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

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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). 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. 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.

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. 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.

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. 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, which have been associated with the biological activity of these molecules.

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. While this issue can be avoided through the use of benzoyl esters or PMB ethers, 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.

While the 2-naphthylmethyl ether (Nap) group has primarily been used as a temporary mask for hydroxyl groups in carbohydrate chemistry, 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.

Fig. 1 Glycosylphosphatidylinositol anchor of the parasite Trypanosoma cruzi.
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-myo-inositol building block 7 started from methyl-glucoside. The primary alcohol was protected with a trityl group, followed by the oxidation of the per-naphthylmethyl methylation using 2-(naphthyl)methyl bromide and NaH (Scheme 1). After removal of the trityl ether, alcohol oxidation per-naphthyl-methylation using 2-(naphthyl)-methyl bromide and protection of the primary alcohol using a TBS group furnished the protected GlcN-Ino 3. Regioselective allylation of 12 using bis(tributyltin) oxide and the selective protection at O-2 as a naphthyl-methyl ether gave building block 7 in 19% overall yield. The Nap group did not affect the process when compared to benzyl ethers and delivered the protected myo-inositol with improved yield.

For the synthesis of GlcN-Ino pseudo-disaccharide 3, the required glucosamine donor 6 was obtained from glucosamine azide 13 by transformation of the anomeric acetyl group into a 2-α-glucosylated myo-inositol moiety. Building blocks 6 and 7 were phosphorylated and global deprotection, will complete the synthesis. The phosphorylation was advanced. Etherification with anisaldehyde dimethylacetal by reacting the generated triol with acetyl groups were removed using Zemplen conditions delivered triol 12. Regioselective allylation of 12 using bis(tributyltin) oxide and the selective protection at O-2 as a naphthyl-methyl ether gave building block 7 in 19% overall yield. The Nap group did not affect the process when compared to benzyl ethers and delivered the protected myo-inositol with improved yield.

For the synthesis of GlcN-Ino pseudo-disaccharide 3, the required glucosamine azide 13 by transformation of the anomeric acetyl group into a trichloroacetimidate using a two-step protocol. Three different conditions were tested to obtain the desired α-glycosidic linkage. Product 14 was obtained in the best α/β ratio (11:1) at room temperature, using Et$_2$O/CH$_2$Cl$_2$ as solvent and TMSOTf as an activator (Scheme 2).

After separation of the α/β isomers of 14 by silica gel chromatography, the acetyl groups were removed using Zemplen conditions and the 4-O and 6-O hydroxyls were protected as a p-methoxybenzylidene acetal by reacting the generated triol with anisaldehyde dimethylether under acidic conditions. Nap protection of the free 3-O position of disaccharide 15, followed by selective opening of the 4,6-acetal group using NaCNBH$_3$ under acidic conditions, and protection of the 4-O position delivered the fully protected 3 in good 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 19 (Scheme 3a).
Acylation 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.23

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 tBuOH 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 oxalyl 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. Methods involving oxidative conditions (DDQ) delivered, which was deallylated using PdCl2 and NaOAc in CH3CO. To complete the synthesis of the fully modified pseudo-dissacharide 25 using 15% TFA, CH3Cl, 0 °C, 15 min, 91%; (b) 5a was oxidized to the corresponding phosphorylated product and hydrolysis with mercury chloride. The resulting free hydroxyl group was phosphorylated with phosphoamidite 4 and oxidized with t-BuOOH to deliver phospholipidated pseudo-disaccharide 26.

In summary, a new strategy to obtain glycosylphosphatidylinositol anchors containing unsaturated lipid chains has been disclosed. The strategy is based on the use of 2-naphthyl-methyl ethers for permanent protection and allyl and PMB ethers as orthogonal groups for masking positions requiring late stage modifications. The synthesis of the required building blocks demonstrated that the size of the Nap group did not affect the reactivity of donors and acceptors and can easily replace benzyl ether in established protocols. We described the synthesis of the complex Glc-Ino pseudo-disaccharide core of the T. cruzi parasite GPI, which bears a lipid moiety, and is modified by a linoleic acid ester and a 2-aminoaphosphoethyamine. We demonstrated the high stability of the Nap group in glycosylation and phosphorylation reactions and its removal under acidic conditions without affecting the phosphorylations, the allyl ether on allylacylglycerol, or the fatty acid ester.
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. Kistersman, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov and J. D. C. Codeé, *J. Org. Chem.*, 2015, 80, 8796–8806.