J|A|C|S
ARTICLES
Published on Web 10/19/2002

Rapid Synthesis of a Glycosylphosphatidylinositol-Based Malaria Vaccine Using Automated Solid-Phase Oligosaccharide Synthesis

Michael C. Hewitt, Daniel A. Snyder, and Peter H. Seeberger*

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received July 2, 2002

Abstract: Described is an automated synthesis of hexasaccharide malarial toxin **1**, currently under development as a malaria vaccine candidate. Using a combination of automated solid-phase methods and solution-phase fragment coupling, the target glycosylphosphatidylinositol was assembled in a matter of days, compared with several weeks for a comparable solution-phase synthesis.

Introduction

Malaria infects 5−10% of humanity each year, killing over 2 million people. Much of the mortality is due to an inflammatory cascade initiated by a malarial toxin, released when parasites rupture the host's red blood cells. Glycosylphosphatidylinositols (GPI) function as a malarial toxin, inducing the inflammatory cytokines TNF-α and IL-1 thought to underlie malarial pathology.[1] We recently demonstrated that anti-GPI vaccination can prevent malarial pathology in an animal model.[2] Mice immunized with chemically synthesized GPI **1** (Figure 1) bound to a carrier protein were substantially protected from death caused by malaria parasites. Between 60 and 75% of vaccinated mice survived, compared to a 0−9% survival rate for unvaccinated mice. While the solution-phase synthesis of **1** allowed us to procure the target significantly faster than through isolation of natural GPI, more-rapid access to **1** is important for the further development of antitoxin malaria vaccines.

The structural complexity of GPIs has attracted the attention of synthetic organic chemists for some time, resulting in a number of elegant total syntheses. The acylglycerol-containing GPI of *Trypanosoma brucei*,[3] ceramide-containing GPI of yeast,[4] and rat brain Thy-1[5] have all been completed using a variety of methodologies and protecting group combinations. Despite the tremendous efforts toward GPI synthesis, no solid-phase or automated syntheses have been reported. Herein, we report a synthesis of malarial toxin **1**, employing our automated solid-phase oligosaccharide synthesizer.[6]

Retrosynthesis

Using our solution phase synthesis as a guide, we contemplated the automated synthesis of **1**.[2] While it would be ideal to prepare the entire carbohydrate skeleton on solid phase, the α linkage between inositol and glucosamine presented a serious impediment to a fully automated approach. Previous GPI syntheses addressed this problem by either separating mixtures of isomers or utilizing α-selective coupling methods followed by protecting group manipulations.[7] Neither of these solutions was amenable to solid phase, which led us to dissect GPI **1** into two fragments: disaccharide **3** not readily accessible on solid phase and tetra-mannosyl fragment **4** rapidly prepared using automated solid-phase methodology (Figure 1). The two fragments could be joined using *n*-pentenyl glycoside coupling[8] or use of trichloroacetimidate **4b**, available by hydrolysis from **4a**.

Results and Discussion

Tetrasaccharide **4a** was accessed on solid phase using four readily available trichloroacetimidate[9] mannose building blocks, **5**−**8** (Scheme 1). The automated synthesis was carried out on our automated oligosaccharide synthesizer using octenediol-functionalized Merrifield resin **9**.[10] Each coupling cycle (Table 1) relied on double glycosylations to ensure high coupling efficiencies and a single deprotection event. Coupling of **5**[2] to

* To whom correspondence should be addressed. E-mail: seeberg@mit.edu.

(1) (a) Schofield, L.; Hackett, F. *J. Exp. Med.* **1993**, *177*, 145. (b) Tachado, S. D.; Schofield, L. *Biochem. Biophys. Res. Commun.* **1994**, *205*, 984. (c) Schofield, L.; Novakovic, S.; Gerold, P.; Schwarz, R. T.; McConville, M. J.; Tachado S. D. *J. Immunol.* **1996**, *156*, 1886. (d) Tachado, S. D.; Gerold, P.; McConville, M. J.; Baldwin, T.; Quilici, D.; Schwarz, R. T.; Schofield, L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4022.

(2) Schofield, L.; Hewitt, M. C.; Evans, K.; Siomos, M.; Seeberger, P. H. *Nature* **2002**, *418*, 785.

(3) (a) Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *234*, 75. (b) Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95. (c) Baeschlin, D. K.; Chaperon, A. R.; Green, L. G.; Hahn, M. J.; Ince, S. J.; Ley, S. V. *Chem.—Eur. J.* **2000**, *6*, 172.

(4) (a) Mayer, T. G.; Kratzer, B.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2177. (b) Mayer, T. G.; Schmidt R. R. *Eur. J. Org. Chem.* **1999**, 1153. (c) Baeschlin, D. K.; Green, L. G.; Hahn, M. G.; Hinzen, B.; Ince, S. J.; Ley, S. V. *Tetrahedron Asymmetry* **2000**, *11*, 173.

(5) (a) Campbell, S.; Fraser-Reid, B. J. *J. Am. Chem. Soc.* **1995**, *117*, 10387. (b) Tailler, D.; Ferrieres, V.; Pekari, K.; Schmidt, R. R. *Tetrahedron Lett.* **1999**, *40*, 679.

(6) (a) Plante, O. J.; Palmacci, E. R.; Seeberger P. H. *Science* **2001**, *291*, 1523. (b) Hewitt, M. C.; Seeberger, P. H. *Org. Lett.* **2001**, *3*, 3699.

(7) Pekari, K.; Tailler, D.; Weingart, R.; Schmidt, R. R. *J. Org. Chem.* **2001**, *66*, 7432.

(8) Fraser-Reid, B.; Udodong, U.; Wu, Z.; Ottoson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927.

(9) For a review, see: Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, 21.

(10) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1881.

10.1021/ja027538k CCC: $22.00 **© 2002 American Chemical Society**

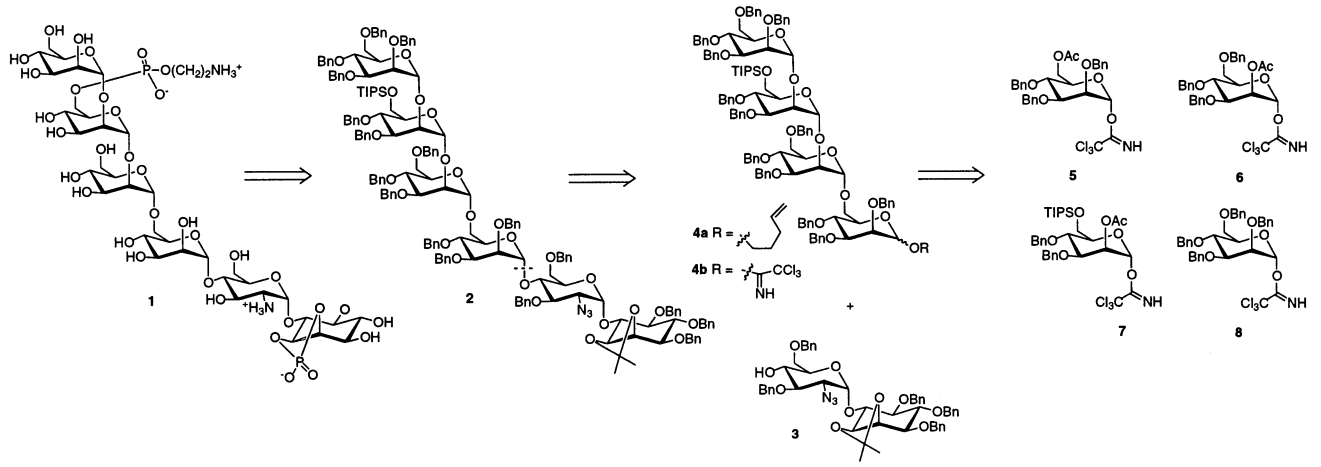


Figure 1. Retrosynthesis of GPI malarial toxin **1**.

Scheme 1

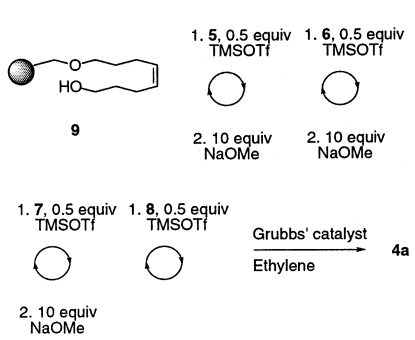


Table 1. Conditions and Reagents for the Automated Synthesis of **4a**

function	reagent	time (min)
glycosylation	5 equiv **5**, **6**, **7**, or **8** and 0.5 equiv TMSOTf	20
wash	CH_2Cl_2	9
glycosylation	5 equiv **5**, **6**, **7**, or **8** and 0.5 equiv TMSOTf	20
wash	CH_2Cl_2	9
deprotection	2 × 10 equiv NaOMe	60
wash	0.2M AcOH/0.2M MeOH/THF	9
wash	THF	9
wash	CH_2Cl_2	9

resin **9** using catalytic TMSOTf was followed by removal of the acetate ester with NaOMe. The lack of stereoselectivity in the coupling of donor **5** and the linker was inconsequential, since the *n*-pentenyl glycoside resulting from the automated synthesis was to serve later as a leaving group during glycosylation.

Elongation of the oligosaccharide chain was achieved using monosaccharide **6**,[4a] followed by deprotection of the 2-acetate by treatment with NaOMe. The coupling of building-block **7**[2] employing catalytic TMSOTf and deprotection with NaOMe proceeded smoothly to create a resin-bound trisaccharide, before the final coupling with **8**.[4a] Cleavage of the octenediol linker using Grubbs' catalyst[11] in an atmosphere of ethylene provided *n*-pentenyl tetrasaccharide **4a**. HPLC analysis of the crude reaction products revealed two major peaks (Figure 2): the desired tetrasaccharide **4a** (44% relative area) and deletion sequences (15% relative area). Purification by HPLC afforded **4a** as the expected α/β mixture at the reducing-end mannose.

(11) (a) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100. (b) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (c) Schmalz, H. G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1833.

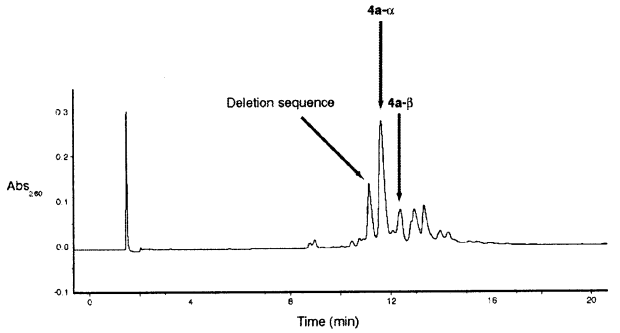
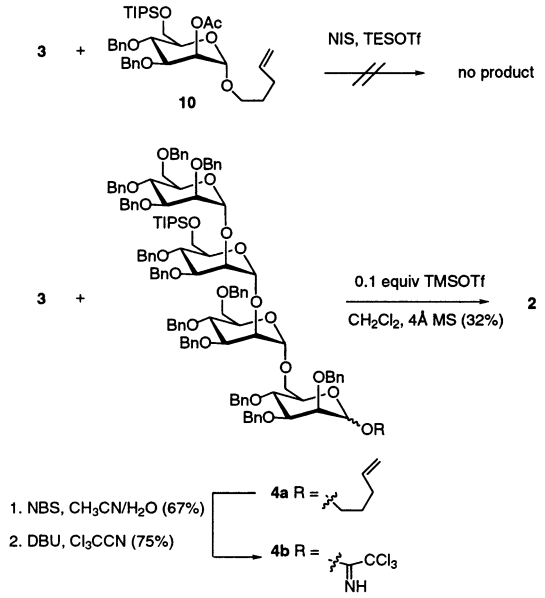


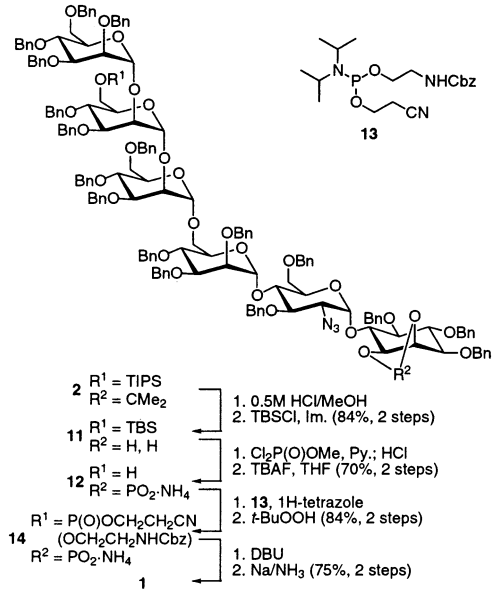
Figure 2. HPLC analysis of automated synthesis of **4a**. Flow rate 1 mL/min, 5→20% EtOAc/hexanes (20 min).

Scheme 2



Prior to attempting the 4+2 coupling, a model coupling between *n*-pentenyl monosaccharide **10** and disaccharide **3** was carried out but failed to produce the desired product (Scheme 2). This led us to examine glycosyl trichloroacetimidate **4b** as a coupling partner. Conversion of **4a** into glycosyl donor **4b** proceeded smoothly over two steps. Reaction of trichloroacetimidate **4b** with disaccharide **3** afforded the desired hexasaccharide **2** in modest yield.

Scheme 3



Hexasaccharide **2** was converted into malarial toxin **1** in the manner previously outlined (Scheme 3).[2] Deprotection of both acid-labile groups and selective silylation of the resultant primary hydroxyl afforded **11**.[12] Treatment with methyldichlorophosphate in pyridine, acidic workup, and exposure to TBAF furnished cyclic phosphate **12**. Reaction with phosphoramidite **13**[4b] and oxidation provided bis-phosphate **14** as a mixture of diastereomers. DBU removed the β-cyanoethoxy group, and global deprotection of the 17 benzyl ethers, the carbamate, and the azide was accomplished simultaneously with Na in NH_3 to afford desired toxin **1**.

Conclusion

We have demonstrated a new method for rapid access to malarial toxin **1**. The minimal structure required for efficacy as an antitoxin vaccine should be easily probed using the techniques disclosed here. Different mannose units can be rapidly synthesized and coupled to disaccharide **3** to generate vaccine precursors for structure/activity-relationship studies. The modular approach used to synthesize **1** can also be applied to other complex GPIs, such as the prion protein GPI.[13] These improvements in the synthesis of GPIs are expected to facilitate studies into the role of these molecules in other biological systems.

Acknowledgment. Financial support from GlaxoSmithKline (Research Scholar Award to P.H.S.), the Alfred P. Sloan Foundation (Scholar Award to P.H.S.), and the NIH (Biotechnology Training Grant for M.C.H.) is gratefully acknowledged. Funding for the MIT-DCIF Inova 501 was provided by the NSF (Grant CHE-9808061). Funding for the MIT-DCIF Avance (DPX) 400 was provided by the NIH (Grant 1S10RR13886-01).

Supporting Information Available: Experimental procedures and NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JA027538K

(12) Frick, W.; Bauer, A.; Bauer, J.; Wied, S.; Müller, G. *Biochemistry* **1998**, *37*, 13421.
(13) Baldwin, M. A.; Burlingame, A. L.; Prusiner, S. B. *Trends Anal. Chem.* **1993**, *12*, 239.