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Synthesis of a Glycosylphosphatidylinositol Anchor Bearing Unsaturated Lipid Chains

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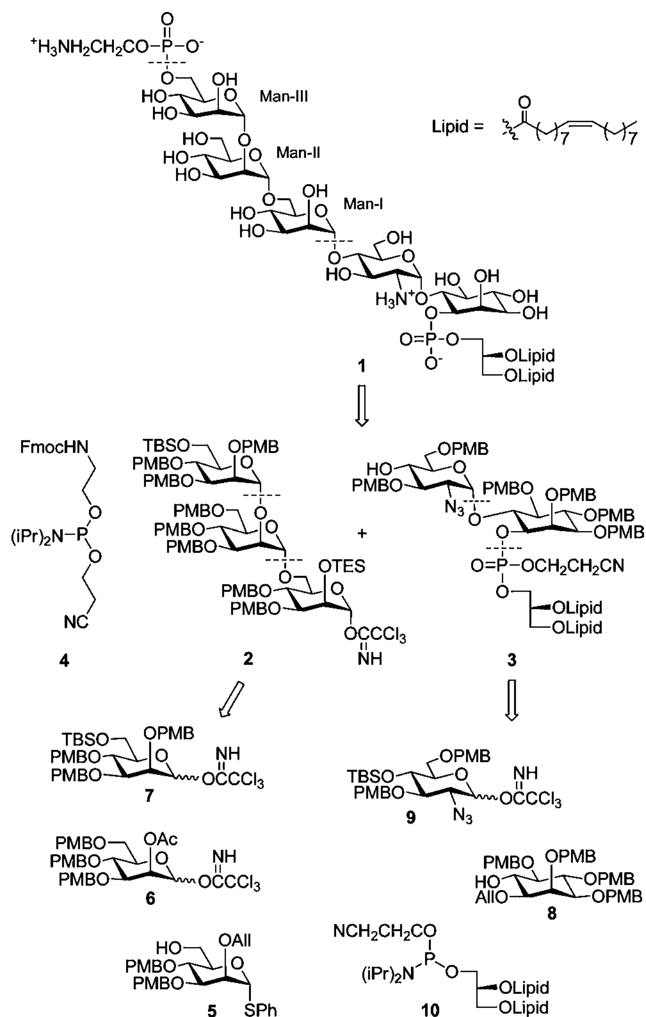
Glycosylphosphatidylinositols (GPIs) are a class of complex glycolipids that are ubiquitously expressed by eukaryotic cells and are operative in many biological processes, most notably the anchorage of extracellular molecules, such as surface proteins and glycoproteins, to the cell membrane.[1] Over 30 GPI anchors have been identified since the *Trypanosoma brucei* variant surface glycoprotein GPI anchor was first characterized by Ferguson and co-workers in 1988.[2] All GPIs identified so far contain the following conserved core: H_2NEt-(P)-6-Manα(1→2)Manα(1→6)Manα(1→4)-GlcNH_2α (1→6)*myo*-inositol-1-(P)-glycerolipid. Structural diversity among GPI anchors arises from additional phosphoethanolamine and carbohydrate moieties linked to various locations of the core, as well as modifications in the lipid chains, which may contain unsaturated bonds that are crucial to bioactivity.[3]

To probe the effects of such structural modifications and better understand the scope and mechanism of GPI anchoring, it is necessary to have access to pure and structurally well-defined samples of GPI anchors, GPI analogues, and functionalized GPIs. Advances in chemical synthesis have enabled the preparation of GPI anchors by several research groups,[4] including ours,[5] but limitations associated with current synthetic strategies prevent the inclusion of some important functional groups. For example, most reported GPI syntheses have employed the benzyl group for permanent hydroxyl protection, which precludes the incorporation of alkene, alkyne, azide, thiol, thioether, and other functionalities that are intolerant to Pd-catalyzed hydrogenolysis. To address this issue, Nikolaev and co-workers[4i] utilized the benzoyl group for permanent protection to achieve the first synthesis of GPI anchors containing unsaturated lipid chains. However, the usage of acyl groups for hydroxyl protection complicates the presence of other esters and/or peptides/glycopeptides in the target molecule due to sensitivity to deprotection with sodium methoxide. For instance, the reported deacylation step in Nikolaev's synthesis gave ≤40% yield, likely a result of degradation of the ester-linked lipid chains.[4i] Ideally, such a precarious and low-yielding step should be avoided, particularly when dealing with valuable and complex late-stage intermediates. Therefore, the efficient synthesis of GPI anchors containing unsaturated lipids and other useful functional groups has essentially remained an unsolved problem.

In this work, we sought to develop a new strategy for the synthesis of functionalized GPIs using the *para*-methoxybenzyl (PMB) group for permanent hydroxyl protection. PMB ethers can be cleaved under mild acidic or oxidative conditions, which we anticipated would provide the flexibility necessary for the incorporation of a wide range of functional groups in target molecules. As a demonstration of the value and feasibility of this methodology in the preparation of functionalized GPIs, as well as other glycoconjugates, we set out to synthesize GPI anchor **1**, which bears unsaturated fatty acid lipid chains. Scheme 1 depicts the retrosynthesis of target molecule **1**. A convergent strategy was designed for the assembly of the target GPI from trimannose **2**, pseudodisaccharide **3**, and phosphorylating reagent **4**. Fragment **2** was stitched together with mannose building blocks **5**−**7**, while **3** was synthesized from inositol derivative **8**, glucosaminyl donor **9**, and phosphoglycerolipid precursor **10**. For the glycosylations, we mainly utilized the Schmidt trichloroacetimidate method,[6] which can be achieved under relatively mild conditions.

Scheme 1. Retrosynthesis of GPI Anchor **1**



Mannose building blocks **5**−**7** were prepared from common mannose derivative **11** (Scheme 2). Thioglycoside alcohol **5** was obtained from known diol **12**[7] via a three-step sequence including stannylene acetal-directed regioselective 3-*O*-methoxybenzylation, 2-*O*-allylation, and regioselective *para*-methoxybenzylidene ring-opening using DIBAL-H. Compound **6** was derived from **11** via orthoester **15**,[8] which after deacetylation-methoxybenzylation was treated with acetic acid to open the orthoester ring, providing a hemiacetal that was then converted to the corresponding imidate. The synthesis of **7** started with BF_3-promoted glycosylation of **11** with allyl alcohol.

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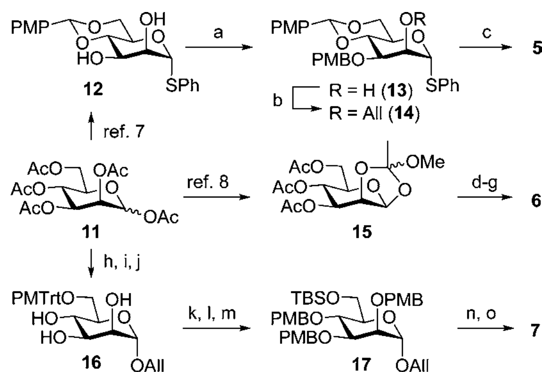
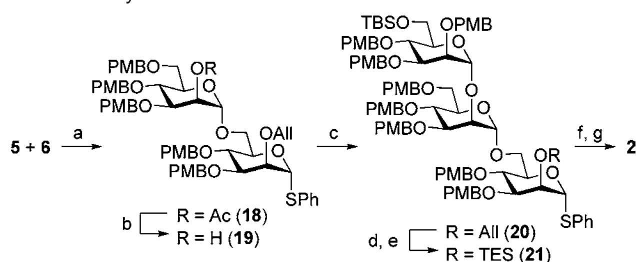
Scheme 2. Synthesis of Mannose Building Blocks **5**−**7**[a]



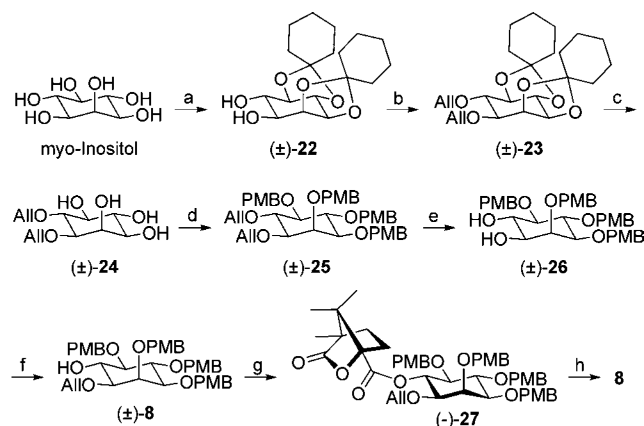
[a] Conditions: (a) Bu_2SnO, toluene, reflux; PMBCl, CsF, DMF. (b) NaH, AllBr, DMF, 68% (two steps). (c) DIBAL-H, CH_2Cl_2, 82%. (d) NaOMe, MeOH. (e) NaH, PMBCl, DMF, 76% (two steps). (f) AcOH-H_2O (1:1), 70%. (g) Cl_3CCN, DBU, CH_2Cl_2, 77%. (h) AllOH, $BF_3 \cdot OEt_2$, MS 4 Å, CH_2Cl_2, 66% (two steps from D-mannose). (i) NaOMe, MeOH. (j) PMTrtCl, pyridine, 66% (two steps). (k) NaH, PMBCl, DMF, 73%. (l) AcOH, H_2O, CH_2Cl_2, 94%. (m) TBSCl, pyridine, 88%. (n) $PdCl_2$, AcOH, NaOAc, CH_2Cl_2, H_2O, 82%. (o) Cl_3CCN, DBU, CH_2Cl_2, 73%.

Scheme 3. Synthesis of Trimannose **2**[a]



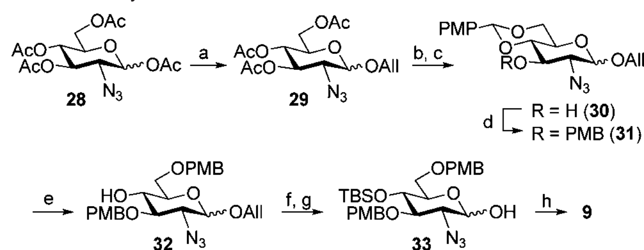
[a] Conditions: (a) TMSOTf (cat.), MS 4 Å, CH_2Cl_2, 0 °C. (b) K_2CO_3, MeOH, 66% (two steps). (c) **7**, TMSOTf (cat.), MS 4 Å, Et_2O, 0 °C, 76%. (d) Ti(O*i*Pr)$_4$, cyclopentylmagnesium chloride, THF, Et_2O, 82%. (e) TESOTf, 2,6-lutidine, CH_2Cl_2, 90%. (f) NIS, AgOTf, TTBP, wet CH_2Cl_2, 74%. (g) Cl_3CCN, DBU, CH_2Cl_2, 88%.

Scheme 4. Synthesis of Inositol **8**[a]



[a] Conditions: (a) cyclohexanone dimethyl ketal, TsOH, DMF, 38%. (b) NaH, AllBr, DMF, 81%. (c) AcCl, CH_2Cl_2, MeOH, 98%. (d) NaH, PMBCl, DMF, 73%. (e) Ti(O*i*Pr)$_4$, cyclohexylmagnesium chloride, THF, Et_2O, 85%. (f) Bu_2SnO, toluene, reflux; AllBr, CsF, DMF, 72%. (g) (1*S*)-(−)-camphanic chloride, DMAP, Et_3N, CH_2Cl_2, 46%. (h) 1 M NaOH, MeOH, THF, 95%.

Scheme 5. Synthesis of Glucosamine **9**[a]



[a] Conditions: (a) AllOH, $SnCl_4$, MS 4 Å, CH_2Cl_2, 81%. (b) NaOMe, MeOH. (c) anisaldehyde dimethyl acetal, CSA, DMF, 77% (two steps). (d) NaH, PMBCl, DMF, 97%. (e) $NaBH_3CN$, dry HCl in Et_2O, MS 4 Å, THF, 71%. (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, 85%. (g) [Ir(COD)-$(PMePh_2)_2$]PF_6, H_2, THF; then $HgCl_2$, HgO, acetone, H_2O, 85%. (h) Cl_3CCN, DBU, CH_2Cl_2, 83%.

Subsequent deacetylation and differentiation of the 6-*O*-position by reaction with *para*-methoxytrityl (PMTrt) chloride afforded triol **16**. Methoxybenzylation of the 2-, 3-, and 4-*O*-positions was followed by swapping the PMTrt group for a *tert*-butyldimethylsilyl (TBS) group to produce **17**. Finally, Pd-catalyzed deallylation revealed the anomeric hydroxyl group, which was transformed into imidate **7**.

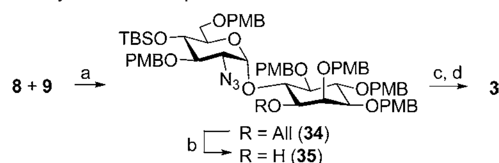
With monosaccharides **5**, **6**, and **7** in hand, the construction of fragment **2** commenced (Scheme 3). The glycosylation of **5** by imidate **6** proceeded smoothly using catalytic TMSOTf in CH_2Cl_2 at 0 °C. The reaction generated only the α-product **18**, which was deacetylated with K_2CO_3/MeOH to give **19** in 66% yield over two steps. Reaction of **19** with imidate **7** under the same conditions gave trimannose **20** in a 5:1 α/β ratio, a figure that was improved to α-only (76% yield) upon switching the solvent from CH_2Cl_2 to Et_2O. Compound **20** was designed as a versatile key intermediate for the synthesis of various GPIs containing a substituent at the Man-I 2-*O*-position. For the synthesis of GPI anchor **1**, however, the allyl group of **20** was replaced with a TES group to ensure later compatibility with the lipidic unsaturated bonds. Thus, **20** was subjected to Cha's deallylation protocol,[9] which proved to be the most effective after attempting several methods. The resulting alcohol then underwent protection by TESOTf to give **21**, which was converted to imidate **2** in two steps, including NIS/AgOTf-promoted hydrolysis of the thioacetal and then treatment of the resulting hemiacetal with Cl_3CCN and DBU.

En route to optically pure inositol **8** (Scheme 4), racemate (±)-**22** was generated from *myo*-inositol using a reported procedure.[10] Here, we protected both the 1- and 6-*O*-positions as allyl ethers to give (±)-**23**, which underwent acid-catalyzed methanolysis of the ketals followed by methoxybenzylation, resulting in (±)-**25**. Deallylation using Cha's method[9] was again effective, providing diol (±)-**26** in 85% yield. Positions 1 and 6 were differentiated at this stage using stannylene acetal-directed selective allylation to protect the 1-*O*-position (72%), and the free 6-*O*-position of (±)-**8** was then used as an anchoring point for chiral resolution. After esterification with (1*S*)-(−)-camphanic chloride, the diastereomeric products were separated by preparative HPLC to afford optically pure (−)-**27**, which was saponified to give inositol alcohol **8** in 44% yield over two steps, including the enantiomeric resolution (maximum yield 50%).[11]

Preparation of glucosaminyl donor **9** (Scheme 5) began with the transformation of known sugar **28**,[12] which contained a nonparticipating azido group at the 2-position, to allyl glycoside **29**. Deacetylation with NaOMe formed a triol that was reacted with *para*-anisaldehyde dimethyl acetal to afford **30**. A PMB group was installed at the 3-*O*-position, and then regioselective opening of the *para*-methoxybenzylidene ring using HCl and $NaBH_3CN$ provided **32**. After usage of TBSOTf to protect the 4-*O*-position, the anomeric allyl ether was removed by a protocol involving Ir-catalyzed isomerization to the corresponding vinyl ether and subsequent hydrolysis using Hg(II).[13] The resulting hemiacetal **33** was readied for glycosylation by conversion to imidate **9**.

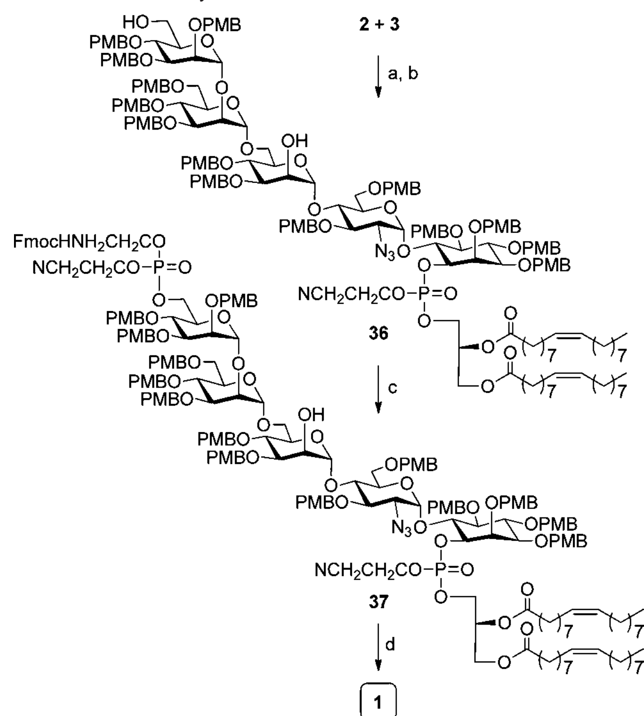
The glycosylation of inositol derivative **8** with glucosaminyl imidate **9** was achieved with catalytic TMSOTf in CH_2Cl_2 at 0 °C to form

Scheme 6. Synthesis of Lipidated Pseudodisaccharide **3**[a]



[a] Conditions: (a) TMSOTf (cat.), MS 4 Å, Et_2O, 44%. (b) [Ir(COD)-($PMePh_2$)$_2$]PF_6, H_2, THF; then $HgCl_2$, HgO, acetone, H_2O, 96%. (c) **10**, tetrazole, CH_3CN, CH_2Cl_2; then *t*BuOOH, −40 °C. (d) $Et_3N\cdot3HF$, THF, CH_3CN, 56% (two steps).

Scheme 7. Assembly of GPI Anchor **1**[a]



[a] Conditions: (a) TMSOTf (cat.), MS 4 Å, CH_2Cl_2, 64%. (b) $Et_3N\cdot3HF$, THF, CH_3CN, 70%. (c) **4**, tetrazole, CH_3CN, CH_2Cl_2; then *t*BuOOH, −40 °C, 50% for **37** (61% BRSM). (d) Zn, AcOH, CH_2Cl_2, 2 h; DBU, CH_2Cl_2 1 h; CH_2Cl_2-TFA (9:1), 1 h, 81% (three steps).

pseudodisaccharide **34** (Scheme 6). While the conversion rate was very good (84%), stereoselectivity slightly favored the undesired β anomer (α/β 1.0:1.6). However, when Et_2O was used as the solvent, a moderately improved stereoselectivity was obtained (α/β 1.2:1.0). After the anomeric mixture was separated by preparative HPLC (separation by silica gel column following the next step was also possible), the Ir/Hg deallylation protocol was utilized to expose the inositol 1-*O*-position, giving **35** in 96% yield. Next, the unsaturated phospholipid was installed by reaction with freshly prepared phosphoramidite **10** under the influence of tetrazole. The intermediate phosphite was selectively oxidized *in situ* to a phosphate using *tert*-butyl hydroperoxide at −40 °C. Exposure of the product to $Et_3N\cdot3HF$ for 5 days removed the presumably hindered 4-*O*-TBS group, which gave compound **3** as a 1:1 diastereomeric mixture, originating from the stereogenic phosphorus atom, in 56% yield over two steps. The resulting mixture was separated by preparative HPLC to facilitate the characterization of **3** and subsequent complex intermediates.

The key step in the final stage of the synthesis was to couple the trimannose and pseudodisaccharide fragments (Scheme 7). Trimannose imidate **2** reacted smoothly with **3** in the presence of catalytic TMSOTf to afford the desired α-pseudopentasaccharide in 64% yield. Overnight treatment of the product with $Et_3N\cdot3HF$ removed the TBS and TES groups to provide diol **36**, of which the anomeric J_{CH} coupling constants confirmed α stereochemistry for all mannose units.[14] Installation of the phosphoethanolamine group by treatment with phosphoramidite **4** for a short period (1 h) was selective for the Man-III 6-*O*-position to give **37**.[15] To obtain the target GPI **1**, a three-step, one-pot deprotection protocol was developed to efficiently remove all of the protecting groups from **37** in 4 h: (i) Zn-mediated reduction of the azide; (ii) removal of the base-labile Fmoc and cyanoethoxy groups with DBU; (iii) hydrolysis of all PMB ethers with 10% TFA. The target GPI anchor **1** was finally obtained in 81% yield after purification with a Sephadex LH-20 column and was characterized with ^{1}H NMR spectroscopy and MALDI-TOF MS.

In summary, a GPI anchor containing unsaturated lipid chains was efficiently synthesized using the PMB group for hydroxyl protection. This represents a potentially generally useful strategy for the design and synthesis of uniquely functionalized GPIs, as well as other complex oligosaccharides, which may not be easily accessible by means of traditional protection methods, such as using benzyl, acetyl, or benzoyl groups. We are currently using this synthetic strategy to prepare various biofunctionalized GPI anchors aimed at probing cell surface GPIomics.

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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) See Supporting Information.
(15) Regiochemistry of **37** was confirmed by ^{1}H/COSY NMR spectroscopy: The Man-I 2-H signal did not shift downfield, as would be expected if phosphorylation occurred at this site.

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