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Synthesis of Truncated Analogues for Studying the Process of Glycosyl Phosphatidylinositol Modification

Franklin John and Tamara L. Hendrickson*

Department of Chemistry, 5101 Cass Avenue, Wayne State University, Detroit, Michigan 48202

tamara.hendrickson@chem.wayne.edu

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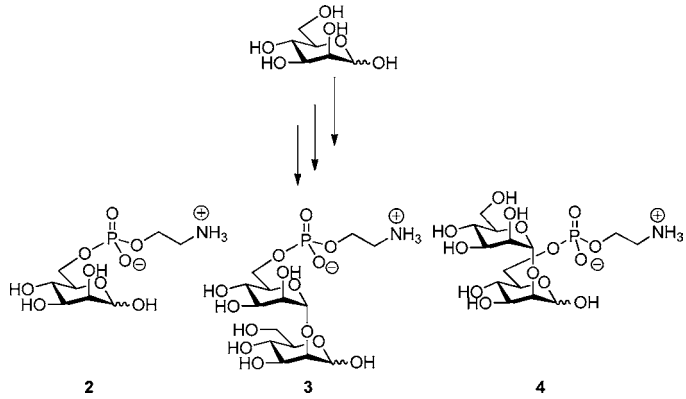
ABSTRACT



Many eukaryotic proteins are modified with a glycosylphosphatidylinositol (GPI) anchor at their C-termini. This post-translational modification causes these proteins to be noncovalently tethered to the plasma membrane. The synthesis of truncated GPI anchor analogues is reported; these compounds were designed for use as soluble substrates for GPI transamidase (GPI-T), the enzyme that appends the GPI anchor onto proteins.

Glycosylphosphatidylinositols (GPIs) represent a class of naturally occurring glycophospholipids that anchor the C-termini of proteins and glycoproteins to the membrane of eukaryotic cells. GPI-anchored proteins mediate many different cellular functions such as signal transduction, cell−cell recognition, and ion transport.[1] The core of the GPI anchor, 6-Man-α-(1→2)-Manα(1→6)-Manα(1→4)-GlcNα(1→6)-*myo*-inosityl-1-phospholipid, is conserved throughout eukaryotes.[1d−f] A phosphoethanolamine attached to C-6 of the ultimate mannose residue forms a bridge between the modified protein and the glycolipid. The GPI anchor from *S. cerevisiae* includes an additional mannose (**1**, Figure 1).[2]

Methods to obtain quantities of pure GPIs from natural sources are inefficient and hindered by complexity and heterogeneity in the oligosaccharide. Chemical synthesis can provide access to both native and novel GPI-anchored protein structures. Most of the synthetic attempts reported to date have focused on the full-length GPI anchor or

(1) (a) Varma, Y.; Hendrickson, T. L. *ChemBioChem* **2010**, *11*, 623. (b) Lakhan, S. E.; Sabharanjak, S.; De, A. *J. Biomed. Sci.* **2009**, *16*, 93. (c) Priola, S. A.; McNally, K. L. *Prions* **2009**, *3*, 134. (d) Paulick, M. G.; Bertozzi, C. R. *Biochemistry* **2008**, *47*, 6991. (e) Nosjean, O.; Briolay, A; Roux, B. *Biochim. Biophys. Acta* **1997**, *1331*, 153. (f) McConville, M. J.; Ferguson, M. A. *Biochem. J.* **1993**, *294*, 305. (g) Homans, S. W.; Ferguson, M. A.; Dwek, R. A.; Rademacher, T. W.; Anand, R.; Williams, A. F. *Nature* **1988**, *333*, 269.

(2) Fankhauser, C.; Homans, S. W.; Thomas-Oates, J. E.; McConville, M. J.; Desponds, C.; Conzelmann, A.; Ferguson, M. A. *J. Biol. Chem.* **1993**, *268*, 26365.

(3) (a) Bosson, R.; Guillas, I.; Vionnet, C.; Roubaty, C.; Conzelmann, A. *Eukaryot. Cell* **2009**, *8*, 306. (b) Imhof, I.; Flury, I.; Vionnet, C.; Roubaty, C.; Egger, D.; Conzelmann, A. *J. Biol. Chem.* **2004**, *279*, 19614.

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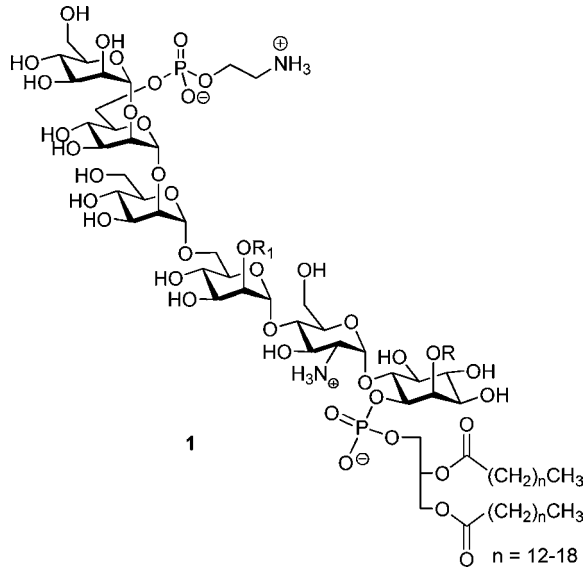


Figure 1. Structure of the yeast GPI anchor **1**. R groups denote sites of known species-specific modifications.[1d,3]

analogues that contain both carbohydrate and lipid functionalities.[4] These lipid-linked compounds are useful for studying the functions of the GPI anchor and anchored proteins, but their amphipathic nature hinders their utility for soluble experiments.[1a,d]

In vivo, GPI anchors are attached to proteins via the action of GPI transamidase (GPI-T); this enzyme catalyzes the replacement of a C-terminal peptide signal sequence with the full-length GPI anchor.[5] Remarkably, the entire GPI anchor substrate can be replaced by potent small nucleophiles like hydrazine and hydroxylamine, if they are presented to GPI-T in sufficiently high concentration.[6] Here we describe the synthesis of three GPI anchor analogues (**2**−**4**, Figure 2), which we predict will be more effective soluble substrates for GPI-T than either hydrazine or hydroxylamine because they more closely resemble the full-length GPI anchor. Peptides modified with a C-terminal disaccharide similar to **3** have been reported previously.[4c]

Compounds **2**−**4** each begin with the site of protein attachment, namely the phosphoethanolamine side chain at

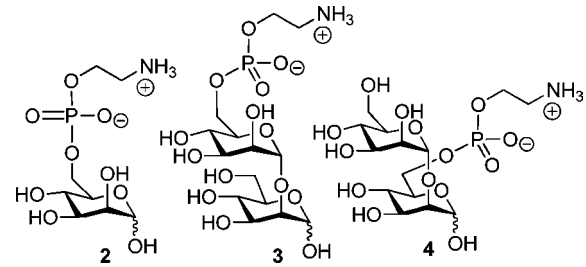


Figure 2. Structures of GPI anchor analogues **2**−**4**.

the 6-position of Man3. Building from this position, each analogue contains one or more mannose residues, based on the structure of the *S. cerevisiae* GPI (**1**). Consequently, they have been truncated to remove the terminal lipid domain, the myo-inositol, and two or more sugar units. These analogues were designed to be used as soluble substrates for GPI-T.

The synthetic strategy adopted here involves the assembly of common building blocks and synthetic routes. Orthogonal protecting groups for the glycosyl donor−acceptor pairs were optimized for yield and to obtain the desired diastereoselectivity in the glycosylation reactions. In order to introduce the phosphoethanolamine moiety onto each glycan, phosphoramidite reagent **5** was synthesized in two steps following published procedure.[7]

Synthesis of GPI anchor analogue **2** started from commercially available 1,2,3,4-tetra-*O*-benzyl-α-D-mannopyranoside **6** (Scheme 1). A standard phosphoramidite coupling reaction employing 4,5-dicyanoimidazole (DCI) as the coupling agent and previously synthesized phosphoramidate **5** resulted in an intermediate phosphite, which was subsequently oxidized in a one-pot reaction to generate **7** in good yield (73%) over two steps.[4a,8] A methanolic solution of formic acid with Pd/C and hydrogen[9] provided the best conditions for cleavage of the benzyl and benzyloxy carbonyl (Cbz) groups in **7** to furnish **2** in quantitative yield (Scheme 1).

Synthesis of GPI analogues **3** and **4** required glycosyl donor−acceptor pairs. The synthesis of mannose building blocks **11** and **13** started from known ortho ester **8**[10] (Scheme 2), obtained from D-mannose. Acid-catalyzed ortho ester deprotection of **8**, followed by acetylation of the intermediate, afforded **9**. Selective deacetylation using hydrazinium acetate afforded **10α,*β*** in excellent yield (83%). Successive treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[4e] almost exclusively afforded α-trichloroacetimidate **11** in 71% yield. Neighboring group participation of the 2-*O*-acetyl group in **11** enables α-selectivity in glycosylation reactions.[4e] Reaction of com-

(4) (a) Paulick, M. G.; Wise, A. R.; Forstner, M. B.; Groves, J. T.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2007**, *129*, 11543. (b) Wu, X.; Guo, Z. *Org. Lett.* **2007**, *9*, 4311. (c) Tanaka, Y.; Nakahara, Y.; Hojo, H; Nakahara, Y. *Tetrahedron* **2003**, *59*, 4059. (d) Xue, J.; Shao, N.; Guo, Z. *J. Org. Chem.* **2003**, *68*, 4020. (e) Mayer, T. G.; Schmidt, R. R. *Eur. J. Org. Chem.* **1999**, *5*, 959. (f) Udodong, U. E.; Madsen, R.; Roberts, C.; Roberts, S. C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1993**, *115*, 7886.

(5) (a) Hong, Y.; Ohishi, K.; Kang, J. Y.; Tanaka, S.; Inoue, N.; Nishimura, J.; Maeda, Y.; Kinoshita, T. *Mol. Biol. Cell* **2003**, *13*, 1780. (b) Chen, R.; Anderson, V.; Hiroi, Y.; Medof, M. E. *J. Cell Biochem.* **2003**, *88*, 1025. (c) Fraering, P.; Imhof, I.; Meyer, U.; Strub, J. M.; van Dorsselaer, A.; Vionnet, C.; Conzelmann, A. *Mol. Biol. Cell* **2001**, *12*, 3295. (d) Amathauer, R.; Kodukula, K.; Gerber, L.; Udenfriend, S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3973.

(6) (a) Ramalingam, S.; Maxwell, S. E.; Medof, M. E.; Chen, R.; Gerber, L. D.; Udenfriend, S. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 7528. (b) Maxwell, S. E.; Ramalingam, S.; Gerber, L. D.; Brink, L.; Udenfriend, S. *J. Biol. Chem.* **1995**, *270*, 19576.

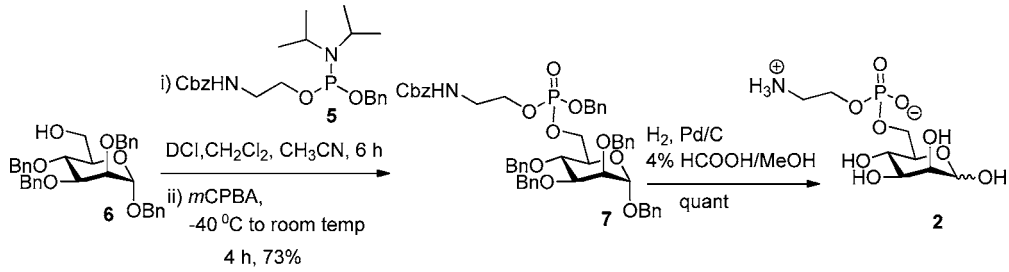
(7) Campbell, A. S.; Fraser-Reid, B. *Bioorg. Med. Chem.* **1994**, *2*, 1209.

(8) Gu, Q.-M.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 8642.

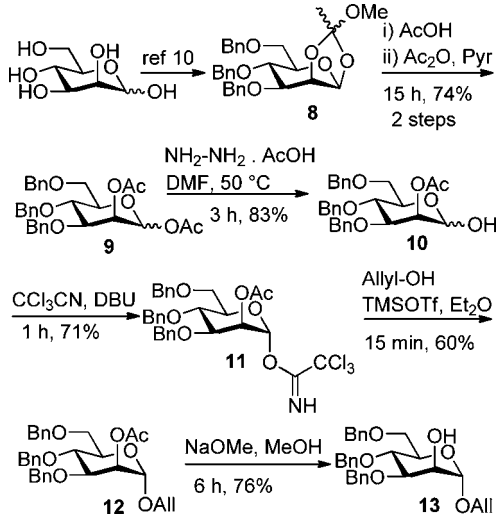
(9) (a) Becker, C. F. W.; Liu, X.; Olschewski, D.; Castelli, R.; Seidel, R.; Seebeger, P. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 8215. (b) Yagi, H.; Thakker, D. R.; Lehr, R. E.; Jerina, D. M. *J. Org. Chem.* **2004**, *69*, 6341.

(10) Beignet, J.; Tiernan, J.; Woo, C. H.; Kariuki, B. M.; Cox, L. R. *J. Org. Chem.* **2004**, *69*, 6341.

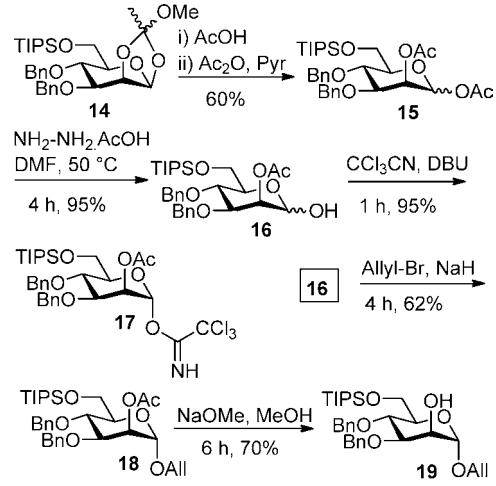
Scheme 1. Synthesis of Monosaccharide **2**



Scheme 2. Synthesis of Building Blocks **11** and **13**



Scheme 3. Synthesis of Building Blocks **17** and **19**



pound **11** with allyl alcohol in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst afforded allyl α-mannoside **12** in 60% yield. 2-*O*-Deacetylation[11] resulted in the formation of compound **13**. Compounds **11** and **13** were obtained in multigram scale in eight and nine steps, respectively.

The synthesis of glycosyl donor−acceptor pairs **17** and **19** began with the acid-catalyzed ortho ester cleavage of known compound **14** (Scheme 3).[12] Acetylation resulted in the formation of 1,2-di-*O*-acetyl derivative **15α,*β*** in 60% yield. Selective 1-*O*-deacetylation with hydrazinium acetate afforded **16α,*β*** in excellent yield (95%). Trichloroacetimidate **17** (α,*β* ≈ 50:1) was prepared from **16** as described above. Compound **16** was also used in the generation of 1-*O*-allyl mannoside **18** via reaction with allyl bromide in the presence of sodium hydride;[4e] ensuing 2-*O*-deacetylation afforded **19**.[11]

Reaction of mannosyl donor **17** with acceptor **13** in the presence of catalytic TMSOTf resulted in formation of the α(1−6)-linked disaccharide **20** in good yield (52%) (Scheme 4). Treatment of **20** with $BF_3{\cdot}OEt_2$ in CH_2Cl_2[13] proved to be most effective for 6-*O*-TIPS deprotection, which afforded **21** in 58% yield. Phosphoramidate **5** was coupled to **21** and oxidized as described above to give **22** in good yield (57%). Cleavage of the 2-*O*-acetyl group[11] afforded **23** in high yield (80%). Selective removal of the allyl group was achieved by acetic acid and $PdCl_2$[4e] to produce disaccharide **24** (70% yield). Final deprotection was carried out in one step under reductive conditions as described above to quantitatively yield GPI analogue **3**.

Synthesis of analogue **4** followed a similar strategy (Scheme 5) beginning with reaction of the donor−acceptor pairs **11** and **19** to produce the α(1−6)-linked disaccharide **25** in good yield (62%). TIPS deprotection[13] afforded **26** in 79% yield, which was coupled to phosphoramidite **5** and oxidized to afford **27** in 60% yield over two steps. Subsequent deprotections furnished **4**.[11]

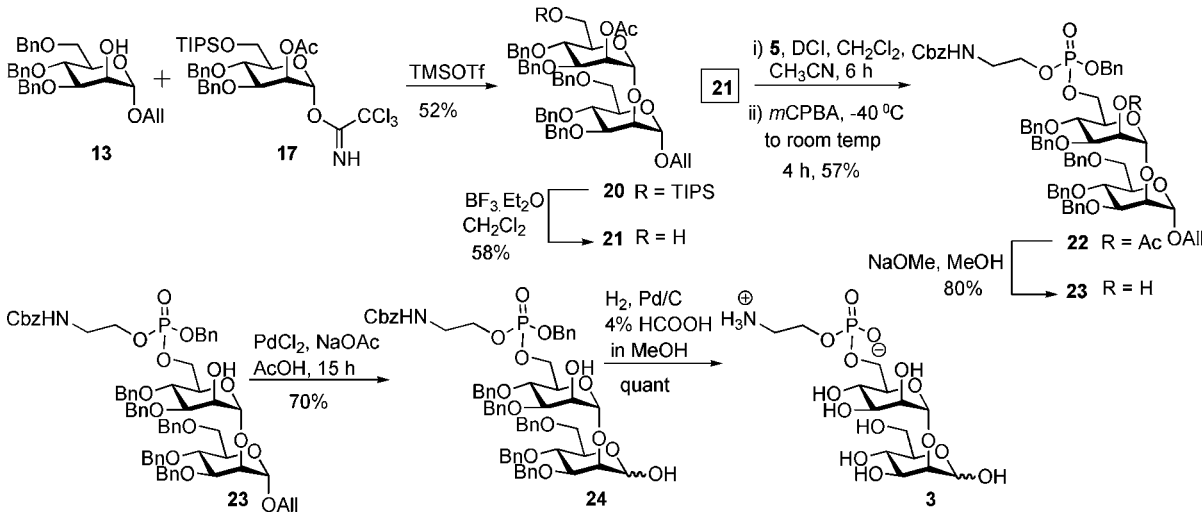
The synthetic strategy described herein will facilitate the synthesis of other truncated GPI analogues with variations within the different mannose positions. Readily accessible synthetic analogues of GPI anchors opens various possibilties for understanding the catalytic activity of the enzyme GPI transamidase (GPI-T). These analogues

(11) Zemplen, G. *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1554.

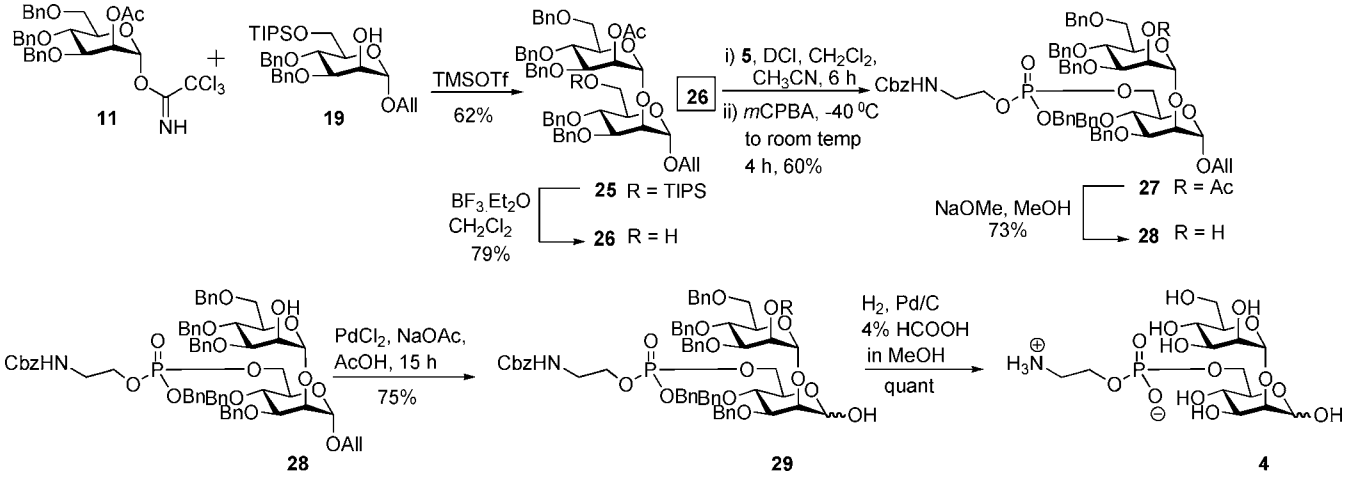
(12) (a) Ravida, A.; Liu, X.; Kovacs, L.; Seeberger, P. H. *Org. Lett.* **2006**, *8*, 1815. (b) Hölemann, A.; Stocker, B. L.; Seeberger, P. *J. Org. Chem.* **2006**, *71*, 8071.

(13) Mabic, S.; Lepoittevin, J. -P. *Synlett* **1994**, *10*, 851.

Scheme 4. Synthesis of Disaccharide **3**



Scheme 5. Synthesis of Disaccharide **4**



lack the lipid moiety, in comparison to other GPI analogues reported so far; their solubility in aqueous media is enhanced. Evaluation of these compounds as substrates for GPI-T is underway.

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Supporting Information Available: Full experimental procedures; ^{1}H and/or ^{13}C NMR spectra and HRMS of compounds **2**−**4**, **7**, and **9**−**29**. This material is available free of charge via the Internet at http://pubs.acs.org.

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