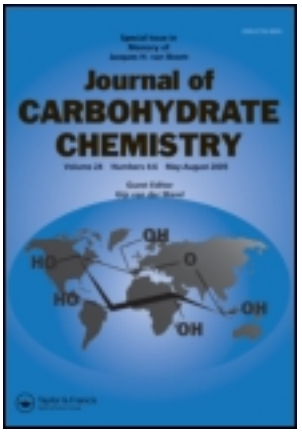

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Charles L. Johnson [a] & Zhongwu Guo [a]
[a] Department of Chemistry , Wayne State University , Detroit , MI , 48202 , USA
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Synthesis of Novel, Fluorescently Tagged Analogs of Glycosylphosphatidylinositol (GPI) Anchors

Charles L. Johnson and Zhongwu Guo

Department of Chemistry, Wayne State University, Detroit, MI 48202, USA

Glycosylphosphatidylinositol (GPI) anchors are a group of complex glycolipids that attach extracellular proteins and glycoproteins to the eukaryotic cell outer membrane. To better understand GPI anchorage, it is necessary to have access to homogeneous, structurally defined, and functionalized GPIs and GPI analogs. In this regard, chemical synthesis is necessary, as GPI anchors are rather scarce and heterogeneous in natural sources. Three GPI analogs with phosphoglycerolipids linked to the pseudodisaccharide core and their fluorescein conjugates were prepared in this work as a small tool set useful for probing how the lipid composition and carbohydrate anomeric configuration may affect the properties of GPI anchors.

Keywords Glycosylphosphatidylinositol; GPI anchor; Phospholipid; Glycolipid; Fluorescein; Fluorescent tag

INTRODUCTION

Glycosylphosphatidylinositol (GPI) anchorage of extracellular membrane proteins and glycoproteins to the cell surface exists in all eukaryotic species.[1–3] It is generally acknowledged that GPIs take part in a number of biological roles such as cell adhesion and recognition,[4] pathogenic infections,[5] signal transduction,[6] and enzymatic reactions on the cell surface.[7] However, relatively little is known about how the GPI structure might affect their biological functions.[8] While all eukaryotic GPI anchors share the same core structure shown in Figure 1,[1–3] they have a great deal of microheterogeneity including variable lipid compositions and additional sugar chains and other functionalities attached to the GPI core glycan. Due to this property, as well as the natural

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Address correspondence to Zhongwu Guo, Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA. E-mail: zwguo@chem.wayne.edu

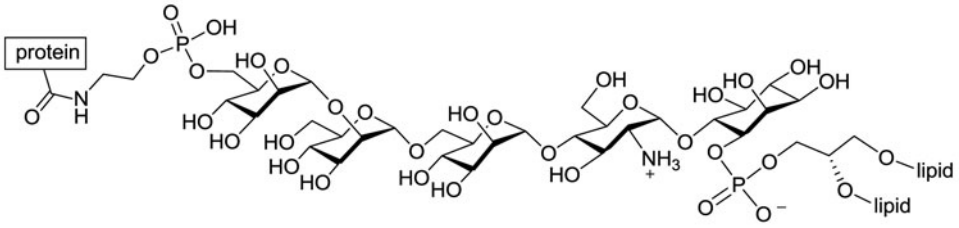


Figure 1: The generic core structure of GPI anchors found on all eukaryotic cells.

scarcity of GPIs in living systems, isolation of pure GPIs from nature for their structural study would be impractical. Chemical synthesis has become the critical means to access homogeneous and structurally defined GPI anchors[9,10] for the better understanding of GPI anchorage. In addition to natural GPI anchors, the synthesis of GPI analogs is also a necessary step for determining the roles, structure–activity relationships, and many other studies of GPI anchors.

In this work, synthetic GPI analogs carrying a fluorescent tag were prepared as a tool set that can be utilized to probe GPI anchorage. The designed target molecules (Fig. 2), which had fluorescein incorporated at the glucosamine 4-*O*-position of the GPI core pseudosaccharide, carried both unsaturated and saturated lipids, **1** and **2**, and with both α- and β-glycosidic linkages, **2** and **3**. We planned to prepare GPI analogs that contained only the core pseudosaccharide as studies have demonstrated that this structural motif was critical for some biological activities of GPI anchors,[11–13] GPI anchor microdomain formation in membranes,[12,14] and GPI biosynthesis.[2] Moreover,

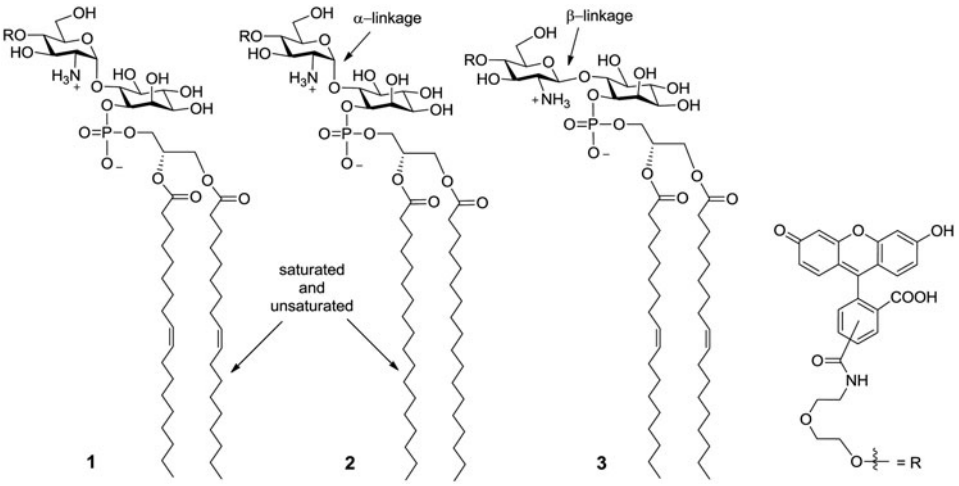
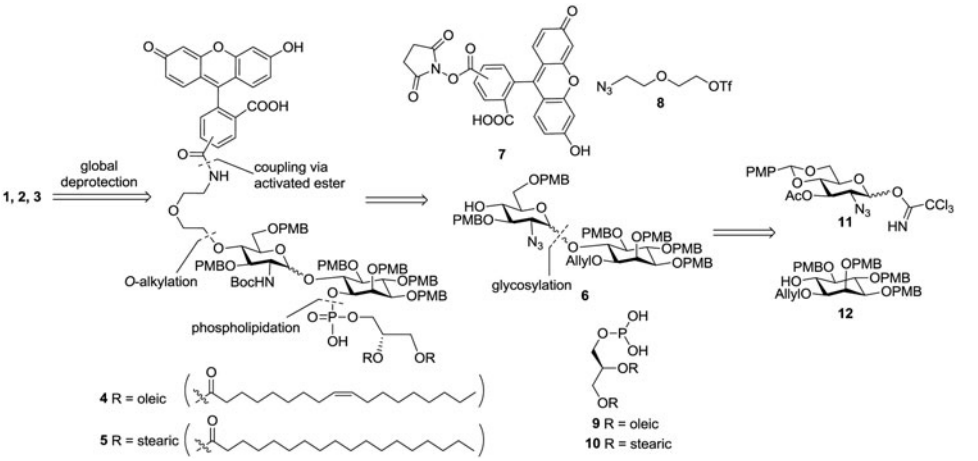


Figure 2: Designed GPI analogs carrying the fluorescein tag.

these GPI analogs can be used to compare with intact GPIs to provide more insight into how variation in the lipid composition and anomeric configuration of the GPI anchor may affect its distribution in lipid bilayers and other studies.

RESULTS AND DISCUSSION

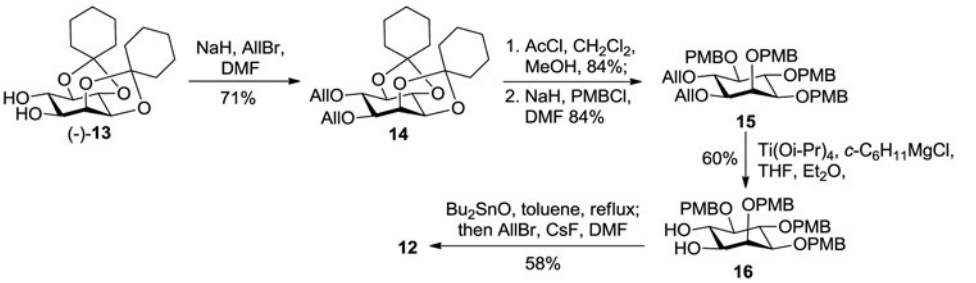
The retrosynthesis of GPI analogs **1**, **2,** and **3** is outlined in Scheme 1. Because some of these synthetic targets contained unsaturated lipids that are incompatible with catalytic hydrogenolysis employed to deprotect benzyl ethers, we planned to use the *para*-methoxybenzyl (PMB) group for global hydroxyl protection.[15,16] Fluorescein would be linked to the GPI pseudodisaccharide via a flexible diethylene glycol analog. Here we design to have an ether linkage, instead of the more easily formed ester or other similar linkages, between the linker and the carbohydrate chain to take advantage of the excellent stability of the ether bond under physiological conditions. The phosphoglycerolipid would be introduced by the 1*H*-phosphonate approach.[17] Finally, the Schmidt glycosylation[18] would be used to couple inositol and glucosamine using imidate **11** as the glycosyl donor. In **11**, the amino group was protected as an azide to facilitate the formation of both α- and β-anomers during the glycosylation reaction.



Scheme 1: Retrosynthesis of the target molecules.

The synthesis of inositol derivative **12** was a significant challenge, achievable via combining two previously developed protocols (Sch. 2).[15,19] First, the optically pure dicyclohexylidene ketal derivative of inositol (-)-**13** was prepared by regioselective protection of *myo*-inositol[20] and then enzymatic resolution of

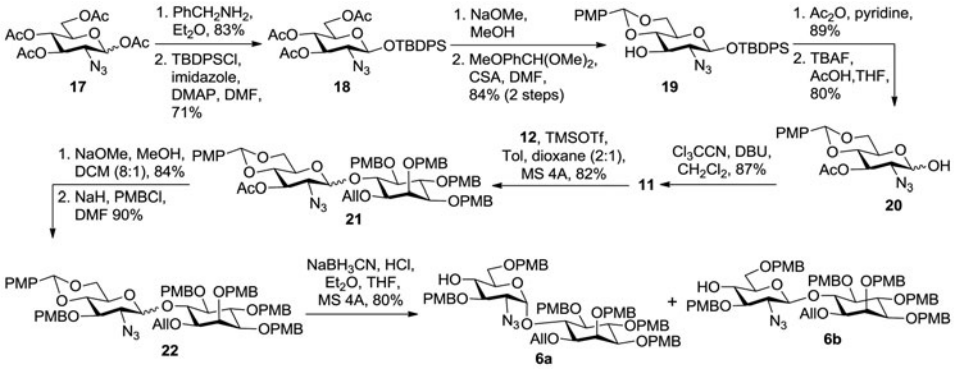
the resulting enantiomers.[19] Thereafter, the 1,6-*O*-positions of **13** were temporarily protected with allyl groups to differentiate them from the remaining inositol positions. The ketal protecting groups of **14** were subsequently removed by acidic methanolysis, which was followed by hydroxyl protection with PMB to afford **15**. To differentiate the inositol 1,6-*O*-positions, the two allyl groups were removed by Cha's method,[21] and the resulting diol **16** was subjected to tin-directed regioselective allylation of the 6-*O*-position to furnish **12**. The final step provided a 3:1 selectivity for the desired regioisomer.



Scheme 2: Synthesis of the inositol derivative 12 (note: bold).

The synthesis of glucosamine derivative **11** (Sch. 3) as a glycosyl donor started from peracetylated azido sugar **17**.[22] Selective removal of the 1-*O*-acetyl group with benzylamine was followed by *tert*-butyldiphenylsilyl (TBDPS) protection of the exposed anomeric position to give **18** as a β-anomer exclusively. The remaining acetyl groups were subsequently removed with sodium methoxide in methanol, which was followed by regioselective protection of the 4,6-*O*-positions with *para*-methoxybenzylidine to provide **19**. Ideally, the remaining free 3-*O*-position in **19** should be protected with a PMB group, but we found that the conventional method to introduce PMB utilizing NaH and PMBCl resulted in the decomposition of **19**, presumably due to the sensitivity of the 1-*O*-TBDPS group to strong basic conditions. To address this issue, the 3-*O*-position was first protected with an acetyl group that was replaced with a PMB group after the glycosylation reaction. Hence, **19** was acetylated with acetic anhydride and pyridine, which was followed by 1-*O*-position deprotection using buffered tetrabutylammonium fluoride (TBAF) to give hemiacetal **20**. Finally, **20** was converted to the corresponding trichloroacetimidate **11** by reaction with trichloroacetonitrile in the presence of 1,8-diazabicycloundec-7-ene (DBU), which was ready to couple with inositol derivative **12**.

Glycosylation of **12** by **11** with trimethylsilyl triflate (TMSOTf) as the promoter afforded a mixture of α- and β- (1.3:1.0) anomers **21** in a very good yield (82%, Sch. 3). However, the two anomers were difficult to separate; therefore,

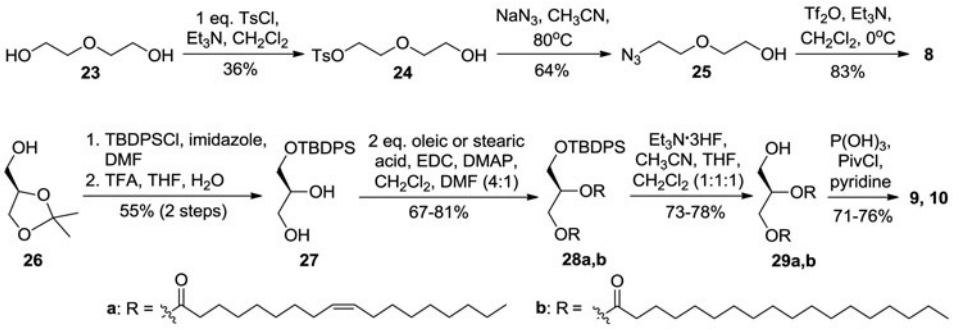


Scheme 3: Synthesis of the pseudodisaccharides.

the purification process was delayed until a later step to facilitate more convenient isomer separation. Before further transformations, the acetyl group at the glucosamine 3-*O*-position in **21** was replaced with a PMB group upon basic deacetylation and PMB-ation to give **22**. Thereafter, regioselective benzylidine acetal ring opening with sodium cyanoborohydride and HCl provided **6a** and **6b** that were readily separable by flash silica gel column chromatography. The anomeric configurations of **6a** and **6b** were confirmed by ^{1}H NMR: the coupling constants of the anomeric protons were 3.6 Hz for **6a** (α-anomer) and 7.9 Hz for **6b** (β-anomer).

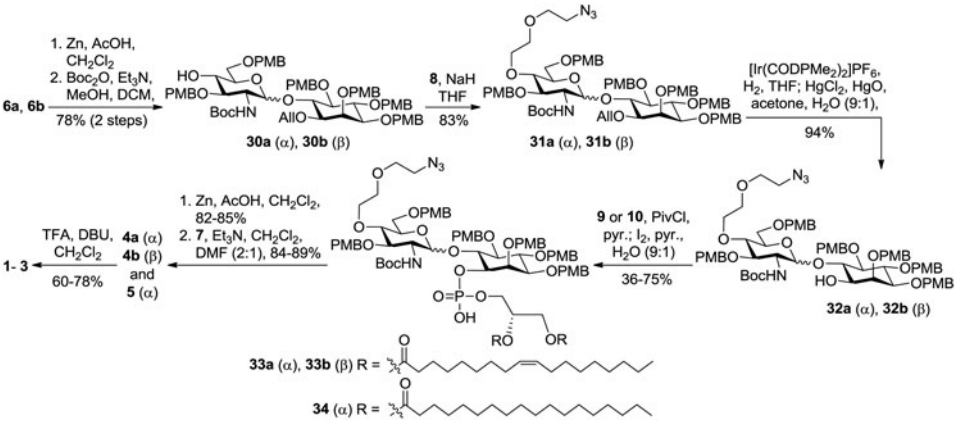
The designed linker **8** for attaching fluorescein to the glucosamine residue was prepared from commercially available diethylene glycol **23** in three separate steps (Sch. 4), including (1) mono-tosylation using 1 equiv. of tosyl chloride, (2) azido substitution of the tosylate using sodium azide, and (3) triflation of the remaining free hydroxyl group. In the meantime, synthesis of 1*H*-phosphonates **9** and **10** began with protecting the free hydroxyl group of commercially available (R)-(-)-2,2-dimethyl-1,3-dioxolane-4-methanol **26** with TBDPS, followed by removal of 2,3-*O*-acetonide under acidic conditions to afford **27** (Sch. 4). Incorporation of oleic acid and stearic acid in **27** was achieved with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the condensation reagent to give **28a** and **28b** in 81% and 67% yields, respectively. Triethylamine trihydrofluoride ($Et_3N\bullet 3HF$), instead of more basic TBAF, was used for TBDPS deprotection in an effort to prevent acyl group migration from the secondary alcohol to the less hindered primary alcohol. Finally, a phosphonate group was attached to the free hydroxyl group in **29a** and **29b** by reacting with phosphonic acid and pivaloyl chloride to produce **9** and **10** in 76% and 71% yields, respectively.

To provide orthogonality to the azido group introduced with linker **8**, the 2-azido group of the glucosamine residue in **6a** and **6b** was reduced using Zn and acetic acid (Sch. 5), and the resulting free amino group was then protected with



Scheme 4: Synthesis of the linker and the phosphorylating reagents.

a *tert*-butoxycarbonyl (Boc) group. It was envisioned that the Boc group could be easily removed under mild acidic conditions along with the PMB groups in the final deprotection step. The addition of linker **8** to the glucosamine 4-*O*-position was realized by reacting **8** with **30a** and **30b** under the influence of sodium hydride, which was followed by removal of the inositol 1-*O*-allyl protection to obtain **32a** and **32b** ready for the attachment of the phospholipid moiety. Phospholipidation of **32a** and **32b** was achieved with excessive **9** or **10** (2.5 equiv.) in the presence of pivaloyl chloride, followed by oxidation in situ with iodine to afford **33a** (75%), **33b** (53%), and **34** (36%, but 76% based on recovered **32a**). The products were rather difficult to separate from the excessive reagent and other side products, which affected the isolated yields.



Scheme 5: Final stage assembly of the target molecules.

The installation of the fluorescent tag was achieved in two steps, including reduction of the linker azide by Zn and acetic acid and reaction of the resulting primary amine with commercial activated ester of fluorescein **7** to give

compounds **4** and **5** in excellent yields. Finally, all of the Boc and PMB protecting groups were smoothly removed using 10% trifluoroacetic acid (TFA) in dichloromethane in the presence of a catalytic amount of DBU to afford the desired target molecules in good yields (60%–78%). It is worth mentioning that the catalytic amount of DBU was necessary for the rapid and complete PMB deprotection, as initial attempts for PMB deprotection using 10%–40% TFA in DCM could not be completed after 4 h of reaction. We believe this may be because of the influence of DBU on the equilibrium of the PMB deprotection reaction that is reversible under anhydrous conditions in an aprotic solvent, and DBU may serve as a base to buffer the reaction and shift it to completion.

CONCLUSIONS

An efficient synthesis was developed for the preparation of fluorescent-tagged GPI analogs. The installed fluorescent tag makes it possible to visualize the GPI analogs, which would facilitate many studies on GPI anchorage, such as the implications of GPI structure variations, including the lipid composition and the anomeric configuration, on the distribution, mobility, and other properties of GPIs in lipids and similar biological systems. The research results should be of particular interest as GPI analogs **1**–**3** contain the GPI components that are the most proximal to the lipid membrane. Moreover, the GPI core pseudodisaccharide is believed to play a pivotal role in the biological function of GPI anchors.

EXPERIMENTAL SECTION

General Methods

Reagents were obtained from commercial sources and used without further purification unless otherwise noted. Anhydrous solvents were obtained from commercial sources or from an alumina column solvent purification system. Molecular sieves were flame dried and used immediately after cooling under dry conditions. Analytical TLC was preformed using glass-backed silica gel 60 Å plates (250 μm thickness). TLC plate detection was done using a UV lamp, by charring with phosphomolybdic acid in EtOH, or by charring with 5% H_2SO_4 in EtOH. Column chromatography was carried out using standard-grade silica gel (40–63 μm particle size). ^{1}H NMR spectra were recorded at 400, 500, or 600 MHz with chemical shifts reported in ppm (δ) relative to $CHCl_3$ (7.26 ppm) or tetramethylsilane (0.00 ppm). ^{13}C NMR spectra were recorded at 100 or 125 MHz relative to the ^{13}C signal of $CDCl_3$ (77.23 ppm). Coupling constants (J) are reported in hertz (Hz). Mass spectrometry was performed using either a Bruker Daltonics Ultraflex MALDI TOF MS or Waters LCT Premier XE high-resolution ESI MS.

tert-Butyldiphenylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside (18)

To **17** (4.84 g, 13.0 mmol) stirring in anhydrous Et_2O (80 mL) under an Ar atmosphere was added benzylamine (21.3 mL, 194.7 mmol). After stirring for 2 h, the reaction was quenched with 1 M aq. HCl solution and then slowly poured into saturated aq. $NaHCO_3$ solution. The aq. layer was extracted three times with Et_2O. Combined organic portions were washed with brine and dried over $MgSO_4$ before being concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give the corresponding hemiacetal intermediate (3.56 g, 83%) as colorless syrup. To a solution of the intermediate (3.56 g, 10.8 mmol), imidazole (1.32 g, 19.4 mmol), and catalytic 4-(dimethylamino)pyridine (DMAP, 16 mg, 0.13 mmol) stirring in anhydrous DMF (70 mL) under an Ar atmosphere was added *tert*-butylchlorodiphenylsilane (5.03 mL, 19.35 mmol). After stirring overnight, DMF was removed under reduced pressure and the remaining residue was diluted with EtOAc before pouring into distilled water. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **18** (4.32 g, 71%) as colorless syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.68 (m, 4H), 7.50–7.26 (m, 6H), 4.98–4.95 (t, $J = 9.3$, Hz, 1H), 4.90–4.85 (t, $J = 9.3$, Hz, 1H), 4.47–4.45 (d, $J = 7.8$ Hz, 1H, 1 position), 4.04–4.00 (dd, $J = 5.2$, 12.4 Hz, 1H), 3.85–3.82 (dd, $J = 2.4$, 12.4Hz, 1H), 3.59–3.55 (dd, 7.8Hz, $J = 10.3$, 1H), 3.29–3.25 (m, 1H), 2.07 (s, 3H), 1.95 (s, 6H), 1.12 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.46, 169.99, 169.60, 135.85, 135.79, 132.12, 130.11, 129.89, 127.68, 127.39, 96.64, 72.65, 71.43, 68.58, 66.23, 61.89, 26.70, 20.68, 20.56, 20.55, 19.09.

tert-Butyldiphenylsilyl2-azido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-D-glucopyranoside, (19)

A solution of **18** (4.32 g, 7.59 mmol) in 0.05 M NaOMe in MeOH (40 mL) was stirred at 0°C for 40 min while being monitored by TLC. The reaction was then neutralized to pH 6–7 with amberlyst acidic resin. The resin was removed by filtration and the solution was condensed under reduced pressure to give the triol as a white solid (3.27 g, 97%) that was used directly for the next step. The crude triol was dissolved in anhydrous DMF under an Ar atmosphere before adding camphor sulfonic acid (176 mg, 0.76 mmol) and *para*-anisaldehyde dimethyl acetal (1.70 mL, 9.87 mmol). The reaction was stirred overnight at rt before dilution with EtOAc and poured into a saturated aq. $NaHCO_3$ solution. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated

under reduced pressure. The remaining residue was purified by silica gel column chromatography to afford **19** (3.59 g, 84%) as colorless syrup. ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.71–7.69 (m, 4H), 7.46–7.26 (m, 6H), 5.42 (s, 1H), 4.53–4.51 (d, $J = 7.6$ Hz, 1H, 1 position), 3.98–3.90 (dd, $J = 4.8$, 10.8 Hz, 1H), 3.78 (s, 3H), 3.61–3.54 (m, 2H), 3.51–3.42 (m, 2H), 3.03–2.99 (m, 1H), 2.56–2.55 (d, $J =$ 2.8 Hz, 1H), 1.12 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.86, 133.03, 132.35, 130.06, 129.87, 129.30, 129.03, 128.22, 127.62, 127.55, 127.41, 114.31, 113.70, 101.85, 97.23, 80.67, 72.16, 69.17, 68.23, 65.96, 55.29, 26.79, 19.11. HR ESI MS: calcd. for $C_{30}H_{36}N_3O_6$ [M + Na]+ m/z, 562.2373; found, 562.2372.

2-Azido-2-deoxy-3-*O*-acetyl-4,6-*O*-(*p*-methoxybenzylidene) -D-glucopyranose (20)

To **19** (2.615 g, 4.66 mmol) stirring in pyridine (20 mL) at 0°C was added acetic anhydride (4.4 mL, 46.6 mmol). The reaction was stirred for 1 h before pyridine and acetic anhydride were removed under reduced pressure. The remaining residue was diluted with EtOAc before it was poured into a saturated aq. $NaHCO_3$ solution. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the remaining residue by silica gel column chromatography gave the acetylated intermediate (2.50 g, 89%) as syrup. To facilitate TBDPS removal, a stock solution was prepared containing 11.9 mL of 1.0 M TBAF in THF and 0.70 mL glacial acetic acid. To the acetylated intermediate (2.49 g, 4.12 mmol) stirring in anhydrous THF at 0°C under an Ar atmosphere was added the freshly prepared, buffered TBAF solution (5.8 mL, 5.48 mmol TBAF). After stirring for another 15 min, the solvent was removed under reduced pressure and the remaining residue was diluted with EtOAc before being poured into distilled water. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **20** as a white solid (1.20 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45–7.30 (m, 2H), 6.94–6.82 (m, 2H), 5.66–5.60 (t, $J = 9.8$ Hz, 1H), 5.48–5.42 (d, $J = 5.4$ Hz, 1H), 5.20–5.31 (t, $J = 9.8$ Hz, 1H), 4.84–4.78 (d, $J = 7.8$ Hz, 1H), 4.41–4.14 (m, 2H), 3.83–3.77 (s, 3H), 3.77–3.67 (m, 1H), 3.65–3.57 (m, 1H), 3.57–3.49 (m, 1H), 3.49–3.42 (dd, 7.8, 9.8 Hz, 1H), 3.37–3.31 (dd, $J = 3.4$, 10.2 Hz, 1H), 3.07–3.01 (s, 1H), 2.15–2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): of α:β mixture, δ 160.13, 132.02, 127.50, 127.42, 114.31, 113.59, 101.65, 101.49, 96.71, 83.18, 79.34, 78.48, 71.13, 68.99, 68.72, 68.28, 66.65, 65.80, 62.26, 55.59, 55.29, 20.87. HR ESI MS: calcd. for $C_{16}H_{19}N_3O_7$ [M+Na]+ m/z, 388.1121; found, 388.1125.

6-*O*-[2-Azido-2-deoxy-3-*O*-acetyl-4,6-*O*-(*p*-methoxybenzylidene)-α,β-glucopyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxy benzyl)-*myo*-inositol (21)

To a solution of **20** (440 mg, 1.20 mmol) and trichloroacetonitrile (1.05 mL, 10.5 mmol) stirring in anhydrous CH_2Cl_2 (8 mL) was added 1 drop of DBU. After stirring for 50 min, the solvent was removed under reduced pressure and the remaining residue was purified with a Et_3N-neutralized silica gel column (1% Et_3N in mobile phase) to give trichloroacetimidate 2-azido-2-deoxy-3-*O*-acetyl-4,6-*O*-(*p*-methoxybenzylidene)-D-glucopyranose (**11**) (503 mg, 82%) as syrup, which was immediately used for the next step of reaction. After a mixture of freshly prepared **11** (503 mg, 0.99 mmol), **12** (400 mg, 0.58 mmol), and flame-dried MS 4 Å (200 mg) in anhydrous toluene and dioxane (3:2, 7 mL) was stirred for 1 h under an Ar atmosphere at rt, it was cooled to −10 °C, and TMSOTf (24 μL, 0.1 mmol) was added while the reaction was monitored by TLC. After stirring for another 25 min, the reaction mixture was neutralized with Et_3N, filtered through celite to remove MS 4 Å, concentrated under reduced pressure, and then purified by silica gel column chromatography to give **21** as an α:β mixture (497 mg, 81%). ^{1}H NMR of α-anomer (400 MHz, $CDCl_3$): δ 7.37–7.27 (m, 6H), 7.27–7.19 (m, 4H), 6.93–6.80 (m, 10H), 5.94–5.83 (m, 1H), 5.43–5.40 (s, 1H), 5.35–5.27 (dd, J = 2.0, 17.6 Hz, 1H), 5.19–5.13 (dd J = 1.5, 10.8 Hz, 1H), 5.13–5.02 (m, 2H), 4.97–4.86 (m, 2H), 4.77–4.74 (d, J = 4.4 Hz, 1H, 1 position), 4.74–4.65 (m, 2H), 4.58–4.50 (m, 2H), 4.37–4.31 (t, J = 9.3 Hz, 1H), 4.28–4.21 (m, 1H), 4.22–4.15 (dd, J = 4.9, 13.2 Hz, 1H), 4.08–3.93 (m, 3H), 3.85–3.75 (m, 12H), 3.76–3.68 (t, J = 10.3 Hz, 1H), 3.59–3.47 (m, 2H), 3.45–3.39 (dd, J = 8.3, 9.8 Hz, 1H), 3.37–3.31 (2.0, 9.8 Hz, 1H), 3.26–3.20 (m, 1H), 3.20–3.14 (dd, J = 2.0, 9.8 Hz, 1H), 2.14–2.10 (s, 3H). ^{13}C NMR of α-anomer (100 MHz, $CDCl_3$): δ 159.27, 159.19, 159.11, 135.19, 130.89, 130.81, 129.90, 129.84, 129.49, 129.42, 129.20, 116.20, 113.94, 113.78, 113.57, 100.82, 83.33, 81.64, 80.54, 78.33, 75.40, 74.08, 72.32, 72.20, 64.44, 55.26, 29.69, 21.05, 20.97, 14.19. MALDI-TOF MS: calcd. for $C_{57}H_{65}N_3NaO_{16}$ [M+Na]+ m/z, 1070.4; found, 1070.1.

6-*O*-[2-Azido-2-deoxy-3-*O*-(*p*-methoxybenzyl)-4,6-*O*-(*p*-methoxy benzylidene)-α,β-D-glucopyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (22)

To **21** (497 mg, 0.47 mmol) stirring in MeOH and CH_2Cl_2 (8:1, 9 mL) at rt was added freshly prepared 2 M NaOMe in MeOH (300 μL, 0.60 mmol). After stirring for 3.5 h, the solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography to give the corresponding deacetylated intermediate (428 mg, 90%). To the intermediate (103 mg, 0.102 mmol) stirring in anhydrous DMF (1.5 mL) under an

Ar atmosphere at 0°C was added NaH (60% dispersion in mineral oil, 8 mg, 0.20 mmol). After stirring for 30 min at 0°C, *p*-methoxybenzyl chloride (27 μL, 0.20 mmol) was added, and the mixture was stirred overnight at rt. DMF was then removed under reduced pressure, and the remaining residue was diluted with EtOAc and poured into distilled water. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **22** (108 mg, 94%). ^{1}H NMR of α-anomer (400 MHz, $CDCl_3$): δ 7.46–7.17 (m, 12H), 7.00–6.76 (m, 12H), 5.94–5.79 (m, 1H), 5.54–5.48 (s, 1H), 5.35–5.25 (dd, 1.5, 17.1 Hz, 1H), 5.18–5.10 (dd, J = 1.0, 10.3 Hz, 1H), 4.96–4.66 (m, 9H), 4.57–4.50 (m, 2H), 4.42–4.13 (m, 3H), 4.10–3.92 (m, 3H), 3.87–3.71 (m, 18H), 3.67–3.59 (t, J = 9.3 Hz, 2H), 3.52–3.44 (m, 2H), 3.41–3.29 (m, 3H), 3.23–3.13 (m, 3H). ^{13}C NMR of α-anomer (100 MHz, $CDCl_3$): δ 159.56, 159.18, 159.14, 159.04, 135.30, 131.99, 130.86, 129.82, 129.59, 129.50, 129.44, 129.19, 116.00, 114.30, 113.76, 113.55, 113.48, 101.21, 83.45, 82.12, 81.67, 80.50, 78.43, 75.26, 75.22, 74.72, 74.07, 74.06, 72.32, 72.25, 70.30, 66.62, 61.79, 55.59, 55,26, 29.69. HR ESI MS: calcd. for $C_{63}H_{71}N_3O_{16}$ [M+Na]+ m/z, 1148.4732; found, 1148.4749.

6-*O*-[2-Azido-2-deoxy-3,6-di-*O*-(*p*-methoxybenzyl)-α,β-D-gluco pyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (6a and 6b)

To a mixture of **22** (209 mg, 0.19 mmol), $NaBH_3CN$ (117 mg, 1.86 mmol), MS 4Å (150 mg), and methyl orange indicator stirring in anhydrous THF (8 mL) at 0°C under an Ar atmosphere was added an HCl solution (1 M in Et_2O) dropwise until pH $\approx$1 was reached (the reaction mixture became a red color as indicated by methyl orange). After stirring for 45 min, the reaction was quenched by adding ice cold saturated aq. $NaHCO_3$ solution. The aq. layer was extracted thre times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel chromatography to give **6a** and **6b** (95.8 mg α-anomer, 72.2 mg β-anomer, 80% overall yield). ^{1}H NMR of **6a** (400 MHz, $CDCl_3$): 7.38–7.29 (m, 4H), 7.29–7.19 (m, 4H), 7.19–7.10 (m, 4H), 6.92–6.81 (m, 8H), 6.81–6.73 (m, 4H), 5.99–5.87 (m, 1H), 5.71–5.68 (d, J = 3.4 Hz, 1H, 1 position), 5.30–5.23 (dd, J = 1.5, 17.1 Hz, 1H), 5.20–5.15 (J = 1.5, 11.7 Hz, 1H), 4.96–4.69 (m, 7H), 4.64–4.51 (m, 3H), 4.39–4.33 (d, J = 11.7 Hz, 1H), 4.24–4.15 (m, 2H), 4.11–3.93 (m, 6H), 3.84–3.72 (m, 18H), 3.72–3.63 (m, 2H), 3.63–3.59 (dd, J = 3.4, 4.9 Hz, 1H), 3.41–3.30 (m, 3H), 3.29–3.25 (m, 2H), 3.23–3.17 (dd, J = 3.4, 10.3 Hz, 1H). ^{13}C NMR of **6a** (100 MHz, $CDCl_3$): δ 159.19, 159.10, 159.04, 158.91, 134.34, 130.42, 129.81, 129.26, 129.15, 127.94, 116.95, 113.93, 113.77, 113.67, 113.57, 97.43, 81.94, 81.72, 81.30, 80.64, 75.30, 75.21, 74.94, 74.45, 73.63, 73.00, 72.38, 72.27, 70.85,

69.28, 68.97, 68.87, 68.75, 62.87, 55.28, 55.26, 55.24, 55.21. HR ESI MS: calcd. for $C_{63}H_{73}N_3O_{16}$ [M+Na]+ m/z, 1150.4889; found, 1150.4911. ^{1}H NMR of **6b** (500 MHz, $CDCl_3$): δ 7.39–7.23 (m, 12H), 6.96–6.78 (m, 12H), 5.92–5.82 (m, 1H), 5.31–5.25 (dd, J = 1.5, 17.4 Hz, 1H), 5.13–5.07 (d, J = 10.7 Hz, 1H), 5.00–4.73 (m, 7H), 4.61–4.47 (m, 4H), 4.41–4.35 (t, J = 9.5 Hz, 1H), 4.30–4.23 (dd, J = 4.9, 13.4 Hz, 1H), 4.09–3.95 (m, 4H), 3.87–3.76 (m, 18H), 3.75–3.70 (dd, J = 4.9, 10.7 Hz, 1H), 3.69–3.64 (m, 2H), 3.57–3.52 (t, J = 9.2 Hz, 1H), 3.40–3.33 (m, 2H), 3.31–3.26 (m, 1H), 3.24–3.18 (m, 2H), 2.89 (s, 1H). ^{13}C NMR of **6b** (125 MHz, $CDCl_3$): δ 159.43, 159.25, 159.18, 159.16, 159.10, 158.90, 135.53, 131.09, 130.88, 130.48, 130.25, 130.00, 129.90, 129.76, 129.52, 129.50, 129.42, 129.23, 115.81, 113.96, 113.81, 113.80, 113.78, 113.76, 113.42, 101.07, 83.76, 82.05, 81.77, 80.45, 78.78, 78.04, 75.29, 75.05, 74.91, 74.65, 73.70, 73.67, 73.51, 72.94, 72.30, 72.13, 69.94, 66.41, 55.28, 55.24. HR ESI MS: calcd. for $C_{63}H_{73}N_3O_{16}$ [M+Na]+ m/z, 1150.4889; found, 1150.4844.

2-(2-Hydroxyethoxy)ethyl-*p*-methylbenzenesulfonate (24)

To a solution of commercially available diethyleneglycol **23** (4.0 g, 37.7 mmol) and *p*-toluenesulfonyl chloride (7.55 g, 39.6 mmol) stirring in anhydrous CH_2Cl_2 under an Ar atmosphere at 0°C was slowly added Et_3N (15.8 mL, 113.1 mmol). After stirring overnight at rt, the reaction was diluted with CH_2Cl_2 and poured into a saturated aq. $NaHCO_3$ solution. The aq. layer was extracted three times with CH_2Cl_2, and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The product mixture was purified by silica gel column chromatography to give **24** (3.56 g, 36%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83–7.76 (d, J = 8.31 Hz, 2H), 7.37–7.30 (d, J = 7.34 Hz, 2H), 4.21–4.15 (m, 2H), 3.70–3.63 (m, 4H), 3.54–3.49 (m, 2H), 2.46–2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.95, 129.84, 127.92, 72.46, 68.54, 61.61, 21.63.

2-(2-Azidoethoxy)ethanol (25)

A solution of **24** (738 mg, 2.84 mmol) and NaN_3 (239 mg, 3.60 mmol) in CH_3CN was stirred for 36 h at 80°C. A white precipitate was formed and removed by filtration. The precipitate was washed three times with cold CH_3CN. The filtrate was evaporated under reduced pressure, diluted with CH_2Cl_2, poured into a saturated aq. $NaHCO_3$ solution, and extracted three times with CH_2Cl_2. The combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **25** (239 mg, 64%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.75–3.69 (m, 2H), 3.69–3.64 (m, 2H), 3.61–3.56 (m, 2H), 3.41–3.36 (m, 2H), 2.59–2.00 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 72.42, 69.99, 61.69, 50.69.

2-(2-Azidoethoxy)ethyl triflouromethanesulfonate (8)

To a solution of **25** (19.5 mg, 148.7 μmol) and Et_3N (3 drops) stirring in anhydrous CH_2Cl_2 (1 mL) at 0°C under an Ar atmosphere was slowly added triflouromethanesulfonic anhydride (60 μL, 354 μmol). After stirring for 25 min, the reaction mixture was diluted with CH_2Cl_2 and poured into saturated aq. $NaHCO_3$ solution. The aq. layer was extracted three times with CH_2Cl_2 and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Compound **8** was obtained (32.6 mg, 83%) as colorless oil and used directly for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.66–4.62 (t, $J = 4.40$ Hz, 2H), 3.85–3.81 (t, $J = 4.40$ Hz, 2H), 3.72–3.68 (t, $J = 4.89$ Hz, 2H), 3.44–3.38 (t, $J = 4.89$ Hz, 2H).

1-*O*-(*tert*-Butyldiphenylsilyl)-*sn*-glycerol (27)

To a solution of commercially available **26** (4.19 g, 31.60 mmol) and imidazole (8.60 g, 126.0 mmol) stirring in anhydrous DMF (20 mL) under an Ar atmosphere at rt was slowly added *tert*-butylchlorodiphenylsilane (16.4 mL, 63.2 mmol). After stirring overnight, the solvent was removed under reduced pressure and the remaining residue was diluted with EtOAc and poured into distilled water. The aq. layer was extracted three times with EtOAc and the combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a solution of the crude TBDPS-protected intermediate stirring in THF and H_2O (4:1, 20 mL) at 0°C was added TFA (1 mL). After stirring for 3 days at rt, the reaction was cooled to 0°C and saturated aq. $NaHCO_3$ solution (100 mL) was added slowly. After stirring for 15 min at 0°C, the aq. layer was extracted three times with EtOAc. The combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **27** (5.77 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.65 (d, $J = 7.3$ Hz, 4H), 7.48–7.37 (m, 6H), 3.85–3.78 (m, 1H), 3.76–3.66 (m, 2H), 3.66–3.60 (m, 1H), 2.65–2.59 (s, 2H), 1.12–1.04 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.52, 132.93, 129.93, 127.85, 71.97, 65.20, 63.84, 26.86, 19.23.

1-*O*-(*tert*-Butyldiphenylsilyl)-2,3-di-*O*-oleoyl-*sn*-glycerol (28a)

To a solution of **27** (532 mg, 1.61 mmol), oleic acid (1.0 g, 3.54 mmol), and catalytic DMAP (19.5 mg 0.16 mmol) stirring in CH_2Cl_2 and DMF (4:1, 16 mL) under an Ar atmosphere at rt was added EDC hydrochloride (679 mg, 3.54 mmol). After stirring overnight, the solvent was removed under reduced

pressure and the remaining residue was diluted with CH_2Cl_2, poured into distilled water, and extracted three times with CH_2Cl_2. The combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **28a** (1.13 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69–7.63 (m, 4H), 7.46–7.35 (m, 6H) 5.39–5.29 (m, 4H), 5.20–5.14 (m, 1H), 4.43–4.38 (dd, J = 4.0, 11.9, 1H), 4.24–4.19 (dd, J = 6.1, 11.6, 1H), 3.80–3.72 (m, 2H), 2.33–2.21 (m, 4H), 2.04–1.97 (m, 8H), 1.63–1.56 (m, 4H), 1.36–1.22 (m, 40H), 1.08–1.03 (s, 9H), 0.90–0.86 (t, 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.33, 172.94, 135.51, 133.04, 133.00, 129.98, 129.80, 129.70, 127.74, 127.72, 71.49, 62.37, 62.31, 34.30, 34.11, 31.92, 29.78, 29.72, 29.53, 29.33, 29.22, 29.20, 29.12, 27.23, 27.18, 26.71, 24.91, 24.87, 22.69, 19.22, 14.12.

1-*O*-(*tert*-Butyldiphenylsilyl)-2,3-di-*O*-stearyl-*sn*-glycerol (28b)

To a solution of **27** (1.258 g, 3.81 mmol), stearic acid (2.40 g, 8.37 mmol), and catalytic DMAP (92.8 mg 0.76 mmol) stirring in CH_2Cl_2 and DMF (4:1, 40 mL) under an Ar atmosphere at rt was added EDC hydrochloride (1.60 g, 8.37 mmol). After stirring overnight, the solvent was removed under reduced pressure. The remaining residue was diluted with CH_2Cl_2, poured into distilled water, and extracted three times with CH_2Cl_2. The combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel column chromatography to give **28b** (2.19g, 67%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.63 (m, 4H), 7.47–7.36 (m, 6H), 5.22–5.15 (m, 1H), 4.45–4.39 (dd, J = 3.91, 11.74 Hz, 1H), 4.26–4.20 (dd, J = 6.36, 11.74 Hz, 1H), 3.83–3.73 (m, 2H), 2.32–2.24 (m, 4H), 1.65–1.55 (m, 4H), 1.38–1.20 (s, 56H), 1.08–1.05 (s, 9H), 0.92–0.87 (t, J = 6.85 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.38, 172.99, 135.57, 135.51, 133.04, 133.00, 129.98, 129.80, 129.70, 127.74, 127.72, 71.49, 62.37, 62.30, 34.32, 34.14, 31.94, 29.72, 29.70, 29.68, 29.65, 29.50, 29.38, 29.31, 29.30, 29.15, 29.13, 26.71, 24.93, 24.89, 22.70, 19.22, 14.12.

2,3-di-*O*-Oleoyl-*sn*-glycerol (29a)

To a solution of **28a** (441 mg, 0.513 mmol) in CH_3CN, THF, and CH_2Cl_2 (1:1:1, 6 mL) stirring under an Ar atmosphere at rt was slowly added $Et_3N{\bullet}3HF$ (1.0 mL). After stirring for 24 h, the reaction mixture was cooled to 0°C and quenched with saturated aq. $NaHCO_3$ solution. After stirring for 30 min at 0°C, the quenched reaction mixture was poured into saturated aq. $NaHCO_3$ solution and extracted three times with CH_2Cl_2. The combined organic portions were dried over $MgSO_4$, concentrated under reduced pressure, and purified by silica gel column chromatography to give **28a** (233 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.40–5.28 (m, 4H), 5.11–5.04 (pent., J = 4.9 Hz,

1H), 4.35–4.28 (dd, $J = 4.9$, 12.2 Hz, 1H), 4.26–4.20 (dd, $J = 5.9$, 12.2 Hz, 1H), 3.74–3.70 (d, 4.89 Hz, 2H), 2.37–2.28 (q, $J = 8.3$ Hz, 4H), 2.06–1.95 (m, 8H), 1.67–1.57 (m, 4H), 1.37–1.20 (m, 40H), 0.91–0.85 (t, $J = 6.4$ Hz, 6H).

1-*O*-(Phosphonooxyl)-2,3-di-*O*-oleoyl-*sn*-glycerol (9)

A mixture of **29a** and phosphonic acid were coevaporated with anhydrous pyridine three times and then dried under reduced pressure for over 2 h. The resulting mixture was dissolved in 5 mL of anhydrous pyridine under an Ar atmosphere before the addition of pivaloyl chloride (20 μL, 0.161 mmol). The reaction mixture was stirred overnight at rt before the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (1% Et_3N in mobile phase) to give **9** as a Et_3N salt (64.5 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.67–11.34 (s, broad, 3H, Et_3NH^+), 7.57–7.53 (s, 0.5H), 5.94–5.90 (s, 0.5H), 5.30–5.20 (m, 4H), 5.15–5.07 (m, 1H), 4.30–4.23 (dd, $J = 4.1$, 15.4 Hz, 1H), 4.11–4.03 (dd, $J = 6.5$, 12.2 Hz, 1H), 4.00–3.92 (m, 2H), 3.09–2.99 (m, 25.5H, Et_3N), 2.25–2.17 (m, 4H), 1.95–1.87 (m, 8H), 1.55–1.45 (m, 4H), 1.35–1.27 (t, $J = 7.3$ Hz, 38.2 Hz, Et_3N), 1.26–1.11 (m, 40H), 0.82–0.75 (t, $J = 6.5$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.23, 172.83, 129.89, 129.62, 69.98, 69.90, 62.26, 62.13, 52.83, 45.89 (Et_3N), 34.14, 33.95, 31.81, 29.67, 29.64, 29.44, 29.23, 29.12, 29.05, 28.99, 27.09, 24.76, 22.60, 14.05, 8.62 (Et_3N). ^{31}P NMR (160 MHz, $CDCl_3$): δ 4.30.

2,3-di-*O*-Stearyl-*sn*-glycerol (29b)

To a solution of **28b** (359 mg, 0.42 mmol) in CH_3CN, THF, and CH_2Cl_2 (1:1:1, 6 mL) stirring under an Ar atmosphere at rt was added $Et_3N{\bullet}3HF$ (1.0 mL). After stirring for 24 h, the reaction mixture was cooled to 0°C and quenched with saturated aq. $NaHCO_3$ solution. After stirring for 30 min at 0°C, the quenched reaction mixture was poured into a saturated aq. $NaHCO_3$ solution and extracted three times with CH_2Cl_2. The combined organic portions were dried over $MgSO_4$, concentrated under reduced pressure, and purified by silica gel column chromatography to give **29b** (206 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12–5.05 (m, 1H), 4.35–4.29 (dd, $J = 4.9$, 12.2 Hz, 1H), 4.27–4.21 (dd, $J = 5.7$, 12.2 Hz, 1H), 3.75–3.71 (d, $J = 5.7$ Hz, 2H), 2.38–2.29 (q, $J = 7.30$ Hz, 4H), 1.66–1.57 (m, 4H), 1.36–1.18 (broad s, 56H), 0.90–0.85 (t, $J = 6.5$ Hz, 6H).

1-*O*-(Phosphonooxyl)-2,3-di-*O*-stearyl-*sn*-glycerol (10)

A mixture of **29b** and phosphonic acid were coevaporated with anhydrous pyridine three times and dried under reduced pressure for over 2 h. The resulting mixture was dissolved in 5 mL of anhydrous pyridine under an Ar atmosphere before the addition of pivaloyl chloride (62 μL, 0.50 mmol). The reaction mixture was stirred overnight at rt. The solvent was removed under

reduced pressure and the residue was purified by silica gel column chromatography (1% Et_3N in mobile phase) to give **10** as an Et_3N salt (161.4 mg, 71%). ^1H NMR (400 MHz, $CDCl_3$): δ 11.81–11.65 (s, broad, 4H), 7.50–7.47 (s, 0.5H), 5.95–5.91 (s, 0.5H), 5.13–5.05 (m, 1H), 4.28–4.21 (dd, J = 3.2, 11.35 Hz, 1H), 4.08–4.01 (dd, J = 6.49, 12.16 Hz, 1H), 3.89–3.83 (dd, J = 5.7, 8.1 Hz, 2H), 3.05–2.97 (m, 34H, Et_3N), 2.21–2.13 (q, J = 7.3 Hz, 4H), 1.52–1.41 (m, 4H), 1.31–1.26 (t, J = 7.3 Hz, 50H, Et_3N), 1.17–1.11 (s, broad, 56H), 0.78–0.73 (t, J = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.29, 172.91, 62.37, 45.78 (Et_3N), 34.20, 33.99, 31.81, 29.60, 29.41, 29.26, 29.02, 24.78, 22.78, 22.58, 14.02, 8.62 (Et_3N). ^{31}P NMR (160 MHz, $CDCl_3$): δ 4.08

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-3,6-di-*O*-(*p*-methoxy benzyl)-α-D-glucopyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (30a) and 6-*O*-[2-*N*-(*tert*-butyl carbamate)-2-deoxy-3,6-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (30b)

To a mixture of **6a** (138 mg, 0.122 mmol) and zinc powder (200 mg, 3.05 mmol) stirring in anhydrous CH_2Cl_2 (7 mL) under an Ar atmosphere at rt was added acetic acid (~100 μL). After stirring for 1 h, the reaction was diluted with CH_2Cl_2, poured into saturated aq. $NaHCO_3$, and extracted four times with CH_2Cl_2. The combined organic portions were dried over $MgSO_4$ and concentrated under reduced pressure. The remaining residue was dissolved in MeOH and CH_2Cl_2 (1:1, 10 mL) before the addition of di-*tert*-butyl dicarbonate (56 μL, 0.244 mmol) and Et_3N (100 μL). After stirring overnight, the solvent was removed under reduced pressure and the remaining residue was purified by silica gel column chromatography to give **30a** (115 mg, 78% two steps). ^1H NMR for **30a** (500 MHz, $CDCl_3$): δ 7.37–7.10 (m, 12H), 6.93–6.77 (m, 12H), 5.96–5.85 (m, 1H), 5.66–5.60 (d, J = 9.8 Hz, 1H), 5.40–5.35 (d, J = 3.1 Hz, 1H, 1 position), 5.33–5.25 (d, J = 17.4 Hz, 1H), 5.22–5.16 (d, J = 10.7 Hz, 1H), 4.91–4.85 (d, J = 10.4 Hz, 1H), 4.85–4.79 (d, J = 9.8 Hz, 1H), 4.79–4.71 (m, 5H), 4.69–4.63 (d, J = 11.0 Hz, 1H), 4.61–4.51 (dd, J = 11.3, 23.2 Hz, 2H), 4.42–4.37 (d, J = 11.3 Hz, 1H), 4.28–4.23 (d, J = 11.6 Hz, 1H), 4.17–4.12 (t, J = 9.8 Hz, 1H), 4.07–3.99 (m, 3H), 3.99–3.88 (m, 3H), 3.87–3.75 (m, 18H), 3.75–3.67 (m, 2H), 3.56–3.43 (m, 3H), 3.38–3.26 (m, 3H), 3.24–3.18 (d, J = 9.5 Hz, 1H), 2.54–2.49 (s, 1H), 1.50–1.40 (s, 9H); ^{13}C NMR for **30a** (125 MHz, $CDCl_3$): δ 155.41, 134.22, 131.04, 131.01, 130.80, 130.41, 130.19, 129.57, 129.50, 129.48, 129.46, 129.25, 129.21, 117.82, 113.79, 113.75, 113.66, 113.60, 99.70, 82.45, 81.83, 80.64, 80.50, 80.43, 79.04, 77.72, 75.33, 75.10, 73.56, 74.36, 73.12, 72.36, 71.82, 71.75, 70.91, 70.66, 69.90, 55.30, 55.26, 55.22, 54.21, 28.55. HR ESI MS: calcd. for $C_{68}H_{83}NO_{18}$ [M+Na]+ m/z, 1224.5508; found, 1224.5511. Compound

6b underwent an analogous procedure to give **30b** (64 mg, 65% two steps). ^{1}H NMR for **30b** (500 MHz, $CDCl_3$): δ 7.36–7.17 (m, 12H), 6.94–6.77 (m, 12H), 5.90–5.81 (m, 1H), 5.28–5.21 (dd, J = 1.5, 18.9 Hz, 1H), 5.09–5.01 (t, J = 9.2 Hz, 2H), 4.89–4.82 (m, 2H, includes 1-pos), 4.82–4.73 (dd, J = 11.6, 20.1 Hz, 2H), 4.71–4.69 (d, J = 10.1 Hz, 1H), 4.65–4.60 (d, J = 11.0 Hz, 1H), 4.58–4.46 (m, 6H), 4.33–4.24 (m, 2H), 4.21–4.15 (t, J = 9.2 Hz, 1H), 4.04–3.93 (m, 3H), 3.83–3.76 (m, 18H), 3.71–3.68 (d, J = 4.6 Hz, 2H), 3.58–3.52 (t, J = 8.9 Hz, 1H), 3.41–3.30 (m, 4H), 3.26–3.19 (m, 1H), 3.19–3.13 (d, J = 9.5 Hz, 1H), 2.69 (s, 1H), 1.44 (s, 9H). ^{13}C NMR for **30b** (125 MHz, $CDCl_3$): δ 159.21, 159.17, 159.13, 158.94, 158.92, 135.65, 131.21, 130.84, 130.52, 130.15, 129.58, 129.55, 129.44, 129.16, 128.17, 115.61, 113.90, 113.83, 113.75, 113.44, 101.35, 81.49, 80.48, 79.36, 79.18, 75.28, 74.61, 73.82, 73.70, 73.41, 72.65, 72.36, 72.12, 70.42, 55.24, 28.47. HR ESI MS: calcd. for $C_{68}H_{83}NO_{18}$ [M+Na]+ m/z, 1224.5508; found 1224.5465.

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy) ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (31a) and 6-*O*-[2-*N*-(*tert*-butylcarbamate)-2-deoxy-4-*O*-(2-(2-azido ethoxy)ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyran osyl]-1-*O*-allyl-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (31b)

To **30a** (114 mg, 94.8 μmol) stirring in anhydrous THF at 0°C was added NaH (23 mg of 60% dispersion in mineral oil, ~570 μmol). After stirring for 30 min, **8** was added from a stock solution containing 103 mg of **8** in anhydrous THF (initially 16.8 mg, 64 μmol) and the reaction was monitored by TLC. Additional 30- to 50-μL portions from the stock solution were added (estimated ~2 eq. with respect to **30a**) until TLC showed a nearly complete reaction (alkylation of Boc-protected amine was observed by HR ESI-MS in initial experiments upon using a larger excess of **8**). The reaction was then diluted with EtOAc, poured into distilled water, and extracted four times with EtOAc. The combined organic portions were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The remaining residue was purified by silica gel chromatography to give **31a** (104 mg, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.11 (m, 12H), 6.91–6.77 (m, 12H), 5.92–5.82 (m, 1H), 5.62–5.56 (d, J = 10.1 Hz, 1H), 5.37–5.33 (d, J = 2.8 Hz, 1 position, 1HH), 5.30–5.23 (d, J = 17.4 Hz, 1H), 5.19–5.13 (d, J = 10.1 Hz, 1H), 4.91–4.85 (d, J = 10.1 Hz, 1H), 4.84–4.80 (d, J = 9.8 Hz, 1H), 4.80–4.70 (m, 5H), 4.70–4.66 (d, J = 11.0 Hz, 1H), 4.61–4.51 (dd, J = 11.6, 23.2 Hz, 2H), 4.50–4.44 (d, J = 11.6 Hz, 1H), 4.23–4.17 (d, J = 11.6 Hz, 1H), 4.16–4.09 (t, J = 9.5 Hz, 1H), 4.06–4.00 (t, J = 9.5, 1H), 4.00–3.93 (m, 3H), 3.93–3.85 (m, 3H), 3.85–3.74 (m,

18H), 3.62–3.52 (m, 5H), 3.52–3.43 (m, 3H), 3.42–3.36 (d, J = 10.7 Hz, 1H), 3.36–3.23 (m, 5H), 3.20–3.13 (d, J = 9.8 Hz, 1H), 1.49–1.38 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.20, 159.08, 159.05, 159.01, 158.95, 155.41, 134.21, 131.13, 131.04, 130.85, 130.81, 130.53, 130.41, 129.56, 129.48, 129.44, 129.25, 129.23, 117.77, 113.77, 113.74, 113,57, 113.54, 99.83, 82.46, 81.85, 80.74, 80.49, 80.43, 78.86, 78.56, 77.91, 75.33, 75.01, 74.48, 69.76, 68.09, 55.29, 55.26, 55.22, 54.85, 50.70, 28.55. HR ESI-MS: calcd. for $C_{72}H_{90}N_4O_{19}$ [M+Na]+ m/z, 1337.6097; found, 1337.6078. Compound **30b** underwent an analogous procedure to give **31b** (51.1 mg, 73%). ^{1}H NMR for **31b** (500 MHz, $CDCl_3$): δ 7.36–7.16 (m, 12H), 6.95–6.73 (m, 12H), 5.89–5.80 (m, 1H), 5.27–5.19 (dd, J = 1.8, 17.4 Hz, 1H), 5.07–4.99 (m, 2H), 4.89–4.44 (m, 12H, includes 1 position), 4.38–4.30 (m, 1H), 4.26–4.19 (t, J = 9.2, 1H), 4.19–4.12 (broad s, 1H), 4.04–3.94 (m, 4H), 3.92–3.86 (m, 2H), 3.85–3.74 (m, 18H), 3.73–3.66 (m, 3H), 3.60–3.54 (m, 2H), 3.54–3.49 (m, 2H), 3.42–3.27 (m, 8H), 3.19–3.13 (d, J = 9.46, 1H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.12, 158.89, 155.46, 135.80, 131.33, 131.24, 130.91, 130.87, 130.74, 130.54, 129.81, 129.58, 129.52, 129.31, 129.16, 128.11, 115.59, 113.91, 113.74, 113.73, 113.68, 113.59, 113.42, 101.39, 83.90, 81.84, 81.56, 80.50, 79.23, 79.02, 78.79, 75.27, 75.21, 74.92, 74.54, 74.36, 73.70, 73.19, 72.57, 72.08, 71.73, 70.79, 69.83, 68.37, 57.68, 55.28, 55.25, 55.23, 55.21, 50.68, 28.47. HR ESI MS: calcd. for $C_{72}H_{90}N_4O_{19}$ [M+Na]+ m/z, 1337.6097; found, 1337.6099.

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy) ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-2,3,4, 5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (32a) and 6-*O*-[2-*N*-(*tert*-butylcarbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy)ethyl) -3,6-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranosyl]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (32b)

A solution of $[Ir(CODPMe_2Ph_2)_2]PF_6$ (6.0 mg, 7 μmol) in anhydrous THF (1.5 mL) was stirred under an H_2 atmosphere until the color of the solution turned from red to pale yellow (~10 min). The H_2 atmosphere was exchanged for Ar and a solution of **31a** in anhydrous THF (1 mL) was added to the catalyst solution. After stirring for 1.5 h, the solvent was removed under reduced pressure and the remaining residue was diluted with acetone and H_2O (9:1, 1.5 mL) followed by the addition of $HgCl_2$ and HgO. After stirring for 10 min, the solvent was removed and the remaining residue was purified by silica gel chromatography to give **32a** (89.5 mg, 90%). ^{1}H NMR for **32a** (500 MHz, $CDCl_3$): δ 7.33–7.18 (m, 12H), 6.19–6.77 (m, 12H), 5.37–5.33 (m, 1H), 5.33–5.29 (d, J = 2.4 Hz, 1H), 5.00–4.95 (d, J = 11.3Hz, 1H), 4.88–4.84 (d, J = 10.4 Hz, 1H), 4.82–4.77 (d, J = 9.8 Hz, 1H), 4.75–4.72 (d, J = 6.1 Hz, 1H), 4.72–4.70 (d, J = 6.4 Hz, 1H), 4.69–4.61 (m, 4H), 4.58–4.53 (d, J = 11.0 Hz,

1H), 4.46–4.41 (d, J = 11.9 Hz, 1H), 4.20–4.15 (d, J = 11.6 Hz, 1H), 4.00–3.95 (t, J = 9.5 Hz, 1H), 3.95–3.91 (m, 1H), 3.90–3.84 (m, 3H), 3.84–3.77 (m, 18H), 3.77–3.75 (m, 1H), 3.62–3.54 (m, 2H), 3.54–3.51 (t, J = 5.5 Hz, 2H), 3.51–3.31 (m, 6H), 3.39–3.33 (m, 1H), 3.31–3.27 (t, J = 5.5 Hz, 2H), 3.27–3.22 (d, J = 8.85 Hz, 1H), 2.95–2.92 (s, 1H), 1.41–1.35 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.97, 130.87, 130.77, 130.36, 130.19, 129.70, 129.61, 129.54, 129.45, 129.32, 113.92, 113.86, 113.73, 113.59, 113.54, 98.96, 81.45, 81.36, 80.80, 80.24, 80.06, 78.37, 75.33, 74.33, 72.90, 72.85, 71.59, 70.79, 69.75, 55.28, 55.26, 55.24, 55.22, 50.68, 42.57, 31.92, 30.85, 29.70, 28.40. HR ESI-MS: calcd. for $C_{69}H_{86}N_4O_{19}$ [M+Na]+ m/z, 1297.5784; found, 1297.5781. Compound **31b** underwent an analogous procedure to give **32b** (59.8 mg, 94%). ^{1}H NMR for **32b** (500 MHz, $CDCl_3$): δ 7.33–7.14 (m, 12H), 6.19–6.76 (m, 12H), 4.94–4.88 (d, J = 10.9 Hz, 1H), 4.88–4.84 (d, J = 10.38, 1H), 4.82–4.75 (dd, J = 11.3, 14.7 Hz, 2H), 4.70–4.59 (m, 3H), 4.56–4.48 (m, 4H), 4.47–4.42 (d, J = 11.6, 1H), 4.40–4.34 (d, J = 7.9 Hz, 1 position), 4.06–4.02 (m, 1H), 4.01–3.86 (m, 4H), 3.81 (s, 6H), 3.79 (s, 3H), 3.78 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H), 3.73–3.60 (m, 5H), 3.60–3.55 (t, J = 4.9, 2H), 3.55–3.44 (m, 4H), 3.42–3.30 (m, 5H), 3.24–3.17 (m, 3H), 1.41 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.24, 159.12, 159.09, 159.08, 158.92, 158.87, 155.38, 131.47, 131.27, 130.93, 130.46, 130.25, 129.84, 129.70, 129.37, 129.24, 128.33, 113.80, 113.74, 113.70, 113.41, 102.39, 82.69, 82.27, 81.73, 81.33, 80.28, 79.65, 78.48, 75.97, 75.30, 74.76, 74.58, 74.46, 73.96, 73.18, 71.93, 71.76, 71.22, 70.70, 69.88, 68.07, 55.25, 55.23, 55.22, 55.20, 50.66, 28.43. HR ESI-MS: calcd. for $C_{69}H_{86}N_4O_{19}$ [M+Na]+ m/z, 1297.5784; found, 1297.5776.

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy) ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (33a) and 6-*O*-[2-*N*-(*tert*-butyl carbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy)ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (33b)

A mixture of **32a** and **9** was coevaporated with anhydrous pyridine three times and then dried under reduced pressure for >2 h. Under an Ar atmosphere at rt, the mixture was dissolved in anhydrous pyridine followed by the addition of pivaloyl chloride. The reaction mixture stirred overnight and was monitored by TLC. The solvent was then removed and the remaining residue was redissolved in a solution of pyridine and water (9:1) containing 22 mg of iodine. After stirring for 5 h, the reaction was quenched with $Na_2S_2O_3$. The solvent was then removed under reduced pressure and the residue was

directly applied to silica gel chromatography to give **33a** (47.6 mg, 75% as an Et_3N salt). ^1H NMR for **33a** (500 MHz, $CDCl_3$), δ 7.37–6.96 (m, 12H), 6.92–6.52 (m, 12H), 5.41–5.30 (m, 5H), 5.27–3.91 (complex, 28H), 3.87–3.69 (m, 18H), 3.66–2.85 (complex, 17H), 2.37–2.07 (complex, 5H), 2.05–1.90 (m, 7H), 1.67–1.56 (m, 4H), 1.36–1.18 (s, broad, 48H), 0.90–0.84 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, resolved signals): δ 167.76, 156.22, 132.42, 130.88, 129.95, 129.69, 129.31, 129.10, 128.78, 127.77, 72.89, 70.52, 69.62, 68.59, 68.14, 55.19, 54.92, 53.42, 50.58, 38.71, 31.92, 31.90, 31.82, 31.00, 30.34, 29.77, 29.70, 29.54, 29.40, 29.31, 29.20, 28.91, 27.22, 24.80, 23.72, 22.98, 22.68, 14.12, 10.96. ^{31}P NMR (160 MHz, $CDCl_3$): δ −2.74. HR ESI-MS: calcd. for $C_{108}H_{157}N_4O_{26}P$ [M-H]- m/z, 1956.0751; found, 1956.0769. Compound **32b** underwent an analogous procedure to give **33b** (17.3 mg, 53% as an Et_3N salt). ^1H NMR for **33b** (500 MHz, $CDCl_3$): δ 11.77 (broad s, 2.4 H, Et_3N^+H), 7.36–7.10 (m, 12H), 6.91–6.72 (m, 12H), 5.42–5.28 (m, 5H), 5.28–5.22 (m, 1H), 5.09–5.01 (d, J = 9.8 Hz, 1H), 4.88–4.79 (m, 3H), 4.79–4.73 (d, J = 7.3 Hz, 1-pos), 4.66–4.44 (m, 8H), 4.42–4.34 (m, 3H), 4.31–4.26 (m, 1H), 4.25–4.18 (m, 2H), 4.17–4.02 (m, 4H), 4.01–3.94 (t, J = 8.6 Hz, 1H), 3.88–3.82 (m, 2H), 3.81–3.71 (m, 18H), 3.69–3.59 (m, 4H), 3.58–3.49 (m, 4H), 3.49–3.42 (m, 3H), 3.42–3.35 (m, 2H), 3.35–3.23 (m, 6H), 3.08–3.01 (m, 12.7 H, Et_3N), 2.26–2.17 (t, J = 7.3 Hz, 4H), 2.04–1.94 (m, 8H), 1.58–1.47 (m, 4H), 1.40 (s, 9H), 1.35–1.29 (t, J = 7.3 Hz, 26.5H, Et_3N), 1.25 (broad s, 39H, overlaped with Et_3N), 0.90–0.84 (t, J = 7.0 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, resolved signals): δ 173.33, 173.01, 159.05, 158.98, 158.88, 158.73, 130.64, 129.95, 129.77, 129.72, 129.59, 129.49, 129.08, 128.26, 113.88, 113.67, 113.65, 113.59, 113.38, 101.11, 81.50, 80.56, 78.99, 78.71, 75.13, 74.60, 74.39, 74.25, 73.16, 71.88, 71.73, 70.73, 70.54, 69.83, 63.02, 55.23, 55.21, 55.18, 55.16, 52.75, 50.64, 45.78 (Et_3N CH_2), 34.21, 34.02, 31.89, 29.78, 29.69, 29.52, 29.31, 29.25, 29.22, 29.17, 29.13, 29.10, 28.48, 27.30, 27.21, 24.85, 24.82, 22.67, 14.12, 8.58 (Et_3N CH_3), 7.80. ^{31}P NMR (160 MHz, $CDCl_3$): δ −1.04. MALDI-TOF MS (negative mode) calcd. for $C_{108}H_{157}N_4O_{26}P$ [M-H]- m/z, 1956.1; found, 1955.8.

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-4-*O*-(2-(2-azidoethoxy) ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-stearyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (34)

A mixture of **32a** and **10** was coevaporated with anhydrous pyridine three times and then dried under reduced pressure for >2 h. Under an Ar atmosphere at rt, the mixture was dissolved in anhydrous pyridine followed by the addition of pivaloyl chloride. The reaction mixture stirred overnight and was monitored by TLC. The solvent was removed and the remaining residue was redissolved in a solution of pyridine and water (9:1) containing 22 mg of

iodine. After stirring for 5 h, the reaction was quenched with $Na_2S_2O_3$. The solvent was removed under reduced pressure and the residue was directly applied to silica gel chromatography to give **34** (15.2 mg, 36% but 76% based on recovered **32a**). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.03 (m, 12H), 6.91–6.62 (m, 12H), 5.43–3.89 (complex, 30H), 3.89–3.62 (m, 18H), 3.60–2.77 (complex, 15H), 2.37–2.03 (complex, 5H), 1.65–1.58 (s, broad, 4H), 1.36–1.09 (s, broad, 56H), 0.93–0.83 (m, 8H). ^{31}P NMR (160 MHz, $CDCl_3$): δ −2.74. HR ESI-MS: calcd. for $C_{108}H_{161}N_4O_{26}P$ [M-H]- *m/z*, 1960.1082; found, 1960.1058.

6-*O*-[2-*N*-(*tert*-Butylcarbamate)-2-deoxy-4-*O*-[2-(2-amidofluoriseneylethoxy)ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (4a), 6-*O*-[2-*N*-(*tert*-butylcarbamate)-2-deoxy-4-*O*-[2-(2-amidofluoriseneylethoxy)ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (4b), and 6-*O*-[2-*N*-(*tert*-butylcarbamate)-2-deoxy-4-*O*-(2-(2-aminoethoxy)ethyl)-3,6-di-*O*-(*p*-methoxybenzyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-stearyl-*sn*-glycerol)-phosphono]-2,3,4,5-tetra-*O*-(*p*-methoxybenzyl)-*myo*-inositol (5)

To **33a** (18.5 mg, 9.4 μmol) stirring in anhydrous CH_2Cl_2 (1.5 mL) was added zinc powder (61.4 mg, 0.94 mmol) and acetic acid (100 μL). The reaction was monitored by MALDI-TOF MS and showed completion after about 6 h. The reaction mixture was filtered and poured into a saturated aq. $NaHCO_3$ solution. The aq. layer was extracted four times with CH_2Cl_2 and the combined organic portions were dried over $MgSO_4$ before being concentrated under reduced pressure. The intermediate (15.5 mg, 8.0 μmol) was used directly for the next step without further purification. The product was dissolved in anhydrous CH_2Cl_2 (800 μL) followed by the addition of **7** (4.2 mg in 400 μL DMF) and one drop of Et_3N. The reaction stirred for 1 h before showing a complete reaction (MALDI-TOF MS). The solvent was removed under reduced pressure before purification by size exclusion chromatography to give **4a** (16.3 mg, 76% for two steps). MALDI-TOF MS (negative mode) for **4a**: calcd. for $C_{129}H_{169}N_2O_{32}P$ $[M-H]^-$ *m/z*, 2288.1; found, 2287.6. Compounds **33b** and **34** underwent an analogous procedure to provided **4b** (5.0 mg, 62% for two steps) and **5** (6.3 mg, 71% for two steps), respectfully. MALDI-TOF MS (negative mode) for **4b**: calcd. for $C_{129}H_{169}N_2O_{32}P$ $[M-H]^-$ *m/z*, 2288.1; found, 2287.6. MALDI-TOF MS (negative mode) for **5**: calcd. for $C_{129}H_{173}N_2O_{32}P$ $[M-H]^-$ *m/z*, 2292.2; found, 2291.5.

6-*O*-[2-Amino-2-deoxy-4-*O*-[2-(2-amidofluoriseneylethoxy)ethyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]-*myo*-inositol (1), 6-*O*-[2-amino-2-deoxy-4-*O*-[2-(2-amidofluoriseneylethoxy)ethyl)-β-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-oleoyl-*sn*-glycerol)-phosphono]-*myo*-inositol (2), and 6-*O*-[2-amino-2-deoxy-4-*O*-[2-(2-amidofluoriseney lethoxy)ethyl)-α-D-glucopyranosyl]-1-*O*-[(2,3-di-*O*-stearyl-*sn*-glycerol)-phosphono]-*myo*-inositol (3)

To **4a** (4.4 mg, 1.9 μmol) stirring in anhydrous CH_2Cl_2 (2 mL) at 0°C was added 1 drop of DBU. After stirring for 1 min, a solution of TFA (0.3 mL) and CH_2Cl_2 (2mL) was slowly added and the reaction was monitored by MALDI-TOF MS. After stirring for 1 h, the solvent and TFA were removed by coevaporation with toluene. The remaining residue was purified by size exclusion chromatography to give **1** (2.2 mg, 78%). ^{1}H NMR (600 MHz, $CDCl_3$:CD_3OD:D_2O [3:3:1], mixture of regioisomers): see supporting information. ^{31}P NMR (160 MHz, $CDCl_3$): δ −0.098. MALDI-TOF MS (negative mode): calcd. for $C_{76}H_{113}N_2O_{24}P$ $[M-H]^-$ m/z, 1467.7; found, 1467.2. Compounds **4b** and **5** underwent an analogous procedure to provide **2** (3.0 mg, 74%) and **3** (2.0 mg, 60%), respectfully. ^{1}H NMR of **2** and **3** (600 MHz, $CDCl_3$:CD_3OD:D_2O [3:3:1], mixture of regioisomers): see supporting information. ^{31}P NMR of **2** (160 MHz, $CDCl_3$): δ −0.050. MALDI-TOF MS (negative mode): calcd. for **2** $C_{76}H_{113}N_2O_{24}P$ $[M-H]^-$ m/z, 1467.7; found, 1467.7. ^{31}P NMR of **3** (160 MHz, $CDCl_3$): δ −0.043. MALDI-TOF MS (negative mode): calcd. for **3** $C_{76}H_{117}N_2O_{24}P$ $[M-H]^-$ m/z, 1471.7; found, 1471.3.

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