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Synthesis of GPI Anchors

A General Method for Synthesis of GPI Anchors Illustrated by the Total Synthesis of the Low-Molecular-Weight Antigen from *Toxoplasma gondii***

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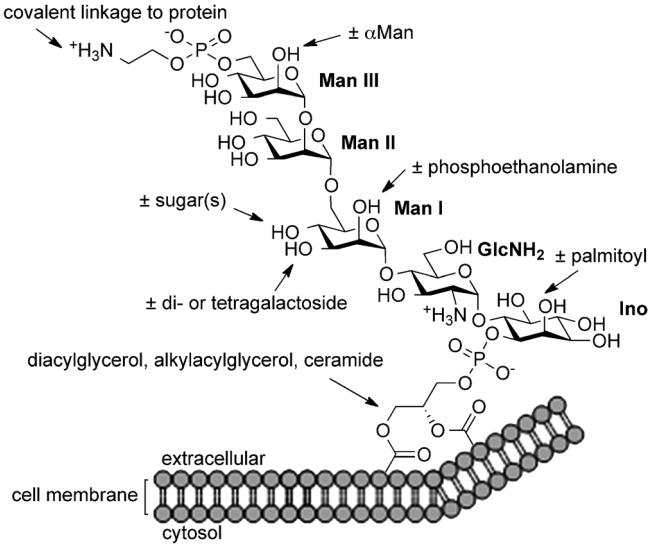
Glycosylphosphatidylinositol (GPI) anchors are a class of naturally occurring glycolipids with a conserved core structure: $H_2N(CH_2)_2OPO_3H$-6Manα1→2Manα1→6Manα1→4GlcNH_2α1→6*myo*-Ino1-OPO_3H-Lipid (Man = mannose; GlcNH_2 = glucosamine; Ino = inositol; Scheme 1).[1] GPI anchors are present in all eukaryotic cells and their structures vary in a species dependent manner. Possible modifications of the core structure include: an extra mannose at the non-reducing end of the linear pseudopentasaccharide, branching at the 3 or 4 position of the mannose I, and additional phosphorylations. There are many known variations of the phospholipid moiety of GPI anchors. Additionally, a fatty acid ester may be present at the 2-position of *myo*-inositol.[2]

Functions of GPI anchors in higher eukaryotic cells stem from their ability to anchor attached proteins to cell membranes and associate them with micro domains, which enables specific interactions with other membrane proteins.[3] This GPI-induced localization is essential for certain cell–cell interactions and signal-transduction processes.[4] Some protozoan parasites, such as *Plasmodium falciparum* or *Toxoplasma gondii*, use free GPI anchors and GPI-anchored proteins to modulate the immune system of their host.[5] For example, GPI **1** (Scheme 2b), also known as the low-molecular-weight antigen, which activates the human immune system during the *T. gondii* infections, is known to be immunogenic and causes antibody production in patients who suffer from toxoplasmosis, although its specific role in these processes is not fully understood yet.[6]



Scheme 1. The conserved core structure of GPI anchors and possible modifications.

In most cases, the function of GPI anchors is unknown beyond protein localization on the outer leaflet of the cell membrane. This lack of knowledge is mainly due to the low availability of pure GPI samples arising from their amphiphilic and heterogeneous character, complicating purification from natural sources. Consequently, detailed structure–activity relationship studies for GPI anchors are not available. The only access to sufficient quantities of structurally defined GPI anchors is by way of chemical synthesis.[7] Although many GPI anchors have been prepared using various synthetic methods and protecting-group strategies, a general unifying route that will enable efficient access to a wide range of GPI anchors has not yet been developed.[8]

Herein, we present a general synthetic route to branched GPI structures (Scheme 2a). Using the interchangeable building blocks **5**–**7** it is in principle possible to prepare a library of orthogonally protected glycans **2** in a few synthetic steps. The protecting-group pattern in the common intermediate **2** enables the synthesis of different GPI anchors and derivatives suitable for biological assays and physical measurements. Structures with an additional mannose, palmitoylated inositol, and different phosphorylation patterns should be easily accessible using this strategy.

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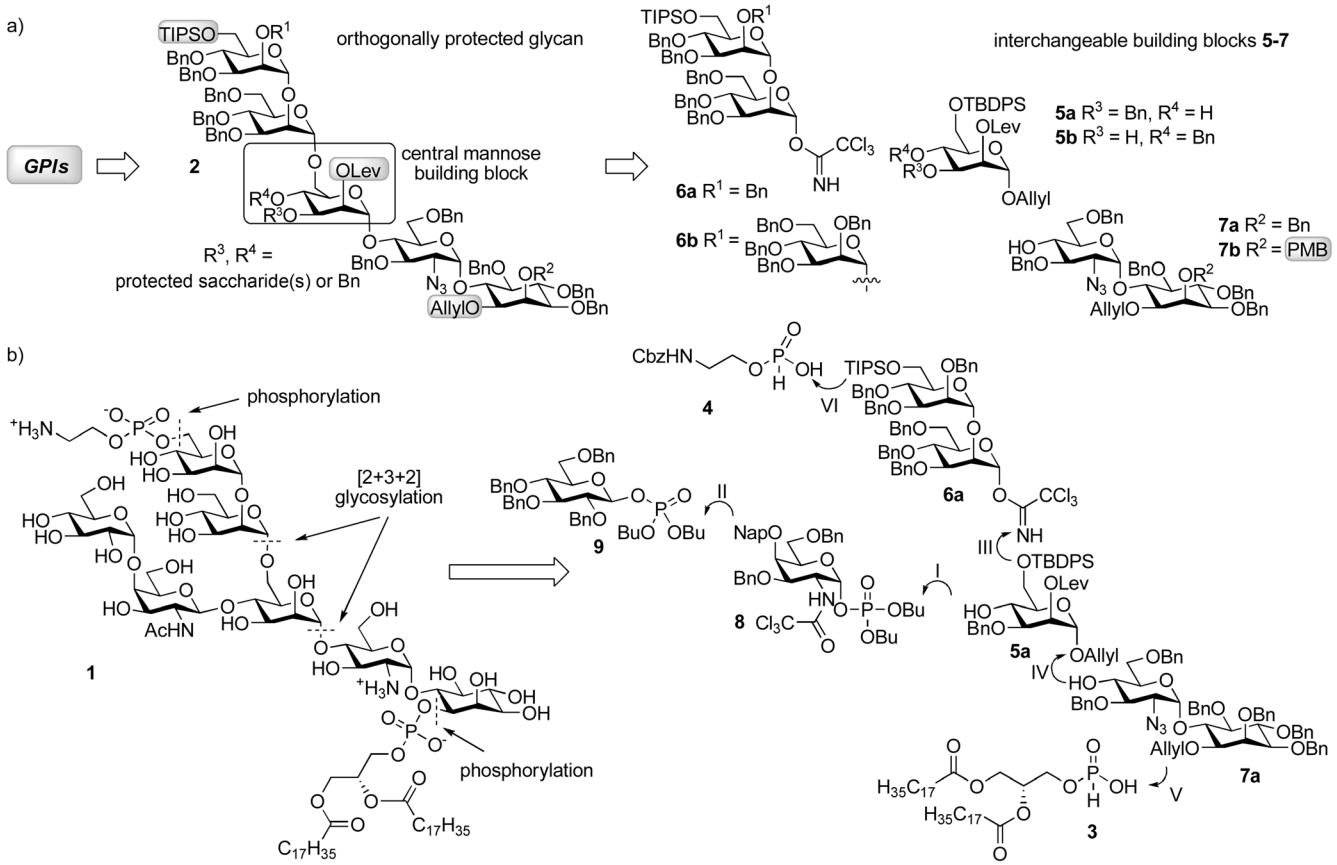
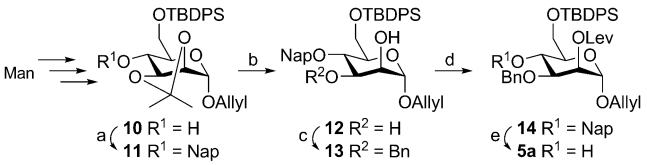


Scheme 2. a) General retrosynthetic analysis for GPI anchors. Orthogonal protecting groups for late-stage modifications are highlighted. b) Retrosynthetic analysis of GPI **1**. Roman numerals indicate the assembly sequence. Ac = acetyl; Bn = benzyl; Bu = butyl; Cbz = benzyloxycarbonyl; Lev = levulinyl; Me = methyl; Nap = 2-naphthylmethyl; Ph = phenyl; PMB = *para*-methoxybenzyl; TBDPS = *tert*-butyldiphenylsilyl; TIPS = triisopropylsilyl.

To demonstrate the feasibility of this approach we have prepared the low-molecular-weight antigen **1** of *T. gondii* (Scheme 2b) and compared this material to isolated GPI anchors of *T. gondii* in binding assays with known monoclonal antibodies.[9]

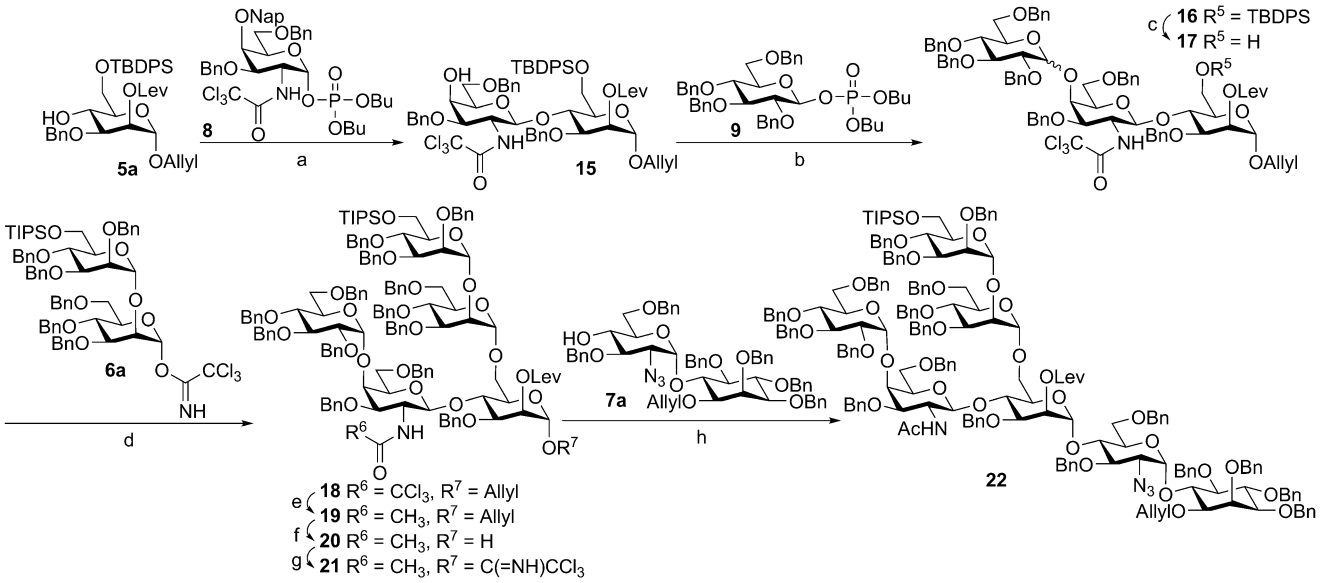
The central mannoside **5a** is the key building block in our retrosynthetic analysis of the GPI **1**. It enables the assembly of a trisaccharide subunit (by glycosylations I and II, Scheme 2b) which is then elongated with dimmanosyl imidate **6a** and pseudodisaccharide **7a**[8c] in a [2+3+2] glycosylation sequence to yield a heptasaccharide. Selective phosphorylations with H-phosphonates **3**[8f] and **4**,[8b] and subsequent global deprotection affords GPI **1**. Initial attempts to directly glycosylate the pentasaccharide core with a variety of *N*-acetylgalactosyl building blocks were unsuccessful, highlighting the importance of the appropriate glycosylation sequence.

The synthesis of building block **5a** commenced with the conversion of D-mannose into the acetal **10**[10] The 4-*O* hydroxy group of **10** was protected as a 2-naphthylmethyl ether[11] (Scheme 3). After removal of the isopropylidene group, diol **12** was selectively protected as a benzyl ether at the 3-position via a stannylene acetal. Levulinate ester protection of the remaining free hydroxy group[12] followed by deprotection of the 2-naphthylmethyl ether afforded orthogonally protected mannoside **5a**.[13] This flexible strategy for the synthesis of **5a** also allowed for the preparation of compound **5b** by switching the protecting groups at the 3- and 4-positions. Building block **5b** enables the synthesis of GPI anchors of *Trypanosoma brucei*, the only GPI structures with a branch at the 3-position of mannose I.[14]



Scheme 3. Synthesis of the key mannoside **5a**. Reaction conditions: a) NapBr, NaH, DMF, 99%; b) 80% $AcOH_{(aq)}$, 65 °C, 72%; c) 1. Bu_2SnO, PhMe, reflux; 2. BnBr, TBAB, 99%; d) LevOH, DIC, DMAP, CH_2Cl_2, 82%; e) DDQ, H_2O, CH_2Cl_2, 96%. DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone; DIC = *N*,*N*′-diisopropylcarbodiimide; DMAP = 4-(dimethylamino)pyridine; DMF = dimethylformamide; TBAB = tetrabutylammonium bromide; THF = tetrahydrofuran.

Synthesis of the orthogonally protected glycan **22** started with a one-pot sequence involving glycosylation of mannose **5a** with galactosyl phosphate **8** followed by oxidative deprotection of the 2-naphthylmethyl ether with DDQ to provide disaccharide **15** in quantitative yield (Scheme 4). Installation of the α-glucoside using glycosyl phosphate **9**[15] in a mixture of thiophene and toluene as solvent gave trisac-

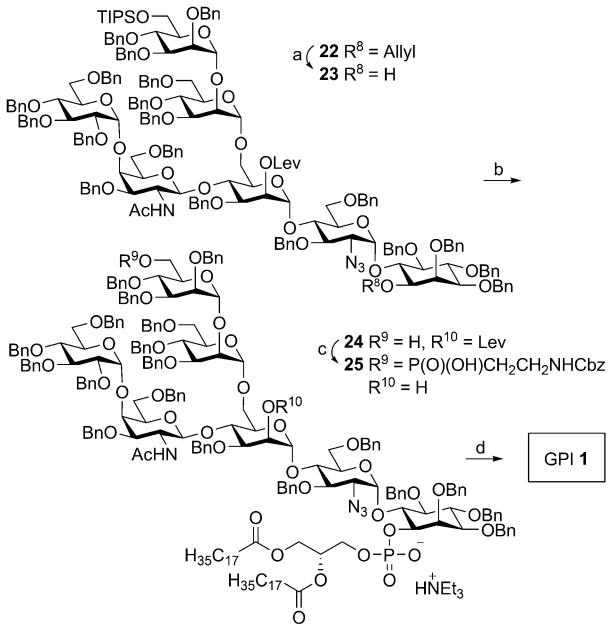


Scheme 4. Synthesis of orthogonally protected glycan **22**. Reaction conditions: a) 1. TMSOTf, CH_2Cl_2, −40 °C; 2. PBS-buffer, DDQ, quant.; b) TBSOTf, thiophene/toluene (2:1), 0 °C, 82% (α:β = 6:1); c) HF-pyridine, THF, 82%; d) TBSOTf, 4 Å MS, thiophene/toluene (1:1), 69%; e) Zn, AcOH, 55 °C, 94%; f) 1. $[Ir(cod)(PPh_2Me)_2]PF_6$, H_2, THF, 2. $HgCl_2$, HgO, H_2O, acetone; g) Cl_3CCN, DBU, CH_2Cl_2, 55% over two steps; h) TMSOTf, toluene, −40 °C, 71%; cod = cyclooctadiene, DBU = 1,3-diazabicyclo[5.4.0]undecene; MS = molecular sieves; PBS = phosphate buffered saline; TBS = *tert*-butyldimethylsilyl; Tf = trifluoromethanesulfonyl; TMS = trimethylsilyl.

charide **16** in 82% yield (α/β ratio 6:1). Use of thiophene, which forms a β-configured intermediate during the glycosylation reaction, led to preferential formation of the desired α-glycoside.[16] However, the anomeric mixture was inseparable by column chromatography. Following HF-pyridine cleavage of the TBDPS ether, glycosylation with imidate **6a** formed branched pentasaccharide **18** (α/β ratio 15:1), which was isolated from the crude mixture of four diastereomers in 69% yield. After reduction of the trichloroacetamide with zinc in acetic acid at 55 °C, the anomeric allyl ether at the reducing end of glycan **19** was removed by isomerization in the presence of iridium catalyst, followed by mild hydrolysis using mercury salts.[8g,17] Hemiacetal **20** was then transformed into trichloracetimidate **21**, which was used in an α-stereoselective [5+2]-glycosylation of pseudodisaccharide **7a** to afford the orthogonally protected glycan **22** in 71% yield.

Removal of the allyl ether from glycan **22** provided, in 80% yield, alcohol **23** (Scheme 5). Phosphonylation via the mixed anhydride prepared in situ from H-phosphonate **3** and pivaloyl chloride, then oxidation by I_2 in aqueous solution[8f,18] and consequent removal of the TIPS group under acidic conditions by treatment with scandium triflate gave glycolipid **24** in 64% yield starting from **23**. The second phosphorylation using H-phosphonate **4** and subsequent hydrazinolysis of the levulinate ester afforded glycolipid **25**, which after hydrogenolysis using palladium on charcoal in a mixture of water, methanol, and chloroform provided GPI **1** in 58% yield starting from **24**.[19]

The synthetic carbohydrate moiety of GPI **1** was printed on appropriately coated glass slides and covalently linked to the surface.[20] This glycoarray was incubated with monoclonal antibodies (mAb), obtained by immunization of mice with *T. gondii* and subsequent subcloning. The mAbT54E10 specifically recognizes GPI **1** while mAbT33F12 recognizes the GPI structure lacking the α-glucose. These experiments were previously performed exclusively with materials isolated from parasites.[6,9] Our binding assays showed that mAbT54E10



Scheme 5. Synthesis of GPI **1**. Reaction conditions: a) 1. $[Ir(cod)(PPh_2Me)_2]PF_6$, H_2, THF 2. $HgCl_2$, HgO, H_2O, acetone, 80%; b) 1. **3**, PivCl, pyridine; 2. I_2, H_2O; 3. Dowex 50WX8 (Na^+), $CHCl_3$, MeOH; 4. $Sc(OTf)_3$, MeCN, $CHCl_3$, 64%; c) 1. **4**, PivCl, pyridine; 2. I_2, H_2O; 3. hydrazine, AcOH, pyridine, $CHCl_3$, 94%; d) H_2, Pd/C, $CHCl_3$/MeOH/H_2O (3:3:1), 62%. Piv = pivaloyl.

strongly binds the carbohydrate moiety of the GPI **1** while mAbT33F12 has lower affinity for this structure. Therefore, we confirmed the structure of the "low-molecular-weight antigen" of *T. gondii* and the selectivity of mAbT54E10 and mAbT33F12 antibodies by total synthesis.[21]

In summary, we have developed a strategy for the synthesis of branched GPI anchors and demonstrated its utility in the first total synthesis of the complete surface antigen **1** of *T. gondii*. We also showed that the synthetic GPI **1** is specifically recognized by monoclonal antibodies. Based on these results, it is possible to develop a microarray diagnostic test to distinguish healthy people from *T. gondii* infected patients if polyclonal antibodies from blood also specifically recognize structure **1**.[22]

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