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Synthesis of a Bifunctionalized Glycosylphosphatidylinositol (GPI) Anchor Useful for the Study of GPI Biology

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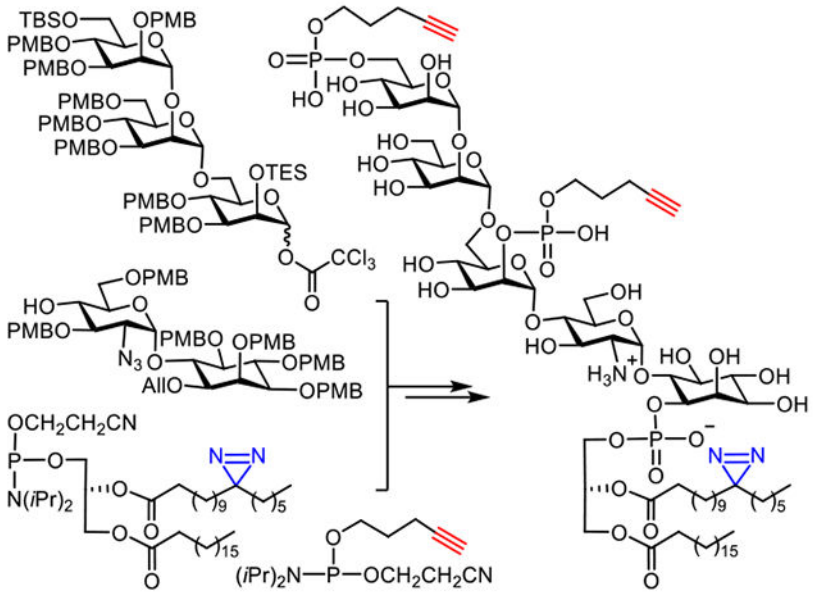
Abstract

A new, bifunctional glycosylphosphatidylinositol (GPI) derivative containing the highly conserved core structure of all natural GPI anchors with a photoactivable diazirine in the lipid chain and clickable alkynes in the glycan was synthesized by a convergent [3 + 2] glycosylation strategy with late stage protecting group manipulation and regioselective phosphorylation. The challenges of this synthesis were due to the presence of several distinctive functional groups in the synthetic target, which complicated the protection tactics, in addition to the inherent difficulties associated with GPI synthesis. This bifunctional GPI derivative can cross-react with molecules in proximity upon photoactivation and be subsequently labeled with other molecular tags via click reaction. Therefore, it should be a valuable probe for biological studies of GPIs, such as analysis of GPI-interacting membrane proteins, and gaining insights into their functional mechanisms.

Graphical Abstract



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A bifunctional glycosylphosphatidylinositol derivative containing photoactivable and clickable groups was synthesized by a convergent strategy.

Keywords

glycosylphosphatidylinositol; carbohydrate; glycolipid; photoactivation; click reaction

Introduction

Glycosylphosphatidylinositol (GPI) attachment to the protein *C*-terminus is one of the most common and important posttranslational protein modifications in eukaryotes.[1] GPI-linked proteins are anchored to the extracellular surface of cell membranes as outer leaflets through the lipid tails of GPIs and play a critical role in various biological and pathological processes,[2] such as signal transduction.[3] However, the lipid chains of all GPI anchors identified thus far, which are typically shorter than 24 carbons, are not long enough to span the entire cell membrane. Therefore, to furnish transmembrane signal transduction and various functions, GPI anchors need to interact with other components in the cell membrane. For example, utilizing fluorescence-labeled analogs of the core structure of GPIs as probes, we have demonstrated that GPIs can selectively interact with specific phosphatidylserine (PS) species in the cell membrane.[4] Despite this and many other progresses in the field, currently, there is still limited information concerning the molecules that interact with GPI anchors and a poor understanding of the functional mechanisms of GPIs and GPI-anchored proteins (GPI-APs). Our research program aims at developing new molecular tools to address this issue and explore GPI anchor-cell membrane interactions.

To this end, we have recently designed and synthesized a bifunctional derivative **1** (Figure 1) of the conserved pseudodisaccharide moiety of GPI anchors,[5] which can be utilized as a probe to study in general the interactions between GPI anchors and the cell membrane, especially the roles of inositol in GPI-cell membrane interactions. However, probe **1** had only a D-glucosamine linked to phosphatidylinositol (PI), while natural GPIs are much more complex. For example, the highly conserved core structure **2** (Figure 1) of all natural GPIs have a tetrasaccharide linked to the inositol 6-*O*-position of PI. It has been demonstrated that the glycans of GPI anchors play a pivotal role in determining the locations and functions of GPI-APs.[6] For instance, replacing the GPI anchor in Thy-1 with a transmembrane polypeptide domain results in a significant change in its function,[7] and altering the GPI anchor moiety of the prion protein (PrP^c) affects its activation and trafficking.[8] To gain more insights into and a better understanding of the functions and the functional mechanisms of GPI anchors in GPI-AP signaling as well as other bioactivities, more sophisticated GPI probes are required. Accordingly, here, we intend to design and develop GPI probes that can more closely resemble natural GPI anchors and establish an efficient method to access such synthetically challenging molecular probes.

Results and Discussion

In this study, we have designed a bifunctional GPI derivative **3** (Figure 1) as a probe that has several important and valuable structural features. First, probe **3** contains the entire core

structure of all natural GPI anchors. Second, it carries two types of functional groups that are orthogonal and can be independently modulated. On the one hand, the diazirine group attached to the lipid in **3** can be photoactivated to cross-react with molecules[9] interacting with it or being in its proximity. On the other hand, the alkynyl groups attached to its glycan can be selectively modified by molecules carrying an azido group via click reactions[10] to facilitate the introduction of other molecular tags, such as affinity tags for the isolation of proteins cross-linked to **3**. Therefore, **3** can be employed to cross-react with and pull-down membrane proteins interacting with GPIs and study the overall role of GPIs in GPI-AP signaling. Third, **3** has two alkynyl phosphate groups attached to the 2-*O*- and 6-*O*-positions of mannose (Man)-I and -III residues, respectively, of the glycan. All GPI-APs have the polypeptide chain linked to the Man-III 6-*O*-position via a phosphoethanolamine (PEA) bridge, and many natural GPI anchors have a PEA linked to the Man-I 2-*O*-position. The sizes of the designed alkynyl phosphate group and PEA are very similar, thus replacing the PEA moieties with alkynyl phosphate groups will cause minimal structural changes. As a result, probe **3** should be able to closely mimic natural GPIs, which is a very useful feature for molecular probes. Fourth, probes **1** and **3** contain the same lipids, thus by comparing the proteins pulled down by these two probes it would be possible to disclose how the inositol residue, PEA moiety, and glycan structure of GPI anchors affect their interactions with the cell membrane.

Our retrosynthetic plan for **3** is outlined in Scheme 1. A significant challenge in the synthesis of molecules like **3** is related to the protecting tactics because the synthetic target contains several fragile functional groups, including diazirine, alkyne and ester, incompatible with the deprotection conditions for protecting groups (*e.g.*, acetyl and benzyl groups) commonly used for the permanent hydroxyl group protection. To address this issue, we decided to employ the *para*-methoxybenzyl (PMB) group for permanent hydroxyl group protection, as PMB ethers are easily cleaved by mild oxidants, such as ceric ammonium nitrate (CAN) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[11] or under mildly acidic conditions,[12] *e.g.*, by using 10% trifluoroacetic acid (TFA). This led to fully protected **4**, in which the amino group of D-glucosamine ($GlcNH_2$) was protected with the *tert*-butylcarbonyl (Boc) group, also removable with mild acids, whereas the phosphate groups are protected with the 2-cyanoethyl group easily removable using mild bases. It was anticipated that their deprotection conditions would not affect the functional groups in **3**. Disconnection of the phosphate linkages in **4**, which meant to install the phosphates at late stages during the synthesis, resulted in the core pseudopentasaccharide skeleton **5** and phosphoramidites **6** and **7**, which were used as phosphorylating reagents and could be readily synthesized by reported procedures.[5] Next, disconnection of the glycosidic bond between Man-I and $GlcNH_2$ in **5** afforded trisaccharide **8** and psudodisaccharide **9**, leading to a convergent [3 + 2] assembly strategy for **5**. Another consideration for this synthetic strategy is that the construction of α-mannosidic linkages is relatively easy, thus the coupling reaction between **8** and **9** was expected to be straightforward.

Scheme 2 illustrates the synthesis of pseudopentasaccharide **5** from **8** and **9**. First, trimannosyl trichloroacetimidate **8** as a glycosyl donor was prepared from **10**, **11**, and **12** by a reported protocol, involving multiple transformations, with an overall yield similar to

that of the literature.[13] In the meantime, pseudodisaccharide **9** was prepared from **13**[13] and **14**.[13] Glycosylation of **14** with **13** in diethyl ether with TMSOTf as the promoter[14] produced a 1:1 mixture of α- and β-anomers, which were difficult to separate. Therefore, the mixture was directly subjected to the next reaction, *i.e.*, fluoride-mediated desilylation using tetrabutylammonium fluoride (TBAF), to afford the desired product **9** that was readily purified by silica gel chromatography as a single isomer in a 39% yield over two steps. The anomeric configuration of **9** was confirmed by its ^{1}H NMR data, which had a small coupling constant (J = 3.6 Hz) between H-1 and H-2 for the $GlcN_3$ residue, while the other product (*i.e.*, β-anomer) had a large coupling constant (J = 7.5 Hz). The glycosylation of **9** with **8** under the promotion of TMSOTf went smoothly to produce **5** in a good yield (58%). The α-configuration of the newly generated mannosyl linkage in **5** was verified by its anomeric $^{1}H–^{13}C$ coupling constant ($J_{H1–C1}$ =170 Hz for all mannosyl residues).

Having **5** in hand, the stage was set for selective manipulations of the temporary protecting groups and consecutive phosphorylation using phosphoramidites **6** and **7** to get **3** (Scheme 3). First, **5** was subjected to a one-pot two-step protocol to remove the allyl (All) group at the inositol 1-*O*-position, including $[Ir(COD)(PMePh_2)_2]PF_6$-mediated olefin rearrangement and subsequently HgO-promoted hydrolysis of the resultant vinyl ether.[15] Thereafter, the azido group was swapped for the Boc-protected amino group upon reduction with dithiol in the presence of diisopropylethylamine (DIPEA) and then chemoselective acylation of the resultant free amine using Boc anhydride and Et_3N to give **15** in a 45% yield over three steps. It is worth noting that, according to our experience and literature results,[16] it is better to swap the azido group for a Boc-protected amino group before inositol 1-*O*-phospholipidation because the phospholipid can sometimes affect the azide reduction, probably due to increased steric hindrance. Subsequently, **15** was phospholipidated at the inositol 1-*O*-position using phosphoramidite **7**, which was prepared by a reported procedure,[5] by the well-established one-pot two-step protocol,[17] utilizing tetrazole as the catalyst to promote the coupling reaction and then *t*-butylperoxide ($tBuO_2H$) to oxidize the resultant phosphine, to provide **16** in a good yield (66%). Compound **16** was obtained as an inseparable mixture of two epimers (in 1:1 ratio) originated from the stereogenic phosphorus atom, which complicated the NMR spectra of **16**. However, both isomers were useful for the synthesis of the final product because the chirality of the phosphorus atom would disappear after deprotection of the phosphate.

Next, the silyl groups protecting Man-I and -III in **16** were removed with $Et_3N{\cdot}3HF$ to provide **17** (78%) that contained two free hydroxyl groups. Double *O*-phosphorylation of **17** by means of the above-described one-pot two-step protocol using a large excess of (12 equiv) phosphoramidite **6**, which was easily prepared from 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite and 4-pentyn-1-ol, went smoothly to produce **4** (67%) as a mixture of multiple diastereomers originated from three phosphorus atoms. As a result, the NMR spectra of **4** became complex, whereas its MS spectrum was clean and clearly verified the successful double phosphorylation. Separation of the different isomers was unnecessary because the chirality of the phosphorus atoms would disappear after phosphate deprotection. Finally, the global deprotection of **4** was accomplished in two steps, including treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to remove all the cyanoethyl

groups protecting the phosphates and then treatment with 10% TFA in dichloromethane to remove the Boc and PMB groups protecting the amino and hydroxyl groups, to finally afford the synthetic target **3** in a 59% yield. The reaction progress was monitored with MS, and the final product was purified by size exclusion chromatography using a Sephadex LH-20 column and characterized with NMR and high-resolution (HR) MS data.

Conclusion

In summary, we have designed and synthesized a new, bifunctional GPI derivative **3** that has a photoactivable diazirine in the lipid and clickable alkynes in the highly conserved core glycan. This represents the first report of a bifunctional GPI derivative containing a photoreactive molecular label and the entire core structure of natural GPI anchors. The challenges of this synthesis are associated with the presence of multiple functional groups in the final synthetic target, in addition to the inherent difficulties of GPI synthesis.[18] The synthetic target was eventually achieved by a streamlined, convergent [3 + 2] glycosylation strategy and late-stage protecting group manipulation and phosphorylation. This strategy may also be applicable to other functionalized GPI derivatives. Compound **3**, which contains the entire core structure of all GPI anchors, should be a better mimic of natural GPI anchors than our first-generation probe **1** containing only a pseudodisaccharide and be useful for the study of GPI biology. Biological studies using both probes (*e.g.*, membrane protein pull-down and proteomic analysis) are in progress in our lab. These probes, as well as other GPI probes that are currently under development in our lab, will form a powerful toolbox. A comparison of the results obtained with these two probes and that obtained with other GPI probes and glycolipids without GPI motifs will provide more insights into the functions of GPI anchors, *e.g.*, how the inositol residue and the glycan structure of GPI anchors affect their interactions with cell membrane and thereby GPI-regulated signal transduction.

Experimental Section

Synthesis of 2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-1-O-allyl-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (9).

To a stirred mixture of glycosyl donor **13** (391 mg, 0.55 mmol), glycosyl acceptor **14** (300 mg, 0.43 mmol), and freshly activated MS 4A (400 mg) in dry Et_2O (6.0 mL) was added TMSOTf (10 μL, 53 μmol) at −10 °C under an N_2 atmosphere. After being stirred for 30 min, the mixture was neutralized with Et_3N, filtered, and concentrated under a vacuum. The residue was subjected to silica gel column chromatography with 10% EtOAc in hexane as the eluent to afford an α,β-mixture of the pseudodisaccharide as colorless syrup. To this anomeric mixture (0.48 g, 0.39 mmol) dissolved in THF (6 mL) was added 1.0 M TBAF in THF (0.58 mL, 0.58 mmol) at 0 °C. The solution was stirred at rt for 3 h and concentrated in vacuum. The residue was purified by silica gel column chromatography with 25-30% EtOAc in hexane as the eluent to provide **9** (187 mg, 39% over 2 steps) and the β-anomer (198 mg, 42%), both as colorless syrup. **9:** R_f = 0.35 (40% EtOAc in Hex). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.36-7.33 (m, 4 H), 7.27-7.14 (m, 8 H), 6.90-6.84 (m, 8 H), 6.80-6.77 (m, 4 H), 5.97 (m, 1 H, All-2), 5.70 (d, J = 3.6 Hz, 1 H, $GlcN_3$-1), 5.29-5.26 (m, 1 H, All-3), 5.20-5.18 (m, 1 H, All-3), 4.94 (d, J = 10.6 Hz, 1 H), 4.89 (d, J = 10.6 Hz, 1 H), 4.84 (d, J = 10.6

Hz, 1 H), 4.80 (d, J = 10.6 Hz, 1 H), 4.76 (s, 2 H), 4.73 (d, J = 10.6 Hz, 1 H), 4.63 (d, J = 10.6 Hz, 1 H), 4.60 (d, J = 11.5, Hz, 1 H), 4.56 (d, J = 11.5, Hz, 1 H), 4.30 (d, J = 11.6 Hz, 1 H), 4.23-4.18 (m, 2 H), 4.07 (t, J = 9.6 Hz, 1 H), 4.03-3.99 (m, 3 H, $GlcN_3$-5), 3.98 (t, J = 2.3 Hz, 1 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.77-3.76 (m, 1 H, $GlcN_3$-3), 3.75 (s, 3 H), 3.70 (td, J = 3.1, 9.9 Hz, 1 H, $GlcN_3$-4), 3.40 (t, J = 9.3 Hz, 1 H), 3.35 (ddd, J = 2.2, 4.3, 9.8 Hz, 2 H), 3.31-3.26 (m, 2 H, $GlcN_3$-6), 3.22 (dd, J = 3.4, 10.4 Hz, 1 H, $GlcN_3$-2), 2.14 (d, J = 3.4 Hz, 1 H, OH). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 159.4, 159.2, 159.1, 159.0 (2 C), 158.9, 134.3, 130.9 (2 C), 130.6, 130.4 (2 C), 130.1, 129.8 (2 C), 129.5 (2 C), 129.4 (2 C), 129.2 (4 C), 129.1 (2 C), 116.9, 113.9 (2 C), 113.8 (2 C), 113.7 (4 C), 113.6 (4 C), 97.4 ($GlcN_3$-1), 81.9, 81.7 ($GlcN_3$-5), 81.3, 80.6, 79.0 ($GlcN_3$-3), 75.3, 75.2, 74.9, 74.4, 73.6, 73.0, 72.5, 72.4, 72.3 ($GlcN_3$-4), 70.8, 69.3, 68.9 ($GlcN_3$-6), 62.9 ($GlcN_3$-2), 55.3 (3 C), 55.2 (3 C). HR ESI-TOF MS calcd for m/z $C_{63}H_{77}N_3O_{16}$ $[M + NH_4]^+$, 1145.5329, found 1145.5375. The β-anomer of **9**: R_f = 0.31 (40% EtOAc in Hex). ^1H NMR ($CDCl_3$, 600 MHz): δ 7.35-7.24 (m, 12 H), 6.92-6.80 (m, 12 H), 5.88-5.82 (m, 1 H, All-2), 5.27-5.24 (m, 1 H, All-3), 5.08-5.06 (m, 1 H, All-3), 4.95 (d, J = 10.0 Hz, 1 H), 4.91 (d, J = 10.2 Hz, 1 H), 4.85-4.82 (m, 3 H, $GlcN_3$-1), 4.76-4.73 (m, 5 H), 4.55-4.53 (m, 3 H), 4.48 (d, J = 11.5, Hz, 1 H), 4.36 (t, J = 9.4 Hz, 1 H), 4.26-4.23 (m, 1 H), 4.04-4.00 (m, 2 H, $GlcN_3$-6), 3.96 (t, J = 2.1 Hz, 1 H), 3.83 (s, 3 H), 3.82 (m, 6 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.72 (dd, J = 4.8, 10.8, Hz, 1 H, $GlcN_3$-4), 3.66-3.63 (m, 2 H), 3.53 (t, J = 9.3 Hz, 1 H, $GlcN_3$-5), 3.36-3.32 (m, 2 H, $GlcN_3$-2), 3.28-3.25 (m, 1 H,), 3.20-3.17 (m, 1 H, $GlcN_3$-3). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 159.4, 159.2, 159.1 (2 C), 159.0, 158.9, 135.5, 131.2, 131.1, 130.9, 130.5, 130.2, 130.0, 129.8 (2 C), 129.7 (2 C), 129.5 (4 C), 129.4 (2 C), 129.2 (2 C), 115.8, 113.9 (2 C), 113.8 (4 C), 113.7 (4 C), 113.4 (2 C), 101.0 ($GlcN_3$-1), 83.7 ($GlcN_3$-5), 82.0 ($GlcN_3$-3), 81.7, 80.4, 78.7, 78.0 ($GlcN_3$-4), 75.2, 75.0, 74.9, 74.6, 73.6, 73.5 (2 C), 73.0, 72.3, 72.1 ($GlcN_3$-6), 70.0, 66.4 ($GlcN_3$-2), 55.3 (3 C), 55.2 (3 C). HR ESI-TOF MS calcd for m/z $C_{63}H_{77}N_3O_{16}$ $[M + NH_4]^+$, 1145.5329, found 1145.5373.

Synthesis of 6-O-tert-butyldimetylsilyl-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→2)-3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→6)-3,4-di-O-(para-methoxybenzyl)-2-O-triethylsilyl-α-D-mannosyl-(1→4)-2-azido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-1-O-allyl-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (5).

A mixture of trichloroacetimidate glycosyl donor **8** (205 mg, 0.11 mmol), glycosyl acceptor **9** (70 mg, 0.06 mmol), and MS 4A (200 mg) in anhydrous CH_2Cl_2 (5 mL) was stirred under an Ar atmosphere at rt for 1 h. After cooling to 0 ºC, TMSOTf (9.0 μL, 0.05 mmol) was added, and the mixture was stirred for 10 min. The reaction was quenched with Et_3N, which was followed by filtration through Celite to remove MS, concentration in vacuum, and purification of the residue by silica gel column chromatography to provide **5** (101 mg, 58%) as colorless syrup. R_f = 0.3 (40% EtOAc in Hex). ^1H NMR (600 MHz, $CDCl_3$): δ 7.33-7.29 (m, 4 H), 7.26-7.05 (m, 24 H), 6.88-6.70 (m, 28 H), 5.94-5.87 (m, 1 H, All-2), 5.76 (d, J = 3.4 Hz, 1 H, $GlcN_3$-1), 5.27-5.24 (m, 1 H, All-3), 5.22 (s, 1 H, Man-III-1), 5.17-5.15 (m, 1 H, All-3), 5.03 (s, 1 H), 4.87 (d, J = 10.8 Hz, 2 H), 4.81-4.71 (m, 10 H, Man-I-1), 4.62 (d, J = 10.2 Hz, 1 H), 4.57-4.51 (m, 5 H), 4.48-4.39 (m, 7 H), 4.35-4.23 (m, 10 H), 4.12-4.08 (m, 2 H), 4.03-3.95 (m, 6 H), 3.88-3.85 (m, 6 H), 3.83 (s, 3H), 3.80 (s, 3

H), 3.79 (s, 3 H), 3.77 (s, 3 H), 3.76 (s, 9 H), 3.75 (s, 3 H), 3.73 (s, 6 H), 3.71-3.70 (m, 3 H), 3.67 (s, 3 H), 3.65 (s, 3 H), 3.63 (s, 3 H), 3.56 (dd, J = 3.8, 11.0 Hz, 1 H), 3.46-3.45 (m, 6 H), 3.38 (t, J = 9.4 Hz, 2 H), 3.33 (d, J = 9.7 Hz, 2 H), 3.25 (dd, J = 3.7, 10.2 Hz, 1 H), 0.87 (s, 9 H, TBS-*t*-Bu), 0.81 (t, J = 7.9 Hz, 9 H, 3×CH_3 TES), 0.48-0.39 (m, 6 H 3×CH_2 TES), 0.05 (s, 3 H, TBS), 0.03 (s, 3 H, TBS). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 159.2, 159.1 (2 C), 159.0 (4 C), 158.9 (6 C), 158.8, 134.3, 131.4, 131.1 (2 C), 131.0, 130.9 (2 C), 130.7 (2 C), 130.6 (2 C), 130.5 (3 C), 130.2, 129.5 (4 C), 129.4 (8 C), 129.3 (10 C), 129.2 (4 C), 129.0 (2 C), 116.9, 113.8 (4 C), 113.7 (8 C), 113.6 (6 C), 113.5 (8 C), 113.4 (2 C), 99.6 (Man-I-1), 98.5 (Man-III-1), 97.3 ($GlcN_3$-1), 81.9, 81.6, 81.1, 80.7, 80.4, 80.0, 79.4 (2 C), 75.3, 75.2, 75.0, 74.6 (2 C), 74.4, 74.3, 74.1, 73.5 (3 C), 73.4, 73.1, 72.8 (2 C), 72.6, 72.4 (2 C), 72.2 (2 C), 72.1, 72.0, 71.9, 71.6, 70.9, 70.8, 70.3, 69.8, 68.8, 68.5, 67.1, 63.4, 62.4, 55.3 (2 C), 55.2 (7 C), 55.1 (3 C), 55.0 (2 C), 26.0 (3 C), 18.3, 6.9 (3 C, TES), 4.9 (3 C, TES), −4.9 (TBS), −5.2 (TBS): HR ESI-TOF MS: calcd for *m/z* $C_{157}H_{199}N_4O_{39}Si_2$ $[M + NH_4]^{2+}$, 1420.1821; found, 1420.1775. Note: Similar to that observed in the literature,[13b] one of the mannose 1-*O*-positions was suppressed in the NMR spectra of this compound, which showed up in all the products after the next step of transformation.

Synthesis of 6-O-tert-butyldimetylsilyl-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→2)-3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→6)-3,4-di-O-(para-methoxybenzyl)-2-O-triethylsilyl-α-D-mannosyl-(1→4)-2-(tert-butoxycarbonyl)amido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol (15).

A solution of $[Ir(COD)(PMePh_2)_2]PF_6$ (2.94 mg, 3.48 μmol) in anhydrous THF (3 mL) was stirred under H_2 at rt until the color turned from red to colorless to pale yellow in 10 min. After the H_2 atmosphere was exchanged with Ar, **5** (75.0 mg, 26.7 μmol) in THF (2.5 mL) was added slowly. TLC showed that the isomerization was complete after stirring at rt for 1 h. The reaction was concentrated in vacuum, and the residue was dissolved in acetone and H_2O (9:1, 4 mL) and treated with $HgCl_2$ (37.2 mg, 0.13 mmol) and HgO (1.0 mg, 3.5 μmol). The mixture was stirred at rt under an Ar atmosphere for 30 min and concentrated in vacuum to remove acetone. The crude product was diluted with CH_2Cl_2 and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuum. To this intermediate (50.0 mg, 18.0 μmol) dissolved in CH_3OH and CH_2Cl_2 (2:1, 3 mL) was added 1,3-propane dithiol (39.0 μL, 0.036 mmol) and DIPEA (63.0 μL, 0.036 mmol) at rt. The solution was stirred at rt for 48 h. After the starting material was completely consumed as indicated by TLC, the reaction mixture was filtered through a Celite pad and concentrated in vacuum. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and brine, and the solution was dried over Na_2SO_4 and concentrated in vacuum. The crude product (37.0 mg, 13.5 μmol) was dissolved in CH_3OH and CH_2Cl_2 (1:1, 3.0 mL), and then trimethylamine (7.5 μl, 0.05 mmol) was added, followed by Boc anhydride (6.2 mg, 0.027 mmol) at rt. After the reaction mixture was stirred for 40 h, it was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuum. The residue was purified with silica gel column chromatography to afford **15** (34.0 mg, 45% over 3 steps) as a colorless syrup. R_f = 0.2 (50% EtOAc in Hex). ^1H NMR (600 MHz, $CDCl_3$): δ 7.27-7.02 (m, 28 H), 6.88-6.70 (m, 28 H), 5.53 (d, J = 9.9 Hz, 1 H, NH), 5.28 (s, 1 H, $GlcN_3$-1), 5.24 (s, 1 H, Man-III-1),

4.98-4.97 (m, 2 H, Man-I-1), 4.85-4.79 (m, 5 H, Man-II-1), 4.72-4.69 (m, 4 H), 4.66-4.57 (m, 5 H), 4.55-4.53 (m, 3 H), 4.50-4.42 (m, 6 H), 4.40 (d, J = 10.6 Hz, 1 H), 4.36-4.23 (m, 8 H), 4.14-4.13 (m, 1 H), 4.07-4.03 (m, 2 H), 3.98-3.95 (m, 3 H), 3.90-3.83 (m, 8 H), 3.82 (s, 3 H), 3.80 (s, 6 H), 3.79 (s, 3 H), 3.76 (s, 3 H), 3.74 (s, 9 H), 3.73 (s, 6 H), 3.73-3.67 (m, 5 H), 3.66 (s, 3 H), 3.65 (s, 3 H), 3.63 (s, 3 H), 3.62 (bs, 4 H), 3.56 (dd, J = 4.4, 11.4 Hz, 2 H), 3.49-3.46 (m, 3 H), 3.42 (d, J = 10.0 Hz, 1 H), 3.39 (dd, J = 1.7, 10.0 Hz, 1 H), 3.33 (t, J = 9.0 Hz, 1 H), 1.27 (s, 9 H, Boc-*t*-Bu), 0.88 (s, 9 H, TBS-*t*-Bu), 0.83 (t, J = 7.8 Hz, 9 H, 3×CH_3 TES), 0.47-0.38 (m, 6 H, 3×CH_2 TES), 0.06 (s, 3 H, TBS), 0.05 (s, 3 H, TBS). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 159.3 (2 C), 159.1, 159.0 (3 C), 158.9 (8 C), 158.8, 131.4, 131.0 (4 C), 130.9, 130.7 (4 C), 130.5, 130.4 (2 C), 130.3, 129.6 (2 C), 129.5 (4 C), 129.4 (4 C), 129.3 (12 C), 129.2 (4 C), 129.0 (2 C), 113.9 (4 C), 113.7 (8 C), 113.6 (12 C), 113.5 (2 C), 113.4 (2 C), 103.2 (Man-I-1), 99.5 (Man-II-1), 98.6 ($GlcN_3$-1), 98.4 (Man-III-1), 81.6 (2 C), 81.2 (2 C), 80.8, 80.5 (2 C), 80.2, 79.4 (2 C), 79.1, 75.2, 74.6 (2 C), 74.5 (2 C), 74.4 (2 C), 74.3 (3 C), 74.2, 73.5, 72.9 (3 C), 72.8 (2 C), 72.7, 72.1, 72.0 (3 C), 71.8, 71.6 (2 C), 71.0 (2 C), 68.8, 67.0, 62.5, 55.3 (4 C), 55.2 (6 C), 55.1 (3 C), 55.0, 28.3 (3 C), 26.0 (3 C), 18.2, 6.9 (3 C, *t*-Bu), 4.8 (3 C, *t*-Bu), −4.9 (TBS), −5.2 (TBS). HR ESI-TOF MS: calcd for *m/z* $C_{159}H_{205}N_2O_{41}Si_2$ $[M + NH_4]^{2+}$, 1437.1940; found, 1437.1925.

Synthesis of 6-O-tert-butyldimetylsilyl-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→2)-3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→6)-3,4-di-O-(para-methoxybenzyl)-2-O-triethylsilyl-α-D-mannosyl-(1→4)-2-(tert-butoxycarbonyl)amido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol 2-cynoethanol (R)-2-O-[11-(3-hexyl-3H-diazirin-3-yl)undecanoyl]-3-O-stearoyl-glycerol phosphate (16).

To a stirred mixture of **15** (36.0 mg, 12.7 μmol), tetrazole (0.45 M in acetonitrile, 0.28 mL, 0.126 mmol), freshly activated MS 4A in anhydrous CH_2Cl_2 and CH_3CN (3:1, 6 mL) was slowly added a solution of freshly prepared phosphoramidite **7** (86 mg in 1 mL of dry CH_2Cl_2, 0.10 mmol) under Ar at rt. After the reaction stirred at rt for 30 min, it was cooled to 0 ºC and *tert*-butyl hydroperoxide (5.5 M solution in decane, 46 μL, 0.25 mmol) was added. The solution was stirred at 0 ºC for 1 h and then warmed to rt and filtered through a Celite pad. The mixture was poured into saturated aqueous $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 three times. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography to give **16** (30 mg, 66%) as syrup and a ~1:1 mixture of two diastereomers. R_f = 0.25 (60% EtOAc in Hex). ^1H NMR (600 MHz, $CDCl_3$): δ 7.27-7.02 (m, 28 H), 6.88-6.69 (m, 28 H), 5.28-5.26 (m, 1 H, $GlcN_3$-1), 5.24-5.21 (m, 2 H, Man-III-1, Gly-2), 5.07-4.94 (m, 1 H, Man-I-1), 4.90-4.87 (m, 2 H), 4.81-4.79 (m, 4 H Man-II-1), 4.74-4.70 (m, 3 H), 4.66-4.60 (m, 3 H), 4.57-4.53 (m, 2 H), 4.49-4.48 (m, 1 H), 4.46-4.43 (m, 3 H), 4.40-4.37 (m, 2 H), 4.36-4.30 (m, 6 H), 4.29-4.18 (m, 6 H), 4.15-4.02 (m, 6 H), 4.0-3.97 (m, 2 H), 3.90-3.84 (m, 6 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 3.78 (s, 3 H), 3.77 (s, 3 H), 3.75 (s, 9 H), 3.74 (s, 6 H), 3.72-3.71 (m, 5 H), 3.65-3.64 (m, 9 H), 3.62-3.55 (m, 3 H), 3.54 (s, 3 H), 3.56 (s, 1 H), 3.47-3.32 (m, 5 H), 2.81-2.78 (m, 1 H), 2.67-2.58 (m, 2 H), 2.40-2.34 (m, 1 H), 2.28-2.26 (m, 4 H), 1.61-1.53 (m, 4 H), 1.35-1.31 (m, 13 H), 1.30-1.26 (m, 50 H), 1.12-1.04 (m, 4 H), 0.90-0.87 (m, 15 H), 0.83 (t, J = 7.8 Hz, 9 H), 0.44-0.39 (m, 6 H), 0.06-0.04 (m, 6 H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 173.1 (C=O), 172.7 (C=O), 159.2 (2 C), 159.1

(2 C), 159.0, 158.9 (5 C), 158.8 (5 C), 131.4, 131.0 (4 C), 130.8, 130.7 (2 C), 130.6 (3 C), 130.4, 129.6 (2 C), 129.4 (6 C), 129.3 (8 C), 129.2 (8 C), 129.1 (2 C), 129.0 (4 C), 113.9 (2 C), 113.8 (6 C), 113.7 (4 C), 113.6 (4 C), 113.5 (8 C), 113.4 (4 C), 103.1 (Man-I-1), 99.5 (Man-II-1), 98.5 (Man-III-1), 98.2 ($GlcN_3$-1), 81.4 (2 C), 81.2, 80.5 (2 C) , 80.1, 79.5 (2 C), 79.4 (2 C), 75.3, 74.6 (2 C), 74.5 (2 C), 74.3 (2 C), 74.2, 73.5 (2 C), 73.1, 72.8 (2 C), 72.6 (2 C), 71.9 (2 C), 71.6 (2 C), 70.9 (2 C), 68.9, 68.7 (2 C, Gly-2), 67.1, 62.5 (2 C), 61.4 (2 C), 55.3 (2 C), 55.2 (5 C), 55.1 (4 C), 55.0 (3 C), 46.4, 45.4, 34.1, 34.0 (3 C), 32.9, 31.9, 31.6, 29.7 (12 C), 29.6 (2 C), 29.5, 29.4, 29.3 (2 C), 29.2, 29.1 (2 C), 28.9, 28.4, 26.0 (3 C), 24.8 (3 C), 23.9, 23.8, 22.7, 22.5, 18.3, 15.5, 14.1 (3 C), 14.0 (2 C), 6.9 (3 C, *t*-Bu), 4.8 (3 C, *t*-Bu), −4.9 (TBS), −5.2 (TBS). $^{31}P\{^1H\}$ NMR ($CDCl_3$, 243 MHz): δ −2.03, −2.77. HR ESI-TOF MS: calcd for *m/z* $C_{201}H_{285}N_6O_{48}PSi_2^{2+}$ $[M + 2\ NH_4]^{2+}$, 1819.9685; found, 1819.9163.

Synthesis of 2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→2)-3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→6)-3,4-di-O-(para-methoxybenzyl)-α-D-mannosyl-(1→4)-2-(tert-butoxycarbonyl)amido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol 2-cynoethanol (R)-2-O-[11-(3-hexyl-3H-diazirin-3-yl)undecanoyl]-3-O-stearoyl-glycerol phosphate (17).

To a solution of **16** (17.0 mg, 4.7 μmol) in anhydrous THF-CH_3CN (1:1, 2 mL) was added Et_3N•3HF (1 mL) under Ar at rt. After stirring for 24 h at rt, the reaction was quenched by dropwise addition of saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography to provide **17** (12.5 mg, 78%) as a ~1:1 mixture of diastereomers and colorless syrup. ^1H NMR (600 MHz, $CDCl_3$): δ 7.22-7.10 (m, 28 H), 6.87-6.71 (m, 28 H), 5.29-5.26 (m, 1 H, Gly-2), 5.22-5.20 (m, 2 H, $GlcN_3$-1, Man-I-1), 5.14 (s, 1 H, Man-III-1), 5.09-5.08 (m, 1 H), 5.00-4.98 (m, 1 H, Man-II-1), 4.90-4.85 (m, 1 H), 4.86-4.67 (m, 8 H), 4.66-4.58 (m, 4 H), 4.57-4.49 (m, 4 H), 4.47-4.40 (m, 7 H), 4.36-4.27 (m, 5 H), 4.25-4.17 (m, 5 H), 4.17-3.97 (m, 7 H), 3.82, (s, 3 H), 3.78 (s, 9 H), 3.77 (s, 3 H), 3.75 (s, 6 H), 3.73 (s, 6 H), 3.71 (s, 6 H), 3.68 (s, 3 H), 3.63 (s, 6 H), 3.51-3.50 (m, 4 H), 3.47-3.30 (m, 8 H), 3.21-3.10 (m, 1 H), 2.79-2.63 (m, 4 H), 2.61-2.55 (m, 1 H), 2.28-2.24 (m, 4 H), 1.53-1.59 (m, 4 H), 1.39-1.38 (m, 5 H), 1.34-1.31 (m, 13 H), 1.26-1.21 (m, 50 H), 1.10-1.02 (m, 4 H), 0.90-0.87 (m, 6 H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 150 MHz): δ 173.0 (C=O), 172.7 (C=O), 159.2 (2 C), 159.1 (6 C), 159.0 (2 C), 158.9 (3 C), 158.8 (2 C), 130.9, 130.8 (2 C), 130.7 (3 C), 130.4 (2 C), 130.3 (2 C), 130.2 (2 C), 130.0 (2 C), 129.5 (8 C), 129.4 (8 C), 129.3 (2 C), 129.2 (2 C), 129.1 (4 C), 129.0 (4 C), 113.8 (4 C), 113.7 (8 C), 113.6 (12 C), 113.5 (4 C), 101.5 (Man-I-1), 99.3 (Man-II-1), 99.2 (Man-III-1), 98.2 ($GlcN_3$-1), 81.4 (2 C), 81.1, 80.1, 80.0, 79.9, 79.6, 79.5 (2 C), 79.3, 75.7, 75.3, 74.8, 74.6 (2 C), 74.5 (2 C), 73.5 (2 C), 72.9 (2 C), 72.7 (2 C), 72.5 (2 C), 72.1, 71.7 (2 C), 71.2, 70.8, 70.4, 69.4 (Gly-2), 68.9, 66.2, 65.4, 62.5 (2 C), 61.4 (2 C), 55.2 (8 C), 55.1 (4 C), 55.0 (2 C), 46.4, 45.6, 45.4, 34.0, 33.9, 32.9, 31.9, 31.5, 29.7 (10 C), 29.6 (2 C), 29.5, 29.4, 29.3, 29.2, 29.1, 28.9, 28.8, 28.5, 28.2, 25.7, 24.8, 23.9, 23.8, 23.7, 23.5, 23.2, 23.0, 22.8, 22.7 (3 C), 22.5, 15.5, 14.1, 14.0. $^{31}P\{^1H\}$ NMR ($CDCl_3$, 243 MHz) δ −2.17, −2.74. HR ESI-TOF MS: calcd for *m/z* $C_{189}H_{257}N_6O_{48}P$ $[M + 2\ NH_4]^{2+}$, 1705.8823; found, 1705.8760.

Synthesis of 6-O-(2-cyanoethyl pent-4-yn-1-yl phosphonyl)-2,3,4-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→2)-3,4,6-tri-O-(para-methoxybenzyl)-α-D-mannosyl-(1→6)-2-O-(2-cyanoethyl pent-4-yn-1-yl phosphonyl)-3,4-di-O-(para-methoxybenzyl)-α-D-mannosyl-(1→4)-2-(tert-butoxycarbonyl)amido-2-deoxy-3,6-di-O-(para-methoxybenzyl)-α-D-glucosyl-(1→6)-2,3,4,5-tetra-O-(para-methoxybenzyl)-myo-inositol 2-cynoethanol (R)-2-O-[11-(3-hexyl-3H-diazirin-3-yl)undecanoyl]-3-O-stearoyl-glycerol phosphate (4).

To a solution of **17** (12 mg, 0.003 mmol) and tetrazole (0.45 M in acetonitrile, 0.157 mL, 0.071 mmol) in anhydrous CH_2Cl_2 and CH_3CN (3:1, 3 mL) under Ar was slowly added a solution of freshly prepared **6** (10.1 mg in 0.5 mL dry CH_2Cl_2, 0.035 mmol) at rt. After the reaction was stirred at rt for 30 min, it was cooled to −40 ºC and *tert*-butyl hydroperoxide (5.5 M solution in decane, 26 μL, 0.142 mmol) as added. The solution was stirred at −40 ºC for 1 h, and then Me_2S (16 μL, 0.213 mmol) was added, and the stirring continued at −40 ºC for 1 h. The mixture was filtered through a Celite pad, and the solution was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 three times. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by silica gel column chromatography to give **4** (9 mg, 67%) as syrup and a mixture of diastereomers. R_f = 0.2 (30% EtOAc in Hex). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27-7.0 (m, 28 H), 6.90-6.67 (m, 28 H), 5.47-5.40 (m, 1 H), 5.28-5.19 (m, 2 H), 5.16-5.13 (m, 1 H), 4.91-4.82 (m, 4 H), 4.81-4.68 (m, 7 H), 4.68-4.45 (m, 10 H), 4.43.−4.27 (m, 11 H), 4.26-4.17 (m, 4 H), 4.17-4.07 (m, 7 H), 4.06-3.96 (m, 5 H), 3.95-3.85 (m, 5 H), 3.82 (s, 3 H), 3.81, (s, 3 H), 3.80 (s, 6 H), 3.78 (s, 3 H), 3.77 (s, 9 H), 3.74 (s, 6 H), 3.68-3.65 (m, 9 H), 3.58 (s, 3 H), 3.56-3.43 (m, 5 H), 3.38-3.31 (m, 1 H), 2.50-2.15 (m, 8 H), 2.10-1.93 (m, 3 H), 1.88-1.74 (m, 2 H), 1.58-1.54 (m, 4 H), 1.45-1.17 (m, 76 H), 1.12-1.02 (m, 4 H), 0.91-0.87 (m, 6 H). HR ESI-TOF MS: calcd for *m/z* $C_{205}H_{277}N_8O_{54}P_3$ $[M + 2\ NH_4]^{2+}$, 1904.9221; found, 1904.9269.

Synthesis of 6-O-(pent-4-yn-1-yl phosphonyl)-α-D-mannosyl-(1→2)-α-D-mannosyl-(1→6)-2-O-(pent-4-yn-1-yl phosphonyl)-α-D-mannosyl-(1→4)-2-amino-2-deoxy-α-D-glucosyl-(1→6)-myo-inositol (R)-2-O-[11-(3-hexyl-3H-diazirin-3-yl)undecanoyl]-3-O-stearoyl-glycerol phosphate (3).

To a solution of **4** (6 mg, 0.002 mmol) in CH_2Cl_2 (500 μL) was added a drop of DBU, and the mixture was stirred at rt for 1 h. Then, 20% TFA in CH_2Cl_2 (500 μL) was directly added to the reaction mixture to give a final concentration of ~10% TFA. After stirring for 30 min, the reaction mixture was co-evaporated with toluene 5 times, and the residue was purified by LH-20 column chromatography using MeOH, $CHCl_3$ and H_2O (3:3:1) as the eluent to give **3** (2.8 mg, 59% over 2 steps) as a glassy solid. ^{1}H NMR (600 MHz, $CDCl_3$:MeOD:H_2O, 3:3:1): δ 5.53 (s, 1 H, $GlcNH_2$-1), 5.27 (m, 1 H, Gly-2), 5.15 (s, 2 H, Man-I-1, Man-II-1), 4.99 (s, 1 H, Man-III-1), 4.23-4.17 (m, 2 H), 4.08 (s, 1 H), 4.04-3.99 (m, 2 H), 3.98-3.92 (m, 4 H), 3.88-3.77 (m, 4 H), 3.70-3.67 (m, 3 H) , 3.62-3.59 (m, 4 H), 3.88-3.77 (m, 4 H), 3.70-3.67 (m, 3 H), 3.62-3.59 (m, 2 H), 3.56-3.51 (m, 2 H), 3.49-3.45 (m, 1H), 2.36 (s, 2 H), 2.67-2.66 (m, 1 H), 2.43 (t, J = 7.4, Hz, 1 H), 2.36 (t, J = 7.4 Hz, 1 H), 2.34-2.28 (m, 4 H), 2.21 (t, J = 2.1 Hz, 1 H), 2.19 (t, J = 2.1 Hz, 1 H), 2.09-2.05 (m, 1 H), 1.89-1.79 (m, 3 H), 1.78-1.71 (m, 2 H), 1.69-1.54 (m, 4 H), 1.38-1.34 (m, 2 H), 1.33-1.24 (m, 50

H), 1.13-1.05 (m, 4 H), 0.92-0.86 (m, 6 H). $^{13}C\{^{1}H\}$ NMR (150 MHz, $CDCl_3$:MeOD:H_2O, 3:3:1): δ 102.3 (Man-III-1), 98.6 (Man-I-1, Man-II-1), 95.2 ($GlcNH_2$-1), 79.2, 77.4 (2 C), 76.8, 76.3, 73.1 (2 C), 72.9 (2 C), 72.8 (2 C), 72.7, 72.2, 71.8 (2 C), 71.0, 70.8 (2 C), 70.6 (2 C), 70.2 (Gly-2), 70.1, 69.8, 69.1 (2 C, CH-acetylene), 67.0, 64.5, 63.6, 62.8 (2 C), 61.1, 59.6, 54.3, 54.0, 48.5, 42.7, 39.0, 37.9, 33.9 (2 C), 32.8, 32.6, 31.6, 29.3 (10 C), 29.2 (2 C), 26.2, 24.8 (2 C), 23.7 (2 C, alpha to diazirine), 23.6, 22.4 (4 C), 22.3 (2 C), 14.4 (2 C), 14.0 (2 C, CH_3). $^{31}P\{^{1}H\}$ NMR ($CDCl_3$, 243 MHz): δ −0.08, −0.09. HR ESI-TOF MS: calcd for *m/z* $C_{79}H_{142}N_3O_{38}P_3$ $[M + 2H]^{2+}$, 916.9237; found, 916.9253.

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References

[1]. Ferguson MAJ, Williams AF, Annu. Rev. Biochem 1988, 57, 285–320. [PubMed: 3052274]

[2]. a)Thomas JR, Dwek RA, Rademacher TW, Biochemistry 1990, 29, 5413–5422; [PubMed: 2143679] b)McConville MJ, Ferguson MAJ, Biochem. J 1993, 294, 305–324; [PubMed: 8373346] c)Marmor MD, Julius M, J. Bio.l Regul. Homeost. Agents 2000, 14, 99–115.

[3]. a)Robinson PJ, in ADP-Ribosylation in Animal Tissues: Structure, Function, and Biology of Mono (ADP-ribosyl) Transferases and Related Enzymes (Eds.: Haag F, Koch-Nolte F), Springer US, Boston, MA, 1997, pp. 365–370;b)Kasahara K, Sanai Y, Glycoconjugate J. 2000, 17, 153–162.

[4]. Raghupathy R, Anilkumar AA, Polley A, Singh PP, Yadav M, Johnson C, Suryawanshi S, Saikam V, Sawant SD, Panda A, Guo Z, Vishwakarma RA, Rao M, Mayor S, Cell 2015, 161, 581–594. [PubMed: 25910209]

[5]. Mullapudi VB, Craig KC, Guo Z, J. Org. Chem 2022, 87, 9419–9425. [PubMed: 35766889]

[6]. a)Treumann A, Lifely MR, Schneider P, Ferguson MAJ, J. Biol. Chem 1995, 270, 6088–6099; [PubMed: 7890742] b)Schroter S, Derr P, Conradt HS, Nimtz M, Hale G, Kirchhoff C, J. Biol. Chem 1999, 274, 29862–29873; [PubMed: 10514467] c)Kim J, Surewicz K, Gambetti P, Surewicz VK, FEBS Lett. 2009, 583, 3671–3675; [PubMed: 19854187] d)Bate C, Tayebi M, Williams A, Biochem. J 2010, 428, 95–101. [PubMed: 20196773]

[7]. Tiveron MC, Nosten-Bertrand M, Jani H, Garnett D, Hirst EM, Grosveld F, Morris RJ, J. Cell Sci 1994, 107 (Pt 7), 1783–1796. [PubMed: 7983148]

[8]. Bate C, Williams A, J. Biol. Chem 2011, 286, 8752–8758. [PubMed: 21212283]

[9]. a)Dubinsky L, Krom BP, Meijler MM, Bioorg. Med. Chem 2012, 20, 554–570; [PubMed: 21778062] b)Moss RA, Acc. Chem. Res 2006, 39, 267–272; [PubMed: 16618094] c)Das J, Chem. Rev 2011, 111, 4405–4417. [PubMed: 21466226]

[10]. a)Kolb HC, Finn MG, Sharpless KB, Angew. Chem. Int. Ed 2001, 40, 2004–2021;b)Takayama Y, Kusamori K, Nishikawa M, Molecules 2019, 24, a172.

[11]. a)Ikeuchi K, Murasawa K, Ohara K, Yamada H, Org. Lett 2019, 21, 6638–6642; [PubMed: 31437002] b)Wright JA, Yu J, Spencer JB, Tetrahedron Lett. 2001, 42, 4033–4036.

[12]. Yan L, Kahne D, Synlett 1995, 523–524.

[13]. a)Swarts BM, Guo Z, Chem Sci 2011, 2, 2342–2352; [PubMed: 22163072] b)Swarts BM, Guo Z, J. Am. Chem. Soc 2010, 132, 6648–6650. [PubMed: 20423078]

[14]. Schmidt RR, Pure Appl. Chem 1989, 61, 1257–1270.

[15]. Oltvoort JJ, Boeckel C. A. A. v., Koning JHD, Boom J. H. v., Synthesis 1981, 305–308.

[16]. Xue J, Guo Z, J. Am. Chem. Soc 2003, 125, 16334–16339. [PubMed: 14692775]

[17]. Bannwarth W, Trzeciak A, Helv. Chim. Acta 1987, 70, 175–186.

[18]. a)Murakata C, Ogawa T, Tetrahedron Lett. 1991, 32, 671–674;b)Murakata C, Ogawa T, Carbohydr. Res 1992, 235, 95–114; [PubMed: 1473115] c)Mayer TG, Kratzer B, Schmidt RR, Angew. Chem. Int. Ed 1994, 33, 2177–2181;d)Mayer TG, Schmidt RR, Eur. J. Org. Chem

1999, 1153–1165;e)Pekari K, Tailler D, Weingart R, Schmidt RR, J. Org. Chem 2001, 66, 7432–7442; [PubMed: 11681958] f)Campbell AS, Fraser-Reid B, J. Am. Chem. Soc 1995, 117, 10387–10388;g)Lu J, Jayaprakash KN, Schlueter U, Fraser-Reid B, J. Am. Chem. Soc 2004, 126, 7540–7547; [PubMed: 15198601] h)Baeschlin DK, Chaperon AR, Charbonneau V, Green LG, Ley SV, Lucking U, Walther E, Angew. Chem. Int. Ed 1998, 37, 3423–3428;i)Ali A, Gowda DC, Vishwakarma RA, Chem. Commun 2005, 519–521;j)Tsai Y-H, Gçtze S, Azzouz N, Hahm HK, Seeberger PH, Silva DV, Angew. Chem. Int. Ed 2011, 50, 9961–9964;k)Tsai YH, Gotze S, Vilotijevic I, Grube M, Silva DV, Seeberger PH, Chem. Sci 2013, 4, 468–481;l)Guerrero PA, Murakami Y, Malik A, Seeberger PH, Kinoshita T, Varón Silva D, ACS Chem. Biol 2021, 16, 2297–2306; [PubMed: 34618440] m)Yashunsky DV, Borodkin VS, Ferguson MAJ, Nikolaev AV, Angew. Chem. Int. Ed 2006, 45, 468–474;n)Burgula S, Swarts BM, Guo Z, Chem. Eur. J 2012, 18, 1194–1201; [PubMed: 22189835] o)Lahmann M, Garegg PJ, Konradsson P, Oscarson S, Can. J. Chem 2002, 80, 1105–1111.

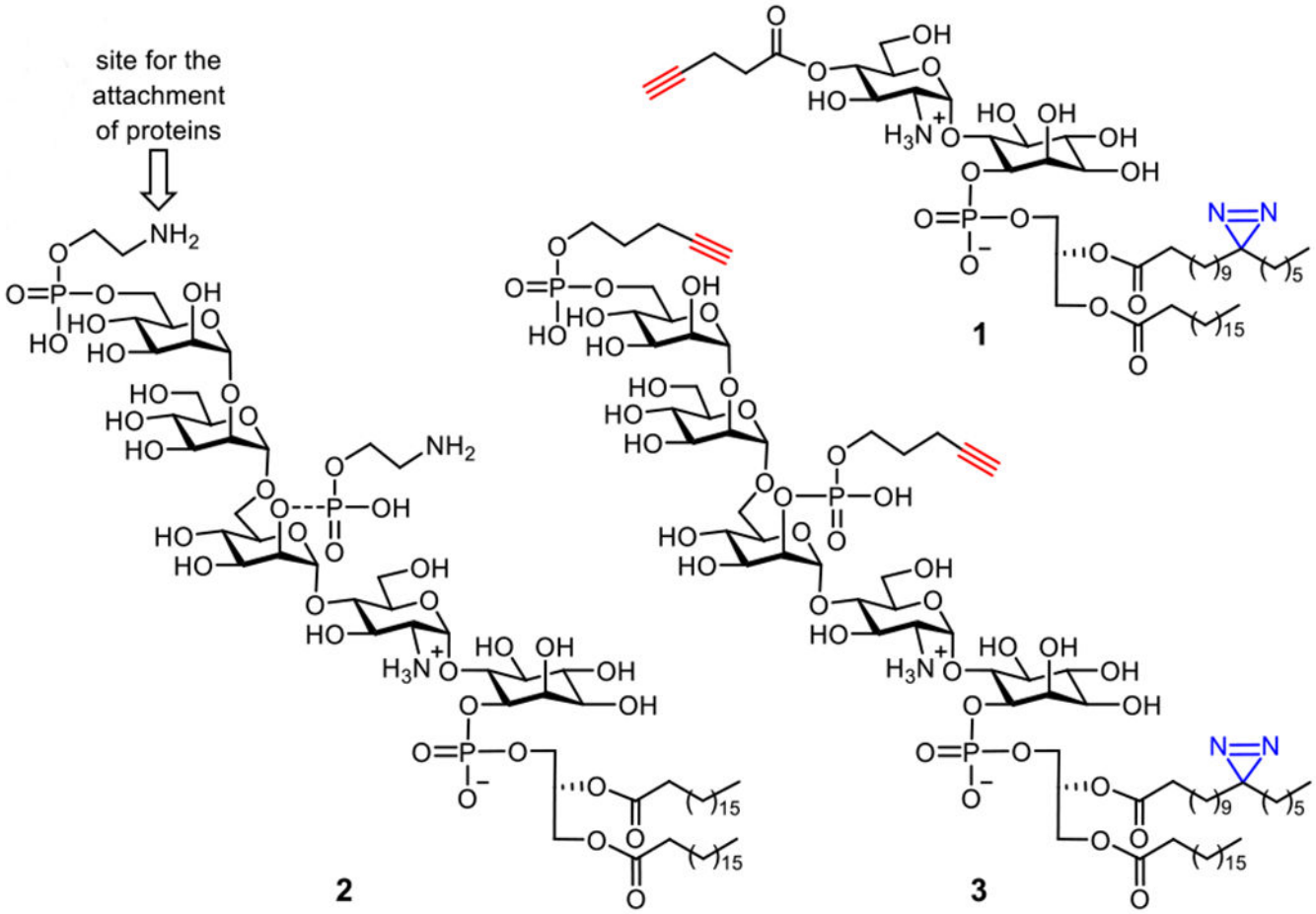
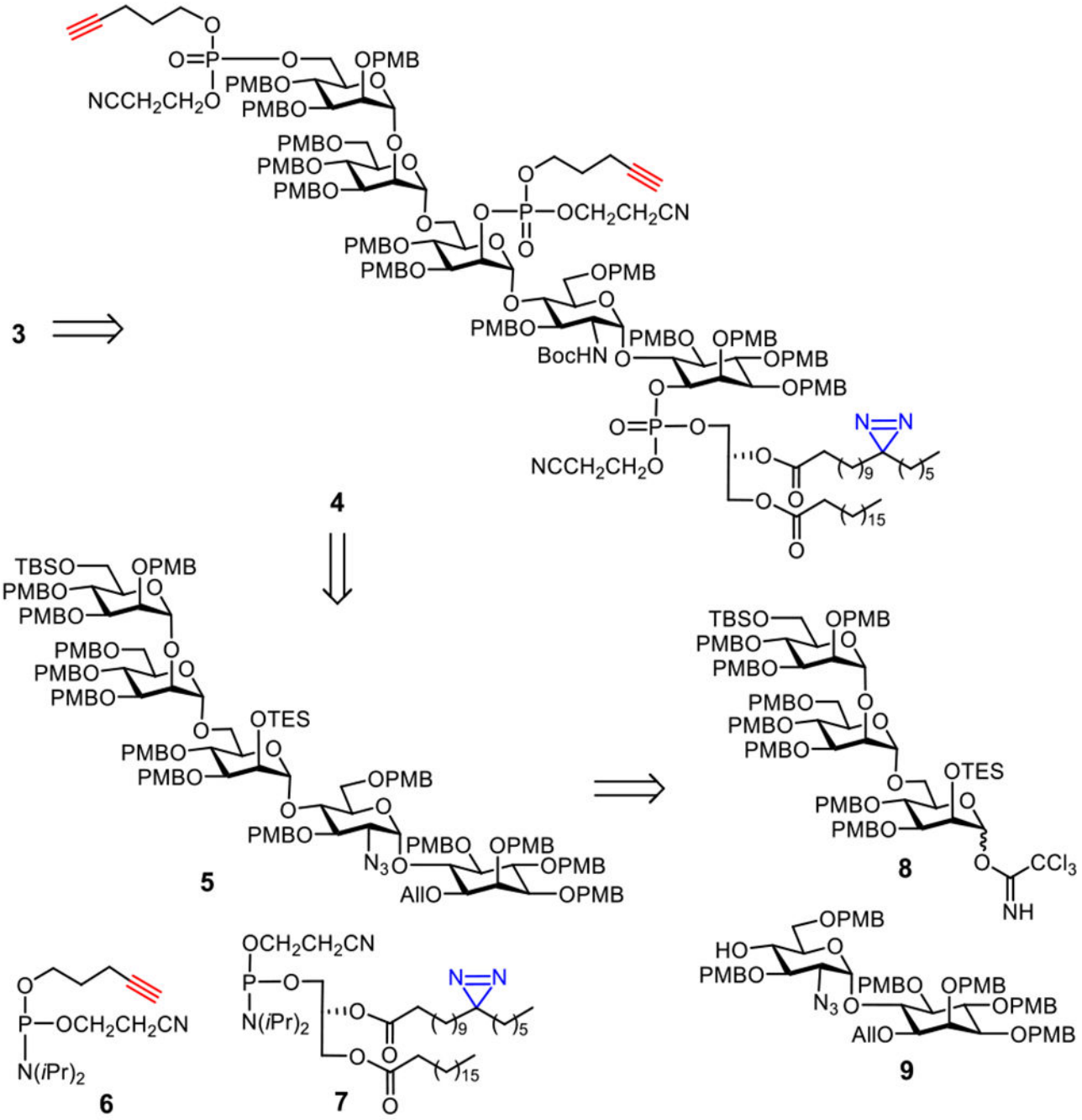
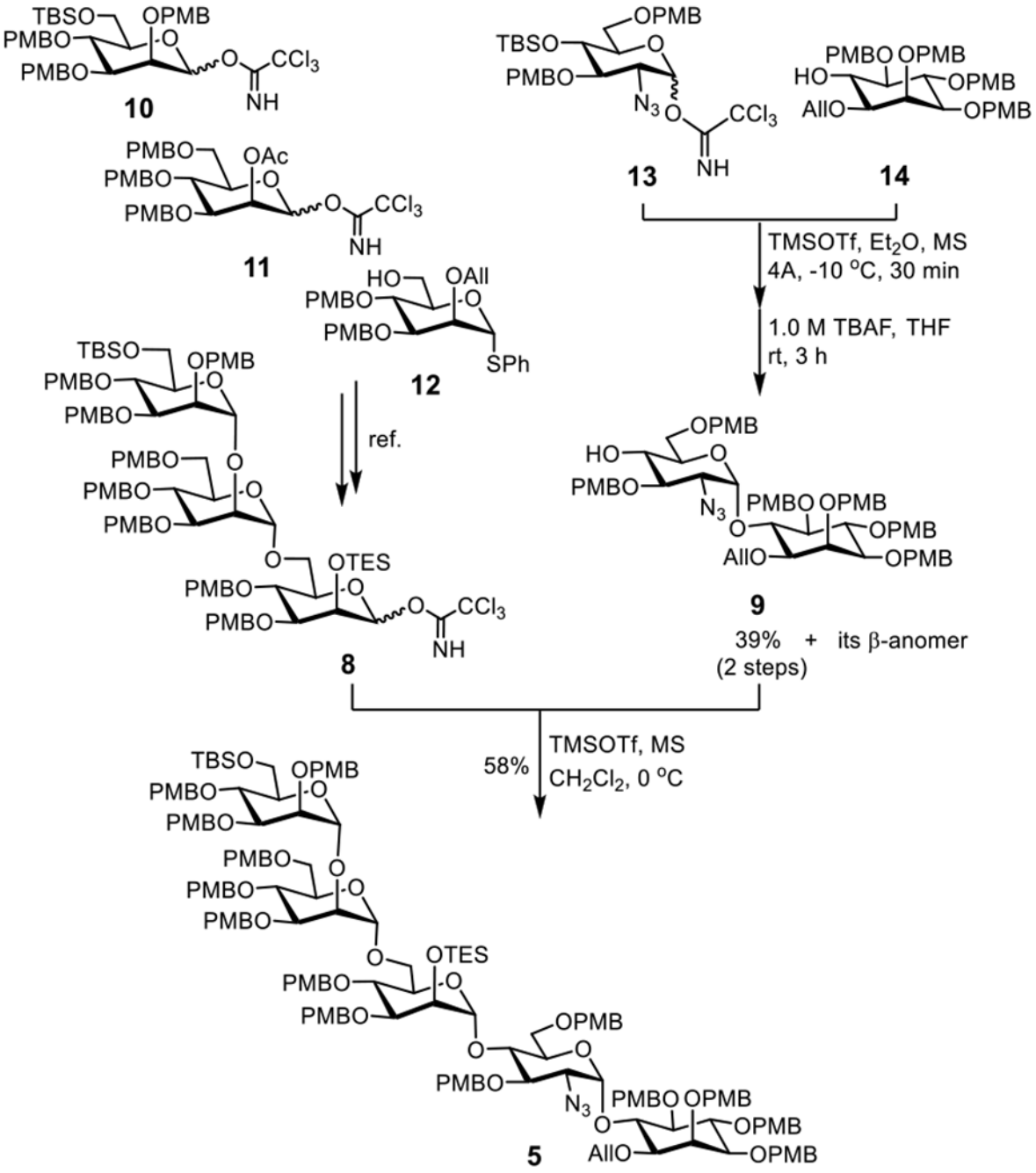


Figure 1.

The structures of reported pseudodisaccharide probe **1**, conserved core structure of all GPI anchors **2**, and the target GPI probe **3** that mimics the GPI core structure and contains two types of orthogonal functional groups, *i.e.*, photoreactive diazirine and clickable alkyne.

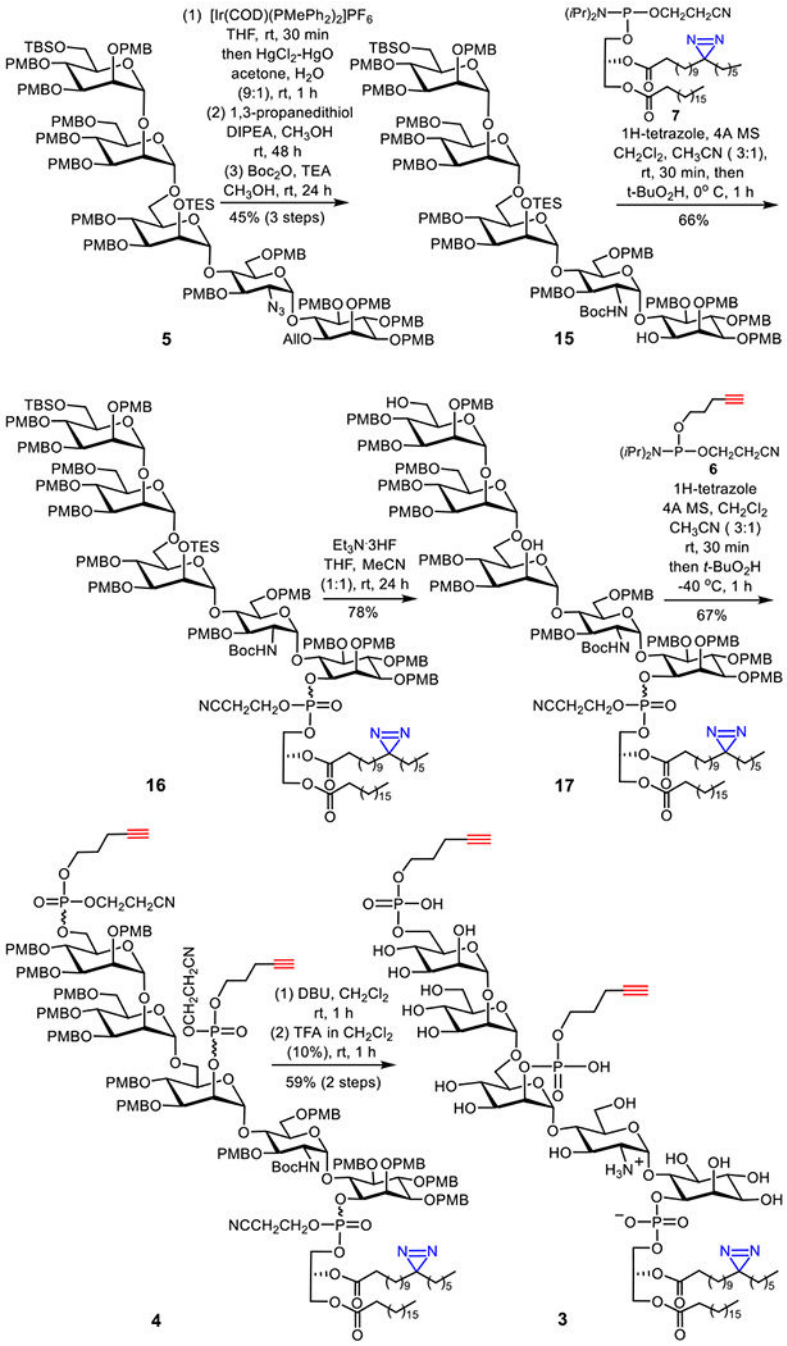


Scheme 1.
Retrosynthesis of the target molecule **3**.



Scheme 2.

Synthesis of the core pseudopentasaccharide **5** of GPI anchors.



Scheme 3.

Sequential phosphorylation of glycan **3** and global deprotection to give target molecule **1**.