoted cleavage of the TBDPS group. To circumvent this problem and improve reactivity with an armed glycosyl donor,[20] trimannose **20** was instead coupled with fully PMB-protected donor **9** by using Schmidt conditions to directly give **22** in an increased yield (Scheme 4B). However, the stereoselectivity of this reaction was low (α/β, 3:2), which was in contrast to previous results obtained in a nearly identical system in which acceptor **7** was glycosylated by a similar 2-*O*-PMB-protected mannosyl donor with complete α stereoselectivity.[15d]

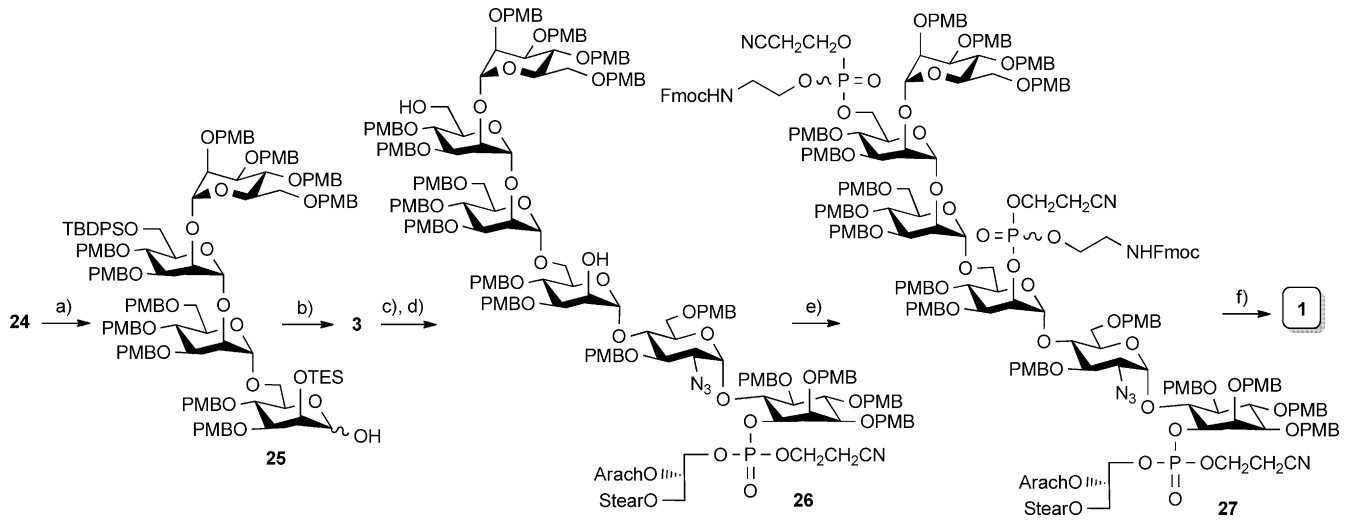
The α- and β-anomers of **22** were inseparable by HPLC on large scale, so we explored conditions for removal of the Man-I 2-*O*-allyl group for two purposes, namely, to improve anomer separation and avoid late-stage deallylation, as the latter is incompatible with the arachidonoyl lipid. Cha's titanium(IV)-mediated deallylation[21] was efficient for this purpose (78% over two steps), whereas other methods were low-yielding. After anomer separation by semipreparative HPLC, the free hydroxyl group of **23** was protected with a TES group (76%) to set up the Man-I 2-*O*- and Man-III 6-*O*-positions for later double phosphorylation.



Scheme 4. Synthesis of tetramannoside **24**. Reagents and conditions: a) **8**, TMSOTf (cat.), MS 4 Å, CH_2Cl_2, 72%; b) NaOMe, MeOH, CH_2Cl_2, 97%; c) **21**, TMSOTf (cat.), MS 4 Å, CH_2Cl_2, 44% (α only); d) NaOMe, MeOH, CH_2Cl_2, 78%; e) **9**, TMSOTf (cat.), MS 4 Å, CH_2Cl_2 (α/β, 3:2); f) $Ti(OiPr)_4$, cyclopentylmagnesium chloride, THF, 78% (two steps); g) TESOTf, Et_3N, CH_2Cl_2, 76%. Tf: trifluoromethanesulfonyl, THF: tetrahydrofuran, TMS: trimethylsilyl.

After pseudodisaccharide **2** and tetramannose thioglycoside **24** were obtained, they were used to construct the target GPI (Scheme 5). Direct glycosylation of **2** by using thioglycoside **24** was inefficient, so **24** was converted to trichloroacetimidate **3** through NIS-promoted hydrolysis of the anomeric thioether (92%) followed by treatment of the intermediate hemiacetal **25** with Cl_3CCN and DBU (99%). The key glycosylation between **3** and pseudodisaccharide **2** under Schmidt conditions gave the desired pseudohexasaccharide, which, after workup but without purification, was directly treated with triethylamine trihydrofluoride to effect desilylation of the Man-I 2-*O*-TES and Man-III 6-*O*-TBS groups. Although the resulting diol **26** was isolated in a moderate yield (39% over two steps) the stereoselectivity of the glycosylation step was excellent (only the α-isomer was observed). Compound **26** was fully characterized, and the stereochemistry of each mannosidic bond was established to be α by using ^{13}C NMR J_{CH} coupling constants: $\delta = 101.9$ ($J_{CH} = 179.7$ Hz, Man-III), 100.5 ($J_{CH} = 173.4$ Hz, Man-I), 99.3 ($J_{CH} = 178.1$ Hz, Man-IV), 99.2 ppm ($J_{CH} = 178.1$ Hz, Man-II).[22]

To install both phosphoethanolamine units simultaneously, diol **26** was doubly phosphorylated by using phosphoramidite **4** in the presence of 1*H*-tetrazole followed by chemoselective oxidation with *t*BuOOH at −40°C. The reaction proceeded in 37% yield to afford **27**, which existed as an inconsequential mixture of diastereomers originating at the newly formed stereogenic phosphates. A monophosphorylated product was also obtained in 15% yield. Finally, compound **27** was globally deprotected in under 3 h by using a three-step, one-pot procedure involving zinc-mediated reduction of $GlcN_3$ to $GlcNH_2$, DBU-promoted cleavage of the cyanoethoxyl and Fmoc groups, and TFA treatment to remove all PMB groups. Following purification by using Sephadex-LH20 size exclusion chromatography, GPI **1** was obtained in 90% yield over the final three steps. The structure



Scheme 5. Completion of GPI **1**. Reagents and conditions: a) NIS, AgOTf, TTBP, wet CH_2Cl_2, 92%; b) Cl_3CCN, DBU, CH_2Cl_2, 99%; c) **2**, TMSOTf (cat.), MS 4 Å, Et_2O; d) $Et_3N{\cdot}3HF$, THF, CH_3CN, 39% (two steps); e) **4**, 1*H*-tetrazole, CH_2Cl_2, CH_3CN; then *t*BuOOH, −40°C, 37%; f) Zn, AcOH, CH_2Cl_2, 1 h; DBU, CH_2Cl_2, 1 h, CH_2Cl_2/TFA (9:1), 30 min, 90% (three steps). NIS: *N*-iodosuccinimide, TTBP: 2,4,6-tri-*tert*-butylpyrimidine.

and homogeneity of the target compound were confirmed by using ^{1}H, ^{31}P and 2D NMR spectroscopy and MALDI mass spectrometry.

Conclusions

The construction of lymphocyte CD52 GPI anchor **1** represents the first total synthesis of a GPI anchor bearing unremodeled PI fatty acids, namely a polyunsaturated arachidonoyl lipid at the *sn*-2 position. GPI **1** and related compounds are useful for studying the impact of lipid structures on the functions of GPIs and GPI-APs. Inclusion of the oxidation- and reduction-sensitive arachidonoyl lipid was made possible by utilizing global PMB protection, which featured a very mild and rapid three-step, one-pot deprotection protocol. Furthermore, two additional GPI core structural modifications present in lymphocyte CD52, specifically additional phosphoethanolamine and mannose units, were incorporated in the target molecule, which further demonstrated the applicability of the PMB protection strategy to the efficient synthesis of highly complex and sensitively functionalized GPI anchors.

Experimental Section

This section contains experimental procedures as well as MS and NMR spectroscopy data for selected key intermediates and the target compound. For all other procedures and characterization data, refer to the Supporting Information.

6-*O*-[2-Azido-2-deoxy-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-[(2-cyanoethoxy)-(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (2): A solution of freshly prepared phosphoramidite **6** (84 mg in 1.0 mL dry CH_2Cl_2, 0.10 mmol) was slowly added to a stirring solution of **5** (40 mg, 0.030 mmol) and 1*H*-tetrazole (0.45 M solution in acetonitrile, 0.44 mL, 0.20 mmol) in anhydrous CH_2Cl_2/CH_3CN (3:1, 6 mL) in the dark under Ar at room temperature. After being stirred at room temperature under Ar for 1 h, the reaction mixture was cooled to −40 °C and treated with *tert*-butyl hydroperoxide (5.5 M solution in decane, 60 μL, 0.33 mmol). The solution was further stirred for 3 h at −20 °C, then Me_2S (49 μL, 0.66 mmol) was added and stirring was continued for an additional 1 h at −20 °C. The mixture was poured into saturated aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification by silica gel column chromatography gave the desired intermediate **13** (briefly characterized by MALDI-MS), which was then dissolved in anhydrous THF/CH_3CN (1:1, 2.5 mL) and treated with $Et_3N{\cdot}3HF$ (0.5 mL) under Ar at room temperature. After being stirred for 13 days at room temperature, the reaction was quenched by dropwise addition of saturated aqueous $NaHCO_3$. The mixture was extracted three times with CH_2Cl_2, and the combined organic layer was dried over Na_2SO_4, concentrated in vacuum, and purified by silica gel column chromatography (hexanes/acetone 2:1) to afford compound **2** (34 mg, 55% over two steps) as an approximately 1:1 diastereomeric mixture originating at phosphorus atom. Semipreparative HPLC (Waters Nova-Pak Silica 6 μm, 300×19 mm, eluent 30% acetone in hexane, 10 mL min^{-1}, isomer **2a** t_R = 13.4 min, isomer **2b** t_R = 13.9 min) was used to separate the mixture. Characterization data for **2a**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.35 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.16–7.12 (m, 4H), 6.90–6.86 (m, 6H), 6.84 (dd, J = 8.5, 1.8 Hz, 2H), 6.79 (dd, J = 8.5, 1.8 Hz, 2H), 6.77 (dd, J = 8.8, 1.8 Hz, 2H), 5.43–5.32 (m, 8H), 5.39 (d, J = 3.7 Hz, 1H, 1-position), 5.25–5.20 (m, 1H), 4.94 (d, J = 10.7 Hz, 1H), 4.92 (d, J = 10.7 Hz, 1H), 4.86 (d, J = 10.4 Hz, 1H), 4.81 (d, J = 11.0 Hz, 1H), 4.79 (d, J = 10.7 Hz, 1H), 4.69 (d, J = 10.4 Hz, 1H), 4.68 (d, J = 11.3 Hz, 1H), 4.67 (d, J = 11.0 Hz, 1H), 4.63–4.59 (m, 2H), 4.42–4.40 (m, 2H), 4.37 (d, J = 11.6 Hz, 1H), 4.36–4.32 (m, 2H), 4.29–4.21 (m, 4H), 4.16–4.12 (m, 1H), 4.12–4.08 (m, 1H), 4.04–4.00 (m, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.77–3.76 (m, 1H), 3.75 (s, 3H), 3.70–3.65 (m, 2H), 3.51 (dd, J = 9.8, 1.5 Hz, 1H), 3.40 (t, J = 9.5 Hz, 1H), 3.29 (ddd, J = 14.6, 10.7, 4.0 Hz, 2H), 3.17 (dd, J = 10.4, 3.7 Hz, 1H), 2.85–2.76 (m, 6H), 2.33–2.27 (m, 4H), 2.10–2.03 (m, 6H), 1.71–1.64 (m, 2H), 1.61–1.55 (m, 2H), 1.39–1.34 (m, 4H), 1.33–1.20 (m, 30H), 0.91–0.84 ppm (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 173.2, 172.6, 159.4, 159.2, 159.15, 159.10, 159.08, 159.03, 130.8, 130.7, 130.5, 130.3, 130.25, 130.23, 130.0, 129.8, 129.5, 129.29, 129.26, 129.04, 128.96, 128.7, 128.6, 128.3, 128.1, 127.8, 127.5, 116.4, 114.0, 113.8, 113.7, 113.65, 113.63, 97.5, 81.2, 80.6, 80.3, 78.3, 76.4, 75.5, 75.3, 74.9, 74.4, 73.0, 72.5, 72.1, 69.7, 69.4, 68.7, 66.0, 62.43, 62.40, 62.3, 61.4, 55.26, 55.25, 55.23, 33.9, 33.5, 31.9, 31.5, 29.72, 29.71, 29.67, 29.5, 29.4, 29.3, 29.1, 27.2, 26.4, 25.63, 25.60, 24.8, 24.7, 22.7, 22.6, 19.7, 19.6, 14.14, 14.09 ppm; ^{31}P NMR ($CDCl_3$, 160 MHz): δ = −1.40 ppm; $[\alpha]_D^{25}$ = +32.0° (c 0.5, $CHCl_3$); HRMS (ESI): calcd for $C_{104}H_{143}N_4NaO_{23}P$ $[M+Na]^+$ m/z: 1869.9778; found, 1869.9824. Isomer **2b** was carried forward to complete the synthesis.

Phenyl [2,3,4,6-tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-2-*O*-allyl-3,4-di-*O*-(*p*-methoxybenzyl)-1-thio-α-D-mannopyranoside (22): A mixture of trichloroacetimidate donor **9** (600 mg, 0.74 mmol), trimannose acceptor **20** (850 mg, 0.49 mmol), and MS 4 Å (300 mg) in anhydrous CH_2Cl_2 (10 mL) was stirred under an Ar atmosphere at room temperature for 1 h. The reaction mixture was cooled to 0 °C, then TMSOTf (13 μL, 0.07 mmol) was added and the reaction was stirred for 10 min. Neutralization with triethylamine was followed by filtration through a Celite pad to remove MS 4 Å and then by concentration under vacuum. The residue was purified by silica gel column chromatography (toluene/EtOAc 10:1) to give an inseparable mixture of α,β-anomers **22**. At this point the isolated yield could not be obtained due to the presence of an inseparable impurity, but a small amount of mixture **22** was subjected to semipreparative HPLC to obtains α,β-anomers for characterization (Waters Nova-Pak Silica 6 μm, 300×19 mm, eluent 33% acetone in hexanes, 10 mL min^{-1}, **22**α t_R = 23.35 min, **22**β t_R = 24.00 min, partially overlapping peaks). Characterization data (listed below for each anomer of **22**) was obtained, but the poor separation made purification by semipreparative HPLC on a larger scale impossible, so we opted to purify after the next step (deallylation) to obtain the isolated yield for the glycosylation–deallylation two-step sequence. Characterization data for **22**α: ^{1}H NMR ($CDCl_3$, 500 MHz, α-isomer): δ = 7.71 (d, J = 6.7 Hz, 2H), 7.67 (dd, J = 7.3, 1.8 Hz, 2H), 7.38 (d, J = 7.6 Hz, 2H), 7.33–7.26 (m, 10H), 7.25–7.20 (m, 6H), 7.18–7.13 (m, 6H), 7.11–6.99 (m, 9H), 6.89 (d, J = 8.5 Hz, 2H), 6.82–6.75 (m, 12H), 6.74–6.72 (m, 6H), 6.70–6.67 (m, 2H), 5.87–5.79 (m, 1H), 5.44 (d, J = 1.2 Hz, 1H, Man-I 1-position), 5.37 (d, J = 1.5 Hz, 1H, Man-III 1-position), 5.30 (d, J = 1.2 Hz, 1H, Man-IV 1-position), 5.24 (dd, J = 17.1, 1.2 Hz, 1H), 5.12 (dd, J = 10.4, 1.2 Hz, 1H), 4.82 (d, J = 1.2 Hz, 1H, Man-II 1-position), 4.81 (d, J = 10.4 Hz, 1H), 4.78 (d, J = 10.4 Hz, 1H), 4.78 (d, J = 10.4 Hz, 1H), 4.75 (d, J = 11.0 Hz, 1H), 4.64–4.56 (m, 5H), 4.53 (d, J = 10.7 Hz, 1H), 4.51–4.48 (m, 2H), 4.47 (d, J = 11.0 Hz, 1H), 4.42 (d, J = 10.4 Hz, 1H), 4.39 (d, J = 11.9 Hz, 1H), 4.38 (d, J = 10.1 Hz, 1H), 4.37 (d, J = 10.4 Hz, 1H), 4.37–4.31 (m, 4H), 4.27–4.25 (m, 2H), 4.16–4.13 (m, 2H), 4.09–4.05 (m, 1H), 4.04–3.98 (m, 2H), 3.96–3.92 (m, 3H), 3.90–3.86 (m, 5H), 3.85–3.83 (m, 2H), 3.83 (s, 3H), 3.83–3.80 (m, 2H), 3.80 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 3.71–3.70 (m, 2H), 3.70 (s, 3H), 3.68–3.64 (m, 2H), 3.66 (s, 3H), 3.60–3.56 (m, 2H), 3.56 (s, 3H), 3.54–3.50 (m, 2H), 0.99 ppm (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, α-isomer): δ = 159.5, 159.4, 159.35, 159.30, 159.2, 159.10, 159.08, 136.1, 135.9, 135.2, 134.9, 134.0, 133.4, 131.5, 131.2, 131.1, 130.9, 130.85, 130.77, 130.7, 130.6, 130.5, 130.4, 130.0, 129.9, 129.8, 129.65, 129.56, 129.5, 129.4, 129.2, 127.93, 127.89, 127.2, 118.0, 114.1, 114.0, 113.94, 113.88, 113.8, 113.74, 113.71, 100.2 (Man-III), 99.5 (Man-IV), 99.4 (Man-II), 86.1 (Man-I), 80.4, 80.1, 79.6, 76.6, 75.0, 74.9, 74.8, 74.7, 74.6, 74.5, 74.4, 74.2, 73.6, 73.2, 72.9, 72.5,

72.4, 72.1, 71.9, 71.7, 71.6, 71.2, 69.0, 68.6, 66.7, 66.0, 63.4, 55.52, 55.48, 55.46, 55.4, 55.34, 55.31, 55.2, 27.1, 19.5 ppm; ^{13}C NMR J_{CH} values (125 MHz): δ = 100.2 (J_{CH} = 176.0 Hz, Man-III), 99.5 (J_{CH} = 176.0 Hz, Man-IV), 99.4 (J_{CH} = 174.0 Hz, Man-II), 86.1 ppm (J_{CH} = 169.0 Hz, Man-I); HRMS (ESI): calcd for $C_{137}H_{156}NaO_{31}SSi$ $[M+Na]^+$ m/z: 2380.0018; found, 2380.0103. Characterization data for **22**β: ^{1}H NMR ($CDCl_3$, 500 MHz, β-isomer): δ = 7.70 (d, J = 6.7 Hz, 2H), 7.64 (d, J = 6.7 Hz, 2H), 7.41 (d, J = 7.3 Hz, 2H), 7.38–7.33 (m, 2H), 7.33–7.29 (m, 8H), 7.26–7.23 (m, 6H), 7.22–7.16 (m, 4H), 7.14–7.07 (m, 8H), 7.05–7.00 (m, 2H), 6.97 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.8 Hz, 2H), 6.82–6.71 (m, 11H), 6.69–6.67 (m, 4H), 6.59 (d, J = 8.5 Hz, 2H), 5.89–5.80 (m, 1H), 5.47 (d, J = 1.2 Hz, 1H, Man-I 1-position), 5.41 (d, J = 1.5 Hz, 1H, Man-III 1-position), 5.25 (dd, J = 17.1, 1.5 Hz, 1H), 5.13 (dd, J = 10.4, 1.2 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 4.84 (d, J = 1.2 Hz, 1H, Man-II 1-position), 4.82 (d, J = 1.2 Hz, 1H, Man-IV 1-position), 4.81 (d, J = 10.1 Hz, 1H), 4.80 (d, J = 10.4 Hz, 1H), 4.75 (d, J = 10.4 Hz, 1H), 4.71 (d, J = 11.0 Hz, 1H), 4.62–4.58 (m, 4H), 4.56–4.52 (m, 1H), 4.50–4.46 (m, 4H), 4.44–4.33 (m, 7H), 4.31–4.23 (m, 3H), 4.19 (dd, J = 9.5, 4.6 Hz, 1H), 4.13–4.09 (m, 1H), 4.08–3.99 (m, 3H), 3.94–3.86 (m, 7H), 3.84–3.82 (m, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 3.80–3.78 (m, 2H), 3.74 (s, 3H), 3.73 (s, 3H), 3.73–3.71 (m, 1H), 3.70 (s, 3H), 3.70 (s, 3H), 3.68–3.66 (m, 1H), 3.65 (s, 3H), 3.65 (s, 3H), 3.60–3.52 (m, 4H), 3.50 (s, 3H), 3.25 (dd, J = 9.2, 2.7 Hz, 1H), 3.13 (dd, J = 9.2, 2.7 Hz, 1H), 0.99 ppm (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz, β-isomer): δ = 159.5, 159.4, 159.28, 159.27, 159.24, 159.22, 159.18, 159.1, 159.0, 136.1, 135.8, 135.3, 134.9, 134.1, 133.6, 131.5, 131.4, 131.3, 131.12, 131.09, 131.0, 130.9, 130.8, 130.72, 130.68, 130.5, 130.4, 130.3, 130.2, 129.88, 129.86, 129.83, 129.81, 129.78, 129.73, 129.66, 129.63, 129.56, 129.5, 129.4, 129.3, 127.84, 127.82, 127.2, 118.0, 114.2, 114.1, 113.9, 113.82, 113.79, 113.78, 113.7, 113.6, 113.5, 99.4 (Man-II), 99.2 (Man-III), 86.0 (Man-I), 82.2 (Man-IV), 80.6, 80.3, 78.0, 77.5, 76.6, 76.0, 75.1, 74.91, 74.87, 74.8, 74.7, 74.6, 74.5, 74.3, 74.2, 73.8, 73.4, 73.2, 73.0, 72.4, 72.1, 71.6, 70.8, 70.1, 69.5, 69.0, 66.7, 63.8, 55.52, 55.47, 55.4, 55.34, 55.31, 55.29, 55.21, 55.17, 27.0, 19.5 ppm; ^{13}C NMR J_{CH} values (125 MHz): δ = 99.4 (J_{CH} = 163.0 Hz, Man-II), 99.2 (J_{CH} = 164.0 Hz, Man-III), 86.0 (J_{CH} = 168.0 Hz, Man-I), 82.2 ppm (J_{CH} = 139.0 Hz, Man-IV); HRMS (ESI) data for β-isomer were consistent with the α-isomer.

Phenyl [2,3,4,6-tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-*O*-(*p*-methoxybenzyl)-1-thio-α-D-mannopyranoside (23): Titanium(IV) isopropoxide (0.76 g in 5.0 mL hexanes, 2.7 mmol) was added to a solution of α,β-mixture **22** (1.3 g, which also contained some inseparable impurity from the previous glycosylation) in anhydrous THF (30 mL). Cyclopentylmagnesium chloride (2.87 M solution in Et_2O, 1.86 mL, 5.36 mmol) was added to the reaction mixture, dropwise under an Ar atmosphere at room temperature over a period of 1 h. After being stirred for an additional 2 h, the reaction mixture was poured into water. The mixture was extracted three times with CH_2Cl_2, and then the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography (toluene/EtOAc 5:1) to give **23** (0.895 g, 78% over two steps). At this stage, the α,β-mixture was purified by semipreparative HPLC (Waters Nova-Pak Silica 6 μm, 300×19 mm, eluent 35% acetone in hexanes, 10 mL min^{-1}, **23** t_R = 36.19 min, β-isomer of **23** t_R = 38.78 min), which showed an anomeric ratio of 3:2. Characterization data for **23**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.74 (dd, J = 6.4, 1.8 Hz, 2H), 7.69 (dd, J = 7.6, 1.8 Hz, 2H), 7.33–7.26 (m, 11H), 7.24–7.22 (m, 6H), 7.19–7.15 (m, 5H), 7.13–7.11 (m, 7H), 7.08 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H), 7.02 (d, J = 7.32 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.83–6.78 (m, 9H), 6.76–6.73 (m, 10H), 5.39 (brs, 1H, Man-I 1-position), 5.37 (brs, 1H, Man-III 1-position), 5.30 (brs, 1H, Man-IV 1-position), 4.86 (d, J = 1.2 Hz, 1H, Man-II 1-position), 4.83 (d, J = 10.4 Hz, 1H), 4.78 (d, J = 9.5 Hz, 1H), 4.76 (d, J = 10.4 Hz, 1H), 4.72 (d, J = 10.7 Hz, 1H), 4.62–4.57 (m, 5H), 4.56 (d, J = 10.4 Hz, 1H), 4.52 (d, J = 10.4 Hz, 1H), 4.50 (d, J = 10.4 Hz, 1H), 4.49 (d, J = 10.7 Hz, 1H), 4.43–4.34 (m, 8H), 4.29–4.26 (m, 2H), 4.15–4.08 (m, 3H), 4.02–3.93 (m, 4H), 3.90–3.86 (m, 4H), 3.85–3.83 (m, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.79–3.76 (m, 2H), 3.78 (s, 3H), 3.76–3.74 (m, 1H), 3.74 (s, 3H), 3.73 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.67 (s, 3H), 3.67–3.65 (m, 2H), 3.61–3.59 (m, 2H), 3.57 (s, 3H), 3.55–3.50 (m, 2H), 2.64 (brs, 1H), 1.00 ppm (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.7, 159.4, 159.30, 159.28, 159.22, 159.17, 159.12, 159.08, 136.1, 135.9, 134.6, 134.1, 133.5, 131.4, 131.23, 131.20, 131.16, 130.9, 130.82, 130.76, 130.62, 130.60, 130.0, 129.92, 129.87, 129.8, 129.65, 129.62, 129.58, 129.54, 129.52, 129.3, 129.2, 127.92, 127.86, 127.3, 114.2, 114.1, 114.0, 113.95, 113.90, 113.85, 113.80, 113.76, 113.7, 100.2 (Man-III), 99.58 (Man-IV), 99.58 (Man-II), 87.5 (Man-I), 80.4, 80.2, 80.1, 79.3, 75.0, 74.93, 74.87, 74.7, 74.6, 74.54, 74.47, 74.3, 73.9, 73.5, 73.2, 73.0, 72.6, 72.5, 72.4, 72.24, 72.19, 71.9, 71.83, 71.76, 71.3, 69.9, 69.0, 68.8, 66.4, 63.4, 55.53, 55.52, 55.49, 55.46, 55.41, 55.40, 55.38, 55.36, 55.32, 55.24, 27.2, 19.5 ppm; ^{13}C NMR J_{CH} values (125 MHz): δ = 100.2 (J_{CH} = 172.5 Hz, Man-III), 99.58 (J_{CH} = 172.5 Hz, Man-IV), 99.58 (J_{CH} = 172.5 Hz, Man-II), 87.5 ppm (J_{CH} = 166.0 Hz, Man-I); $[\alpha]_D^{25}$ = +49.8° (c 0.5, $CHCl_3$); HRMS (ESI): calcd for $C_{134}H_{152}NaO_{31}SSi$ $[M+Na]^+$ m/z: 2339.9705; found, 2339.9761.

Phenyl [2,3,4,6-tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-*O*-(*p*-methoxybenzyl)-2-*O*-triethylsilyl-1-thio-α-D-mannopyranoside (24): TESOTf (29 μL, 0.13 mmol) was added slowly to a solution of tetramannose **23** (20 mg, 8.6 μmol) and triethylamine (36 μL, 0.26 mmol) in anhydrous CH_2Cl_2 (1.5 mL) and stirred under Ar at 0 °C. After being stirred for 2 h while being warmed to room temperature, the reaction mixture was poured into saturated aqueous $NaHCO_3$, which was then extracted three times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, concentrated under vacuum, and purified by silica gel column chromatography (toluene/EtOAc 8:1) to afford **24** (16 mg, 76%) as syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.77 (dd, J = 6.4, 1.5 Hz, 2H), 7.73 (dd, J = 7.6, 1.5 Hz, 2H), 7.45–7.42 (m, 3H), 7.40–7.30 (m, 9H), 7.28–7.21 (m, 6H), 7.22–7.15 (m, 7H), 7.16–7.12 (m, 5H), 7.10–7.02 (m, 5H), 6.91 (d, J = 8.5 Hz, 2H), 6.87–6.81 (m, 7H), 6.81–6.76 (m, 8H), 6.76–6.74 (m, 3H), 5.42 (brs, 1H, Man-III 1-position), 5.34 (d, J = 1.2 Hz, 1H, Man-IV 1-position), 5.31 (d, J = 1.2 Hz, 1H, Man-I 1-position), 4.85 (d, J = 1.2 Hz, 1H, Man-II 1-position), 4.84 (d, J = 10.4 Hz, 1H), 4.81 (d, J = 10.4 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.78 (d, J = 11.0 Hz, 1H), 4.69 (d, J = 11.3 Hz, 1H), 4.66 (d, J = 11.9 Hz, 1H), 4.64 (d, J = 11.6 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.6 Hz, 1H), 4.58 (d, J = 11.0 Hz, 1H), 4.56 (d, J = 10.4 Hz, 1H), 4.55 (d, J = 11.3 Hz, 1H), 4.54 (d, J = 11.9 Hz, 1H), 4.52 (d, J = 11.0 Hz, 1H), 4.46 (d, J = 10.7 Hz, 1H), 4.43 (d, J = 10.4 Hz, 1H), 4.42 (d, J = 10.4 Hz, 1H), 4.42 (d, J = 11.0 Hz, 1H), 4.41 (d, J = 10.4 Hz, 1H), 4.40 (d, J = 10.4 Hz, 1H), 4.38 (d, J = 10.7 Hz, 1H), 4.37 (d, J = 11.0 Hz, 1H), 4.29 (d, J = 2.0 Hz, 2H), 4.27 (brs, 1H), 4.20 (t, J = 7.6 Hz, 1H), 4.15 (dd, J = 2.0, 1.5 Hz, 1H), 4.06 (t, J = 9.5 Hz, 1H), 4.01–3.95 (m, 3H), 3.95–3.91 (m, 3H), 3.91–3.86 (m, 5H), 3.84 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.81–3.77 (m, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.69–3.65 (m, 2H), 3.60 (s, 3H), 3.60–3.53 (m, 2H), 1.03 (s, 9H), 0.95 (t, J = 7.9 Hz, 9H), 0.60 ppm (distorted q, J = 7.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.4, 159.35, 159.33, 159.24, 159.21, 159.18, 159.13, 159.12, 136.1, 135.9, 135.3, 134.1, 133.4, 131.5, 131.32, 131.27, 131.15, 131.12, 130.9, 130.80, 130.78, 130.7, 130.62, 130.59, 129.9, 129.80, 129.78, 129.7, 129.64, 129.58, 129.55, 129.54, 129.4, 129.31, 129.26, 128.0, 127.9, 127.3, 114.1, 114.02, 113.99, 113.96, 113.91, 113.88, 113.86, 113.79, 113.75, 100.2 (Man-III), 99.6 (Man-IV), 99.2 (Man-II), 89.7 (Man-I), 80.7, 80.15, 80.14, 80.10, 75.03, 74.97, 74.7, 74.6, 74.5, 74.4, 74.3, 73.7, 73.2, 72.9, 72.55, 72.49, 72.4, 72.2, 72.1, 72.0, 71.7, 71.5, 71.4, 68.8, 68.6, 67.0, 63.4, 55.52, 55.51, 55.49, 55.46, 55.42, 55.40, 55.39, 55.33, 55.2, 27.2, 19.5, 7.2, 5.3 ppm; $[\alpha]_D^{25}$ = +40.0° (c 0.5, $CHCl_3$); HRMS (ESI): calcd for $C_{140}H_{166}NaO_{31}SSi_2$ $[M+Na]^+$ m/z: 2454.0570; found, 2454.0669.

[2,3,4,6-Tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-3,4-di-*O*-(*p*-methoxybenzyl)-2-*O*-triethylsilyl-D-mannopyranose (25): 2,4,6-Tri-*tert*-butylpyrimidine (26 mg, 0.11 mmol), *N*-iodosuccinimide (16 mg, 0.07 mmol), and silver triflate (18 mg, 0.07 mmol) were added to a solution of tetramannose thioglycoside **24** (88 mg, 0.036 mmol) in wet CH_2Cl_2 (5 mL) at 0 °C. After being stirred for 2 h at room temperature, saturated aqueous $Na_2S_2O_3$ was added at 0 °C and the reaction mixture

was stirred for an additional 30 min while being warmed to room temperature. The reaction mixture was extracted three times with CH_2Cl_2, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (toluene/EtOAc 5:1) to afford tetramannose hemiacetal **25** (78 mg, 92%) as syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.78 (dd, J = 7.3, 1.5 Hz, 2H), 7.75 (dd, J = 7.0, 1.5 Hz, 2H), 7.39–7.32 (m, 8H), 7.31–7.24 (m, 8H), 7.22–7.19 (m, 5H), 7.18–7.12 (m, 5H), 7.06 (dd, J = 8.5, 2.7 Hz, 4H), 6.87 (d, J = 8.8 Hz, 2H), 6.85 (d, J = 7.9 Hz, 2H), 6.84 (d, J = 7.9 Hz, 2H), 6.83 (d, J = 7.0 Hz, 2H), 6.82 (d, J = 7.0 Hz, 2H), 6.81–6.75 (m, 10H), 5.33 (brs, 1H, Man-III 1-position), 5.28 (brs, 1H, Man-IV 1-position), 4.85 (d, J = 2.1 Hz, 1H, Man-II 1-position), 4.84 (d, J = 10.4 Hz, 1H), 4.83 (brs, 1H, Man-I 1-position), 4.79 (d, J = 10.7 Hz, 1H), 4.75 (d, J = 10.7 Hz, 1H), 4.68 (d, J = 10.7 Hz, 1H), 4.65 (d, J = 11.3 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.58–4.51 (m, 6H), 4.50–4.38 (m, 6H), 4.33 (d, J = 10.4 Hz, 1H), 4.31 (d, J = 11.3 Hz, 1H), 4.29 (d, J = 11.9 Hz, 1H), 4.25 (d, J = 10.4 Hz, 1H), 4.21 (dd, J = 2.0, 1.2 Hz, 1H), 4.12 (dd, J = 2.5, 1.5 Hz, 1H), 4.00–3.94 (m, 5H), 3.93–3.85 (m, 6H), 3.82 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.79–3.77 (m, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 3.74 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.68 (s, 3H), 3.67–3.60 (m, 5H), 3.59–3.51 (m, 2H), 2.39 (brs, 1H), 1.05 (s, 9H), 0.95 (t, J = 7.9 Hz, 9H), 0.58 ppm (distorted q, J = 7.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 159.43, 159.40, 159.36, 159.32, 159.26, 159.22, 159.19, 159.17, 159.14, 136.2, 135.9, 134.1, 133.4, 131.3, 131.2, 131.1, 130.9, 130.81, 130.76, 130.5, 129.8, 129.75, 129.70, 129.67, 129.64, 129.5, 129.3, 128.0, 127.9, 114.05, 114.01, 113.95, 113.93, 113.90, 113.82, 113.79, 113.77, 99.70 (Man-III), 99.70 (Man-IV), 99.5 (Man-II), 95.1 (Man-I), 80.1, 80.0, 74.9, 74.85, 74.77, 74.7, 74.5, 73.4, 73.2, 73.0, 72.5, 72.4, 72.2, 72.0, 71.9, 71.85, 71.80, 70.4, 69.6, 69.1, 68.8, 63.3, 55.49, 55.48, 55.46, 55.43, 55.40, 55.37, 55.35, 27.2, 19.5, 7.2, 5.2 ppm; HRMS (ESI): calcd for $C_{134}H_{162}NaO_{32}Si_2$ $[M+Na]^+$ m/z: 2362.0485; found, 2362.0591.

6-*O*-{[2,3,4,6-Tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-azido-2-deoxy-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]}-1-*O*-[(2-cyanoethoxy)-(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (26): DBU (1 drop) and trichloroacetonitrile (0.3 mL) were added to a solution of tetramannose hemiacetal **25** (78 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5 mL). After being stirred under an Ar atmosphere at 0°C for 30 min, the reaction mixture was concentrated under vacuum and purified with a triethylamine-treated neutral silica gel column to furnish tetramannose donor **3** (82 mg, 99%). A mixture of the resulting trichloroacetimidate **3** (44 mg, 0.02 mmol), acceptor **2** (10 mg, 5.95 μmol), and MS 4 Å (10 mg) in anhydrous Et_2O (4 mL) was stirred under an Ar atmosphere at room temperature for 1 h. The reaction mixture was cooled to 0°C, TMSOTf (0.05 M solution in anhydrous Et_2O, 40 μL) was added, and the reaction was stirred, overnight, while being warmed to room temperature. The reaction was neutralized by addition of saturated aqueous $NaHCO_3$. After filtration through a Celite pad to remove MS 4 Å, the mixture was extracted three times with CH_2Cl_2, and the combined organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residue was partially purified by silica gel column chromatography to give the intermediate α-pseudohexasaccharide, which was taken directly to the next step. $Et_3N{\cdot}3HF$ (0.5 mL) was added to a solution of the crude material (13.7 mg) in anhydrous THF/CH_3CN (1:1, 1 mL) under an Ar atmosphere at room temperature. After being stirred for 7 days at room temperature, the reaction was quenched by dropwise addition of saturated aqueous $NaHCO_3$ at 0°C. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography (toluene/EtOAc 2.5:1) to give diol **26** (8.1 mg, 39% for two steps) as syrup. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.34–7.26 (m, 10H), 7.25–7.15 (m, 15H), 7.13–7.07 (m, 9H), 7.02 (d, J = 8.5 Hz, 2H), 6.89–6.84 (m, 8H), 6.81–6.78 (m, 8H), 6.76–6.72 (m, 16H), 5.43–5.30 (m, 8H), 5.36 (d, J = 3.7 Hz, 1H, $GlcN_3$ 1-position), 5.30–5.26 (m, 1H, glyceride CH), 5.21 (d, J = 2.4 Hz, 1H, Man-III 1-position), 5.21 (d, J = 2.4 Hz, 1H, Man-IV 1-position), 5.13 (brs, 1H, Man-I 1-position), 4.97 (d, J = 11.0 Hz, 1H), 4.96 (brs, 1H, Man-II 1-position), 4.92 (dd, J = 10.4, 2.5 Hz, 1H), 4.85 (d, J = 10.4 Hz, 1H), 4.84 (d, J = 9.2 Hz, 1H), 4.81–4.71 (m, 5H), 4.69–4.62 (m, 4H), 4.60–4.56 (m, 4H), 4.56–4.50 (m, 4H), 4.49–4.45 (m, 5H), 4.44–4.39 (m, 4H), 4.39–4.34 (m, 4H), 4.33–4.30 (m, 2H), 4.29–4.27 (m, 2H), 4.25–4.15 (m, 8H), 4.04–3.96 (m, 4H), 3.86–3.81 (m, 7H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.70 (s, 3H), 3.70 (s, 3H), 3.70–3.67 (m, 2H), 3.67 (s, 3H), 3.66–3.64 (m, 3H), 3.63 (s, 3H), 3.61 (s, 3H), 3.62–3.56 (m, 2H), 3.57 (s, 3H), 3.57–3.52 (m, 3H), 3.51–3.43 (m, 4H), 3.40–3.36 (m, 2H), 3.36 (s, 3H), 3.22 (dd, J = 10.7, 2.1 Hz, 1H), 3.16 (dd, J = 9.8, 3.7 Hz, 1H), 3.09 (ddd, J = 10.7, 2.1, 0.9 Hz, 1H), 2.84–2.77 (m, 6H), 2.68–2.63 (m, 2H), 2.59 (brs, 1H), 2.37–2.32 (m, 2H), 2.30–2.26 (m, 2H), 2.12–2.08 (m, 2H), 2.08–2.03 (m, 2H), 1.72–1.65 (m, 3H), 1.61–1.55 (m, 2H), 1.39–1.33 (m, 4H), 1.32–1.22 (m, 30H), 0.91–0.87 ppm (m, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 173.5, 172.9, 159.6, 159.4, 159.35, 159.32, 159.26, 159.22, 159.17, 159.15, 159.14, 131.09, 131.07, 131.02, 130.92, 130.89, 130.84, 130.7, 130.6, 130.49, 130.47, 130.3, 129.87, 129.83, 129.81, 129.80, 129.77, 129.75, 129.74, 129.72, 129.6, 129.5, 129.3, 129.25, 129.22, 129.1, 129.0, 128.8, 128.6, 128.5, 128.3, 128.1, 127.7, 116.6, 114.12, 114.10, 114.05, 114.04, 114.01, 113.96, 113.93, 113.90, 113.84, 113.83, 113.79, 113.70, 101.9 (Man-III), 100.5 (Man-I), 99.3 (Man-IV), 99.2 (Man-II), 98.0 ($GlcN_3$), 81.4, 80.7, 80.6, 79.8, 79.7, 79.5, 79.4, 78.5, 76.42, 76.41, 75.65, 75.56, 75.3, 75.0, 74.9, 74.7, 74.65, 74.59, 74.5, 74.3, 74.2, 73.90, 73.88, 73.6, 73.19, 73.15, 73.13, 73.0, 72.8, 72.58, 72.53, 72.48, 72.40, 71.8, 71.64, 71.59, 71.4, 70.05, 70.01, 69.7, 69.0, 68.8, 68.7, 68.2, 66.5, 66.0, 64.9, 63.25, 63.23, 62.51, 62.48, 62.45, 62.40, 62.37, 61.7, 55.51, 55.48, 55.46, 55.44, 55.42, 55.40, 55.37, 55.35, 55.33, 55.27, 55.23, 55.20, 41.3, 34.2, 33.8, 32.2, 31.7, 30.0, 29.9, 29.8, 29.62, 29.58, 29.4, 27.5, 26.70, 26.67, 26.65, 25.9, 25.8, 25.1, 24.9, 22.9, 22.8, 19.85, 19.78, 14.4, 14.3 ppm. Configurations of anomeric positions were established as α by coupled ^{13}C NMR J_{CH} values (125 MHz): δ = 101.9 (J_{CH} = 179.7 Hz, Man-III), 100.5 (J_{CH} = 173.4 Hz, Man-I), 99.3 (J_{CH} = 178.1 Hz, Man-IV), 99.2 ppm (J_{CH} = 178.1 Hz, Man-II); ^{31}P NMR ($CDCl_3$, 160 MHz): δ = −1.79 ppm; $[\alpha]_D^{25}$ = +43.0° (*c* 0.4, $CHCl_3$); HRMS (ESI): calcd for $C_{216}H_{271}N_4NaO_{54}P$ $[M+Na]^+$ m/z: 3838.8218; found, 3838.8269.

6-*O*-{[2,3,4,6-Tetra-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[6-*O*-[(2-cyanoethoxy)-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-phosphono]]-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→2)-[3,4,6-tri-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→6)-[2-*O*-[(2-cyanoethoxy)-[2-(9-fluorenylmethoxycarbonylamino)ethyl]-phosphono]]-3,4-di-*O*-(*p*-methoxybenzyl)-α-D-mannopyranosyl]-(1→4)-[2-azido-2-deoxy-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]}-1-*O*-[(2-cyanoethoxy)-(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (27): Freshly prepared phosphoramidite **4** (15.5 mg in 500 μL dry CH_2Cl_2, 0.03 mmol) was added to a stirring solution of pseudohexasaccharide **26** (4.1 mg, 1.0 μmol) and MS 4 Å (5 mg) in anhydrous CH_2Cl_2/CH_3CN (3:1, 1.5 mL). Then, 1*H*-tetrazole (0.45 M solution in acetonitrile, 143 μL, 0.06 mmol) was added under Ar at room temperature. After being stirred at room temperature under Ar, overnight, the reaction mixture was cooled to −40°C and treated with *tert*-butyl hydroperoxide (5.5 M solution in decane 23 μL, 0.13 mmol). The solution was further stirred for 1 h at −40°C, after which Me_2S (19 μL, 0.26 mmol) was added and stirring was continued for an additional 1 h at −40°C. After passage through a Celite pad to remove MS 4 Å, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum. Purification of the crude material by silica gel column chromatography afforded diphosphorylated GPI **27** and also the Man-III monophosphorylated GPI. Both compounds were further purified by using Sephadex LH-20 size exclusion chromatography ($CHCl_3$/MeOH, 3:1) to provide **27** (1.85 mg, 37%) and a monophosphorylated compound (0.7 mg, 15%), both of which existed as complex diastereomeric mixtures resulting from the newly formed stereogenic phosphates. This was inconsequential as the following global deprotection step rendered the phosphorus atoms nonstereogenic. Compound **27**: ^{1}H NMR ($CDCl_3$, 500 MHz, resolved signals): δ = 7.73–7.70 (m, 4H), 7.55–7.48 (m, 4H), 7.40–7.28 (m, 10H), 7.26–7.19 (m, 12H), 7.18–7.10 (m, 12H), 7.09–7.04 (m, 4H), 7.01–6.95 (m,

4H), 6.87–6.80 (m, 10H), 6.80–6.69 (m, 24H), 5.55 (brs, 1H, Man-I 1-position), 5.41–5.31 (m, 8H), 5.35 (brs, 1H, $GlcN_3$ 1-position), 5.30–5.26 (m, 1H, glyceride CH), 5.10 (s, 1H, Man-III 1-position), 4.88 (s, 1H, Man-IV 1-position), 4.84 (s, 1H, Man-II 1-position), 2.84–2.76 (m, 6H), 2.64–2.58 (m, 2H), 2.33–2.20 (m, 8H), 2.08–2.00 (m, 4H), 1.72–1.54 (m, 4H), 1.38–1.20 (m, 34H), 0.90–0.87 ppm (m, 6H); ^{31}P NMR ($CDCl_3$, 160 MHz): $\delta = -0.78, -0.82, -1.26, -1.29, -1.64, -1.65, -1.85, -2.25, -2.26, -2.69, -2.71, -3.00, -3.05$ ppm; MALDI-ToF MS (positive mode): calcd For $C_{256}H_{310}N_8NaO_{64}P_3$ $[M+H+Na]^+$ m/z: 4636.0; found, 4636.5. Monophosphorylated compound: ^1H NMR ($CDCl_3$, 500 MHz, resolved signals): $\delta =$ 7.73–7.70 (m, 2H), 7.53–7.51 (m, 2H), 7.36–7.28 (m, 10H), 7.26–7.21 (m, 6H), 7.20–7.13 (m, 10H), 7.12–7.05 (m, 8H), 7.04–6.98 (m, 4H), 6.90–6.82 (m, 10H), 6.81–6.70 (m, 24H), 5.40–5.30 (m, 9H, includes glyceride CH), 5.24 (brs, 2H), 5.15 (brs, 2H), 4.95 (brs, 1H), 2.83–2.77 (m, 6H), 2.65–2.60 (m, 2H), 2.35–2.21 (m, 6H), 2.07–2.00 (m, 4H), 1.70–1.55 (m, 4H), 1.37–1.20 (m, 34H), 0.90–0.85 ppm (m, 6H); ^{31}P NMR ($CDCl_3$, 160 MHz): $\delta = -0.72, -1.29, -2.14, -2.30, -2.62, -2.64, -2.91, -2.99$ ppm. MALDI-ToF MS (positive mode): calcd For $C_{236}H_{292}N_6NaO_{59}P_2$ $[M+2H+Na]^+$ m/z: 4238.9; found, 4238.4.

6-*O*-{[α-D-Mannopyranosyl]-(1→2)-[6-*O*-[(2-aminoethyl)-phosphono]-α-D-mannopyranosyl]-(1→2)-[α-D-mannopyranosyl]-(1→6)-[2-*O*-[(2-aminoethyl)-phosphono]-α-D-mannopyranosyl]-(1→4)-[2-amino-2-deoxy-α-D-glucopyranosyl]}-1-*O*-[(2-*O*-arachidonoyl-3-*O*-stearoyl-*sn*-glycerol)-phosphono]-*myo*-inositol (1): Acetic acid (1 drop) and zinc powder (10 mg) were added to a solution of pseudohexasaccharide **27** (1.85 mg, 0.40 μmol) in CH_2Cl_2 (500 μL). After being vigorously stirred for 1 h at room temperature, MALDI-ToF MS showed complete reduction of the azide; MALDI-ToF MS (positive mode): calcd For $C_{256}H_{311}N_6NaO_{64}P_3$ $[M+Na]^+$
m/z: 4609.0; found, 4609.0. The mixture was filtered through a Celite plug and condensed in vacuum to remove acetic acid, and the resulting residue was redissolved in CH_2Cl_2 (500 μL) and treated with DBU (2 μL). The solution stirred for 1 h, after which MALDI-ToF MS confirmed removal of the Fmoc and cyanoethoxyl protecting groups; MALDI-ToF MS (negative mode): calcd for $C_{217}H_{281}N_3O_{60}P_3$ $[M-H]^-$ m/z: 3981.8; found, 3981.6. Then, 20% TFA in CH_2Cl_2 (500 μL) was added directly to the reaction mixture at 0 °C, to give a final concentration of approximately 10% TFA. After being stirred for 30 min at room temperature, the reaction was co-evaporated with toluene 5 times. Purification of the crude product by Sephadex LH-20 size exclusion chromatography ($CHCl_3$/MeOH/H_2O, 3:3:1) afforded GPI **1** (0.70 mg, 90%). ^1H NMR ($CDCl_3$/CD_3OD/D_2O 3:3:1, 500 MHz, anomeric region): $\delta = 5.46$ (1 H Man-I), 5.42–5.32 (8H, arachidonoyl lipid sp^2-CH), 5.25 (1H, glyceride CH), 5.24 (1H, $GlcNH_2$-1), 5.04 (1H, Man-III), 4.98 (1H, Man-IV), 4.98 ppm (1H, Man-II); ^{13}C NMR ($CDCl_3$/CD_3OD/D_2O 3:3:1, 125 MHz, anomeric region): $\delta = 100.3$ (Man-IV), 100.3 (Man-II), 101.5 ($GlcNH_2$-1), 99.5 (Man-III), 97.5 ppm (Man-I); ^{31}P NMR ($CDCl_3$, 160 MHz): $\delta = 4.37, 3.59, 3.51$ ppm; MALDI-ToF MS (negative mode): calcd for $C_{81}H_{145}N_3O_{43}P_3$ $[M-H]^-$ m/z: 1940.8; found, 1940.7.

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