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First synthesis of a malarial prototype: a fully lipidated and phosphorylated GPI membrane anchor

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Abstract—A strategy is described for syntheses of a fully lipidated and phosphorylated prototype of the GPI of *Plasmodium falciparum*, the causative agent of lethal cerebral, drug-resistant malaria. Orthoesters, prepared in four steps from D-mannose, and methyl α-D-glucopyranoside are the key starting materials. The latter furnishes the inositol moiety using Bender's procedure, while the former gives the other four units of the pseudo-pentasaccharide. The strategy for installing the three biologically important acyl units of the phosphoinositide has been worked out. The critical, biosynthetically important C2-O-acyl group of the inositol is exceptionally stable, showing no tendency to migrate to the *cis*-related C3-OH in several test substrates.

The insurgence of cerebral malaria that traditionally claims 2 to 3 million lives annually, mainly in tropical 'Third World' nations,[1] now threatens the northern and southern temperate zones.[2] This development has accelerated attention to the causative parasite, *Plasmodium falciparum*, prompting determination of its genome,[3] and development of various drugs and vaccine candidates.[4]

Seminal 1992 publications by Playfair and co-workers implicated phosphoinositide antigens,[5] and subsequent studies[6] culminated in the assignment of the glycosylphosphatidylinositol (GPI) candidate structure **1a** by Schwarz and co-workers.[7] Subsequent refinement by Gowda and co-workers has shown that the lipid residues of **1a**, exhibit wide structural variability, which profoundly affect biological activity.[8] In this manuscript, we report the synthesis and properties of a malarial GPI prototype, **1b**, in which the crucially important lipid residues of the phosphoinositol moiety have been incorporated by a strategy that should be readily extended to other such heavily lipidated GPIs.

Although GPIs have a conserved glycan core comprising units I → V of **1**, there are significant differences in how they are assembled in nature. By using cell-free systems to study the most readily available GPI, namely the variant surface glycoprotein (VSG) of *Tyrpanosoma brucei*, Güther and Ferguson have established a parasite biosynthetic pathway,[9] which enables comparisons to be drawn with the mammalian pathway deduced by Kinoshita and Inoue.[10] A crucial difference is that in mammals inositol acylation *necessarily* precedes addition of the first mannose (i.e., unit I). However, in the case of *T. brucei*, the mannosylation does not require prior inositol acylation.[11]

Inositol acylation therefore enforces a subtle distinction between mammalian and parasite biosynthetic pathways that has provided an opportunity for therapeutic intervention.[12]

Further attention to lipidation arises from the fact that the inositol acyl group does not normally persist in mature GPIs. However, reacylation is sometimes seen, notably in human derived GPI-anchored proteins such as CD52,[13] and AchE.[14] In these compounds, as in the case of **1a**, the inositol's acyl group was assigned at C2 because a cyclic phosphate was not produced[15] upon treatment with phospholipase C (PLC). However, acyl migration to the *cis*-related C3-OH of **1** is a possibility and such an occurrence would compromise the above-mentioned PLC diagnosis.

Several total syntheses of GPI have been reported,[16] none of which has had to confront phospholipidation as well as inositol acylation.[17] Anticipating that the latter feature would present new challenges, we carried out

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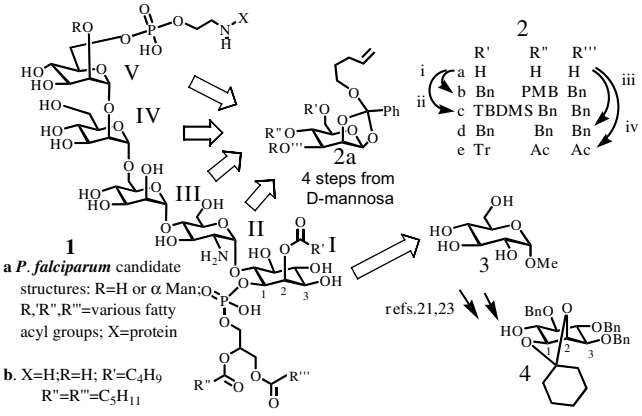
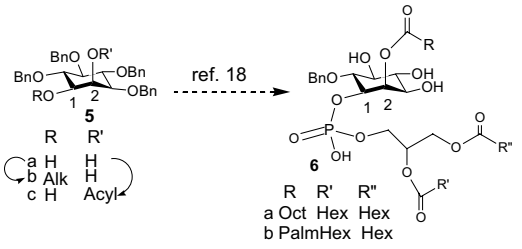


Scheme 1. Reagents and conditions: (i) (a) $(n\text{-}Bu_3Sn)_2O$, benzene/$PhCH_2Br/Bu_4NI$; (b) PMBCl/NaH/DMF; (ii) $PhCH_2Br/NaH/Bu_4NI$; (iii) TBDMSCl/THF/imidazole; (iv) Ph_3CCl/pyridine/Ac_2O.



Scheme 2.

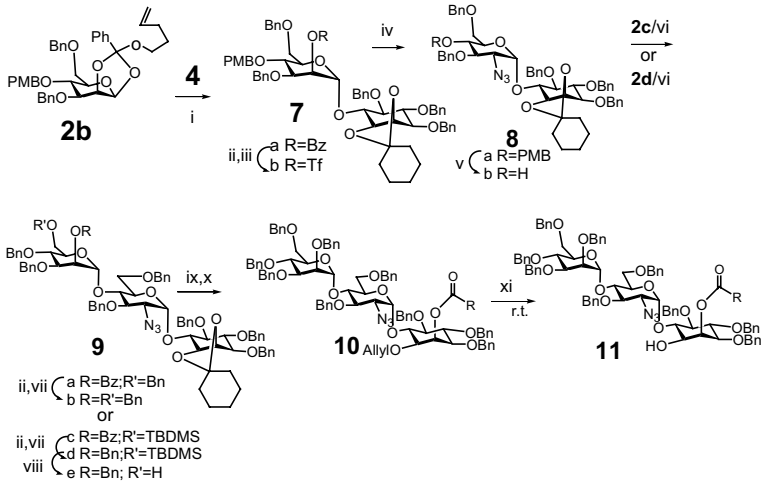
model studies on the inositol moiety by itself.[18] Nearly 40 years ago, Angyal and Tate had shown that the vicinal 1, 2-OH groups of **5a**, could be differentiated by chemoselective reactions with alkylating and acylating agents giving rise to **5b** and **5c**, respectively, as major products.[19] The desired acylation therefore could not be achieved directly, and so protection/deprotection steps, featuring tin-mediated chemistry,[20] were applied, which paved the way to the O2-acylated inositols **6a** and **6b**. As shown in Scheme 2 this strategy, which has also been reported by Guo and co-workers,[17] was unreliable, indicating that a different approach would be needed. A more advanced substrate was chosen for testing, and this was developed in Scheme 3.

In keeping with the retrosynthetic plan in Scheme 1, the inositol moiety was obtained from methyl α-D-glucopyranoside **3**,[21] while the 'rest' of **1**, came from the *n*-pentenyl orthoester (NPOE) **2a**.[22] Coupling of **2b** with **4**[23] (Scheme 3) under the agency of ytterbium(III)triflate $[Yb(OTf)_3]$[24] and NIS gave a pseudo-disaccharide, **7a**, the lone benzoate of which was replaced by triflate, **7b**, so as to employ Deshong's novel azide displacement to give **8a**.[25]

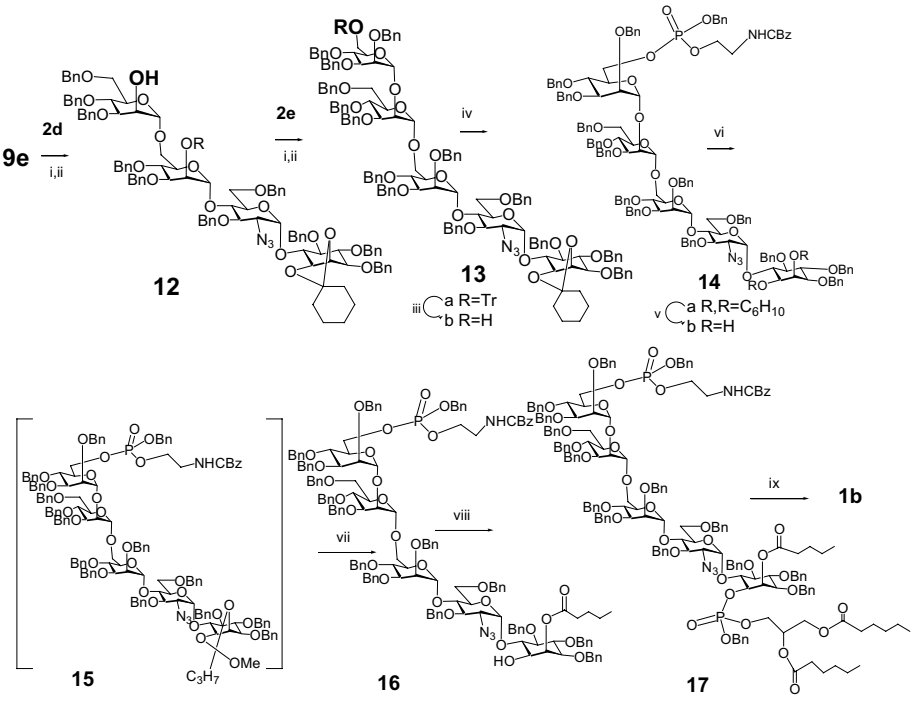
Removal of PMB to give **8b**, followed by efficient (79%) reaction with NPOE **2c**, paved the way to the pseudo-trisaccharide **9a** and thence **9b**. Tin-mediated chemistry[20] was applied to effect O1-allylation and subsequent O2-acylation gave **10a** and **b**. However, attempts to remove the 1-*O*-allyl group under standard conditions gave **11a** in poor yield, and in the case of **11b**, there was gross decomposition.

A different approach was therefore needed for substrates more elaborate than **6**. The pseudo-trisaccharide **9c** (Scheme 3) obtained from **8a** by use of NPOE **2d**, led to **9d**, and the related acceptor **9e** was coupled to the third NPOE, **2d** (Scheme 4) followed by debenzoylation gave pseudo-tetrasaccharide acceptor **12**.

The next step was very problematic. We had hoped to use the 3,4-di-*O*-benzyl NPOE **2c** again, but it failed to couple with acceptor **12**. Several probing experiments revealed that acylated NPOEs worked better. Accord-



Scheme 3. Reagents and conditions: (i) NIS/Yb(OTf)$_3$/0.3 equiv/CH_2Cl_2/0 °C (99%); (ii) NaOMe/MeOH/CH_2Cl_2 (87%); (iii) Tf_2O/PyDMAP/CH_2Cl_2 (93%); (iv) $TMSN_3$/TBAF/THF (39%); (v) $BF_3{\cdot}Et_2O/CH_2Cl_2$ (86%); (vi) NIS/$BF_3{\cdot}Et_2O/CH_2Cl_2$/0 °C; (vii) BnBr/NaH/$Bu_4NI$; (viii) TBAF/MS(4 Å)/THF (83%); (ix) CSA/ethylene glycol (68%); (x) (a) Bu_2SnO, toluene, then DMF, −10 °C allyl bromide, CsF, 18 h (54%); (b) RCOCl, pyridine; (xi) $PdCl_2$, HOAc, H_2O, NaOAc.



Scheme 4. Reagents and conditions: (i) $NIS/BF_3{\cdot}Et_2O/CH_2Cl_2/0\,^{\circ}C$; (ii) (a) $NaOMe/MeOH/CH_2Cl_2$; (b) $BnBr/NaH/Bu_4NI$; (iii) $HCOOH/Et_2O$ (60%); (iv) $CbzNH(CH_2)_2OP(OBn)N(i\text{-}Pr)_2$/1H-tetrazole/$CH_2Cl_2$; (v) CSA/ethylene glycol; (vi) $C_4H_9C(OMe)_3$, CSA, CH_3CN, 1 h (90%); (vii) $Yb(OTf)_3$, CH_2Cl_2, 0 °C, 1 h (50%); (viii) $C_5H_{11}COOCH_2CH(OCOC_5H_{11})CH_2OP(OBn)N(i\text{-}Pr)_2$/1H-tetrazole, −40 °C/mcpba (66%); (ix) Pearlman's catalyst, atm pressure.

ingly, the tritylated diester **2e** afforded the pseudo-pentasaccharide **13** (75%). Protecting group adjustments preceded installation of the phosphoethanolamine complex[26] and liberation of the inositol's *cis*-diol **14b**.

Given the poor yields in the conversion of **10** into **11**, the stereoelectronic decomposition of cyclic orthoesters pioneered by King and Allbutt[27] was an attractive option. Indeed, treatment of diol **14b** with trimethyl orthovalerate afforded the cyclic orthoester **15**, which was usually not isolated, but treated directly with ytterbium(III)triflate[24] to give the desired β-hydroxyester **16**,[28] as the major regioisomer in keeping with expectations.[27] Reaction with the readily prepared[26] diacylatedglycerylphosphoamidite gave the fully lipidated material **17**.[28] Debenzylation by atmospheric hydrogenolysis over Pearlman's catalyst finally gave **1b**.[28]

With respect to the crucial question of migration of the inositol's acyl group, we have examined three substrates: (1) 2-*O*-pentanoyl *myo*inositol, (2) the fully lipidated pseudo-disaccharide corresponding to units I and II of **17**, and (3) the synthetic material **1b**. In the first, ^1H NMR easily gave no evidence of any acyl migration over several months. The same conclusions were reached for the other two substrates, based on the signal at 5.43 ppm that is assignable to the equatorial proton α to the acyl group. (As a point of interest, we have also synthesized the regioisomer of **1b** with the inositol's C1 and C2 functionalities reversed. The axial proton α to the acyl group comes to much higher field ~4.7 ppm.)

Acknowledgements

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28. Supporting spectroscopic data for key compounds: For **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 3H, $-CH_3$), 1.34 (m, 2H, $-CH_2$), 1.63 (m, 2H, $-CH_2$), 2.40 (t, 2H, $-CCH_2-$), 3.18–5.06 (m, 66H), 5.266 (d, 1H), 5.31 (d, 1H), 5.70 (t, 1H, H-2, inositol), 7.06–7.31 (m, 70H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.82, 22.18, 27.26, 29.77 (pentyl), 46.12, 65.19, 66.55, 66.79, 68.83, 69.69, 71.96, 72.80, 73.10, 73.69, 74.15, 74.48, 74.87, 75.21, 76.87, 77.37, 78.358, 79.57, 79.99, 80.68, 80.91, 95.13, 98.89, 99.10, 100.06, 110.81, 126.83, 127.04, 127.14, 127.27, 127.33, 127.42, 127.49, 127.62, 127.69, 127.76, 127.86, 127.94, 128.05, 128.12, 128.15, 128.20, 128.25, 128.30, 128.37, 137.59, 138.01, 138.18, 138.29, 138.44, 138.67. For **17**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (m, 9H, 2× $-CH_3$), 1.26 (m, 10H, $-CH_2$), 1.57 (m, 6H, $-CH_2$), 2.26 (m, 6H, 3-$COCH_2-$), 3.17–5.21 (m, 72H), 5.47 (m, 1H, anomeric H), 5.89 (t, 1H, H-2, inositol). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.80, 13.99, 22.11, 22.36, 24.56, 39.77, 31.24, 31.295, 33.99, 34.01, 61.63, 66.59, 66.82, 68.89, 69.47, 69.98, 71.98, 72.23, 73.156, 74.15, 74.48, 74.88, 75.03, 76.59, 77.20, 79.62, 97.11, 98.67, 98.99, 99.28, 126.98, 127.08, 127.20, 127.30, 127.36, 127.42, 127.53, 127.55, 127.62, 127.71, 127.78, 127.86, 127.93, 127.98, 128.07, 128.13, 128.17, 128.27, 128.34, 128.44, 128.49, 128.56, 136.44, 137.62, 137.73, 137.99, 138.14, 138.31, 138.44, 138.58, 138.81, 172.31, 172.58, 172.99 (C═O). ^{31}P NMR (126 MHz, $CDCl_3$) δ −0.49, −0.469, 0.21, 0.37, 7.48, 7.56, 7.83, 7.87. MS calcd 2985.28, found 3010.4 (M+Na).
For **1b**: ^{1}H NMR (300 MHz, D_2O): δ 0.89 (m, 9H, 3× $-CH_3$), 1.31 (m, 10H, $-CH_2-$),1.64 (m, 6H, $-CH_2-$), 2.43–2.53 (m, 6H, $-CO-CH_2-$), 3.30–4.38 (m, 38H, mannose, inositol), 5.03 (1H), 5.12 (1H), 5.22 (1H, anomeric H), 5.54 (1H, H-2, inositol). MS (FAB+, 1385.7 M+H; 1407.9 M+Na).