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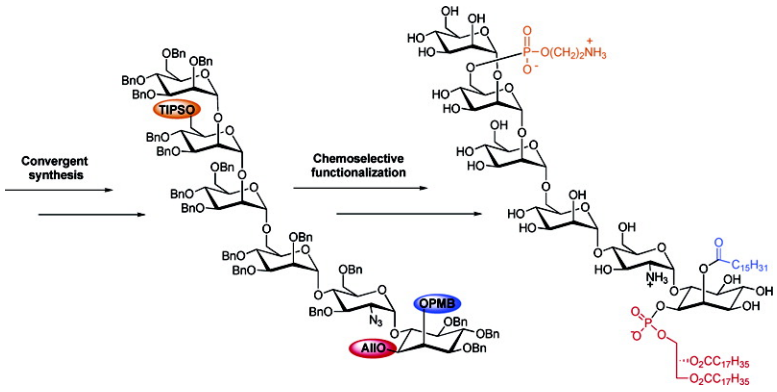

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Convergent Synthesis of a Fully Lipidated Glycosylphosphatidylinositol Anchor of *Plasmodium falciparum*

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Malaria is a devastating parasitic disease that threatens 40% of the world's population and claims more than two million lives each year, mostly young children in developing countries.[1] Using chemical synthesis, we identified the glycan part of the malaria toxin, a cell surface glycosylphosphatidylinositol (GPI) from *P. falciparum* that is responsible for mortality by malaria[2] and demonstrated that a synthetic glycan can serve as an effective antitoxin vaccine in a rodent model.[3] The glycan alone, while sufficient for vaccine development, is not toxic. To elucidate the role of GPIs in malaria pathology and signal transduction and to investigate GPI biosynthesis, access to the pure, naturally occurring, lipidated malarial GPI will be of paramount importance.

GPIs are among the most complex classes of natural products as they combine lipids, carbohydrates, and peptides. The highly complex nature of GPIs renders their isolation a daunting task. Diligent work has yielded a proposed consensus structure (Figure 1) although the exact nature of the lipid portion remains elusive.[4] To determine the exact structure of the malarial GPI responsible for the toxic effect and establish a structure−function relationship, the synthesis of GPI glycans containing a variety of lipids will be required.[5]

Here, we report the first total synthesis of fully lipidated malarial GPI **1** using a convergent yet versatile synthetic approach.[6] Three variable sites exist on the GPI glycan backbone; inositol is lipidated and serves as attachment site for phospholipids, and the C6 hydroxyl group of the penultimate mannose contains a phosphate ethanolamine group. Mindful of the structural diversity points, the hallmark of our synthetic approach is the convergent assembly of a glycan containing three differentially protected hydroxyl groups in anticipation of late stage acylation and phosphorylation (Figure 1). Thus, rapid access to various GPIs with different lipid moieties can be achieved.

GPI **1** will be derived by [4 + 2] coupling of a tetramannoside and a glucosamine−inositol pseudodisaccharide. On the basis of our experience with the synthesis of GPI glycans,[7] triispropylsilyl ether and allyl ether groups were chosen to mark the two phosphorylation sites. Selection of the protecting group for the C2 hydroxyl group of inositol revealed the complexities associated with the synthesis of highly complex glycans; a DDQ-labile *p*-(3,4-dimethoxyphenyl)benzyl ether that had served well in the synthesis of a disaccharide model could not be selectively removed when operating on a hexasaccharide.[8] Extensive trials established the *p*-methoxybenzyl (PMB) group as the most reliable mode of protection for the inositol C2 hydroxyl in the context of complex molecule construction.

Following the strategic decisions, the assembly of GPI **1** commenced with the stereoselective synthesis of the α-linked glucosamine−inositol pseudodisaccharide. Regioselective alkylation with 4-methoxybenzyl chloride resulted in the differentiation of the two hydroxyl groups in inositol **2** to afford **3**. The notoriously difficult glycosylation of **3** was carried out with glucosamine

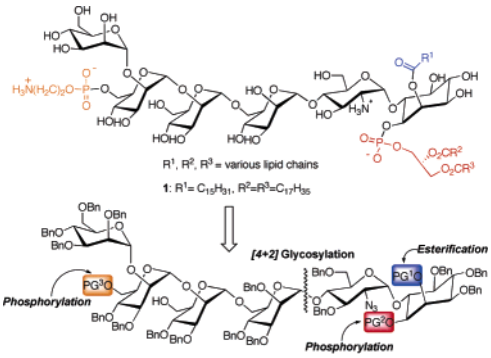
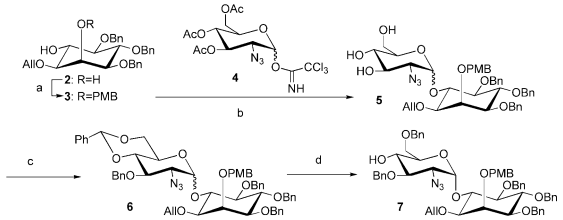


Figure 1. Consensus structure and retrosynthetic analysis of lipidated *P. falciparum* GPIs.

Scheme 1. Preparation of Glucosamine−Inositol Disaccharide **7**[a]



[a] Reagents and yields: (a) PMBCl, NaH, TBAI, DMF, 68%; (b) i. **4**, TMSOTf, CH_2Cl_2, −30 °C, ii. NaOMe/MeOH, 89% (α:β =4: 1); (c) i. $PhCH(OMe)_2$, CSA, CH_3CN, ii. BnBr, NaH, DMF, 74%; (d) $NaCNBH_3$, HCl/Et_2O, THF, 43% (α-isomer).

trichloroacetimidate **4** to furnish preferentially the desired α-linked disaccharide (α:β = 4:1). The three acetate groups on the glucosamine moiety, crucial in directing the stereochemistry of the glycosylation reaction, were removed to give disaccharide **5** in 89% yield (two steps). Installation of the appropriate protecting group pattern by formation of the 4,6-*O*-benzylidene group and benzylation of the C3 hydroxyl was completed by the regioselective opening of the 4,6-*O*-benzylidene to afford the differentially protected pseudodisaccharide **7**. At this stage, the α- and β-isomers can be readily separated by silica gel column chromatography.

The union of disaccharide **7** and tetramannoside **8**,[7] readily available in multigram quantities, furnished hexasaccharide **9** in excellent yield under complete stereocontrol by virtue of a neighboring benzoyl ester protecting group. Having served their purpose, the ester groups in **9** were subsequently replaced by benzyl ethers to afford nearly 1 g of hexasaccharide **11**. Hexasaccharide **11** is the key for the preparation of fully lipidated GPI anchors as it contains three orthogonal protecting groups for further elaboration.

Decoration of glycan backbone **11** with lipids, phospholipids, and phosphate ethanolamine can be performed in varying order. Careful evaluation of the order of functionalization regarding transformation efficiency and ease of purification revealed that removal of the PMB group and lipidation, followed by deallylation

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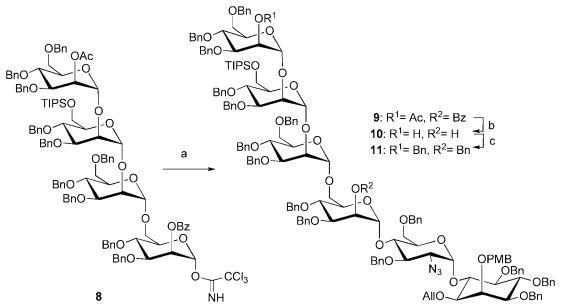
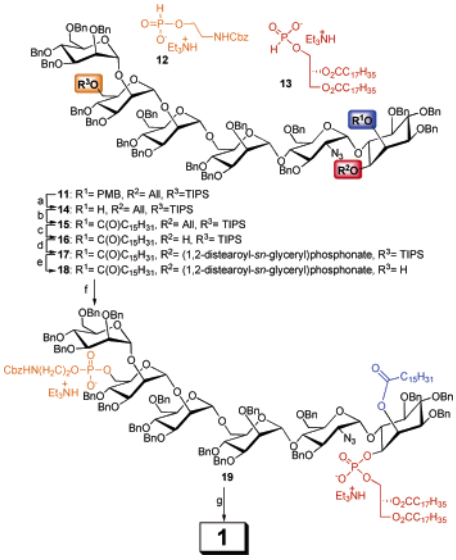
Scheme 2. Assembly of the Hexasaccharide Backbone[a]



[a] Reagents and conditions: (a) **7**, TMSOTf, CH_2Cl_2, −40 °C, 94%; (b) NaOMe/MeOH, 50 °C, 77%; (c) NaH, BnBr, DMF, 91%.

Scheme 3. Chemoselective Functionalization of Hexasaccharide **11** and Synthesis of GPI Anchor **1**[a]



[a] Reagents and conditions: (a) CAN, CH_3CN/PhMe/H_2O, 76%; (b) $C_{15}H_{31}COOH$, DCC, DMAP, CH_2Cl_2, 98%; (c) $PdCl_2$, NaOAc, AcOH/H_2O, 52%; (d) i. **13**, PivCl, pyridine, ii. I_2, Py/H_2O, 72%; (e) $Sc(OTf)_3$, CH_3CN, H_2O, 40 °C, 77%; (f) i. **12**, PivCl, pyridine, ii. I_2, pyridine/H_2O, 94%; (g) H_2, $Pd(OH)_2$/C, $CHCl_3$/MeOH/H_2O, 94%.

and introduction of the phospholipid prior to the removal of the silyl ether followed by installation of the phosphate ethanolamine was best. The PMB ether was cleaved using cerium ammonium nitrate (CAN) to afford hexasaccharide **14** in 76% yield.[9] The C2 hydroxyl group of inositol was esterified with palmitic acid by DCC activation to furnish **15**. The following deallylation proved to be more challenging, and careful control of the reaction time was crucial to the success of this transformation. Treatment with a large excess of $PdCl_2$ in acetate buffer furnished **16** in moderate yield,[10] but it completely suppressed acyl migration. Installation of the phospholipid chain was achieved by phosphorylation using H-phosphonate **13**. Coupling of **13** by activation with pivaloyl chloride was followed by in situ oxidation to furnish hexasaccharide **17** in 72% yield. This mode of phosphorylation was compatible with the azide group and avoided the problems encountered by others using phosphoramidite reagents.[5f] Removal of the silyl ether was most efficiently achieved by exposure to scandium (III) triflate in the presence of traces of water. Finally, the phosphate ethanolamine was introduced by coupling H-phosphonate **12** and glycolipid **18** and in situ oxidation. Thus, fully protected, lipidated GPI hexasaccharide **19** was obtained in pure form as the bistriethylammonium salt in 94% yield.[9]

After establishing the structural integrity of **19**, hydrogenolysis using Pearlmann's catalyst in a mixture of solvents required 32 h at ambient temperature to ensure the complete removal of all of the benzyl ether groups. After lyophilization, lipidated GPI **1** was harvested as an amorphous white solid in excellent yield. Final product **1** is very poorly soluble in organic solvents other than DMSO, but methods to ascertain identity and purity of the product were established.[9]

In conclusion, we have developed a highly convergent synthetic strategy to access fully functionalized GPI anchors, as demonstrated by the first total synthesis of the *P. falciparum* GPI. Strategic placement of three orthogonal protecting groups for the late stage installation of three side chains on the GPI hexasaccharide backbone was key to the success of the synthesis. The synthetic GPI **1** is currently used as a molecular probe for the study of malarial pathogenesis and aspects of fundamental immunology. The strategy reported here is the basis for the preparation of various GPIs with different lipid side chains for biological applications.

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Supporting Information Available: Experimental procedures and spectral copies (^1H, ^{13}C, ^{31}P, HSQC, MS) of all new compounds and full citation for ref 4b. This material is available free of charge via the Internet at http://pubs.acs.org.

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