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Synthesis of a cell-permeable analogue of a glycosylphosphatidylinositol (GPI) intermediate that is toxic to the living bloodstream form of *Trypanosoma brucei*

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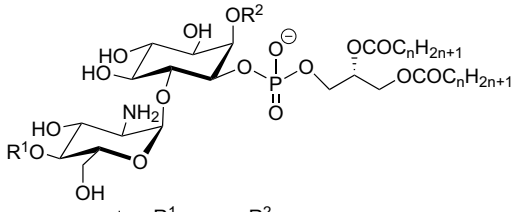
Abstract—The synthesis of two cell-permeable analogues related to a GPI intermediate is described for studies with living trypanosomes and human (HeLa) cells. One of the analogues is metabolised by the former GPI pathway and is toxic to the parasite *Trypanosoma brucei* but not to human cells.

The World Health Organisation (WHO)[1] estimates that sleeping sickness threatens over 60 million people in 36 countries of sub-Saharan Africa. African sleeping sickness is a debilitating and often fatal disease caused by trypanosomes transmitted by the tsetse fly. These extracellular protozoan parasites are able to survive in the human bloodstream by virtue of a dense cell-surface coat composed of variant surface glycoprotein (VSG).[2] This dense palisade of VSG is attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors.[3] It is our contention that disruption of GPI biosynthesis would seriously impair the parasite's ability to survive in the host.[4,5] However, it must be stressed that parasite-specific inhibitors of GPI biosynthesis are required since essential GPI-anchored glycoproteins are found in mammals.[6] The GPI biosynthetic pathway in mammalian cells appears to be broadly similar to that of the bloodstream form of *Trypanosoma brucei*, the causative agent of African sleeping sickness. One major difference between the parasite and mammalian pathways is the relative order of D-mannosylation and inositol acylation of α-D-Glc*p*N-(1→6)-PI (**1**, Fig. 1). *T. brucei* strictly D-mannosylates α-D-Glc*p*N-(1→6)-PI (**1**) to α-D-Man*p*-(1→4)-α-D-Glc*p*N-(1→6)-PI



	R^1	R^2
1	H	H
2	α-D-Man*p*	H
3	α-D-Man*p*	$COC_{15}H_{31}$

Figure 1.

(**2**) prior to inositol acylation (→**3**), whereas the reverse holds for the mammalian pathway.[3]

We have already synthesised an extensive library of various analogues of α-D-Glc*p*N-(1→6)-PI (**1**)[7] in order to ascertain the substrate specificities of *T. brucei* and human (HeLa) GPI biosynthetic enzymes in cell-free systems.[5,8] For the purpose of this Letter, the following results are relevant: (a) α-D-Gal*p*N-(1→6)-PI (**4**, Fig. 2) was not metabolised by either the trypanosomal or the mammalian system, presumably due to epimerisation of the hydroxyl group at the site of D-mannosylation; (b) the inositol-methylated analogue **5** is D-mannosylated by the *T. brucei* GPI pathway but not by the

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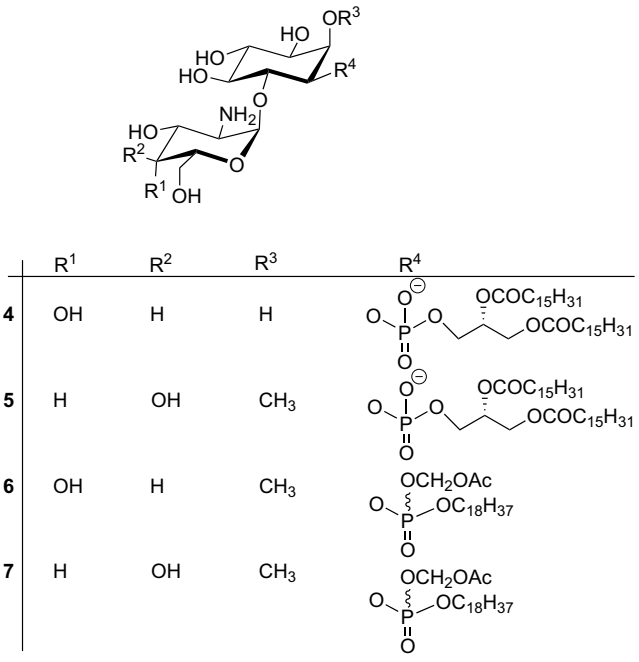
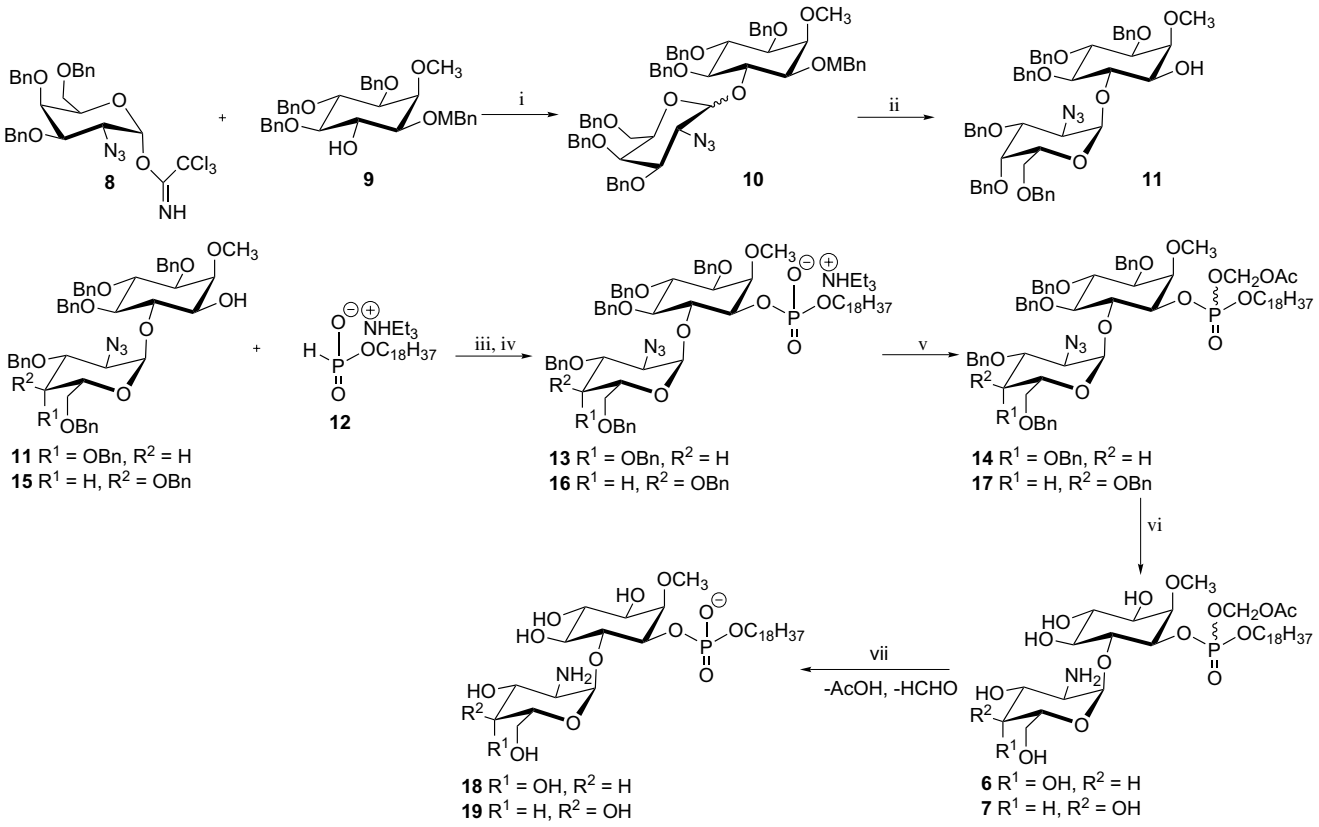


Figure 2.

human (HeLa) GPI pathway; (c) only those GPI analogues bearing a negatively charged phosphoric diester linkage are metabolised by the *T. brucei* cell-free system.[9] However, the negative charge on the phosphoric diester prevents these analogues from diffusing across the plasma membrane of living trypanosomes.

Bearing in mind these results in designing potential inhibitors of the *T. brucei* GPI pathway, we synthesised the neutral phosphoric triesters **6** and **7** (Fig. 2) possessing a bioactivatable acetoxymethyl (AM) protecting group.[10] It was anticipated that these neutral phosphoric triesters would diffuse across the plasma membrane of living trypanosomes and revert to the charged phosphoric diesters through the action of resident cytosolic esterase(s).[11] The closely related analogues **6** and **7** contain a C18 alkyl chain in place of the phospholipase-sensitive diacylglycerol of the natural substrate **1**, and are inositol-methylated (cf. **5**) to prevent substrate recognition by human GPI D-mannosyltransferases. Of the two, only the D-glucosamine-containing analogue **7** should be metabolised by living trypanosomes, whereas the D-galactosamine-containing analogue **6** functions as a non-metabolisable control.

The synthesis of the D-galactosamine-containing analogue **6** began by coupling the known trichloroacetimidate **8**[12] with the inositol acceptor **9**[7c] in the presence of a catalytic amount of TMSOTf to furnish the pseudodisaccharide **10** as a roughly 1:1 mixture of anomers (Scheme 1). The methoxybenzyl protecting group was next removed with trifluoroacetic acid to give, after radial-band chromatography, the desired α anomer **11** in 34% yield. Compounds **11** and **12**[7e] reacted in the



Scheme 1. Reagents and conditions: (i) TMSOTf, MS 4 Å, Et_2O, rt, 45 min, 80%; (ii) TFA, CH_2Cl_2, rt, 1 h, 34%; (iii) PivCl, pyridine, rt, 1 h; (iv) I_2, pyridine/H_2O {19:1}, rt, 45 min; 43% for **13**, 80% for **16**; (v) DIEA, acetoxymethyl bromide, THF, rt, overnight; 65% for **14**, 94% for **17**; (vi) H_2, 20% $Pd(OH)_2/C$, 1:1 THF–MeOH, 3 atm, rt, 2 h; 95% for **6**, 96% for **7**; (vii) cytosolic esterase(s).

presence of 3 equiv of pivaloyl chloride[13,14] to give the phosphoric diester **13** (isolated as the triethylammonium salt) following oxidation of the intermediate phosphoric diester with iodine in wet pyridine. Treatment of **13** with acetoxymethyl bromide in THF in the presence of DIEA afforded the AM derivative **14** as a mixture of two diastereomers. Finally, hydrogenolysis of the benzyl groups, with concurrent reduction of the azido group, provided **6** as a mixture of diastereomers.

An identical sequence of reactions was used to synthesise the D-glucosamine-containing analogue **7** (**15**[7c]→**16**→**17**→**7**).

Details of in vivo studies with the AM-protected GPI analogues **6** and **7** have been published elsewhere.[9] It suffices here to note that both **6** and **7** fulfilled our expectations and, after diffusing across the plasma membrane, were hydrolysed by the cytosolic esterase(s) of living trypanosomes to the charged phosphoric diesters **18** and **19**, respectively. Only the D-glucosamine-containing analogue **19** is subsequently metabolised and this resulted in killing of the trypanosomes in a concentration- and time-dependant manner.[15] The mechanism for killing the parasite is not clear, but there are strong indications[9] that a metabolite of **19** that accumulates within the endoplasmic reticulum, possibly D-Manp_3-α-D-Glc*p*N-(1→6)-(2-*O*-methylinositol)-P-C18, is responsible. We also showed that the deprotected form **19** is parasite specific since it is neither a substrate nor an inhibitor in a HeLa cell-free system. These results and the complete lack of toxicity of the non-metabolisable D-galactosamine-containing analogue **18** provide the first unambiguous chemical validation of the *T. brucei* GPI pathway as a drug target.

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Supplementary data

Experimental procedures and partial characterisation data for all the compounds synthesised in this Letter are available online. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.08.112.

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