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Short Note

6*R*/*S*-deutero-α-D-mannopyranoside 1-phosphate

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Abstract: 6*R*/*S*-deutero-α-D-mannopyranoside 1-phosphate was synthesised from a C6 aldehydic mannose thioglycoside donor in four steps. Using $NaBD_4$ as the reductant, isotopic enrichment at C6 was achieved and the resultant C6-deuterated material was converted through to the glycosyl 1-phosphate using a protection/glycosylation/deprotection sequence. The product was fully characterised by ^{1}H, ^{13}C, ^{31}P and 2D NMR, alongside MS analysis.

Keywords: mannose; glycosyl-phosphate; deuterium; isotope; NMR

1. Introduction

Glycosyl 1-phosphates are key intermediates in carbohydrate primary metabolism and are utilised by microorganisms to form polyphosphate architectures that constitute keys parts of their extracellular capsule and cell walls [1–5]. They serve as precursors to sugar-nucleotides [6,7], the sugar-donor components utilised by glycosyltransferases in the assembly of oligosaccharides and glycans and have played a key role in the development of glycosylated natural-product-based therapeutics [8]. Additionally, glycosyl 1-phosphates have been used as substrates for glycoside phosphorylases (a rapidly expanding family of CAZy enzymes) for the synthesis of oligosaccharide targets [9,10] and also play important technological roles in the food and detergent sectors [11–14].

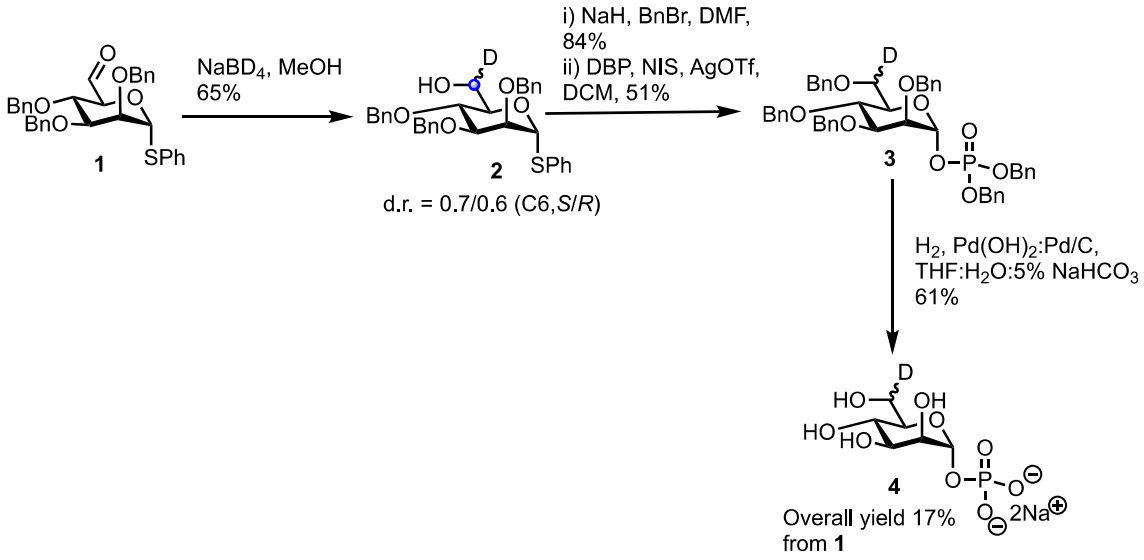
A chemical synthesis approach to these target molecules allows modification of the native structure, rendering a capability to then interrogate the biosynthetic enzymes/processes that utilise them. This is typified by regio- and stereoselective deuteration, which has proven the underpinning for the elucidation of biosynthetic mechanisms involving carbohydrates and has also been used to confirm assignments of their NMR and mass spectra [15,16]. Carbohydrates diastereoselectively deuterated at C6 have provided important chemical tools [17], illustrated for mannose by a study reported by Tanner concerning the synthesis of ADP-[6''-^{2}H]-D,D-Hep for elucidating the mechanism of ADP-L-glycero-D-manno-heptose 6-epimerase [18]. As part of wider project concerning the chemical synthesis of modified mannose 1-phosphates and derived sugar-nucleotides [19], we required access to the title compound to establish a proof of concept methodology for incorporating C6 deuterium. Herein we provide our record of its synthesis and full characterisation from *S*-phenyl thioglycoside C6 aldehyde (**1**).

2. Results

Our synthetic route began from D-mannose which was appropriately transformed into C6-aldehyde thioglycoside (**1**) using established procedures [19]. We next completed a reduction of (**1**) with $NaBD_4$ to deliver (**2**) in 65% yield (Scheme 1). ^{1}H and ^{13}C NMR analyses of (**2**) were unable to unambiguously distinguish the diastereomeric ratio of the product mixture, nor were the diastereoisomers separable

by TLC. We thus completed a small-scale synthesis of the 1,6-anhydro derivative of (**2**), using NBS to activate the thioglycoside and close the C6-OH onto the anomeric centre. 2D-HSQC data for this compound showed the expected correlation between H_6 and C_6 for both diastereoisomers, with a ^1J ^{13}C-^{2}H coupling of 23.1 Hz. ^{1}H NMR data provided a clear resolution of the diastereomeric anhydro-sugar mixture at H_6 and an expected almost equal product ratio from the $NaBD_4$ reduction (0.7:0.6, *endo*/*exo*) which could be extrapolated back to give the indicative diastereomeric ratio at C6 for (**2**) [0.7/0.6, *S*/*R*].

We next completed our route to (**4**) containing a C6-deuterium. Accordingly, alcohol (**2**) was protected at C6 with a benzyl group in good yield (84%) and the required 1-phosphate installed (in protected form) using dibenzylphosphate (DBP) as the acceptor under thioglycoside activation conditions (NIS/AgOTf) in satisfactory 51% yield to deliver (**3**). ^{1}H and ^{31}P NMR for (**3**) confirmed the presence of an anomeric phosphate with the characteristic doublet of doublets observed for H_1 coupling to H_2 and ^{31}P ($^3J_{H1\text{-}H2}$ = 1.9 Hz, $^3J_{H1\text{-}P}$ = 6.1 Hz). Finally, a global hydrogenolysis using H_2 with Pd/C and $Pd(OH)_2$/C was completed, providing the title compound in a moderate 61% yield. Analytical data collected for (**4**) supported the structural assignment and gave an indicative level of purity. Copies of NMR, and MS data are included in Supplementary Materials.



Scheme 1. Synthesis of 6*R*/*S*-deutero-α-D-mannopyranoside 1-phosphate (**4**) from C6-aldehyde (**1**).

3. Materials and Methods

3.1. General

All reagents and solvents which were available commercially were purchased from Acros (Geel, Belgium), Alfa Aesar (Heysham, UK), Fisher Scientific (Geel, Belgium), or Sigma Aldrich (Gillingham, UK). All reactions in non-aqueous solvents were conducted in oven dried glassware under a nitrogen atmosphere with a magnetic stirring device. Solvents were purified by passing through activated alumina columns and used directly from a Pure Solv-MD solvent purification system and were transferred under nitrogen. Reactions requiring low temperatures used the following cooling baths: −30 °C (dry ice/acetone), −15 °C (NaCl/ice/water) and 0 °C (ice/water). Infra-red spectra were recorded neat on a Perkin Elmer Spectrum 100 FT-IR spectrometer; selected absorbtion frequencies (ν_{max}) are reported in cm^{-1}. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C spectra at 100 MHz, respectively, using a Bruker AVIII400 spectrometer. ^{1}H NMR signals were assigned with the aid of gDQCOSY. ^{13}C NMR signals were assigned with the aid of gHSQCAD. Coupling constants are reported in Hertz. Chemical shifts (δ, in ppm) are standardised against the deuterated solvent peak. NMR data were analysed using Nucleomatica iNMR software. ^{1}H NMR splitting patterns were assigned as follows:

s (singlet), d (doublet), app. t (apparent triplet), t (triplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), or m (multiplet and/or multiple resonances). For ^{13}C NMR data quaternary carbons are indicated as C_q. Reactions were followed by thin layer chromatography (TLC) using Merck silica gel 60F254 analytical plates (aluminium support) and were developed using standard visualising agents: Short wave UV radiation (245 nm) and 5% sulfuric acid in methanol/Δ. Purification *via* flash column chromatography was conducted using silica gel 60 (0.043–0.063 mm). MS and HRMS (ESI) were obtained on a Waters (Xevo, G2-XS TOF) spectrometers using a methanol mobile phase. Purification via ion exchange chromatography was conducted on Bio-Rad Biologic LP system using a Bio-Scale Mini UNOsphere Q (strong anion exchange) cartridge (5 mL): flow rate (1.5 mL/min), 0→90% 1.0 M $(NH_4)HCO_3$ over 28 min.

*3.2. Phenyl 2,3,4-tri-O-benzyl-6R/S-deutero-1-thio-α-D-mannopyranoside (**2**)*

To a stirred solution of (**1**) [19] (0.75 g, 1.38 mmol, 1.0 equiv.) in dry MeOH (6.0 mL) at −15 °C was added a solution of $NaBD_4$ (60 mg, 1.44 mmol, 2.0 equiv.) in MeOH (2.0 mL) dropwise over 5 min and the reaction mixture was allowed to warm to room temperature gradually. After 3 h, the reaction temperature was returned to −15 °C at which point the pH was adjusted to between 5 and 7 with 2.0 M HCl (3.0 mL). The solvent was removed under reduced pressure and the aqueous residue was extracted with EtOAc (2 × 10 mL), washed with saturated aq. $NaHCO_3$ (10 mL solution), H_2O (15 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification using silica gel flash column chromatography (hexane/EtOAc, 8:2) gave (**2**) (0.25 g, 0.46 mmol, 65%) as a pale-yellow oil; R_f 0.3 (hexane/EtOAc 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.41-7.23 (20 H, m, Ar-*H*), 5.51 (1 H, d, *J* = 1.5 Hz, H_1), 4.96 (1 H, d, *J* = 10.9 Hz, OC*H*$_2$Ph, C4), 4.70–4.61 (5 H, m, 1H of OC*H*$_2$Ph, C4, 2 × OC*H*$_2$Ph, C3 and C2), 4.14–4.09 (1 H, m, H_5), 4.07–3.97 (1 H, m, H_4), 3.89 (1 H, dd, *J* = 9.2, 3.0 Hz, H_2), 3.79 (1 H, dd, *J* = 9.2, 3.0 Hz, H_3), 3.76–3.71 (1 H, m, H_6); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 138.2 (C_q), 138.0 (C_q), 137.8 (C_q), 133.9 (C_q), 131.8 (*C*HAr), 129.1 (*C*HAr), 128.4 (*C*HAr), 128.1 (*C*HAr), 127.9 (*C*HAr), 127.8 (*C*HAr), 85.9 (C1), 80.0 (C3), 76.3 (C2), 75.3 (OCH_2Ph), 74.7 (C4), 72.3 (C5), 72.3 (OCH_2Ph), 71.7 (OCH_2Ph), 62.0 (C6); **HRMS** (ES^+) *m*/*z* found: $(M + NH_4)^+$ 561.2529 $C_{33}H_{37}DO_5SN$ requires $(M + NH_4)^+$ 561.2528; **IR** ν_{max} (neat)/cm^{-1}: 3484 (br), 3030 (w), 2870 (m), 1084 (s), 736 (s).

*3.3. Dibenzyl (2,3,4,6-tetra-O-benzyl-6R/S-deutero-1-phosphate-α-D-mannopyranoside) (**3**)*

To a stirred solution of (**2**) (1.0 g, 1.84 mmol, 1.0 equiv.) in dry *N*,*N*-dimethylformamide (16 mL) at 0 °C was added sodium hydride (83 mg, 60% dispersion in mineral oil, 2.20 mmol, 1.2 equiv.) portionwise over 2 min. The resulting mixture was stirred for 30 min at 0 °C, whereupon benzyl bromide (0.24 mL, 2.02 mmol, 1.1 equiv.) was added dropwise over 2 min and stirred for 2 h at 0 °C. Excess sodium hydride was destroyed by careful addition of MeOH (4.0 mL) and the solvent was removed under reduced pressure. The crude residue was partitioned between CH_2Cl_2 (8.0 mL) and H_2O (8.0 mL). The layers were separated and the organic layer was washed with brine (8.0 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography (hexane/EtOAc, 8:2) afforded phenyl 2,3,4,6-tetra-*O*-benzyl-6*R*/*S*-deutero-1-thio-α-D-mannopyranoside (0.99 g, 1.55 mmol, 84%) as a yellow oil. R_f 0.41 (hexane/EtOAc, 3:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.44–7.07 (25 H, m, Ar-*H*), 5.68 (1 H, d, *J* = 1.5 Hz, H_1), 4.95 (d, *J* = 10.8 Hz, 1H, OC*H*$_2$Ph, C4), 4.77 (1H, d, *J* = 12.4 Hz, 1H, OC*H*$_2$Ph, C2), 4.71–4.64 (m, 4H, 1H of OC*H*$_2$Ph, C2 and C6, OC*H*$_2$Ph C3), 4.58 (d, *J* = 10.8 Hz, 1H, OC*H*$_2$Ph, C4), 4.54–4.51 (m, 1H, OC*H*$_2$Ph, C6), 4.31-4.21 (1 H, m, H_5), 4.07 (1 H, t, *J* = 9.7 Hz, H_4), 4.00 (1 H, dd, *J* = 3.0, 1.5 Hz, H_2), 3.86 (1 H, dd, *J* = 9.7, 3.0 Hz, H_3), 3.77 (d, *J* = 5.1 Hz, H_{6S}), 3.73 (d, *J* = 1.8 Hz, H_{6R}); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 138.4 (C_q), 138.3 (C_q), 138.2 (C_q), 137.9 (C_q), 134.4 (C_q), 128.6 (*C*HAr), 128.3 (*C*HAr), 128.3 (*C*HAr), 128.0 (*C*HAr), 127.9 (*C*HAr), 127.8 (*C*HAr), 127.7 *C*HAr), 127.6 (*C*HAr), 127.5 (*C*HAr), 126.8 (*C*HAr), 126.0 (*C*HAr), 85.3 (C1), 80.2 (C3), 77.2 (C2), 75.2 (C4), 75.3 (OCH_2Ph), 72.8 (C5), 72.4 (OCH_2Ph), 72.2 (OCH_2Ph), 71.7 (OCH_2Ph), 62.8 (C6); **HRMS** (ES^+) *m*/*z* found: $(M + NH_4)^+$ 651.3015 $C_{40}H_{39}DO_5NH_4$ requires $(M + NH_4)^+$, 651.3017; **IR** ν_{max} (neat)/cm^{-1}: 3029 (w), 2863 (w), 1082 (s), 733 (s).

Phenyl 2,3,4,6-tetra-*O*-benzyl-6*R*/*S*-deutero-1-thio-α-D-mannopyranoside (0.27 g, 0.42 mmol, 1.0 equiv.) was co-evaporated with toluene (3 × 6 mL) and dissolved in dry CH_2Cl_2 (7.0 mL). Dibenzyl phosphate (0.14 g, 0.50 mmol, 1.2 equiv.) and powdered 4 Å molecular sieves were added to the mixture at room temperature and stirred for 40 min. *N*-Iodosuccinamide (0.14 g, 0.63 mmol, 1.5 equiv.) and silver trifluoromethanesulfonate (53 mg, 0.21 mmol, 0.5 equiv.) were added sequentially at −30 °C. The reaction mixture was stirred for 3 h as the temperature was raised from −30 to 0 °C and monitored to completion by TLC (hexane/EtOAc, 1:1). The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (4.0 mL) and filtered through Celite®. The filtrate was diluted with CH_2Cl_2 (4 mL), washed with saturated aqueous $NaHCO_3$ (4.0 mL) and brine (4.0 mL). The layers were separated and the organic layer dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by silica gel column chromatography (hexane/EtOAc, 8:2) furnished (**3**) (0.11 g, 0.13 mmol, 51%) as a clear oil. R_f 0.34 (hexane/EtOAc, 1:1); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37–7.22 (28 H, m, Ar-*H*), 7.19–7.13 (2 H, m, Ar-*H*), 5.77 (1 H, dd, *J* = 6.1, 1.9 Hz, H_1), 5.02 (2 H, d, *J* = 8.2 Hz, P(O)OC*H*$_2$Ph), 4.96 (2 H, d, *J* = 8.4 Hz, P(O)OC*H*$_2$Ph), 4.86 (1 H, d, *J* = 10.8 Hz, 1H of OC*H*$_2$Ph, C4), 4.62–4.41 (7 H, m, OC*H*$_2$Ph, 1H of OC*H*$_2$Ph, C4 and OC*H*$_2$Ph C2, C3 and C6), 4.03 (1 H, app. td, *J* = 9.7, 2.4 Hz, H_4), 3.89 (1 H, dd, *J* = 9.8, 4.5 Hz, H_5), 3.81 (1 H, dd, *J* = 9.4, 3.0 Hz, H_3), 3.72–3.66 (2 H, m, H_2, H_6); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 138.4 (C_q), 138.3 (C_q), 138.2 (C_q), 138.1 (C_q) 137.9 (C_q), 134.4 (C_q), 128.6 (*C*HAr), 128.3 (*C*HAr), 128.3 (*C*HAr), 128.0 (*C*HAr), 127.9 (*C*HAr), 127.8 (*C*HAr), 127.7 (*C*HAr), 127.6 (*C*HAr), 127.5 (*C*HAr), 126.8 (*C*HAr), 126.0 (*C*HAr), 96.1 (d, $J_{C\text{-}P}$ = 6.1 Hz, C1), 78.8 (C3), 75.5 (OCH_2Ph), 75.2 (C2), 74.1 (C4), 73.7 (C5), 73.1 (OCH_2Ph), 73.6 (OCH_2Ph), 73.3 (OCH_2Ph), 72.4 (OCH_2Ph), 72.2 (OCH_2Ph), 62.8 (C6); **^{31}P NMR** (160 MHz, $CDCl_3$) δ −2.84; **HRMS** (ES^+) *m*/*z* found: $(M + NH_4)^+$ 819.3542 $C_{48}H_{52}DO_9PN$ requires $(M + NH_4)^+$ 819.3540; **IR** ν_{max} (neat)/cm^{-1}: 3030 (w), 2923 (w), 1454 (s), 1095 (s), 733 (s).

3.4. R/S-deutero-α-D-mannopyranoside-1-phosphate (4)

Dibenzyl (2,3,4,6-tetra-*O*-benzyl-6*R*/*S*-deutero-1-phosphate-α-D-mannopyranoside) (**3**) (0.15 g, 0.18 mmol, 1.0 equiv.) was dissolved in THF:H_2O (2 mL, 1:1, *v*/*v*) at room temperature. To the solution was added $NaHCO_3$ (38 mg, 0.45 mmol) followed by $Pd(OH)_2$/C:Pd/C (0.15 g, 4:1). The reaction mixture was transferred to a Parr vessel and placed under a hydrogen atmosphere (5 bar) for 3 days. The progress of the reaction was monitored by TLC (CH_3CN:H_2O:NH_4OH 7:2:1). The catalyst was then removed by filtration through Celite® and the filtrate concentrated under reduced pressure and passed through a G25 Sephadex cartridge, eluting with H_2O. The crude product was applied to a strong anion exchange column as detailed in the General Experimental section. The sugar-containing fractions were pooled, passed over a Na^+ exchange resin (IR-120H^+) and freeze-dried to afford (**4**) (30 mg, 0.11 mmol, 61%) as a white amorphous powder. **^{1}H NMR** (400 MHz, D_2O) δ 5.32 (1 H, dd, *J* = 8.0, 2.0 Hz, H_1), 3.89 (1 H, app. t, *J* = 2.6 Hz, H_2), 3.84 (1 H, dd, *J* = 9.7, 3.2 Hz, H_3), 3.80–3.68 (2 H, m, H_6, H_5), 3.58 (1 H, t, *J* = 9.7 Hz, H_4); **^{13}C NMR** (100 MHz, D_2O) δ 95.1 (C1), 72.9 (C5), 69.9 (C2), 69.3 (C3), 65.7 (C4), 60.0 (C6); δ **^{31}P NMR** (160 MHz, D_2O) δ −1.36; **HRMS** (ES^+) *m*/*z* found: $(M + H)^+$ 307.0135 $C_6H_{10}DNa_2O_9P$ requires $(M + H)^+$ 307.0130; **IR** ν_{max} (neat)/cm^{-1}: 3218 (w), 1593 (s), 1352 (s), 1093 (s), 950.

Supplementary Materials: NMR data for (**2**) (**3**) and (**4**) are available online.

Author Contributions: G.J.M conceived and designed the experiments; S.A. performed the experiments and analysed the data with G.J.M; G.J.M. wrote the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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