A new approach to construct full-length glycosylphosphatidylinositols of parasitic protozoa and [4-deoxy-Man-III]-GPI analogues†

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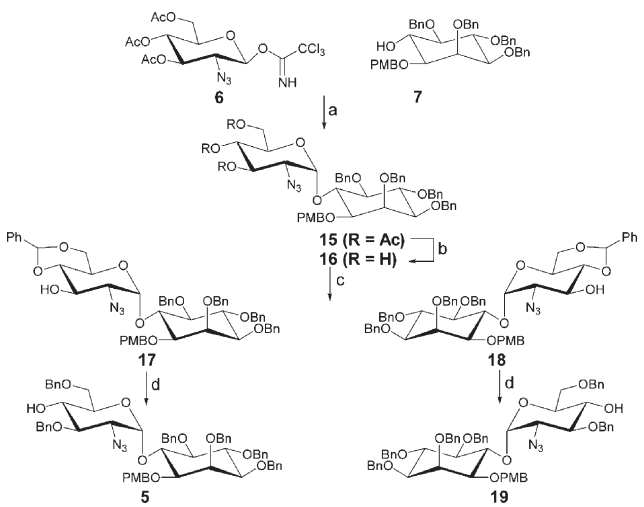
Received (in Cambridge, UK) 17th September 2004, Accepted 13th October 2004
First published as an Advance Article on the web 2nd December 2004
DOI: 10.1039/b414119a

A new [2 + 2 + 2] approach to construct GPI molecules through the efficient synthesis of glucosamine-inositol and tetramannose intermediates led to a total synthesis of a GPI-anchor of *Trypanosoma cruzi*, and also afforded a key intermediate for the synthesis of valuable [4-deoxy-Man-III]-GPI analogues.

Since the discovery[1] of the glycosylphosphatidylinositol (GPI) anchor as a novel and alternative mode of membrane-association of cell-surface proteins, the biology of these complex glycolipids has remained in focus.[2] Subsequently, several GPI-anchors and protein-free GPIs have been isolated across the eukaryotic species including humans. Surprisingly, the GPIs are produced in high abundance by protozoan parasites (*Trypanosoma, Leishmania* and Malaria) compared to that in higher organisms, and are essential virulence factors that allow these parasites to infect, proliferate and subvert the host immune system. Marked differences in the structure and biosynthesis of GPIs from the parasites and human cells have been identified[2] providing valuable targets[3] for drug and vaccine design. Even among the parasites, various species express GPIs with subtle structural differences that manifest in remarkable and, at times, opposing biological functions in the host.

Their structural complexity and biological function have inspired widespread chemical interest and a number of synthetic approaches towards GPIs (Thy-1, yeast, *T. brucei*, sperm CD-52, *Leishmania* and *P. falciparum*) have been reported.[4] However, despite the concerted efforts of several leading groups, the total synthesis of a full-length GPI-anchor remains a daunting task, which is further complicated by the presence of (a) structural and functional differences among the species and (b) significant micro-heterogeneity in their lipid and glycan domains. Arguably, the most demanding aspect of GPI synthesis has been to access suitably protected glucosamine-inositol intermediates requiring an optically pure protected D-*myo*-inositol acceptor and a 2-azido-2-deoxyglucosyl donor. This has mainly been done[4] either by the painstaking resolution of *bis*-cyclo hexylidene-*myo*-inositols using costly camphanate auxiliaries/enzymes or through a multi-step synthesis from D-glucose by the Ferrier reaction. In our ongoing investigations[5] into the chemical biology of the GPI molecules, such an intermediate was required for fluorescent GPI analogues. Instead of following the reported methods based on the *a priori* resolution of *myo*-inositol, we reasoned, based on structural modeling, that if sufficient strain is built through a cyclic protective group, the azidoglycosyl unit itself could function as an efficient chiral auxiliary on the way to GPIs, making a number of early steps redundant. To test this proposition, the racemic 1-*O*-PMB-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol **7**[6] was glycosylated with a 2-azido glycosyl donor **6**[4b] to get pseudodisaccharide **15** (Scheme 1), which on deacylation (**16**) and benzylidenation, gave 4,6-cyclic acetal protected disaccharide. To our surprise, this quantitative reaction led to a clean separation (R_f difference of 0.1) of two enantiomeric disaccharides **17** and **18** by a simple silica column (for a comparison of their NMR spectra, see Fig. S1 of ESI†). The next two steps, benzylation at 3-OH and regioselective opening of benzylidene acetal by $NaCNBH_3$, provided the key building block **5** and the undesired isomer **19**. The spectral and $[\alpha]_D$ data of **17** and **5** were identical to that reported[6] for the compounds prepared by an alternative route. The method also worked with 2-*O*-allyl-1-*O*-PMB-3,4,5-tri-*O*-benzyl- and 1-*O*-allyl-2,3,4,5-tetra-*O*-benzyl inositols showing its generality. Since good separation was obtained for **17/18** and their benzylated pairs, benzylidene-protected donors can also be used. A resolution at disaccharide level was tried[7] earlier but not used due to a problem in separation.

The multi-gram scale synthesis of **5** encouraged us to apply this new method to the total synthesis of structurally and biologically challenging GPIs. For this, we decided to construct a GPI anchor of the *Trypanosoma cruzi* IG7 antigen[8a] (Fig. 1) because (a) synthesis of the GPIs from this parasite has not been reported and (b) purified materials from *T. cruzi* have shown[8b] extraordinary pro-inflammatory activities (*p*M), comparable to those of bacterial



Scheme 1 (a) TMSOTf, CH_2Cl_2, 0 °C; (b) NaOMe, MeOH; (c) $PhCH(OCH_3)_2$, CH_3CN; (d) BnBr, NaH, DMF; HCl–Et_2O, $NaCNBH_3$.

† Electronic supplementary information (ESI) available: experimental and characterisation data for key compounds. See http://www.rsc.org/suppdata/cc/b4/b414119a/

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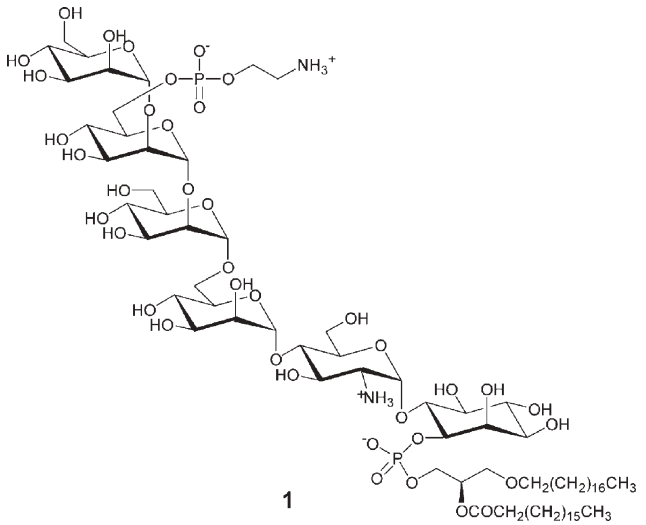
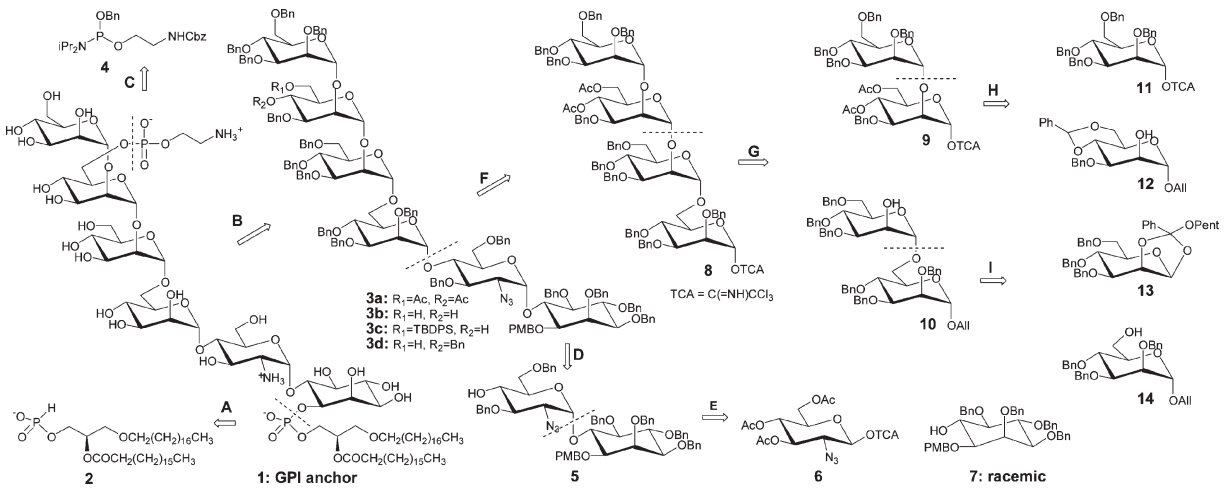


Fig. 1 Proposed backbone structure of the GPI anchor of *T. cruzi*.

LPS (lipopolysaccharide). The issues regarding the structural features responsible for such unusual activity can only be resolved through synthesis. Herein, we present a new strategy for the synthesis of parasitic GPIs (Scheme 2, retrosynthetic analysis). The synthetic design also accommodates a feature that allows access to valuable [4-deoxy-Man-III]-GPI analogues to address a fundamental biological question: why proteins are transferred only to the 6-OH of a Man-III residue[9] and what happens if the conformation of this residue is disturbed by 4-deoxygenation.

After the efficient access to glucosamine-inositol intermediate **5**, we designed a new and convergent [2 + 2] approach for the construction of the tetramannose building block **8**. This was prepared from two protected mannobiosides, the activated donor **9** and acceptor **10**. The donor **9** was prepared by the coupling of allyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannoside (**12**, prepared in 4 steps from D-Man) with 2,3,4,6-tetra-*O*-benzyl-α-D-mannosyl trichloroacetimidate (**11**, made from D-Man). The glycosylation (TMSOTf, DCM, −20 °C) went smoothly and the product was taken to the next stage: simultaneous removal of anomeric allyl and 4,6-benzylidine groups (KO_tBu, DMSO, 80 °C; 1M HCl–acetone, 1 : 9, 60 °C). The per-acetylation of the resultant triol, selective removal of the anomeric acetyl (Me_2NH, MeCN, −20 °C) followed by Schmidt activation (CCl_3CN, DBU) provided the desired mannobiose **9**. It needs to be mentioned that the two acetyls at positions 4- and 6-OH were deliberately placed keeping in view our future target, the [4-deoxy-Man-III]-GPI analogue. Lower mannobiose **10** was prepared by the glycosylation (TESTf, NIS) of allyl-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**14**, from D-Man in 4 steps) with 3,4,6,-tri-*O*-benzyl-β-D-man-1,2-pent-4-enylorthobenzoate **13**[4c] followed by the removal of benzoyl from the 2-position.

Having both mannobiose donor **9** and acceptor **10** in hand, further glycosylation, which required considerable optimization, provided a fully protected tetramannose in an acceptable 65% yield. This, after anomeric allyl-removal (palladium chloride, NaOAc, AcOH–water, rt) and activation (CCl_3CN, DBU), afforded the desired tetramannose donor **8**. To our satisfaction, the next critical step of the [4 + 2] glycosylation of glucosamine-inositol **5** with the above tetramannose **8** went smoothly (TMSOTf, DCM, 0 °C, 70%) to provide a pseudohexasaccharide **3a** as the central point for both the GPI-anchor as well as the deoxy-GPI analogues. For the synthesis of the GPI-anchor, two acetyls were first removed and the primary 6-OH of the diol **3b** was silylated (TBDPSCl, imidazole) to get **3c**, followed by benzylation of the 4-OH (BnBr, NaH) and TBDPS removal (TBAF, THF) to obtain the pseudohexasaccharide acceptor **3d** ready for phosphorylation with ethanolamine. A part of the diol **3b** (with free 4- and 6-OH of the Man-III) was used for 4-deoxygenation ($CSCl_2$,Bu_3SnH, AIBN) by Barton's cyclic-thiocarbonate method for further synthesis of [4-deoxy-Man-III]-GPI probes. The coupling of **3d** with NHCbz-ethanolamine-phosphoramidite[4c] (**4**) was carried out with 1*H*-tetrazole followed by *m*CPBA oxidation. Now the PMB group from the 1-position of the *myo*-inositol residue was removed (CAN, MeCN–DCM–H_2O) and the product was phospholipidated with 1-*O*-alkyl$_{18:0}$-2-*O*-acyl$_{18:0}$-*sn*-glycero-*H*-phosphonate (**2**)[4a] by pivaloyl chloride/I_2-oxidation, to provide a fully-protected GPI-anchor. The final step involved global deprotection and azide-reduction by hydrogenolysis ($Pd(OH)_2$, DCM–MeOH–H_2O, H_2) to the target GPI anchor.



Scheme 2 Retrosynthetic analysis showing key building blocks and intermediates.

In conclusion, we designed a new and efficient approach for the synthesis of GPIs, and showed its utility by accomplishing a total synthesis of a full-length GPI of *T. cruzi*. This synthesis represents the first step in our efforts aiming at related GPIs (*e.g.* with unsaturated fatty-acids at *sn*-2-glycerol and aminoethyl phosphonate on a GlcN residue) to probe their biology. The synthesis also afforded key intermediates for valuable [4-deoxy-Man-III]-GPI analogues and the GPIs of the malaria parasite, which will be reported along with the relevant biological studies.

The work was supported by DST, India (SR/S5/OC-11/2002) and NIAID, NIH-USA (RX4220-105 of ROI A141139-05).

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