COMMUNICATION

ChemComm
www.rsc.org/chemcomm

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

Peter H. Seeberger†,* Regina L. Soucy, Yong-Uk Kwon†, Daniel A. Snyder and Takuya Kanemitsu
Department of Chemistry: Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail: seeberger@org.chem.ethz.ch; Fax: +41) 1-633123; Tel: (+41) 1-6332103

Received (in Cambridge, UK) 14th May 2004, Accepted 1st June 2004
First published as an Advance Article on the web 9th July 2004

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.

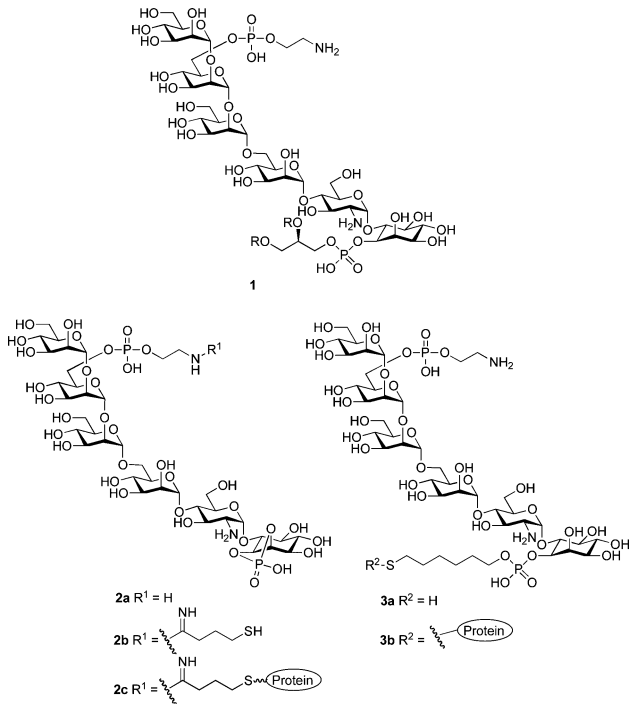
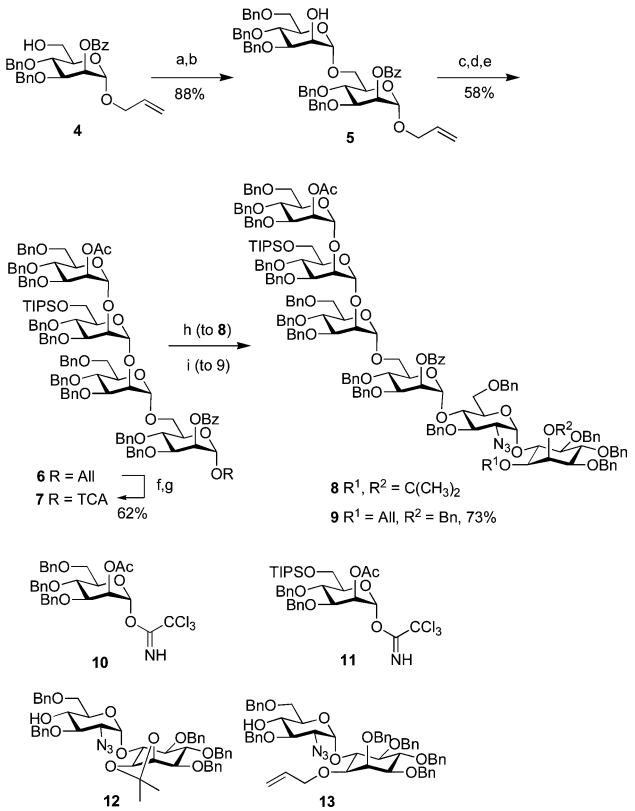


Fig. 1 Malarial GPI (**1**) and model vaccine constructs (**2c** and **3b**).

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,



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.

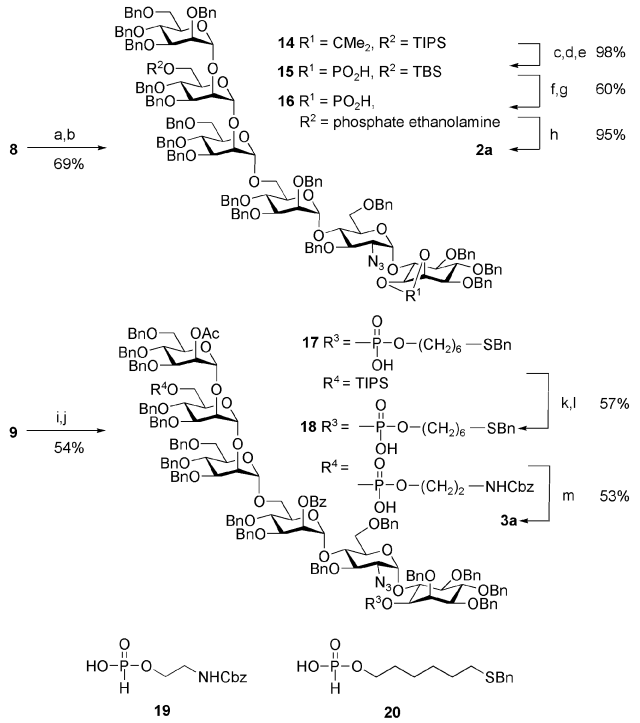
† New Address: Laboratory for Organic Chemistry, ETH Hönggerberg, HCI F315, Wolfgang-Pauli-Str. 10, CH-8093 Zürich, Switzerland

DOI: 10.1039/b407323a

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.

This research was supported by GlaxoSmithKline (Scholar Award to P.H.S.), the Alfred P. Sloan Foundation (Fellowship to P.H.S.) and Merck & Co (Academic Development Award to P.H.S). Y.-U. Kwon thanks Korea Science and Engineering Foundation (KOSEF) for financial support (Postdoctoral Fellowship).

Notes and references

1 WHO, Division of Control of Tropical Diseases, *World Health Stat. Q.*, 1992, **45**, 257.
2 M. F. Good, *Nat. Rev. Immunol.*, 2001, **1**, 117.
3 B. Kuberan and R. J. Linhardt, *Curr. Org. Chem.*, 2000, **4**, 653.
4 L. Schofield, M. C. Hewitt, K. Evans, M. Siomos and P. H. Seeberger, *Nature*, 2002, **418**, 785.
5 A. Guha-Niyogi, D. R. Sullivan and S. J. Turco, *Glycobiol.*, 2001, **11**, 45R.
6 (*a*) L. Schofield and F. Hackett, *J. Exp. Med.*, 1993, **177**, 145; (*b*) S. D. Tachado and L. Schofield, *Biochem. Biophys. Res. Commun.*, 1994, **205**, 984; (*c*) L. Schofield, S. Novakovic, P. Gerold, R. T. Schwarz, M. J. McConville and S. D. Tachado, *J. Immunol.*, 1996, **156**, 1886; (*d*) S. D. Tachado, P. Gerold, R. T. Schwarz, M. J. McConville and L. Schofield, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 4022.
7 M. C. Hewitt, D. A. Snyder and P. H. Seeberger, *J. Am. Chem. Soc.*, 2002, **124**, 13434.
8 (*a*) C. Murakata and T. Ogawa, *Carb. Res.*, 1992, **235**, 95; (*b*) U. E. Udodong, R. Madsen, C. Roberts and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1993, **115**, 7886; (*c*) T. G. Mayer, B. Kratzer and R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 2177; (*d*) A. S. Campbell and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 10387; (*e*) D. K. Baeschlin, A. R. Chaperon, L. G. Green, M. G. Hahn, S. J. Ince and S. V. Ley, *Chem. Eur. J.*, 2000, **6**, 172; (*f*) K. Pekari, D. Tailler, R. Weingart and R. R. Schmidt, *J. Org. Chem.*, 2001, **66**, 7432; (*g*) K. Pekari and R. R. Schmidt, *J. Org. Chem.*, 2003, **68**, 1295.
9 (*a*) D. R. Tolan and R. R. Traut, *J. Biol. Chem.*, 1981, **256**, 10129; (*b*) C. A. Alagon and T. P. King, *Biochemistry*, 1980, **19**, 4341.
10 C. Evans, P. H. Seeberger and L. Schofield, unpublished results.