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Synthesis of a Glycosylphosphatidylinositol Anchor Derived from *Leishmania donovani* That Can Be Functionalized by Cu-Catalyzed Azide−Alkyne Cycloadditions

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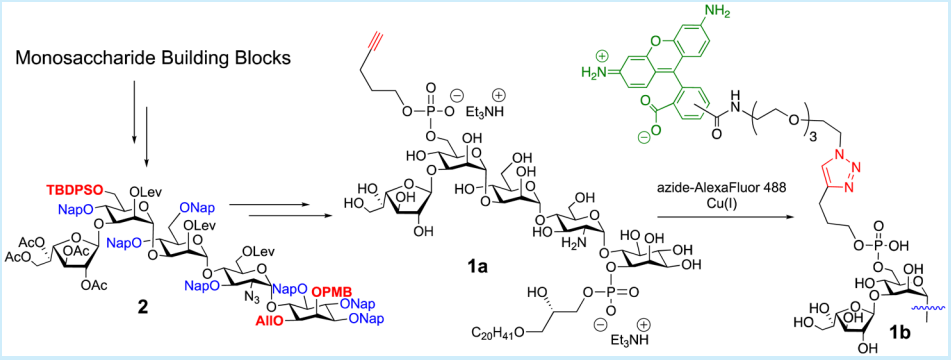
Supporting Information



ABSTRACT: A flexible assembly strategy has been developed for the synthesis of *Leishmania donovani* GPI anchors that bear a clickable alkyne tag. This strategy is based on the use of the 2-naphthylmethyl (Nap) ethers and levulinoyl (Lev) ester for permanent protection of hydroxyls. Removal of seven Nap ethers by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone made it possible to prepare GPIs having an alkyne functionality that could be modified by Cu(I)-catalyzed [3 + 2] cycloadditions to install tags for imaging studies.

The protozoan parasite *Leishmania donovani* causes fatal visceral leishmaniasis, which afflicts millions of people worldwide.[1] All parasites of the Trypanosomatid family, including *Leishmania*, express a highly complex lipophosphoglycan (LPG),[2] which plays critical pleiotropic roles in parasite survival and infectivity in both the sandfly vector and the mammalian host. LPG circumvents the lysis of the parasite by the host complement system.[3] It facilitates intracellular parasite survival by interfering with the pro-inflammatory host cell responses via binding of Toll-like receptor (TLR) 2 and 4 of macrophages and NK cells.[4] It also blocks the assembly of NADPH oxidase and prevents recruitment of proton ATPases at the phagosomal membrane.[5] This function has been attributed to the localization of LPG at the membrane of the *Leishmania*-containing phagosome.

A priority for understanding the disease process of *L. donovani* is to elucidate the structure−activity relationship of LPG. This membrane-bound glycoconjugate is composed of four distinct domains including a phosphatidylinositol lipid anchor, a phosphosaccharide core, a repeating phosphorylated saccharide region, and a terminating oligosaccharide cap.[1] The first two domains are also termed the glycosylphosphatidylinositol (GPI)-anchor motif, which is highly conserved among all the *Leishmania* LPGs.

Here, we report a facile strategy for the chemical synthesis of a GPI anchor derived from *L. donovani* (e.g., compound **1a**) bearing an alkyne tag, which facilitated the attachment of various tags such as fluorophores (e.g., compound **1b**).[6] The GPI anchor domain of **1** has an unusual structure and consists of an alkyl phosphatidylinositol having a single saturated aliphatic chain. It is expected that the synthetic compounds will make it possible to establish correlations between molecular structure and the intracellular localization of LPG in host cells. Another emerging area of research is "GPIomics", which seeks to develop proteomic tools for identifying proteins carrying a GPI anchor as a posttranslational modification. Such research also requires tagged GPI derivatives.[7] Well-defined GPI fragments will also make it possible to establish which domains are responsible for modulation of TLR responsiveness.[8]

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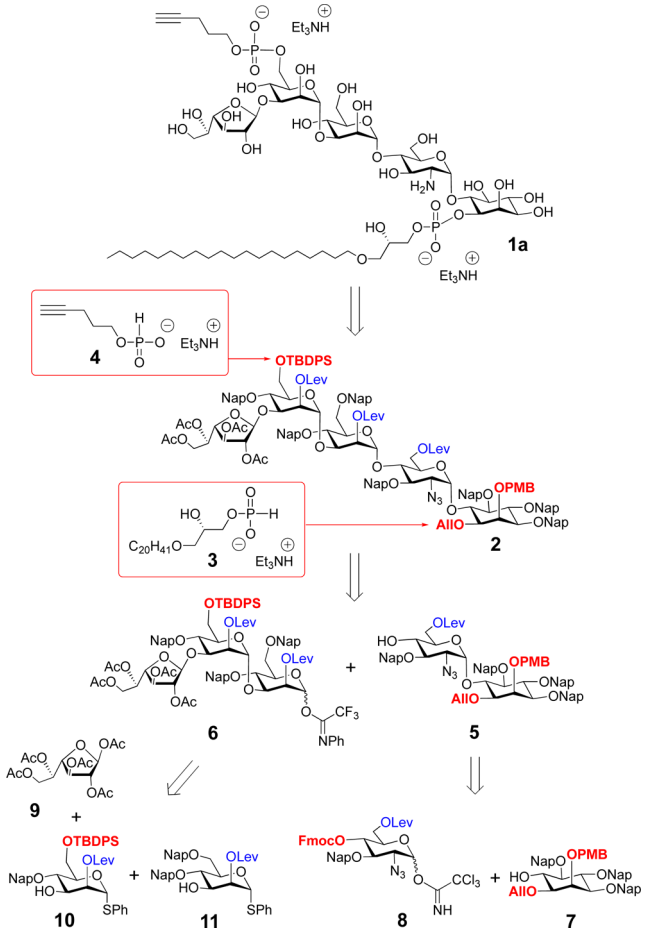
The preparation of compounds such as **1a** (Scheme 1) is challenging because benzyl ethers, which are commonly employed as permanent protecting groups for the preparation of GPI anchors,[8] are not compatible with the presence of the alkyne moiety. Benzoyl esters have been used as a permanent protecting group for GPI synthesis.[9] However, such a protecting group reduces glycosyl donor and acceptor reactivities, and their removal is not compatible with the presence of fatty esters. Guo and co-workers have pioneered the use of *p*-methoxybenzyl (PMB) ethers for the permanent protection of alcohols of GPI intermediates.[6d,10] Multiple PMB ethers could be removed by treatment with trifluoroacetic acid (TFA) in DCM, and these conditions were compatible with the presence of unsaturated functionalities. This strategy did, however, not use glycosyl donors having a C-2 ester for controlling 1,2-*trans* anomeric selectivities in glycosylations.[11] Furthermore, PMB ethers are rather fragile in acid-catalyzed glycosylations, which may cause difficulties when highly complex compounds need to be assembled. The 2-naphthylmethyl (Nap) ether is emerging as an attractive alternative of the PMB ether[12] and recently was employed as a permanent protecting group in the synthesis of a GPI pseudo-disaccharide.[13] In this case, removal was accomplished under acidic conditions using TFA in toluene. Compounds such as **1a**, which contain acid-sensitive glycosidic linkages, are not stable to treatment under moderately strong acidic conditions, and hence, this approach does not provide a reliable entry into compounds such as **1a**.

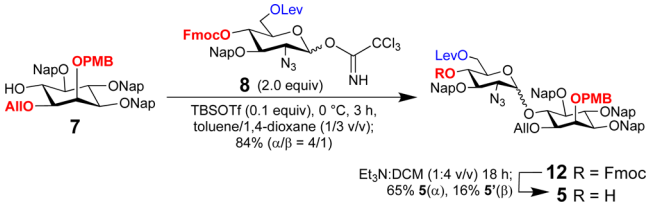
Scheme 1. Retrosynthetic Analysis of the Target GPI 1a



We report here a strategy for the preparation of GPI anchor derived pseudo-oligosaccharides, such as **1a**, employing Nap ethers and Lev esters for permanent protection. Surprisingly, as many as seven Nap ethers could be cleanly removed by oxidation with DDQ using an appropriate solvent mixture. Lev esters were used for permanent protection of mannosyl donors and have as attractive features that when present at C-2 can perform neighboring group participation to selectively introduce 1,2-*trans* glycosides, while they can be removed under neutral conditions using hydrazine acetate[14] without affecting fatty acids that are commonly part of GPI structures. Furthermore, PMB, allyl (All), and *tert*-butyldiphenylsilyl (TBDPS) ethers were used as a set of temporary protecting groups that could be selectively removed at a later stage of the synthesis to allow further functionalization with phosphate diesters. On the basis of these considerations, target compound **1a** was assembled from monosaccharide building blocks **7**–**11** (Scheme 1).

The assembly of **1a** commenced with the synthesis of the α-linked pseudo-disaccharide **12** (Scheme 2), which required the preparation of an optically pure inositol derivative and the formation of an α-glycosidic bond between glucosamine and inositol, which is notoriously difficult due to the formation of anomeric mixtures.[8] To address this challenge, inositol derivative **7** was prepared using an optimized method described by Fraser-Reid and co-workers[15] (see the SI for details). To achieve a glycosylation with acceptable α-selectivity, 2-azido-2-deoxy-D-glucosyl donor **8** was employed in view of possible remote participation of the Lev ester at C-6.[16] First, the glycosylation of inositol derivative **7** (see the SI) with glucosaminyl imidate **8** (see the SI) was carried out in the presence of catalytic TBSOTf in DCM at −30 °C, and while the pseudo-disaccharide **12** could be isolated in acceptable yield (74%), the anomeric outcome was unfavorable (α/β = 1.0/1.3). The use of diethyl ether as the reaction solvent did not substantially improve the stereoselective outcome of the glycosylation (α/β = 1/1); however, by employing toluene/1,4-dioxane (1:3 v/v),[17] the pseudo-disaccharide **12** was isolated in 84% yield mainly as the α-anomer (α/β = 4/1). After removal of the Fmoc protecting group using triethylamine, the anomers could readily be separated by silica gel column chromatography to give anomerically pure **5**. The glycosylation and Fmoc removal could be carried out as a one-pot, two-step procedure allowing the preparation of pseudo-disaccharide **5** in multigram quantities.

Scheme 2. Synthesis of Pseudo-Disaccharide 5



Monosaccharides **9**–**11** (see the SI for details) were employed for the assembly of trisaccharide donor **6** (Scheme 3). Compound **10** was used as a versatile key intermediate that would make it possible to prepare various GPIs having a substituent at the C-6 position of Man-II. The glycosylation of **10** with anomeric acetate **9** proceeded smoothly using $SnCl_4$ as the promoter in DCM to give disaccharide **13** in good yield. Thioglycoside **13** was converted into *N*-phenyl trifluoracetimi-

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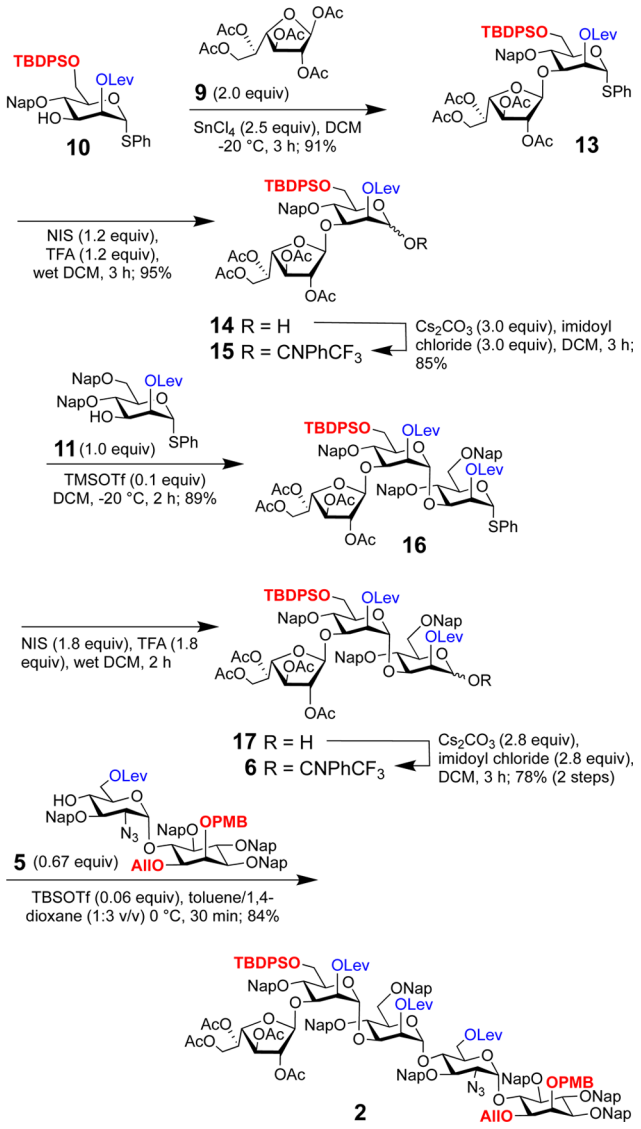
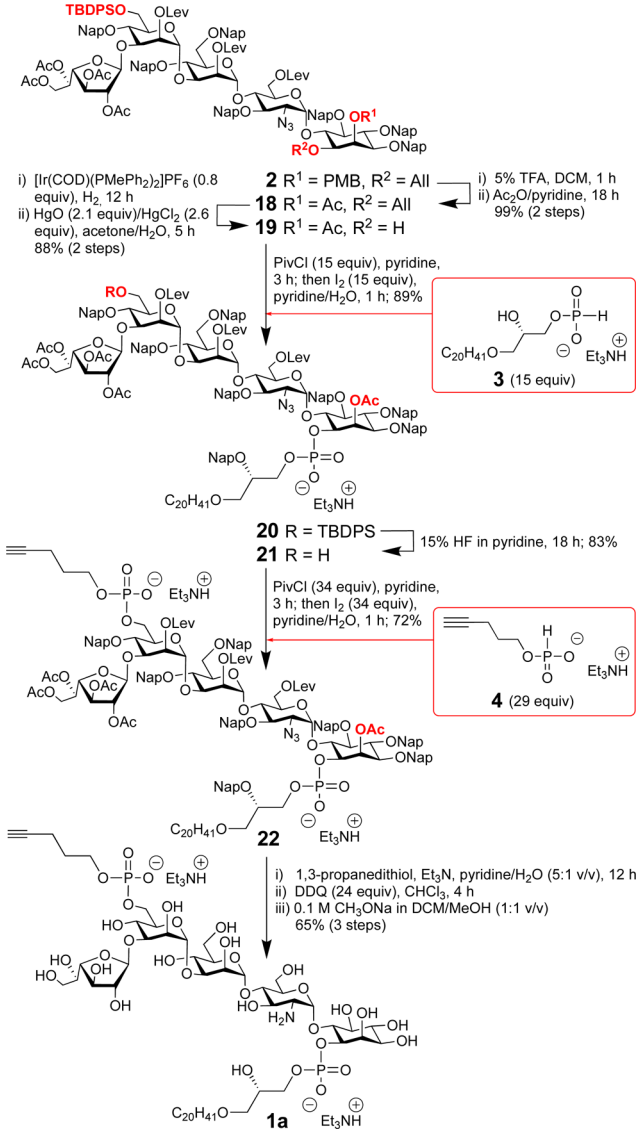
Scheme 3. Synthesis of the Saccharide Scaffold 2



Scheme 4. Synthesis of the Target GPI Anchor 1a



date **15** by treatment with a mixture of NIS and TFA in wet DCM followed by reaction of the resulting lactol with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of Cs_2CO_3.[18] A TMSOTf-mediated glycosylation of **15** with **11** in DCM at −20 °C gave trisaccharide **16** as only the α-anomer due to the presence of the Lev ester at C-2 of the glycosyl donor. Compound **16** was converted into *N*-phenyl trifluoracetimidate **6** using a standard two-step procedure.

The glycosylation of pseudo-disaccharide **5** with **6** was challenging due to the low reactivity of the C-4 hydroxyl of the glucosamine moiety. A glycosylation using TMSOTf (0.1 equiv) as the activator in DCM at 0 °C did not provide the desired product but resulted in the hydrolysis of the donor along with partial loss of the PMB protecting group of the acceptor. A lower reaction temperature (−50 °C) avoided the PMB loss, but the yield of **2** was modest (47%). Fortunately, the use of TBSOTf (0.1 equiv)[6d] as the promoter in toluene/1,4-dioxane (1:3, v/v)[17] at 0 °C gave the desired pseudo-pentasaccharide **2** in 84% yield.

Next, attention was focused on the further modification of **2** with phosphodiesters at C-2 of the inositol moiety and C-6 of the distal mannoside (Scheme 4). Selective cleavage of the PMB ether of **2** exposed a hydroxyl group that could be esterified with fatty acids to give entry into a range of important GPIs.[2] Target GPI derivative **1** does, however, not contain a lipid at this position, and therefore, the PMB ether was cleaved with 5% TFA in DCM and the resulting hydroxyl temporarily protected as an acetyl ester by treatment with acetic anhydride in pyridine to give **18**. Next, the allyl ether of **18** was removed by a two-step procedure involving isomerization with $[Ir(COD)(PMePh_2)_2]$-PF_6 that was activated by H_2, followed by cleavage of the resulting vinyl ether by employing $HgO/HgCl_2$ in acetone/H_2O[19] to give **19** in 88% yield. Installation of the phospholipid was achieved by using *H*-phosphonate **3**[20] (see the SI for details), which was activated with pivaloyl chloride (PivCl) followed by in situ oxidation with I_2 to furnish glycolipid **20** in 89% yield. Subsequent removal of the TBDPS group by treatment with diluted HF·pyridine gave alcohol **21** (83%), which was modified by an alkynyl phosphate by coupling with *H*-phosphonate **4** (see the SI) in the presence of PivCl followed by in situ oxidation with I_2 to give lipidated **22** as the triethylammonium salt (72%).

The deprotection sequence commenced with the reduction of the azide using 1,3-propanedithiol in the presence of Et_3N[21] to give the corresponding amine. Oxidative removal of the seven

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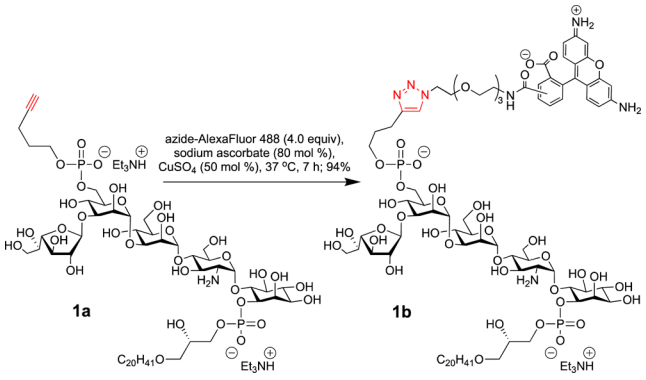
Nap ethers was carried out in chloroform in the presence of excess of DDQ, and gratifyingly, the reaction was fast and efficient and provided the expected glycolipid product. The latter compound was treated with sodium methoxide in DCM/MeOH (0.1 mol/L) to remove the remaining ester protecting groups to give target compound **1a** in an overall yield of 65% over three steps. Alternatively, the Lev esters could be cleaved with hydrazine acetate while leaving other esters intact, and such an approach may give entry to GPI anchors having base-sensitive fatty acids.

To demonstrate that the alkynyl moiety of GPI **1a** could be modified by probes, azide−AlexaFluor 488 was conjugated with **1a** by a Cu(I)-catalyzed [3 + 2] cycloaddition under standard conditions to provide **1b** (Scheme 5), which could potentially be used for imaging studies.

Scheme 5. Conjugation of Azide−AlexaFluor 488 to 1a



In summary, a synthesis of an *L. donovani* GPI anchor bearing a clickable alkyne tag (**1a**) has been accomplished using Nap ethers and Lev esters as permanent hydroxyl protecting groups. This approach allowed the incorporation of functionalities such as alkynes that are incompatible with the use of benzyl ethers for permanent protection. By using the Cu(I)-catalyzed [3 + 2] cycloaddition of **1a** with azide−AlexaFluor 488, **1b** was furnished; it is available for further biological studies. This flexible strategy is expected to be adaptable to other GPI anchors, especially those having unsaturated fatty acids.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01703.

Experimental procedures and NMR spectra for the synthesis of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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