Synthesis of glycosylphosphatidylinositol (GPI)-anchor glycolipids bearing unsaturated lipids†

B.-Y. Lee,a P. H. Seebergerab and D. Varon Silvaa

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2-Naphthyl-methyl ethers as permanent protecting groups are readily removed under acidic conditions and are key to the synthesis of complex glycosylphosphatidylinositol anchors containing unsaturated lipids. The total synthesis of the GPI pseudo-disaccharide core found on the surface of the Trypanosoma cruzi parasite serves to illustrate the power of the strategy.

GPIs are complex glycolipids that are ubiquitous in eukaryotic cells and have a common pseudo-pentasaccharide core structure 6-O-NEtP-Man1-2-Man1-6-Man1-4-GlcN1-6-Ino-1-P (Fig. 1).1 This conserved core is generally modified by additional phosphorylations, glycosylations, or acylations at the 2-O position of the myo-inositol in a cell-type dependent manner.2 GPIs are heterogeneous in the glycan as well as the lipid part. Inositol can bear sn-1-alkyl-2-acylglycerol, sn-1,2-diacylglycerol, or ceramide adorned with lipid chains of different lengths and degrees of saturation.3

Chagas disease, caused by the parasite Trypanosoma cruzi (T. cruzi), is a major public health problem in Latin America, infects around 7–8 million persons worldwide, and causes more than 10 000 deaths each year.4 There is no vaccine for this disease and the two drugs available for treatment are used sparingly due to their cost, side effects, and low antiparasitic activity in patients with chronic infections.5

The cell surface of T. cruzi contains a high concentration of glycosylphosphatidylinositol (GPI) molecules, which exhibit proinflammatory activities comparable to bacterial lipopolysaccharides and are predominately attached to highly glycosylated mucins and phosphoglycans.6,7 Structural hallmarks of these GPIs are a glycan branch of galactoses, a T. cruzi specific 2-aminoethylphosphonate (2-AEP) unit at the 6-O position of the glucosamine residue and the presence of unsaturated fatty acids in the phospholipid,8,9 which have been associated with the biological activity of these molecules.6,10

Isolation of homogeneous GPIs is extremely difficult due to the heterogeneity and amphiphilic character of both the glycan and lipid. Biological evaluation of T. cruzi GPIs and potential applications for the diagnosis and prevention of Chagas disease require synthetic GPIs bearing unsaturated lipids. Most synthetic strategies for GPI glycolipids use benzyl ethers as permanent carbohydrate protecting groups. However, the reductive conditions required for benzyl ether removal are not compatible with the double bonds present in the lipid moiety.11 While this issue can be avoided through the use of benzoyl esters or PMB ethers,12,13 their use in this application has been limited due to the saponification of the fatty acid esters during base-mediated benzoyl ester removal and the low stability of PMB ethers under the mild acidic conditions commonly used for glycosylations.13–15

While the 2-naphthylmethyl ether (Nap) group has primarily been used as a temporary mask for hydroxyl groups in carbohydrate chemistry,16,17 its stability during glycosylation reactions and its orthogonality to silyl ethers, acyl esters, and even PMB ethers make it the ideal group for permanent protection during GPI synthesis.

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Herein, we report an efficient strategy for the synthesis of GPIs bearing unsaturated lipids. The key of our strategy is the use of stable 2-naphthylmethyl ethers for permanent hydroxyl group protection, which are readily removed using acidic conditions. The strategy is illustrated for the synthesis of a portion of the GPI anchor from *T. cruzi*.

We considered an assembly sequence based on our recently reported strategy for accessing GPIs with saturated lipids, in which the glycan is assembled first and the phosphorylations are installed at a late-stage of the synthesis. In this strategy, Nap ethers replaced the benzyl ethers as permanent protecting groups and the Allyl and PMB ethers were included as temporary protecting groups for positions requiring phosphorylation, while an azide served as a masked amine.

To obtain the bis-phosphorylated GPI pseudo-disaccharide, a number of building blocks were envisioned branching from the core glycan fragment containing Nap ethers as permanent protecting groups. For the subsequent phosphorylations, building blocks 4 and 5 were necessitated (Fig. 2). To assemble the glycan moiety, building blocks 6 and 7 were designed to generate the desired α-glucosylated myo-inositol. Through a series of protecting group manipulations, the pseudo-disaccharide glycan GlcN-Ino 3 was obtained with both a PMB and an allyl-protecting group. Selective step-wise removal of the PMB and allyl ethers, followed by phosphorylations and global deprotection, will complete the synthesis.

Synthesis of the protected optically pure α-myoinositol building block 7 started from methyl-glucoside. The primary alcohol was protected with a trityl group, followed by the overnight per-naphthylmethylmethylation using 2-(naphthyl)methyl bromide and NaH (Scheme 1). After removal of the trityl ether, alcohol oxidation with SO_3·Py-pyridine complex to the aldehyde, and subsequent per-naphthyl-methylation using 2-(naphthyl)methyl bromide and alcohol was protected with a trityl group, followed by the overnight hydrolysis of the isopropylidene acetal and subsequent protection of the primary alcohol using a TBS group furnished in 19% overall yield (Scheme 2).

With the glycan part in hand, the synthesis of the phosphorylated building blocks 4 and 5 was advanced. Etherification of alcohol 17 using 1-bromooctadecane and NaH, followed by the hydrolysis of the isopropylidene acetal and subsequent protection of the primary alcohol using a TBS group furnished (Scheme 3a).

**Scheme 1** Synthesis of protected myo-inositol. 7. Reaction conditions: (a) NapBr, NaH, DMF, rt, overnight, 85%; (b) p-TsOH, MeOH, rt, 16 h, 90%; (c) i. SO_3·Py, DIPA, DMSO, CH_2Cl_2, 0 °C, 1 h, ii. Ac_2O, K_2CO_3, MeCN, reflux, 4 h, 80% (2 steps); (d) i. Hg(OII)Cl, acetone/H_2O, rt, 1 h, ii. NaOAc, NaCl, 0 °C to rt, 12 h; (e) NaBH(OAc)_3, MeCN, AcOH, rt, 12 h, 68% (2 steps); (f) NaOMe, MeOH, rt, 30 min; (g) i. (Bu_3Sn)_2O, toluene, reflux, 5 h, ii. AlBr, TBAI, toluene 65 °C, 17 h, 68% (20% recovered starting material); (h) NapBr, NaH, DMF, −20 °C to 0 °C, 2 h, 67%.

**Scheme 2** Synthesis of GlcN pseudo-disaccharide. 3. Reaction conditions: (a) i. NH_3(g), CH_3CN, 0 °C to rt, 1 h; ii. CCl_4CN, DBU, CH_2Cl_2, 0 °C, 15 min (5% recovery); (b) CCl_4CN, 0 °C, 1 h, 87%; (c) TMSOTf, Et_3O/CH_2Cl_2(6:1), 0 °C, 1 h, 85%; (d) TMSOTf, Et_3O/CH_2Cl_2(6:1), 0 °C, 1 h, 85%; (e) NaOMe, MeOH, rt, 1 h, ii. Anisaldehyde dimethylacetal, CSA, DMF, rt, overnight, 86% (2 steps); (f) NapBr, NaH, TBAI, DMF, rt, overnight, 88%; (g) NaCNBH_3, TFA, THF/CH_2Cl_2, rt, 9 h, 80%; (h) NapBr, NaH, DMF, rt, overnight, 82%. PMP: p-methoxyphenyl.
Acetylation of alcohol 19 with linoleic acid using DCC/DMAP and deprotection of the silyl group provided the desired allylacylglycerol 20 (Scheme 3). To obtain the required phosphoamidite building block, glycerol derivative 20 was transformed into 4 using the commercially available bis(diisopropylamino)(2-cyanoethoxy)phosphine and 1H-tetrazole.22

Ethyl-(2-azidoethyl)phosphonochloridate (5) and bis(chloro)-(2-azidoethyl)phosphonate (5a) were obtained from commercially available diethyl (2-bromoethyl)phosphonate. Starting with the conversion of bromide into the corresponding azide 21,12 the obtained ethyl phosphonate 21 was converted into chlorophosphonate 5 using a two-step protocol. First, ethylphosphonate 21 was hydrolyzed with LiBr to provide phosphonic acid mono ethyl ester 22.23 Which was converted into 5 by treatment with oxalyl chloride.24 Phosphonodichloridate 5a was synthesized from ethyl phosphonate 21 via silylated intermediate 23, which underwent reaction with oxaly chloride.25

Two phosphorylation sequences of 3 were evaluated. In the first case, the PMB group of pseudo-disaccharide 3 was selectively removed under acidic conditions and without affecting the Nap groups to obtain alcohol 24.26 This compound was further phosphorylated with 5 in the presence of 1H-tetrazole and DIPEA. Following reduction of the azides using DTT/DIPEA and amine protection with Boc anhydride the phosphonate compound 26 was delivered, which was deallylated using PdCl₂ and NaOAc in AcOH. To complete the synthesis of the fully modified pseudo-disaccharide, the free hydroxyl group of 27 was phosphorylated with 4 and oxidized to the corresponding phosphorylated product 28 by using tert-butylhydroperoxide (Scheme 4).

Removal of the protecting groups from 2-aminophospholipids for permanent protection and allyl and PMB ethers as orthogonal groups for masking positions requiring late stage ethers for permanent protection and allyl and PMB ethers as orthogonal groups for masking positions requiring late stage.
Notes and references


26 During the preparation of this report a new method for the acidic removal of the Nap group was published. Although we did not try this method in our compounds, it is an additional method for global deprotection. see: A. G. Volbeda, H. A. V. Kistemaker, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov and J. D. C. Codée, *J. Org. Chem.*, 2015, 80, 8796–8806.