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A convergent, versatile route to two synthetic conjugate anti-toxin malaria vaccines

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The synthesis of two glycosylphosphatidyl inositol (GPI) glycans that constitute the malaria toxin and promising anti-toxin vaccine constructs using a scalable route is described.

Malaria infects 5–10% of humanity, and kills up to three million people each year, mostly children in Africa.[1] Current malaria treatments are often impractical in many endemic areas, and drug resistance is a growing problem. At the same time, there is still no effective malaria vaccine.[2] Conjugate carbohydrate vaccines have shown great utility as public health tools in preventing the infection of children by *Haemophilus influenzae* type b and *Streptococcus pneumoniae*.[3] We have previously demonstrated the efficacy of anti-toxin vaccination in a mouse model of malaria.[4] Here, we report the development of a general and practical synthesis strategy for access to defined malaria toxin structures, and its application to the synthesis of a second-generation vaccine.

The malaria parasite, *Plasmodium falciparum*, expresses a large amount of glycosylphosphatidylinositol (GPI) in protein anchored and free form on the cell surface.[5] Mounting evidence suggests that the proinflammatory-cytokine cascade triggered by this GPI is responsible for much of malaria's morbidity and mortality.[6] Vaccination with synthetic GPI produces anti-GPI antibodies, which neutralize this toxin and result in host survival.[4] Based on the GPI toxin **1**, our initial vaccine candidate **2a** was designed and conjugated to a carrier protein (Fig. 1).[4,7] However, as the native toxin is linked to the cell membrane *via* an inositol phosphate diester, we reasoned that presenting the antigen in the proper orientation, as in **3a**/**3b**, could result in better vaccination.

To obtain ready access to large quantities of **2a** and **3a**, we devised a convergent, modular synthesis, involving a minimum of late-stage modification, and using robust chemistry throughout. Assembly proceeded *via* a key 4+2 glycosylation, which allowed for the same tetrasaccharide building block to be used in generation of both **2a** and **3a**. In addition to the two inositol-containing disaccharides **12** and **13**, three mannose synthons **4**, **10** and **11** were used (Scheme 1). After the completion of the hexamers, the phosphate diester functions were installed using H-phosphonates **19** and **20** prior to global deprotection and conjugation (Scheme 2).

This synthesis built on previous efforts towards GPI structures by our[4,7] and other laboratories.[8] Solution-phase methods are considerably less rapid when compared to our automated assembly but allow for ready scale-up, an important consideration in preparation for preclinical and clinical trials.

The production of the key tetramannose trichloroacetimidate building-block **7** started from C2-benzoyl mannose **4** (see Scheme 1). Glycosylation of **4** with **10**, followed immediately by selective removal of the 2-*O*-acetate in the presence of the benzoate ester using acetyl chloride in methanol, provided disaccharide alcohol **5**. Repetition of this maneuver, first using donor **11** and then **10** again,

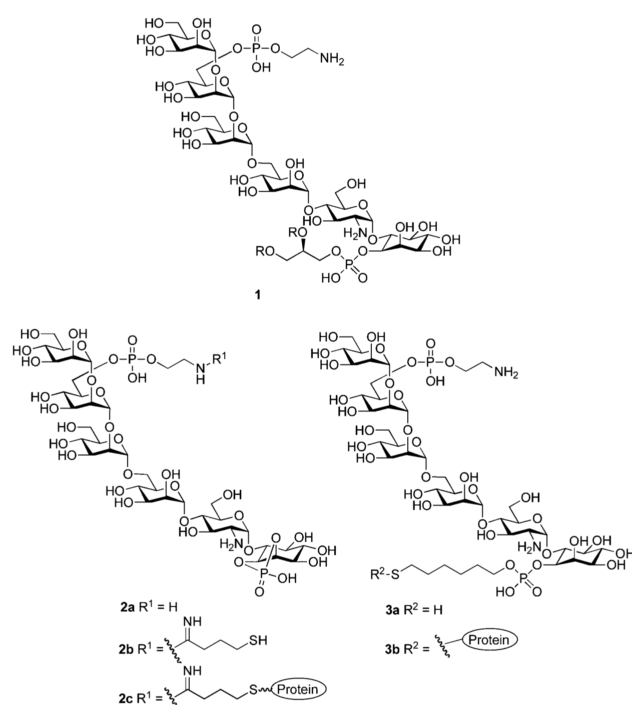
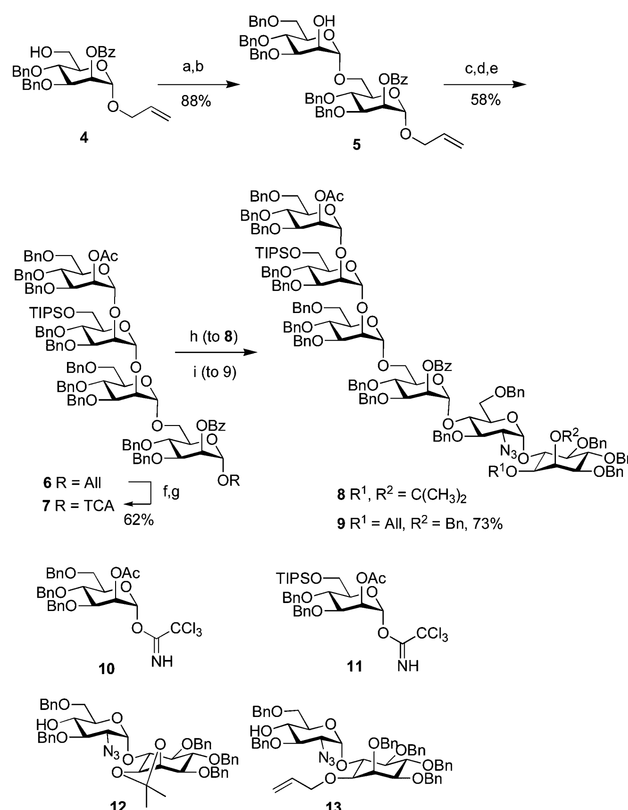


Fig. 1 Malarial GPI (**1**) and model vaccine constructs (**2c** and **3b**).



Scheme 1 (a) **10**, TMSOTf; (b) AcCl, MeOH; (c) **11**, TMSOTf; (d) $Mg(OMe)_2$, MeOH; (e) **10**, TMSOTf; (f) $PdCl_2$, NaOAc, HOAc, H_2O; (g) Cl_3CCN, DBU; (h) **12**, TMSOTf; (i) **13**, TMSOTf.

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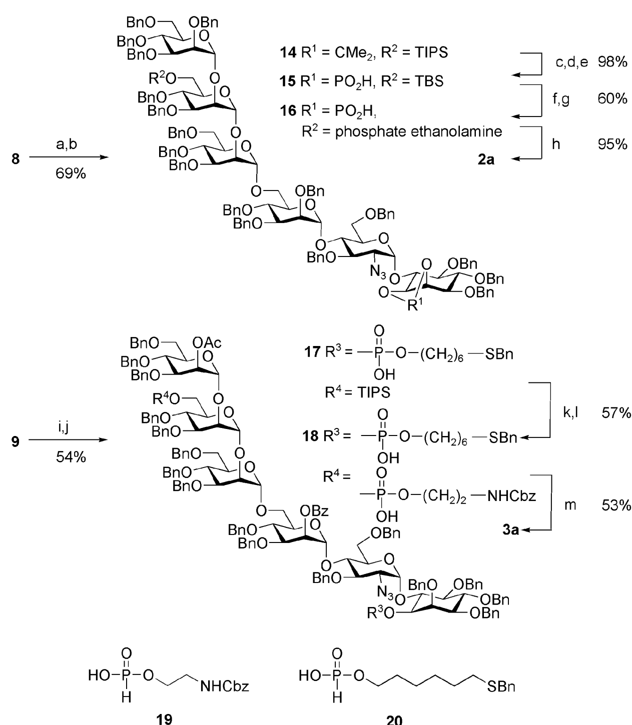
provided fully-protected tetrasaccharide **6**. Removal of the anomeric allyl group with $PdCl_2$ in wet acetic acid, was followed by the formation of glycosyl trichloroacetimidate **7** using Cl_3CCN/DBU. Demonstrating the scalability of this chemistry, central tetrasaccharide **7** was produced readily on a 20 g scale. Coupling of **7** with **12** afforded hexamer **8** *en route* to **2a**. The union of **7** and **13** provided **9** to be elaborated into **3a**. It should be noted that the 2-*O*-benzoate resulted in a significantly improved glycosylating agent when compared to a tetrasaccharide containing the 2-*O*-benzyl ether used previously: 85% yield as opposed to 39%.

The ester functions of hexamer **8** were first replaced with benzyl ethers to fashion **14** (see Scheme 2). Elaboration of **14** as reported previously furnished **15**.[7] Desilylation and phosphorylation using H-phosphonate **19** provided fully-protected intermediate **16**. Global deprotection in one step using $Pd(OH)_2$ was followed by reaction of the primary amine with 2-iminothiolane,[9] to generate thiol **2b**, ready for coupling to maleimide-activated BSA and formation of model vaccine **2c**.[4]

Removal of the allyl ether from hexasaccharide **9** (see Scheme 2), using $PdCl_2$ in wet acetic acid was followed by phosphorylation with **20** to give **17**. The TIPS ether was cleaved using $Sc(OTf)_3$, and the ethanolaminephosphate linker was installed using H-phosphonate **19**, yielding fully-protected **18**. Global deprotection was accomplished by the removal of ester groups using sodium methoxide in methanol and subsequent Birch reaction using sodium in ammonia to afford **3a**, ready for conjugation to maleimide-functionalized BSA, giving the new model vaccine **3b**.



Scheme 2 (a) MeOH, NaOMe; (b) BnBr, NaH; (c)TsOH, MeOH; (d) TBSCl, Im.; (e) Cl_2PO_2Me, Py.; (f) TBAF; (g) 1. **19**, PivCl, pyridine; 2. I_2; (h) $Pd(OH)_2$, H_2; (i) $Sc(OTf)_3$, H_2O; (j) 1. **20**, PivCl, pyridine; 2. I_2; (k) $PdCl_2$, NaOAc, HOAc, H_2O; (l) 1. **19**, PivCl, pyridine; 2. I_2; (m) 1. NaOMe, MeOH; 2. Na, NH_3.

The products of these syntheses (**2b** and **3a**) were attached to BSA both as a model for attachment to the antigenic proteins desired for vaccination, and to produce useful substrates for ELISA tests for anti-GPI IGs in both naturally immune and vaccinated individuals.[10] Work is currently underway to determine, *via* rodent trials, the best carrier protein and adjuvants for the vaccines. Also, we are engaged in synthetic studies producing a variety of substructures of the GPIs, to be used in determining the minimum antigen structure necessary to produce good immune response. The method presented here has 14 steps and provides **3a** in 6.4% overall yield.

In conclusion, we have demonstrated the development of a practical synthesis of malarial GPI structures, and applied these methods to the generation of conjugate anti-toxin malaria vaccines from fully synthetic oligosaccharides, resulting in more efficient access both to previously tested and second-generation vaccines.

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