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Synthesis of 3′-, 4′- and 6′-deoxy and other analogues of D-glucosaminylphosphatidylinositol

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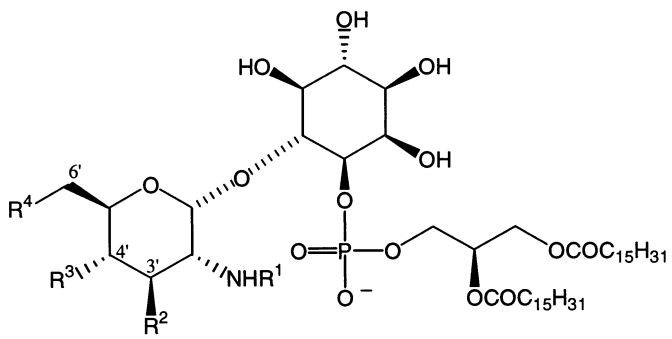
Abstract—Deoxy and other analogues of D-glucosaminylphosphatidylinositol **1** have been synthesised and tested as substrates or inhibitors of a de-*N*-acetylase and mannosyltransferase (MT-1) involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) membrane anchor of the parasite *Trypanosoma brucei*.

The ability of the parasite *Trypanosoma brucei* to evade a mammalian host's immune response is due to a uniformly dense protective coat[1] made up of $\sim 10^7$ copies of a 55 kDa variant surface glycoprotein (VSG) on the cell surface.[2] This surface coating of VSGs not only protects the parasite from lysis by the alternative- and lectin-mediated complement pathways of the host, but also, by the process of antigenic variation, from the humoral immune response. The VSGs of *T. brucei* form homodimers attached to the cell surface by means of glycosylphosphatidylinositol (GPI) membrane anchors.[3] The absolute dependence of the trypanosome on its VSG coat, and the dependence of the latter on its GPI anchors, makes the parasite GPI biosynthetic pathway an attractive therapeutic target. However, in the view of the presence and importance of GPI-anchored glycoproteins in mammals,[4] parasite-specific inhibitors of GPI biosynthetic enzymes are desirable. With this in mind, we have sought to define the substrate specificities of two enzymes, viz. a de-*N*-acetylase and a mannosyltransferase (MT-1), that exert their catalytic activities early on in the GPI biosynthetic pathway.[3,5]

In systematically extending our earlier studies,[6] it became necessary to test the 3′-, 4′- and 6′-deoxy analogues of **1** as potential substrate/inhibitors of these enzymes. Having outlined the synthesis of the requisite glycosyl donors in the preceding Letter, we now proceed to the synthesis of the monodeoxygenated GPI analogues **2**–**4** and a related 4-*O*-methyl analogue **5**.

Coupling of the 2-azido-2,3-dideoxyglycosyl fluoride **6**[7] with the D-*myo*-inositol derivative **7**[8] in dry toluene:1,4-dioxane (3:1) in the presence of zirconocene dichloride and silver perchlorate afforded the α-(1→6)-linked pseudodisaccharide **8** ($J_{1',2'} = 3.0$ Hz) in 35% yield after



	R^2	R^3	R^4
1ab	HO	HO	HO
2ab	H	HO	HO
3ab	HO	H	HO
4ab	HO	HO	H
5ab	HO	MeO	HO

a series R^1 = H; **b** series R^1 = Ac

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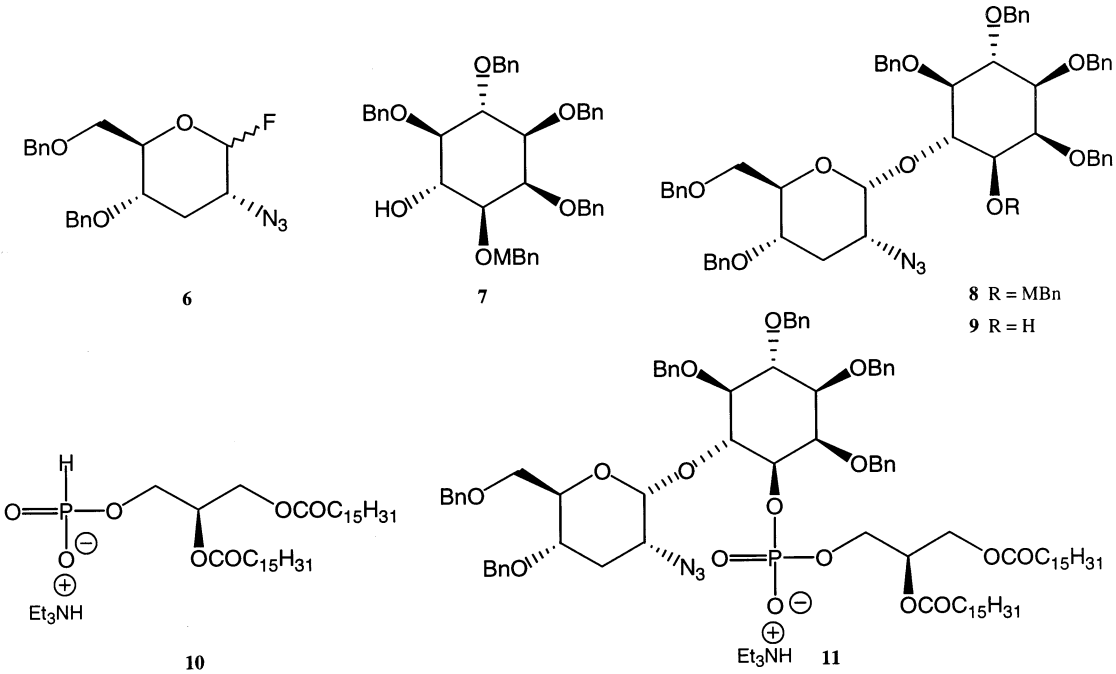


6 7 8 R = MBn 9 R = H 10 11

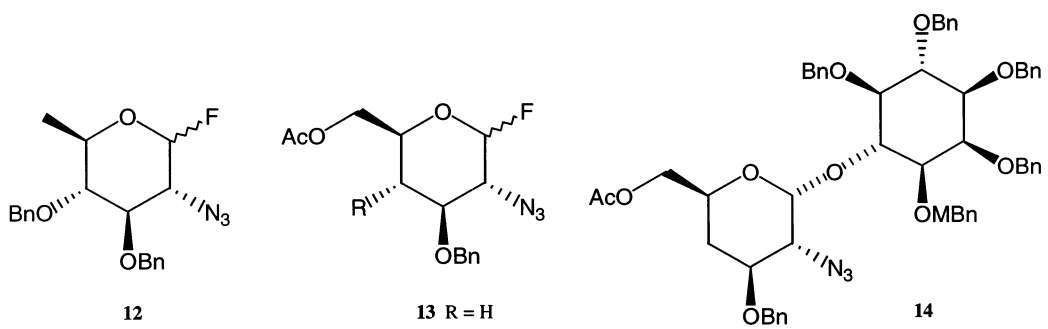
radial-band chromatography. Demethoxybenzylation with 90% trifluoroacetic acid then gave the alcohol **9**. Activation of the H-phosphonate **10**[9] with 3 equivalents of pivaloyl chloride in pyridine and coupling with **9** furnished the phosphodiester **11** (isolated as the triethylammonium salt) after oxidation with iodine. Finally, removal of the benzyl groups and simultaneous conversion of the azido group into an amino group was achieved by hydrogenolysis with 10% palladium hydroxide on carbon and gave 3-dGlc*p*N-PI **2a**.[10]

An identical approach was used to incorporate the 2-azido-2,6-dideoxyglycosyl fluoride **12**[7] into 6dGlc*p*N-PI **4a**[10] ($J_{1',2'} = 4.1$ Hz), following coupling with the D-*myo*-inositol derivative **7**.[8]

Coupling of the 2-azido-2,4-dideoxyglycosyl fluoride **13**[7] with **7**[8] provided the pseudodisaccharide derivative **14** ($J_{1',2'} = 3.6$ Hz) in 35.5% yield, which, following de-*O*-acetylation and benzylation at the 6′-position, was converted into 4dGlc*p*N-PI **3a**[10] in the usual way. An analogous procedure, commencing with the glycosyl fluoride **15**,[7] provided Glc*p*N4Me-PI **5a**[10] ($J_{1',2'} = 3.7$ Hz). This and the dGlc*p*N-PI analogues were readily transformed into the corresponding *N*-acetyl derivatives **2b**–**5b** on treatment with acetic anhydride (tritiated for studies with the de-*N*-acetylase) in 2:1 THF–MeOH containing 2% triethylamine.

Details of the enzymic studies[11] with the Glc*p*N(Ac)-PI analogues **2**–**5** will be published elsewhere in due course, so that only a résumé of the results is provided here. Neither of the 3′-deoxy analogues **2b** or **2a** is a substrate for either the *T. brucei* de-*N*-acetylase or MT-1, respectively, unlike the 6′-deoxy analogues **4** which are good substrates for their respective enzymes. Because the 4′-position is unavailable, neither **3a** nor **5a** can function as a substrate for MT-1, which transfers a D-mannose residue to this position from dolichol phosphate D-mannose, but both of them are potential inhibitors of MT-1. Of the two, 4dGlc*p*N-PI **3a** proved to be a considerably better inhibitor than Glc*p*N4Me-PI **5a**, which showed only weak inhibition.

These results are highly significant in the design of the next generation of inhibitors of the de-*N*-acetylase and MT-1 of the bloodstream form of *T. brucei*. For instance, they show that neither enzyme tolerates the removal of 3′-OH from Glc*p*N(Ac)-PI **1**, whereas the removal of 6′-OH, as with 6dGlc*p*N(Ac)-PI **4**, leaves an entirely acceptable substrate and augers well for the



12 13 R = H 15 R = OMe 14

introduction of fluorogenic substituents at the 6′-position as biological studies progress. Also encouraging is the strong inhibition of MT-1 shown by 4dGlc*p*N-PI **3a**.

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10. ES-MS(−) data: **2a** *m/z* 954.3000 [100%, $M-Et_3N-H]^-$ (requires *m/z* 954.5919); **3a** *m/z* 954.4623 [100%, $M-Et_3N-H]^-$ (requires *m/z* 954.5919); **4a** *m/z* 954.1000 [100%, $M-Et_3N-H]^-$ (requires *m/z* 954.5919); **5a** *m/z* 984.2988 [100%, $M-Et_3N-H]^-$ (requires *m/z* 984.6025). These compounds also exhibited ^1H and ^{31}P NMR spectra compatible with the assigned structures.
11. These studies were conducted with a trypanosomal cell-free system to which the various dGlc*p*N(Ac)-PI analogues were added as exogenous substrates. The action of the de-*N*-acetylase was followed by the release of acetic-$^3H_3$ acid from analogues with tritium labelling of the *N*-acetyl group, whilst that of MT-1 was assessed from the extent of transfer of the tritium label from dolichol phosphate D-mannose-2-^3H to the various analogues. The analogues were also tested for their ability to inhibit the processing of exogenous synthetic Glc*p*N(Ac)-PI **1** by the trypanosomal cell-free system.