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Analogues of D-glucosaminylphosphatidylinositol: synthesis of the glycosyl donors

Alexander P. Dix,[a] Charles N. Borissow,[a] Michael A. J. Ferguson[b] and John S. Brimacombe[a,*]

[a]*Department of Chemistry, University of Dundee, Dundee DD1 4HN, UK*
[b]*Department of Biochemistry, University of Dundee, Dundee DD1 4HN, UK*

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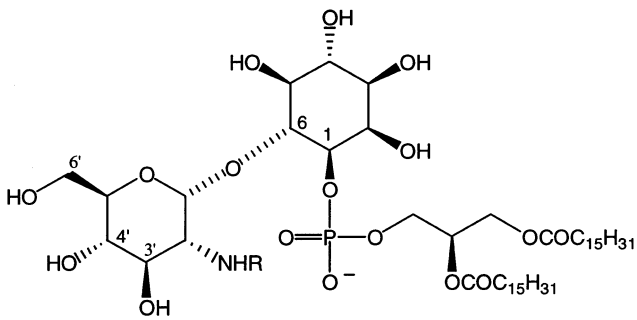
Abstract—Appropriately protected deoxy and other analogues of 2-azido-2-deoxy-D-glucopyranosyl fluoride have been synthesised preparatory to inclusion into analogues of the D-glucosaminylphosphatidylinositol **1**. © 2000 Elsevier Science Ltd. All rights reserved.

The essential features of glycosylphosphatidylinositol (GPI) biosynthesis in the bloodstream form of *Trypanosoma brucei* (the causative agent of African sleeping sickness) have been established[1] and start with the transfer of *N*-acetylglucosamine (GlcNAc) to phosphatidylinositol (PI) to form α-D-Glc*p*NAc-(1→6)-PI, which is then de-*N*-acetylated to produce α-D-Glc*p*N-(1→6)-PI. This step, catalysed by a de-*N*-acetylase, is a prerequisite for further processing which, in *T. brucei*, entails the addition of the first of three D-mannose residues to form α-D-Man*p*-(1→4)-α-D-Glc*p*N-(1→6)-PI. The mannosyltransferase (MT-1) catalysing this step has a requirement for dolichol phosphate D-mannose. Further processing is required[1] before the fully assembled GPI precursor is transferred en bloc to newly synthesised protein and thereafter to the plasma membrane where it forms part of the dense protective coating of the trypanosome. The substrate requirements of the de-*N*-acetylase and MT-1 of *T. brucei* have been probed[2] extensively in our laboratories utilising substrate analogues in which changes and deletions have been effected in the various components of the D-glucosaminylphosphatidylinositol **1**.[3] In furtherance of these studies, 3′-, 4′- and 6′-deoxy and other analogues of **1** were required to be tested as substrate analogues/inhibitors of these enzymes. This Letter outlines the synthesis of the various glycosyl donors required for coupling with an appropriately protected D-*myo*-inositol acceptor,[3] whilst the following Letter describes the completed syntheses and, briefly, the results of the enzymic studies.



1 R = Ac or H

The synthetic strategy is dictated by the need to locate a non-participating azido group (a masked amino group) at C-2 of the glycosyl donor, invariably the glycosyl fluoride, to promote the formation of the requisite α-glycosidic linkage in subsequent coupling reactions.

Either of two routes provided the 2-azido-2,3-dideoxyglycosyl fluoride **5**.[4] An azide displacement on the tosylate **3**, prepared from the known alcohol **2**,[5] furnished the 2-azido compound **4**, which was transformed straightforwardly into the glycosyl fluoride **5** (Scheme 1). Alternatively, a Michael reaction of the enone **6**[6] with azide ion resulted, after equilibration, in a 2:1 mixture of the azido ketones **7** and **8**, respectively (Scheme 2). Reduction of the mixture of **7** and **8** with sodium borohydride gave, after careful radial-band chromatography,[7] ethyl 2-azido-2,3-dideoxy-α-D-*ribo*-hexopyranoside **9**[6] as one of the products. Thereafter,

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* Corresponding author. Fax: (44) 1382-345 517; e-mail: j.s.brimacombe@dundee.ac.uk

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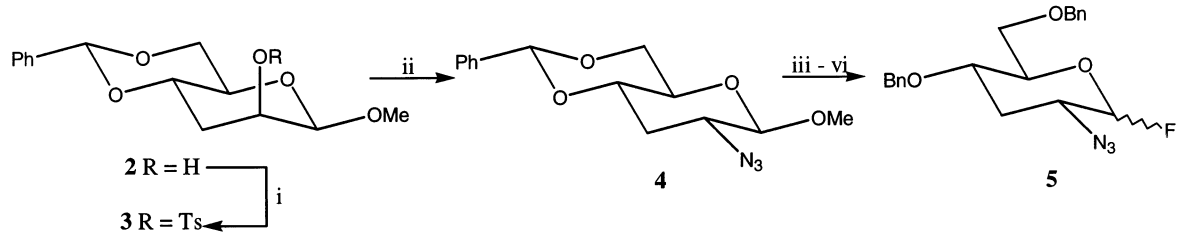
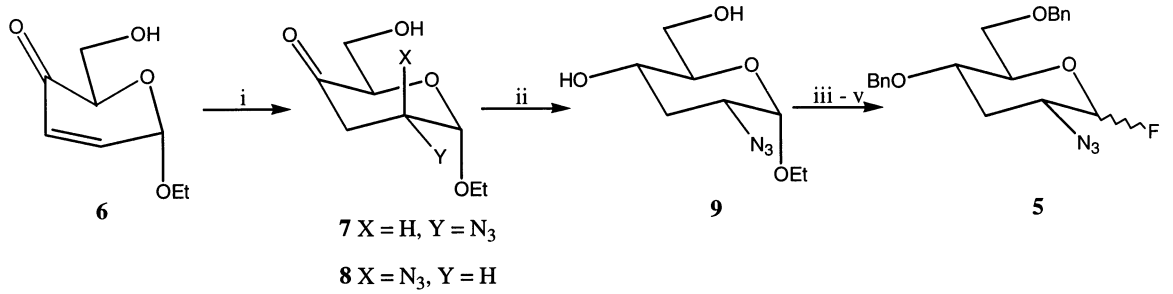
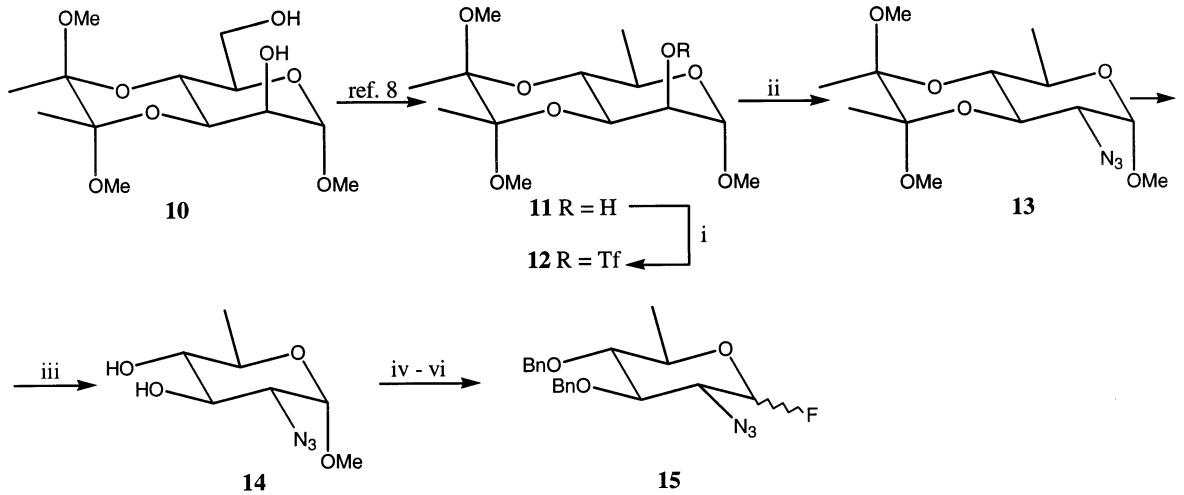


Scheme 1. Reagents: i, TsCl, pyridine; ii, NaN_3, 18-crown-6-ether, DMF, 60°C; iii, 70% AcOH; iv, BnBr, NaH, DMF; v, 1 M HCl, 80% AcOH; vi, DAST, 1,2-dichloroethane



Scheme 2. Reagents: i, NaN_3, AcOH, H_2O; ii, (a) $NaBH_4$, MeOH; (b) radial-band chromatography; iii, BnBr, NaH, DMF; iv, 1 M HCl, 80% AcOH; v, DAST, 1,2-dichloroethane



Scheme 3. Reagents: i, Tf_2O, pyridine; ii, NaN_3, DMF, 70°C; iii, 90% CF_3CO_2H; iv, BnBr, NaH, DMF; v, 1 M HCl, 80% AcOH; vi, DAST, 1,2-dichloroethane

benzylation, acid hydrolysis and treatment of the released hemiacetal with DAST produced the glycosyl fluoride **5**.

Our approach to the 2-azido-2,6-dideoxyglycosyl fluoride **15**[4] followed a literature procedure[8] from the 3,4-diacetal **10** through to the 6-deoxy analogue **11**, which, in turn, was converted into the triflate **12** (Scheme 3). Despite the presence of the α-glycosidic substituent,[9] the triflate **12** reacted with azide ion in hot DMF to give the crystalline 2-azido compound **13**[4,10] in 58% yield (over the two steps from **11**). Deacetalation then provided the 3,4-diol **14**, which was transformed into the glycosyl fluoride **15** without incident.

The 1,6-anhydro compound **17**,[11] with the 2-azido group already installed, provided access to the 2-azido-2,4-dideoxyglycosyl fluoride **22**[4] (Scheme 4). This key compound, which is available in a few steps from D-glucal **16**, was converted into the imidazolide **18** and thence, by radical-induced deoxygenation,[12] into the 4-deoxy derivative **19**.[13] Gratifyingly, there was no significant reduction of the reducible azido group[14] in this step. Subsequent acetolysis provided the diacetate **20** (α:β ~ 3:1), which was transformed, via the hemiacetal **21**, into the glycosyl fluoride **22**. This approach also provided the 4-*O*-methyl analogue **24** by way of the methylated derivative **23** obtained from **17**.

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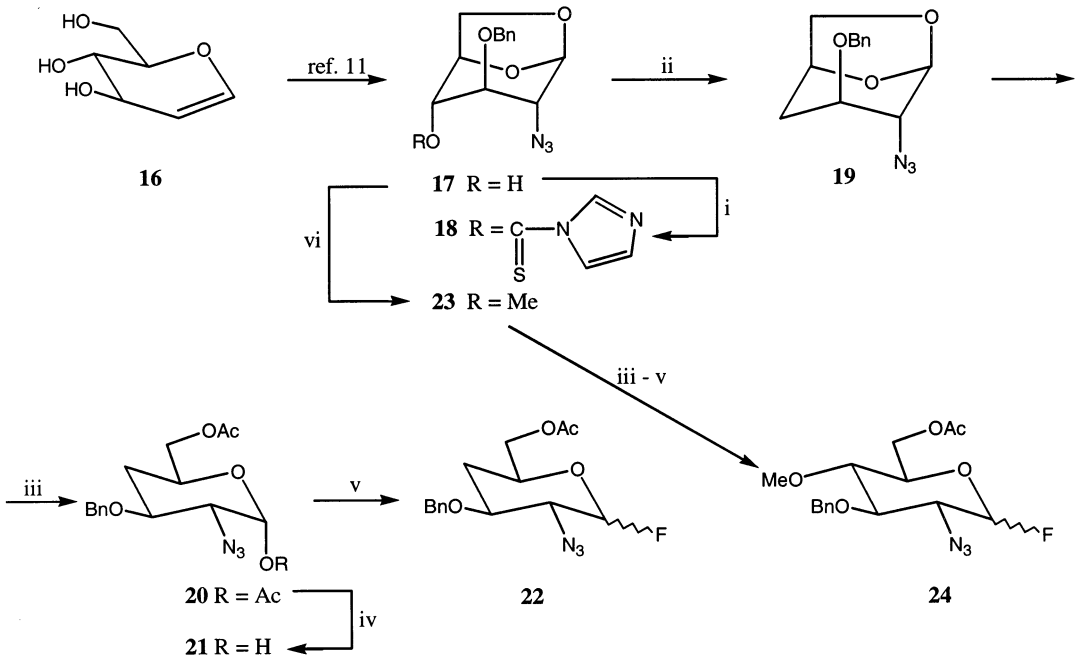

Scheme 4. Reagents: i, 1,1-Thiocarbonyldiimidazole, toluene; ii, Bu_3SnH, AIBN, benzene, 80°C; iii, Ac_2O, CF_3CO_2H; iv, Me_2NH, MeCN; v, DAST, 1,2-dichloroethane; vi, MeI, NaH, DMF

References

1. McConville, M. J.; Ferguson, M. A. J. *Biochem. J.* **1993**, *294*, 305.
2. Milne, K. G.; Field, R. A.; Masterson, W. J.; Cottaz, S.; Brimacombe, J. S.; Ferguson, M. A. J. *J. Biol. Chem.* **1994**, *269*, 16403; Smith, T. K.; Cottaz, S.; Brimacombe, J. S.; Ferguson, M. A. J. *J. Biol. Chem.* **1996**, *271*, 6476; Sharma, D. K.; Smith, T. K.; Crossman, A.; Brimacombe, J. S.; Ferguson, M. A. J. *Biochem. J.* **1997**, *328*, 171; Smith, T. K.; Sharma, D. K.; Crossman, A.; Dix, A.; Brimacombe, J. S.; Ferguson, M. A. J. *EMBO J.* **1997**, *16*, 6667; Sharma, D. K.; Smith, T. K; Weller, C. T.; Crossman, A.; Brimacombe, J. S.; Ferguson, M. A. J. *Glycobiology* **1999**, *9*, 415; Smith, T. K.; Sharma, D. K.; Crossman, A.; Brimacombe, J. S.; Ferguson, M. A. J. *EMBO J.* **1999**, *18*, 5922.
3. Cottaz, S.; Brimacombe, J. S.; Ferguson, M. A. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2945.
4. Satisfactory analytical and/or spectroscopic data were obtained on all new compounds.
5. Probert, M. A.; Zhang, J.; Bundle, D. R. *Carbohydr. Res.* **1996**, *296*, 149.
6. Jegon, E.; Cleophax, J.; Leboul, J.; Gero, S. D. *Carbohydr. Res.* **1975**, *45*, 323.
7. This procedure required sacrificial cuts and so resulted in a low yield (18%) of the pure compound **9**, whose identity was confirmed by conversion (i, $Pd(OH)_2/C$, H_2, MeOH; ii, Ac_2O, MeOH) into ethyl 2-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranoside, mp 184–187°C; lit. (Ref. 6), mp 185–187°C.
8. Ley, S. V.; Owen, D. R.; Wessen, K. E. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2805.
9. Richardson, A. C. *Carbohydr. Res.* **1969**, *10*, 395.
10. Mp 88–89°C (from methanol), $[\alpha]_D$+298 (*c* 1, $CHCl_3$), ν_{max} 2100 cm^{-1} (N_3).
11. Tailler, D.; Jacquinet, J.-C.; Noirot, A.-M.; Beau, J.-M. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3163.
12. Rasmussen, J. R.; Slinger, C. J.; Kordish, R. J.; Newman-Evans, D. D. *J. Org. Chem.* **1981**, *46*, 4843.
13. The presence of the 4-deoxy group was clearly indicated by distinctive signals, each corresponding to one proton, centred at δ 1.79 and 2.17, J_{gem} 15 Hz, in the ^1H NMR spectrum ($CDCl_3$), whilst the presence of the azido group was signalled by an IR absorption at ν_{max} 2100 cm^{-1}.
14. Hays, D. S.; Fu, G. C. *J. Org. Chem.* **1998**, *63*, 2796.