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Note

Synthesis of a partially protected 1D-6-*O*-(2-azido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol: a useful precursor of glycosylphosphatidylinositols and related compounds ☆

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Glycoconjugates on the cell surface of parasitic protozoa of the trypanosomatidae (including, for example, African trypanosomes and *Leishmania spp.*) have a crucial role in determining parasite survival and infectivity [1]. Many glycoconjugates are attached to the plasma membrane by means of glycosylphosphatidylinositol (GPI) anchors [1,2], whose principal function is to provide a stable association of protein or oligosaccharide with the lipid bilayer. A key, early step in the biosynthesis of the GPI membrane anchor of bloodstream forms of *Trypanosoma brucei*, an African protozoan parasite that causes a disease related to sleeping sickness in domestic cattle, involves the de-*N*-acetylation of 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1 → 6)-phosphatidylinositol (**1**) [3]. The de-*N*-acetylase responsible for this step has been partially purified [4] through the agency of the synthetic analogue **2** (tritium-labelled in the *N*-acetyl group) [5], which is added as an exogenous substrate to detergent-solubilised cell extracts. With the partial purification

☆ Parasite Glycoconjugates. Part 2. For Part 1, see ref. [5].

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of this de-*N*-acetylase, it is now possible to probe its substrate specificity and, in particular, to seek to inhibit its action in expectation that disruption of the GPI biosynthetic pathway in vivo would seriously impair the parasite's ability to survive in the host's bloodstream.

The partially benzylated glycosyl-inositol **3** is a useful intermediate in the synthesis of **2** [5] and other analogues, such as **4**, which can be tested [4] as potential substrates for, or inhibitors of, the *Trypanosoma brucei* de-*N*-acetylase. It is also of interest as an intermediate for the synthesis of putative insulin mimetics such as 1D-6-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol 1-phosphate (**5**) [6]. We have recently described [5] a synthesis of **3** from enantiomerically pure 1D-3-*O*-benzyl-1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (**6**) [7], and now report an alternative route from **6** to **3**, the early stages of which are strategically similar to one reported by van Boom's group [8] in preparing 1D-6-*O*-α-D-mannopyranosyl-*myo*-inositol from *rac*-**6**. Our preference to start from enantio-pure **6** stems from the ease of preparation and crystallisation of the (*S*)-camphanoyl derivative used in the optical resolution process [5,7], possibly forestalling a tedious chromatographic separation if optical resolution was left to a later stage.

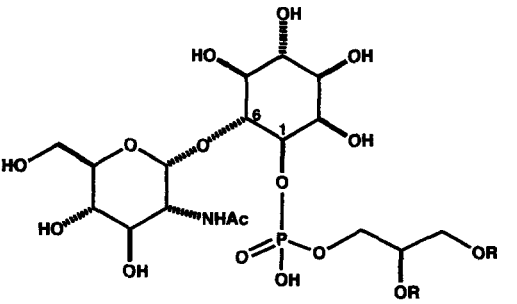
Conventional 4-methoxybenzylation of **6** gave **7**, from which the less stable *trans*-fused 4,5-*O*-cyclohexylidene group was removed selectively (→ **8**) by transacetalation with ethylene glycol [5,8]. Benzylation of **8** then afforded **9**, which rapidly lost the 4-methoxybenzyl group (→ **10**) on treatment with boron trifluoride etherate in dichloromethane. Compound **10** was glycosylated with the trichloroacetimidate donor **11** using trimethylsilyl trifluoromethanesulfonate as promoter to give, after removal of the 1,2-*O*-cyclohexylidene group with aq tetrafluoroboric acid, a mixture of the α-coupled product **13** (46% yield, $J_{H-1',H-2'} = 3.4$ Hz) and the corresponding β-coupled product **14** (25% yield, $J_{H-1',H-2'} = 8.6$ Hz). The individual products were obtained in crystalline form after flash-column chromatography. It is of interest to note that glycosylation of *rac*-**10** with the 6-acetylated compound **12**, using the same promoter, is α-stereospecific and also yielded **13** after further manipulation of the temporary protecting groups and resolution of the diastereoisomers [9].

Regioselective 4-methoxybenzylation of the equatorial HO-1 group of the *cis*-diol **13**, via the 1,2-*O*-dibutylstannylidene derivative, gave **15**. Conventional benzylation of the remaining HO-2 group produced **16**, which was smoothly converted into **3** on treatment with trifluoroacetic acid in dichloromethane. As mentioned earlier, compound **3** provides a convenient springboard for the synthesis of the glycosylphosphatidylinositol **2** [5] and other substrate analogues such as **4** [10], which have been instrumental in establishing that the lipid component has an important role in substrate recognition and/or presentation of the substrate to the *Trypanosoma brucei* de-*N*-acetylase in detergent micelles [4].

Experimental

General methods.—Melting points were measured on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were obtained using a Perkin–Elmer 141 polarimeter at ambient temperature. ^{1}H NMR spectra were recorded for solutions in $CDCl_3$

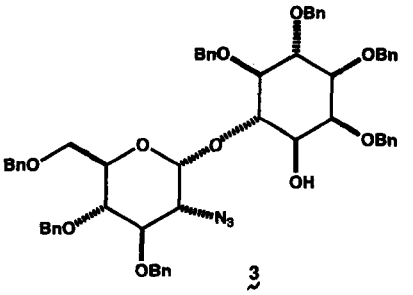
at 200 MHz (Bruker AM 200) with Me_4Si (δ 0) as an internal standard. IR spectra were recorded using KBr discs with a Perkin–Elmer Model 298 spectrometer. FABMS were measured in the positive ionisation mode with a VG250/70 SE instrument on samples



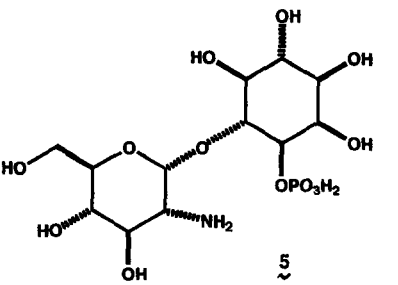
1 R = fatty acid residues (> $COC_{13}H_{27}$)

2 R = $COC_{15}H_{31}$ (palmitoyl)

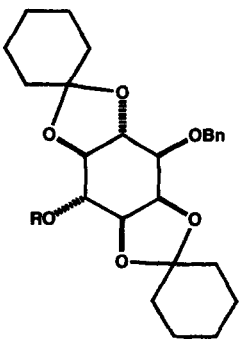
4 R = H



3

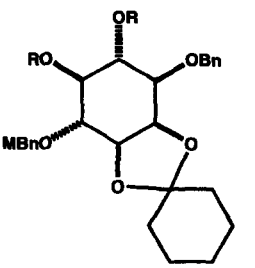


5



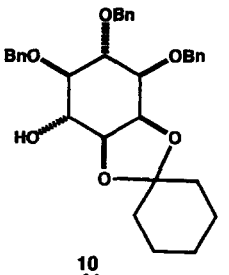
6 R = H

7 R = MBn = 4-methoxybenzyl

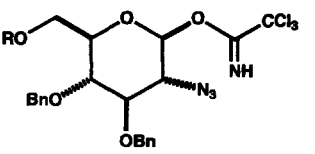


8 R = H

9 R = Bn

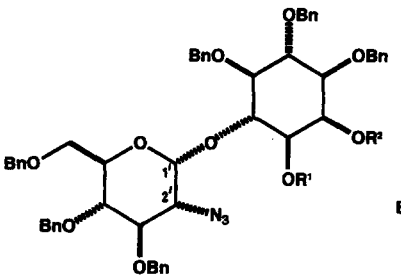


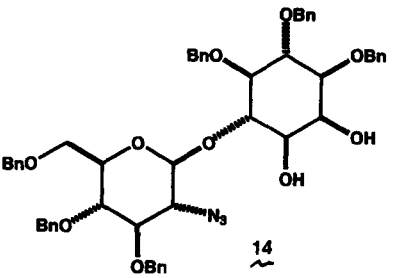
10



11 R = Bn

12 R = Ac





13 $R^1 = R^2 = H$

14

15 R^1 = MBn, $R^2 = H$

16 R^1 = MBn, R^2 = Bn

suspended in a matrix of thioglycerol–glycerol. Flash-column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck); TLC was performed on Silica Gel $60F_{254}$ (Merck) with detection by charring with dilute H_2SO_4. Light petroleum refers to the fraction having the boiling range 60–80°C.

1D-3-O-Benzyl-1,2:4,5-di-O-cyclohexylidene-6-O-(4-methoxybenzyl)-myo-*inositol* (**7**). —To a cooled (0°C) solution of enantio-pure **6** [5,7] (3.0 g, 7 mmol) in DMF (50 mL) was added NaH (0.2 g, 8.33 mmol), KI (0.31 g, 1.87 mmol), and 4-methoxybenzyl chloride (1.2 mL, 8.26 mmol). The reaction mixture was stirred at room temperature for 1.5 h before MeOH was added to decompose the excess of NaH. The resulting solution was diluted with Et_2O, washed in turn with H_2O and saturated aq NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography (2:1 cyclohexane–Et_2O) of the residue gave **7** (3.76 g, 98%), mp 154–155°C (from Et_2O–hexane); $[\alpha]_D$ −57° (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{33}H_{42}O_7$: C, 72.0; H, 7.7. Found: C, 71.8; H, 7.6. The ^1H NMR spectrum ($CDCl_3$) of enantio-pure **7** was indistinguishable from that of the racemic compound [8].

*1D-3-O-Benzyl-1,2-O-cyclohexylidene-6-O-(4-methoxybenzyl)-*myo*-inositol* (**8**).—A solution of **7** (1.4 g, 2.54 mmol) and ethylene glycol (1.6 mL, 28.7 mmol) in a mixture of $CHCl_3$ (15 mL) and MeCN (13.5 mL) containing *p*-toluenesulfonic acid monohydrate (45 mg) was stirred at room temperature for 20 min before the reaction was stopped by the addition of Et_3N (0.25 mL). After dilution with $CHCl_3$, the resulting solution was washed in turn with saturated aq $NaHCO_3$ and H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography (1:2 Me_2CO–cyclohexane) of the residue gave unreacted **7** (0.55 g, 39%) followed by **8** (0.68 g, 57%), mp 146–147°C (from Me_2CO–hexane); $[\alpha]_D$ + 0.6° (*c* 2, $CHCl_3$). Anal. Calcd for $C_{27}H_{34}O_7$: C, 68.9; H, 7.3. Found: C, 68.7; H, 7.1. The ^1H NMR spectrum ($CDCl_3$) of enantio-pure **8** was indistinguishable from that of the racemic compound [8]. The unreacted material was recycled, raising the overall yield of **8** to 82%.

*1D-3,4,5-Tri-O-benzyl-1,2-O-cyclohexylidene-*myo*-inositol* (**10**).—Benzyl bromide (0.375 mL, 3.15 mmol) was added gradually to a stirred and cooled (0°C) solution of the diol **8** (0.68 g, 1.45 mmol) in DMF (25 mL) that had been pretreated with NaH (0.15 g, 6.25 mmol). The reaction mixture was stirred at room temperature overnight, MeOH was then added to destroy the excess of NaH, and the resulting solution was extracted with Et_2O. The ethereal extract was washed twice with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. TLC (1:1 Et_2O–cyclohexane) showed the residue to contain **9** and a less polar impurity. To a solution of the residue in dry CH_2Cl_2 (20 mL) was added $BF_3 \cdot Et_2O$ (1 mL), whereafter the solution was stirred at room temperature for 5 min and then neutralised with Et_3N. The resulting solution was washed twice with saturated aq NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Flash-column chromatography (2:1 cyclohexane–Et_2O) of the residue gave **10** (0.62 g, 81%), mp 96–98°C (from Et_2O–hexane); $[\alpha]_D$ −18° (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{33}H_{38}O_6$: C, 74.7; H, 7.2. Found: C, 74.9; H, 7.1. The ^1H NMR spectrum ($CDCl_3$) of enantio-pure **10** was indistinguishable from that of the racemic compound [8].

2-Azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl trichloroacetimidate (**11**).—A solution of 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose [11] (0.25 g, 0.53 mmol) and trichloroacetonitrile (0.175 mL, 1.74 mmol) in dry CH_2Cl_2 (3.5 mL) containing K_2CO_3 (0.07 g, 0.51 mmol) was stirred vigorously at room temperature for 4 h and then filtered through a short column of silica gel with washing with CH_2Cl_2. The combined eluent was concentrated under reduced pressure to give **11** (0.177 g, 54%), mp 103–104°C (from Et_2O–cyclohexane); $[\alpha]_D$ −3° (*c* 1.15, $CHCl_3$); ν_{max} 3320(NH), 2100(N_3), and 1665 cm^{-1} (C=N). ^1H NMR ($CDCl_3$): δ (inter alia) 3.53–4.91 (m, 12 H), 5.62 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 7.15–7.35 (m, 15 H, 3 × C_6H_5), and 8.75 (s, 1 H, NH). Anal. Calcd for $C_{29}H_{29}Cl_3N_4O_5$: C, 56.2; H, 4.7; Cl, 17.2; N, 9.0. Found: C, 56.5; H, 4.8; Cl, 16.9; N, 9.1. Flash-column chromatography (4:1 cyclohexane–Et_2O) of the mother liquors afforded a further quantity (0.102 g) of **11**, bringing the combined yield to 85%.

*1D-6-O-(2-Azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-O-benzyl-*myo*-inositol* (**13**).—To a cooled (−55°C) and stirred solution of the inositol derivative

10 (0.1 g, 0.19 mmol) and the imidate **11** (0.13 g, 0.21 mmol) in dry Et_2O (2.5 mL) containing powdered 3 Å molecular sieves (0.25 g) under N_2 was added dropwise a 0.02 M solution of trimethylsilyl trifluoromethanesulfonate in dry CH_2Cl_2 (0.2 mL). Stirring was continued at −55°C for 0.5 h, whereafter the reaction mixture was neutralised with Et_3N (0.5 mL) and percolated through a short column of silica gel with washing with Et_2O. The combined eluent was concentrated under reduced pressure, and a solution of the residue in MeCN (5 mL) containing tetrafluoroboric acid (0.05 mL of a 42% solution in H_2O) was stirred at room temperature for 0.5 h, neutralised with Et_3N (0.1 mL), and concentrated under reduced pressure. Flash-column chromatography (2:1 light petroleum–EtOAc) gave first the α anomer **13** (79 mg, 46%), mp 123–124°C (from Et_2O–hexane); $[\alpha]_D$ +36° (*c* 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$): δ (inter alia) 2.54 (br s, 1 H, OH), 3.02 (dd, 1 H, H-6′a), 3.27 (dd, 1 H, H-6′b), 5.35 (d, 1 H, $J_{1',2'} = 3.4$ Hz, H-1′), and 6.99–7.35 (m, 30 H, $6 \times C_6H_5$). Anal. Calcd for $C_{54}H_{57}N_3O_{10}$: C, 71.4; H, 6.3; N, 4.6. Found: C, 71.25; H, 6.3; N, 4.5. Lit [9], mp 125–126°C; $[\alpha]_D$ + 39.3° (*c* 1, $CHCl_3$). Continued elution gave the β anomer **14** (43 mg, 25%), mp 117–118°C (from Et_2O–hexane); $[\alpha]_D$ −21° (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$): δ (inter alia) 4.53 (d, 1 H, $J_{1'2'} = 8.6$ Hz, H-1′). Found: C, 70.9; H, 6.3; N, 4.2.

1D-6-O-(2-Azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-3,4,5-tri-O-benzyl-1-O-(4-methoxybenzyl)-myo-inositol (**15**).—A mixture of **13** (70 mg, 77 μmol) and dibutyltin oxide (27 mg, 108 μmol) in dry MeOH (2.5 mL) was boiled under reflux for 2 h, then cooled, and the resulting solution concentrated under reduced pressure. Toluene was added to, and distilled from, the residue twice. A mixture of the residue, CsF (16 mg, 0.105 mmol), KI (17 mg, 0.1 mmol), and 4-methoxybenzyl chloride (15 μL, 0.11 mmol) in DMF (2.2 mL) was stirred under N_2 at room temperature overnight, and the solvent was then removed under reduced pressure. Flash-column chromatography (1:1 Et_2O–cyclohexane) of the residue gave **15** (39 mg, 49%), $[\alpha]_D$ +26° (*c* 2.1, $CHCl_3$). ^1H NMR ($CDCl_3$): 2.43 (s, 1 H, 1-OH), 3.18 (dd, 2 H, H-6′a, 6′b), 3.28 (dd, 1 H, $J_{1',2'} = 3.7$, $J_{2',3'} = 10.2$ Hz, H-2′), 3.39 (t, 1 H, $J_{4,5} = J_{5,6} = 9.2$ Hz, H-5), 3.40 (dd, 1 H, $J_{1,2} = 2.9$, $J_{1,6} = 9.2$ Hz, H-1), 3.50 (dd, 1 H, $J_{2,3} = 2.5$, $J_{3,4} = 9.3$ Hz, H-3), 3.69 (t, 1 H, $J_{3'4'} = J_{4'5'} = 9.8$ Hz, H-4′), 3.80 (s, 3 H, OCH_3), 3.95 (t, 1 H, H-3′), 4.02 (m, 1 H, H-5′), 4.04 (t, 1 H, H-6), 4.13 (t, 1 H, H-4), 4.20 (t, 1 H, H-2), 4.26–5.00 (m, 14 H, $7 \times CH_2C_6H_5$), 5.69 (d, 1 H, H-1′), and 6.85–7.38 (m, 34 H, aromatic Hs). Positive ion FABMS: *m/z* 1050.5 $(M + Na)^+$.

1D-6-O-(2-Azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,5-tetra-O-benzyl-myo-inositol (**3**).—Benzyl bromide (3 μL, 25 μmol) was added to a solution of **15** (20 mg, 19.5 μmol) in DMF (1 mL) containing NaH (2 mg, 83 μmol), whereafter the mixture was stirred at room temperature for 1 h and then diluted with Et_2O. The ethereal solution was washed with saturated aq NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Toluene was added to, and distilled from, the crude heptabenzyl derivative **16** (~ 21 mg), which was then dissolved in CH_2Cl_2 (2 mL) and treated with CF_3CO_2H (20 μL) at room temperature for 1 h. After neutralisation with Et_3N (0.1 mL), the solution was concentrated under reduced pressure. Flash-column chromatography (25:1 toluene–butanone) of the residue gave **3** (13 mg, 67%), $[\alpha]_D$ +44° (*c* 1, $CHCl_3$), which was identical in all respects with the compound prepared previously [5].

Acknowledgments

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