**Exploring anomeric glycosylation of phosphoric acid: optimisation and scope for non-native substrates**

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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.[1-4] They serve as precursors to sugar nucleotides,[5-6] 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.[7] Additionally, glycosyl 1-phosphates have been used as substrates for glycoside phosphorylases, a rapidly expanding[8] family of CAZy enzymes for the synthesis of oligosaccharide targets[9] and also play important technological roles in the food and detergent sectors.[10-11]

In order to access these significant materials, a variety of chemical and chemoenzymatic strategies have been developed. Chemoenzymatic methods frequently involve glycosyl kinases[12-15] although phosphomutase enzymes have also been explored.[16] From a chemical perspective, several synthetic options exist to create glycosyl 1-phosphates, most commonly *via* anomeric glycosylation or hemi-acetal deprotonation and reaction with a suitable phosphorous electrophile.[2,17] These approaches both present a capability to modify native glycosyl 1-phosphate structures, enabling to then interrogate the biosynthetic enzymes and processes that utilise them.[18]

 As part of a program pursuing the synthesis of glycosyl 1-phosphate and sugar nucleotide chemical tools,[19-21] the MacDonald method for accessing anomeric 1-phosphates became of interest. This method has been successfully used by several groups, including for non-native systems.[22-27] Originally published in 1962,[28] the procedure uses elevated temperature and low pressure to form a melt of crystalline phosphoric acid and a peracetylated sugar, glycosylating the anomeric position and releasing AcOH. This is followed by ester hydrolysis in the same pot to deliver the deprotected 1-phosphate. (*Scheme 1*). However, the reaction can be low yielding, requires a significant excess of H3PO4 (10 equiv.) and purification of the product(s) is not facile. The capabilities of this transformation as a simple method for accessing modified 1-phosphates, quickly, from acetylated precursors required investigation to optimise the reaction and explore its scope further.



*Scheme 1*. Original MacDonald phosphorylation conditions to access D-Gal 1-phosphate **2** from per-acetylated precursor **1**.

The crystalline phosphoric acid reagent is extremely hygroscopic and whilst received from suppliers as an opaque, crystalline solid, it readily forms a paste as hydration upon opening the container to the atmosphere is unavoidable. This is problematic for the ensuing reaction as water can compete with phosphate in the anomeric substitution reaction, which produces the corresponding hemi-acetal by-product, reducing the final yield.

 In order to avoid this, a glove box (or commercially available glove bag) was used for the anomeric phosphorylation experiments. Hence, ten equivalents of phosphoric acid and 500 mg (one equivalent) of **1** were transferred to a Schlenk tube under an atmosphere of nitrogen. The closed tube containing the reactants was then transferred to a dual line manifold and the solid mixture heated to a melt (50 °C) under vacuum (*Scheme 2*). Once the reaction was complete, as monitored by TLC analysis (3 h), the acetyl groups were saponified in the same pot and, following work-up, **2** was isolated as a white solid. Examination of **2** by 1H and 31P NMR showed >90% conversion to the desired 1-phosphate: 1H δ 5.31 (dd, *J* = 7.3, 3.6 Hz, H1). The only impurity observed was a trace amount of the hemi-acetal byproduct (<10%). As necessary, this could easily be removed using a strong anion exchange column to elute the uncharged species with water, followed by an ammonium bicarbonate eluent to release **2** in a much improved 68% final yield (after freeze-drying), compared to the original procedure.



*Scheme 2*. Phosphorylation of per-acetylated D-Gal **1**.

Encouraged by these improvements to the yield and purity of the reaction outcome, other reaction parameters in the conversion of **1** to **2** were targeted. These findings are summarised in Table 1.

*Table 1.* Optimisation of per-acetyl D-Galanomericphosphorylation

|  |
| --- |
|  |
| Entry | Equivalents H3PO4 | Temperature(°C)# | Yield(%) | Scale(mg **1**) | Time(h) |
| 1 | 10.0 | 50 | 68 | 500 | 3 |
| 2 | 10.0 | 50 | 65 | 100 | 3 |
| 3 | 10.0 | 50 | 69 | 50 | 3 |
| 4 | 10.0 | 40 | 62 | 80 | 3 |
| 5 | 5.0 | 40 | 67 | 300 | 3 |
| 6 | 5.0 | 35 | 49 | 200 | 3 |

#Temperature of the heating block, not the internal reaction temperature.

The initial reaction with commercially available **1** was conducted on 500 mg scale. However, for more exotic, non-native substrates, material availability is often a limiting factor and so a reduction in the scale of the reactions, down to ranges between 50 and 100 mg, was investigated first. Pleasingly, little effect was observed on the isolated yield (Table 1, entries 2 and 3). Generally, this phosphorylation is carried out at a temperature ranging between 50 and 60 °C (to form the melt and assist in removing AcOH under vacuum). The reaction was conducted successfully at 40 °C (Table 1, entry 4) using a high vacuum line pressure of 1.6 mbar, also noting that the required melt failed to form efficiently when the temperature was lowered further to 30 °C. Finally, the equivalents of phosphoric acid required was successfully reduced from ten to five (Table 1, entries 5 and 6), observing this to strike the best balance between reaction time (increasing this led to blackening of the melt and thus reduced yields) and full conversion of starting material by TLC at 40 °C. With an improved procedure for this phosphorylation in hand a series of monosaccharide substrates was selected for evaluation. The results of these experiments are presented in Table 2 and discussed thereafter.

*Table 2.* Exploring substrate scope for anomericphosphorylation

|  |
| --- |
|  |
| Entry | Substrate | Product | Yield(%) | Scale(mg acetate) |
| 1 |  |  | 41 | 200 |
| 2 |  |  | 0decomp. | 200 |
| 3 |  |  | 55 | 250 |
| 4 |  |  | 66 | 250 |
| 5 |  |  | 0 (85£) | 400 |
| 6 |  |  | 72\* | 54 |
| 7 |  |  | 42 | 45 |
| 8 |  |  | 65 | 100 |
| 9 |  |  | 43$ | 100 |

Conditions used were initially the same as Table 1, entry 5. £See discussion for formation of cyclic product **13** below. \* formed as a 1:0.3 mixture with **13**. $Observed as the disulfide.

For per-acetyl-D-glucose **3**,anomeric phosphorylation proceeded smoothly, and the target 1-phosphate **4** was isolated in 41% yield (*Table 2*, entry 1). This was lower than observed for **2** and was attributed to the melt requiring a higher temperature (60 °C) to effect full conversion, which led to a blackening of the reaction mixture. The reaction for 2-deoxy glucose derivative **5** showed only decomposition by TLC after 3 h at a temperature of 60 °C, with the reaction melt turning black almost immediately (*Table 2*, entry 2). After several repeats, no conversion into **6** was detected, concluding **5** to be a poor substrate for these anomeric phosphorylation conditions. Finally, for these simple glycosyl 1-phosphates the D-manno compound **8** was isolated in satisfactory 55% yield from **7**, noting that the reaction temperature was successfully lowered to 45 °C (*Table 2*, entry 3).

 A 5-*C*-methyl mannose derivative was evaluated next to establish if a steric effect from a C5-axial methyl group would influence the anomeric selectivity of phosphorylation. Following the procedure reported by Davis *et al,*[29] 5-*C*-methyl-D-mannose was accessed and acetylation of the free sugar using either Ac2O/pyridine or Ac2O/H2SO4 gave **9**. Anomeric phosphorylation was accomplished in good yield (66%) to deliver **10**. (*Table 2*, Entry 4). Formation of an α/β 1-phosphate mixture was observed by 1H NMR [1H δ 5.27 (dd, *J* = 9.1, 2.2 Hz, H1α), 5.19 (dd, *J* = 8.6, 1.1 Hz, H1β)] with a 2:1 preference for formation of the α-anomer. This finding indicated that a sterically encumbering C5-Me group introduced a competing pathway for formation of the β 1-phosphate. To question whether the formation of **10** was influenced by the ratio of anomeric mixture used as starting material the ratio of α-acetate in **9** was increased to 50% from 33% (accomplished by acetylation with Ac2O/H2SO4 instead of Ac2O/Pyr). However, this made only a small difference to the observed α/β ratios in the product (α/β-**10** 2:1, from a 1:2 α/β ratio in **9** and α/β-**10** 2.5:1, from a 1:1 α/β ratio in **9**).

A series of C6-halogenated analogues (*Table 2*, entries 5-7) were targeted next. Starting from C6-bromide **11** (accessed *via* an Appel reaction from the corresponding C6-alcohol[30]), exposure to the established conditions showed a smooth conversion to one product in 85% yield, which is proposed to be cyclic phosphate **13**, not the expected 1-phosphate **12** (*Scheme 3*). HRMS analysis for **13** showed no bromine isotope pattern and inspection of the 1H NMR data showed an apparent triplet in the anomeric region [δ 5.20 (app. t, *J* = 6.9 Hz)]. This indicated that the 3*J*P-H1 and 3*J*H2-H1 coupling constants were now almost equal and distinct from the characteristic doublet of doublets for H1 in a 1-phosphate e.g. as seen for **8** [δ 5.23 (dd, *J* = 8.6P-H1, 1.3H1-H2 Hz].In addition, the apparent triplet usually observed for H4 of 4C1 mannose derivatives was now a doublet of doublets [δ 4.20 (dd, *J* = 6.0, 2.8 Hz)], leading us to conclude that **13** had adopted an alternative solution state conformation. Inspection of the data for H6 showed the distinctive doublet of doublets to be absent for one of these protons as only the diastereotopic 2*J* coupling was observed [δ 4.10 (d, *J* = 10.9 Hz, H6*exo*).A very small (not always detectable) 3*J* H6-5 coupling exists only as the dihedral angle between these two protons approaches 90°, which can occur in a locked bicyclic system. Finally, 31P-1H HMBC data for **13** demonstrated 3*J*P,H coupling to both H1 and H6*exo*, confirming cyclisation through C1/C6.

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*Scheme 3*. Attempted phosphorylation of C6-halo D-mannose derivatives. Anomeric configuration of **13** is tentatively assigned as the β-configuration.

In accounting for this unexpected finding, it can be considered that phosphorylation at C1 of **11** occurred, followed by an intramolecular substitution of the C6 bromo derivative through anomeric phosphate oxygen (*Scheme 4a*). However, in the absence of X-ray crystallographic data the anomeric configuration of **13** is unconfirmed. As evidenced by the examples highlighted in *Table 2*, α-phosphorylation is the predominant product using this method (no β 1-phosphate was observed using **7** as a substrate) but β-linked products can be obtained, as evidenced for **10**. It is possible that under the acidic reaction conditions interconversion between the α and β 1-phosphates occurs, allowing for irreversible cyclic phosphate formation of the β anomer, when a suitable C6 electrophile is present,. It remains unconfirmed as to whether this cyclisation could occur during the saponification process (the protected 1-phosphates were not isolated), however no such products were observed for native substrates and the strongly alkaline conditions would likely displace a C6-halide to the parent C6-OH. 1H NMR data for **13** showed a 3*J*H1-H2 coupling constant of 6.9 Hz, which is small for an H1-H2 axial-axial coupling. DFT calculations were completed for α- and β-configurations of **13** (*Scheme 4b*, β-phosphate shown) which showed a -24.3 kcal/mol preference for the β and an H1-H2 dihedral angle of 40.3°. This correlates well to the experimentally observed 3*J*H1-H2 coupling, whereas the dihedral angle obtained for the α-linked system was 163.5° (see SI), which would give rise to a much larger coupling. To the best of our knowledge this is the first report of an *O*1,*O*6 7-membered cyclic phosphate; an *O*1,*O*3 system was previously reported for D-glucose.[31]

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*Scheme 4. a)* Possible mechanism for the formation of cyclic phosphate β**-13** *b)* DFT energy minimised 3CO conformation for β**-13**.

To investigate this further (in pursuit of C6-halogenated mannose 1-phosphates), the phosphorylation of the corresponding C6-fluoro derivative **14**[32]was completed (*Table 2*, entry 6). As expected with this inferior leaving group, using the same conditions seen for **11**, reaction with **14** led to the desired glycosyl 1-phosphate **15**. However, the bicyclic phosphate **13** was still observed, in a three to one (**15**:**13**) ratio, as adjudged by 1H NMR (*Scheme 3*). Intramolecular SN2 reactions with aliphatic fluoride are uncommon, but have been described for substrates with reduced conformational flexibility.[33] Lowering the temperature for this reaction to 30 °C gave the same ratio of products and increasing it to 60 °C reduced the amount of **15** formed (**15**:**13**, 1:1). For comparative purposes, a C6-chloride was synthesised (see Experimental) and, when subject to phosphorylation, was fully converted to **13**. These findings suggest that the identity of the halogen is (predictably) key to the rate of competing nucleophilic substitution at C6.

 In light of the positive result obtained for accessing **15**, phosphorylation of C6-deoxy-*gem*-difluoro D-mannose tetraacetate **16** was attempted (*Table 2*, entry 7). Pleasingly, **17** was isolatedin a moderate yield of 42%. This 6-*gem*-difluoro substituted material was unreactive to phosphorylation at 40 °C and conversion was instead completed at 60 °C and over 6 h. This inevitably induced significant reaction blackening/decomposition, hence the lower yield. It is however encouraging that such an electronically deactivated substrate can be converted to its glycosyl 1-phosphate using this simple process from the tetraacetate.

Investigating other C6-substituted substrates, phosphorylation of 6-deoxy-6-azido D-mannose **18** proceeded smoothly on 100 mg scale and in 65% yield to deliver **19** (*Table 2*, entry 8). A C6-thioacetate **20** was accessed from the C6-chloro derivative by nucleophilic substitution using KSAc and enabled an attempted synthesis of 6-thio 1-phosphate **21** (*Table 2*, entry 9). The yield for this reaction was lower than that of the native mannose compound **8** (43% compared to 55%) and disulfide formation for **21** was observedby HRMS and 1H NMR. Purification of this material was further complicated by the free hemi-acetal by-product also forming a mixed disulfide with **21**. Any hemi-acetal by-product was normally removed by anion exchange chromatography, but due to the charged nature of this mixed disulfide it was not possible to isolate pure amounts of **21**. H1 in the disulfide form of **21** could clearly be observed by 1H NMR, alongside H1 of the mixed disulfide in a ratio of 2.5:1 for **21**. Attempts to reduce these disulfides with TCEP (to enable removal of the hemi-acetal) were unsuccessful.

**Conclusions**

An improved access to glycosyl 1-phosphates has been developed, formed using a melt of an acetylated precursor with H3PO4 under high vacuum. Through preparation of the reaction components in a glove box and reducing the reaction temperature and equivalents of H3PO4, small scale access to the preparation of native and non-native glycosyl 1-phosphates in good yields and with facile purification is enabled. Additionally, the formation of an unexpected *O*1,*O*6-cyclic phosphate is reported when good leaving groups (Br, Cl) at C6 of the starting material are included. These anomeric phosphates will serve as key tools for the study of enzymes and biochemical processes that utilise glycosyl 1-phosphates, for example, phosphorylases and uridylyltransferases.

**Acknowledgments**

Keele University are thanked for PhD studentship funding to LB and ED. The Engineering and Physical Sciences Research Council (EPSRC, EP/P000762/1) and the Biotechnology and Biological Sciences Research Council (BBSRC, BB/L013762/1) are thanked for project grant funding. We also thank the EPSRC (core capability EP/K039466/1) for funding and the EPSRC UK National Mass Spectrometry Facility (NMSF) at Swansea University.

**Experimental**

***General Experimental***

All reagents and solvents which were available commercially were purchased from Acros, Alfa Aesar, Fisher Scientific, or Sigma Aldrich. 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. 1H NMR spectra were recorded at 600 or 400 MHz and 13C spectra at 100 MHz respectively using a Bruker AVIII400 spectrometer. 1H NMR signals were assigned with the aid of gDQCOSY. 13C 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 or Mestrenova software. 1H NMR splitting patterns were assigned as follows: br s (broad singlet), 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). 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). Optical activities were recorded on automatic polarimeter Rudolph autopol I or Bellingham and Stanley ADP430 (concentration in g/100mL). MS and HRMS (ESI) were obtained on Waters (Xevo, G2-XS TOF) or Waters Micromass LCT spectrometers using a methanol mobile phase. High resolution (ESI) spectra were obtained on a Xevo, G2-XS TOF mass spectrometer. HRMS was obtained using a lock-mass to adjust the calibrated mass. Phosphorylation reactions were prepared in a MBRAUN LABstar glove box.

***General procedure anomeric phosphorylation***

The acetylated sugar (1.0 equiv.) was weighed into a pre-dried and weighed Schlenk tube and dried under high vacuum for 1 h. H3PO4 (5.0 equiv.) was weighed out inside a glove box, transferred to the Schlenk tube and the tube sealed under N2. The tube was placed under N2 on a double manifold and heated under high vacuum at 50 °C with gentle stirring. In most instances a yellow-gold melt was formed during this time and all reactions were monitored to completion by TLC (petroleum ether/EtOAc, 1/2) for the formation of a baseline (Rf = 0) spot (1-phosphate) with any hemi-acetal side product generally at Rf = 0.5. The resultant melt was allowed to cool to room temperature then reconstituted in anhydrous THF. This was then added slowly to a stirred solution of 1.0 M LiOH (concentration = 0.025 M with respect to starting material) at 0 °C and stirred until saponification was complete by TLC analysis (MeCN/H2O/AcOH, 3/1/0.1), typically 48-72 h. The reaction mixture was filtered through a Whatman GA filter under vacuum and rinsed with deionised water. The resultant filtrate was then neutralised through the addition of AmberliteTM 120 ion-exchange resin (H+ form) to pH = 7, filtered and concentrated under reduced pressure. The crude residue was then triturated with MeOH, centrifuged and the supernatant removed The pellet was purified by dissolving in deionised water and passed through a Bio-Scale Mini UNOsphere Q (strong-anion exchange) cartridge [Column Volume (CV) = 5 mL], eluting with 3 CV of deionised water followed by 3 CV of 1.0 M NH4HCO3 solution. Fractions containing the glycosyl-1-phosphate were collected and lyophilised repeatedly to remove residual NH4HCO3 and deliver the target material.

*α-D-Galactose 1-phosphate bis ammonium salt* **2**

**1** (300 mg, 0.77 mmol) gave **2** (152 mg, 0.52 mmol, 67%).

**1H NMR** (400 MHz, D2O) δ 5.31 (1H, dd, *J1,P* = 7.3, *J1,2* = 3.6 Hz, H1), 4.01 (1H, dd, *J* = 7.0, 4.9 Hz, H5), 3.82 (1H, d, *J* = 2.7 Hz, H4), 3.74 (1H, dd, *J* = 10.2, 3.4 Hz, H3), 3.57 (3H, m, H2, H6a and H6b); **13C NMR** (101 MHz, D2O) δ 94.1 (C1), 71.0 (C5), 69.6 (C3), 69.0 (C4), 68.7 (C2), 61.4 (C6); **31P{1H} NMR** (162 MHz, D2O) δ 2.56. Data matched those previously reported.[28]

*α-D-Glucose 1-phosphate bis sodium salt* **4**

**3** (200 mg, 0.51 mmol) gave **4** (54 mg, 0.20 mmol, 41%). Following purification as described in the General Experimental, the material was treated with DOWEX-IR-120 (Na+ form) to obtain the bis-sodium salt form for comparison to reported data.**1H NMR** (400 MHz, D2O) 5.44 (1H, dd, *J1,P* = 7.4, *J1,2* = 3.4 Hz, H1), 3.92 (1H, ddd, *J* = 10.0, 5.2, 2.1 Hz, H5), 3.86 (1H, dd, *J* = 12.2, 2.2 Hz, H6b), 3.75 (2H, dt, *J* = 12.3, 7.3 Hz, H3, H6a), 3.47 (ddd, *J* = 9.7, 3.3, 1.6 Hz, H2), 3.38 (1H, app. t, *J* = 9.6 Hz, H4); **13C NMR** (101 MHz, D2O) δ 93.5 (C1), 73.1 (C3), 72.2 (C5), 71.9 (C2), 69.7 (C4), 60.7 (C6); **31P NMR** (161 MHz, D2O) δ2.45 (1P, d, *J*P,H = 7.4 Hz). Data matched those previously reported.[28]

*2-Deoxy-1,3,4,6-tetra-O-acetyl α/β-D-glucose* **5**

To a stirred solution of 2-deoxy-D-glucose (1.0 g, 6.1 mmol, 1.0 equiv.) in anhydrous pyridine (12 mL) at 0 °C was added dropwise acetic anhydride (4.6 mL, 48.7 mmol, 8.0 equiv.). The reaction mixture was warmed slowly to room temperature then stirred for 15 h, whereby TLC analysis (petroleum ether/EtOAc, 1/1) indicated complete conversion of starting material to a higher Rf spot. The reaction mixture was poured onto iced water (100 mL) and diluted with EtOAc (75 mL). The organic layer was washed successively with 1.0 M HCl, saturated aqueous NaHCO3 solution, water and brine (75 mL each). The aqueous layer was re-extracted with EtOAc (100 mL) and the combined organic layers dried (MgSO4), filtered and concentrated under reduced pressure. The resultant oil was co-evaporated with toluene (3 × 20 mL) to afford **5** as a white solid (1.84 g, 5.54 mmol, 91%). Rf  0.74 (petroleum ether/EtOAc, 1/1); **1H NMR** (400 MHz, CDCl3) 5:1 β/α, δ(β-anomer)5.77 (1H, dd, *J* = 10.0, 2.3 Hz, H1), 5.06-4.97 (2H, m, H3, H4), 4.29 (1H, dd, *J* = 12.4, 4.7 Hz, H6a), 4.06 (1H, dd, *J* = 12.4, 2.2 Hz, H6b), 3.72 (1H, ddd, *J* = 9.3, 4.6, 2.2 Hz, H5), 2.32 (1H, ddd, *J* = 12.6, 4.9, 2.3 Hz, H2), 2.09 (3H, s, C(O)C*H*3), 2.06 (3H, s, C(O)C*H*3), 2.02 (3H, s, C(O)C*H*3), 2.01 (3H, s, C(O)C*H*3); **13C NMR** (100 MHz, CDCl3) δ(β-anomer)170.8 (C=O), 170.2 (C=O), 169.8 (C=O), 168.9 (C=O), 91.2 (C1), 72.9 (C5), 70.2 (C4), 68.4 (C3), 62.1 (C6), 34.8 (C2), 21.0 (C(O)*C*H3), 20.9 (C(O)*C*H3), 20.9 (C(O)*C*H3), 20.8 (C(O)*C*H3); HRMS *m/z* (ESI+) Found: (M+Na)+ 355.1010, C14H20O9Na requires355.1005. Data matched those previously reported.[34]

*α-D-Mannose 1-phosphate bis sodium salt* **8**

**7** (250 mg, 0.64 mmol) gave **8** (91 mg, 0.35 mmol, 55%). Reaction temperature was 45 °C, following purification as described in the General Experimental, the material was treated with DOWEX-IR-120 (Na+ form) to obtain the bis-sodium salt form for comparison to reported data. Rf 0.40 (acetonitrile/water/NH4OH, 2/1/0.1); [α]$\begin{matrix}26\\D\end{matrix}$ +22.2 (*c* = 0.45, H2O); **1H NMR** (400 MHz, D2O) δ5.23 (1H, d, *J*1,P = 8.5 Hz, H1), 3.87-3.74 (4H, m, H2, H3, H5, H6a), 3.66-3.59 (1H, m, H6b) 3.50 (1H, app. t, *J* = 9.6 Hz, H4); **13C NMR** (100 MHz, D2O) δ 95.0 (C1), 73.0, 71.2, 70.2, 67.2 (C4), 61.3 (C6); **31P NMR** (161 MHz, D2O) δ 2.00 (1P, d, *J*P,H = 8.5 Hz); **HRMS** *m/z* (ES-)Found: (M-H)− 259.0224, C6H11O9 requires259.0231. Data matched those previously reported.[35]

*1,2,3,4,6-Penta-O-acetyl-5-C-Methyl-α/β-D-mannose* **9**

Acetylation using Ac2O/pyridine

5-*C*-methyl-α/β-D-mannose[29] (1.1 g, 5.8 mmol, 1.0 equiv.) was stirred in acetic anhydride (20 mL) and pyridine (40 mL) for 18 hours at room temperature under a nitrogen atmosphere. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3 x 20 mL). TLC (3% MeOH in DCM) showed four spots (Rf 0.39–0.75) corresponding to the diastereomeric pyranoside and furanoside forms. This residue was purified by silica gel column chromatography to afford **9** as an inseparable mixture of pyranosides and α-furanoside(>93% pyranoside by 1H NMR integration and a 58:35 β:α pyranoside ratio)as an off-white solid (688 mg, 1.73 mmol, 67%). The β-furanosidewas separable as a yellow, flaky solid (91 mg, 0.23 mmol, 4%). **1H NMR** (400 MHz, CDCl3) δ α-pyranoside6.11 (1H, d, *J* = 2.3 Hz, H1), 5.58 (1H, d, *J* = 10.5 Hz, H4), 5.25 (2H, dd, *J* = 10.2, 3.3 Hz, H2, H3), 4.11−3.95 (2H, m, H6a+b), 2.02 (3H, s, (C(O)*C*H3), 2.06 (3H, s, (C(O)*C*H3), 2.12 (3H, s, (C(O)*C*H3), 2.14 (3H, s, (C(O)*C*H3), 2.17 (3H, s, (C(O)*C*H3), 1.39 (3H, s, CH3); β-pyranoside6.06 (1H, d, *J* = 1.4 Hz, H1), 5.48 (1H, d, *J* = 10.4 Hz, H4), 5.25 (2H, dd, *J* = 10.2, 3.3 Hz, H2, H3), 4.11−3.95 (2H, m, H6a+b) 2.01 (3H, s, (C(O)*C*H3), 2.05 (3H, s, (C(O)*C*H3), 2.10 (3H, s, (C(O)*C*H3), 2.12 (3H, s, (C(O)*C*H3), 2.21 (3H, s, (C(O)*C*H3), 1.40 (3H, s, CH3); α-furanoside5.41 (1H, s, H1), 5.21 (1H, m, H2) 4.99 (1H, dd, *J* = 5.6, 1.8 Hz, H3), 4.91 (1H, d, *J* = 1.8 Hz, H4), 4.35, 3.56 (2H, q, *J* = 7.7 Hz, H6a+6b), 2.19 (3H, s, (C(O)*C*H3), 2.15 (3H, s, (C(O)*C*H3), 2.07 (3H, s, (C(O)*C*H3), 1.98 (3H, s, (C(O)*C*H3), 1.34 (3H, s, CH3), β-furanoside6.25 (1H, d, *J* = 3.2 Hz, H1), 5.71–5.68 (1H, m, H3), 5.41 (1H, dd, *J* = 5.1, 3.2 Hz, H4), 4.27 (1H, d, *J* = 4.3 Hz, H2), 4.15–4.14 (2H, m, H6a+b), 2.11 (3H, s, (C(O)*C*H3)), 2.10–2.10 (6H, m, (C(O)*C*H3), 2.07 (3H, s, (C(O)*C*H3), 1.29 (3H, s, CH3); **13C NMR** (101 MHz, CDCl3) For pyranoses δ 170.6, 170.5, 170.2, 170.0, 169.9, 169.7, 169.5, 169.4, 168.6, 168.4, 91.1 (C1β), 86.8 (C1α), 78.2 (C5β), 76.1 (C5α), 68.4, 68.3, 67.3 (C6α), 67.2 (C6β), 66.5, 21.2, 20.8, 20.8, 20.8, 20.7, 20.6, 19.3 (CH3-β), 15.0 (CH3-α); **HRMS** *m/z* (ES+) Found: (M+Na)+ 427.1215, C17H24O11Na requires (M+Na)+ 427.1217.

Acetylation using Ac2O/H2SO4

To a stirred mixture of Ac2O (850 µL, 9.0 mmol, 10.3 equiv.) and 5-*C*-methyl-α/β-D-mannose[29] (170 mg, 0.87 mmol, 1.0 equiv.), H2SO4 (1 drop) was added at 0 °C, under an atmosphere of N2. The solution was stirred for 10 min at 0 °C and then allowed to warm to room temperature and stirred for a further 45 min. The mixture was then diluted with ice–water (30 mL), and the organic phase extracted with EtOAc (50 mL). The extract was washed with water (3 × 20 mL), sat. aq. NaHCO3 solution (30 mL), dried over MgSO4, filtered, and the solvent evaporated to dryness to yield **9** as diastereomeric pyranoside and furanoside forms as a pale-yellow viscous oil (9.9 g, 0.69 mmol, 79%). 1H NMR integration showed >83% pyranoside and a 43:40 β:α pyranoside ratio. **13C-GATED** (101 MHz; CDCl3): 91.1 (*1JC1-H1* = 176 Hz, C1α), 86.8 (*1JC1’-H1’* = 164 Hz, C1β).Other analytical data matched those presented above and as previously reported.[29]

*5-C-Methyl-α/β-D-mannose 1-phosphate bis ammonium salt* **10**

From **9** (250 mg, 0.69 mmol) gave **10** (141 mg, 0.46 mmol, 66%).

**1H NMR** (400 MHz, D2O) δ α-anomer5.27 (1H, dd, *J1,P* = 9.1, *J1,2* = 2.2 Hz, H1), 4.09 (1H, dd, *J* = 10.2, 3.3 Hz, H3), 3.93–3.91 (1H, m, H2), 3.78 (1H, d, *J* = 10.2 Hz, H4), 3.39 (2H, q, *J* = 11.9 Hz, H6a+b); β-anomerδ 5.19 (1H, dd, *J* = 8.6, 1.1 Hz, H1), 3.96 (1H, d, *J* = 2.8 Hz, H2), 3.84 (1H, dd, *J* = 10.2, 3.4 Hz, H3), 3.64 (1H, d, *J* = 10.2 Hz, H4), 3.54–3.45 (2H, m, H6a+b); **13C NMR** (101 MHz, D2O) δ α-anomer95.8 (d, *J* = 3.9 Hz), 79.8, 68.0, 67.2, 66.7, 66.0, 18.0; **31P {1H} NMR** (162 MHz, D2O) δ1.88, 2.31; **HRMS** *m/z* (ES-)Found: (M-H)− 273.0385, C7H14O9Prequires273.0381.

*1,2,3,4-Tetra-O-acetyl-6-bromo-6-deoxy-β-D-mannose* **11**

Triphenylphosphine (2.25 g, 8.60 mmol, 2.0 equiv.) was added to a stirred solution of 1,2,3,4-tetra-O-acetyl-β-D-mannose[30](1.50 g, 4.30 mmol, 1.0 equiv.) in anhydrous pyridine (43 mL) at room temperature under a N2 atmosphere. The reaction mixture was cooled to 0 °C and carbon tetrabromide (3.08 g, 9.30 mmol, 2.2 equiv.) was added portion wise over 5 min. After 16 h at rt, TLC analysis (hexane/EtOAc, 3/1) showed complete conversion of starting material to a higher Rf value spot. The solvent was removed under reduced pressure and the residue re-suspended in EtOAc (30 mL), washed with H2O (20 mL) and brine (20 mL). The organic phase was dried over MgSO4, filtered and concentrated under vacuum. Purification using silica gel column chromatography (hexane/EtOAc, 8:2) furnished **11** (1.52 g, 3.70 mmol, 86%) as a pale yellow solid. Rf 0.77 (petroleum ether/EtOAc, 1/1); [α]$\begin{matrix}26\\D\end{matrix} $ -14.6 (*c* = 0.55, CHCl3); **1H NMR** (400 MHz, CDCl3) δ5.89 (1H, d, *J* = 1.1 Hz, H1), 5.47 (1H, dd, *J* = 3.2, 1.2 Hz, H2), 5.27 (1H, appt, *J* = 9.7 Hz, H4), 5.13 (1H, ddd, *J* = 9.8, 3.2, 1.2 Hz, H3), 3.82 (1H, ddd, *J* = 9.4, 6.2, 3.0 Hz, H5), 3.50 (1H, dd, *J* = 11.4, 3.0 Hz, H6a), 3.42 (1H, dd, *J* = 11.5, 6.4 Hz, H6b), 2.20 (3H, s, C(O)OC*H*3), 2.10 (3H, s, C(O)OC*H*3), 2.07 (3H, s, C(O)OC*H*3), 1.99 (3H, s, C(O)OC*H*3); **13C NMR** (100 MHz, CDCl3) δ170.3 (C=O), 169.9 (C=O), 169.7 (C=O), 168.5 (C=O), 90.4 (C1), 74.5 (C5), 70.6 (C3), 68.3 (C2), 68.1 (C4), 30.3 (C6), 20.9 (2C, C(O)*C*H3), 20.8 (2C, C(O)*C*H3), 20.6 (2C, C(O)*C*H3); **HRMS** *m/z* (ESI+) Found: (M+Na)+ 433.0115, C14H19BrO9Na requires (M+Na)*+* 433.0110. Previously reported data were for the α-anomer.[36]

*1,2,3,4-Tetra-O-acetyl-6-chloro-6-deoxy-β-D-mannose*

To a stirred solution of *1,2,3,4-tetra-O-acetyl-β-D-mannose*[30](500 mg, 1.44 mmol, 1.0 equiv.) and triphenylphosphine (640 mg, 2.45 mmol, 1.7 equiv.) in anhydrous dichloromethane (14 mL) under inert N2 atmosphere at 0 °C was added dropwise carbon tetrachloride (0.24 mL, 2.45 mmol, 1.7 equiv.). The reaction mixture was stirred at 0 °C for 30 min then warmed gradually to room temperature. The reaction mixture was then heated to 40 °C for 6 h, whereby TLC analysis (petroleum ether/EtOAc, 1/1) showed conversion of starting material to a higher Rf value spot. The reaction mixture was poured onto distilled water (30 mL) and diluted with dichloromethane (35 mL). The organic layer was washed with distilled water (2 × 30 mL) and brine (30 mL) then the aqueous layer was re-extracted with dichloromethane (35 mL). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with petroleum ether/EtOAc (4/1, 3/1, 1/1) to afford the title compound (320 mg, 0.82 mmol, 61%) as a white foam. Rf 0.78 (petroleum ether/EtOAc, 1/1); [α]$\begin{matrix}26\\D\end{matrix}$ -13.3 (*c* 0.75, CHCl3); **1H NMR** (400 MHz, CDCl3) δ5.87 (1H, d, *J* = 0.7 Hz, H1), 5.44 (1H, d, *J* = 2.3 Hz, H2), 5.26 (1H, app. t, *J* = 9.7 Hz, H4), 5.12 (1H, dd, *J* = 9.9, 3.2 Hz, H3), 3.84-3.78 (1H, m, H5), 3.64 (1H, dd, *J* = 12.2, 2.9 Hz, H6b), 3.57 (1H, dd, *J* = 12.2, 5.8 Hz, H6a), 2.16 (3H, s, C(O)OC*H*3), 2.07 (3H, s, C(O)OC*H*3), 2.04 (3H, s, C(O)OC*H*3), 1.96 (3H, s, C(O)OC*H*3); **13C NMR** (100 MHz, CDCl3) δ170.2 (C=O), 169.8 (C=O), 169.6 (C=O), 168.4 (C=O), 90.3 (C1), 74.7 (C5), 70.5 (C3), 68.2 (C2), 66.9 (C4), 42.9 (C6), 20.8 (C(O)*C*H3), 20.7 (2C 2 × C(O)*C*H3), 20.6 (C(O)*C*H3); **HRMS** *m/z* (ESI+) Found: (M+Na)+ 389.0633, C14H19ClO9Na requires [M+Na]+389.0616.

*O1,O6-Hydroxyhosphoryl-D-mannopyranose ammonium salt* **13**

Synthesised from **11**

**11** (400 mg, 0.97 mmol) gave **13** (200 mg, 0.82 mmol, 85%).

Rf = 0.30 (MeCN/H2O/AcOH, 2/1/0.1); [α]$\begin{matrix}26\\D\end{matrix}$ +3.93 (*c* 0.25, H2O); **1H NMR** δ (600 MHz, D2O) δ 5.20 (1H, app. t, *J1,P&1,2* = 6.9 Hz, H1), 4.29 (1H, app. t, *J =* 2.8 Hz, H5), 4.20 (1H, dd, *J* = 6.0, 2.8 Hz, H4), 4.14 (1H, br. d, *J* = 6.5 Hz, H3), 4.10 (1H, d, *J* = 10.9 Hz, H6a)**,** 3.96 (1H, dd, *J* = 10.8, 3.0 Hz, H6b), 3.76 (1H, dd, *J* = 6.6, 1.2 Hz, H2); **13C NMR** (125 MHz, D2O) δ 96.5 (C1, d, 3*JC,P* = 3.2 Hz), 76.9 (C3), 75.3 (C5), 70.3 (C2, d, 4*JC,P* = 4.4 Hz), 70.0 (C4), 68.9 (C6); **31P {1H} NMR** (162 MHz, D2O) δ 2.41; **HRMS** *m/z* (ESI-) Found: (M-H)- 241.0116, C6H10O8P requires (M-H)- 241.0119.

Synthesised from 6-chloro-6-deoxy-1,2,3,4-tetra-*O*-acetyl-β-D-mannose

**6-chloro-6-deoxy-1,2,3,4-tetra-*O*-acetyl-β-D-mannose** (150 mg, 0.41 mmol) gave **13** (15 mg, 0.07 mmol, 17%). Analytical data matched those above.

*1,2,3,4-Tetra-O-acetyl-6-deoxy-6-fluoro-β-D-mannopyranose* **14**

Synthesised from 1,2,3,4-tetra-*O*-acetyl-β-D-mannose,[30] according to literature procedures.[32]

*6-Deoxy-6-fluoro-α-D-mannose 1-phosphate bis ammonium salt* **15**

**14** (54 mg, 0.15 mmol) gave **15** (40 mg, 0.15 mmol, 72%, **15**:**13** = 1.0:0.3).

Data for **15**: **1H {19F} NMR** (600 MHz, D2O) δ 5.26 (dd, *J1,P* = 8.5, *J1,2 =*1.0 Hz, H1), 4.62 (1H, dd, *J* = 10.5, 3.1 Hz, H6a), 4.55 (1H, dd, *J* = 10.6, 1.8 Hz, H6b), 3.92-3.85 (3H, m, H2, H3, H5), 3.70 (1H, app. t, *J* = 9.7 Hz, H4); **13C NMR** (101 MHz; D2O) δ 95.0 (d, *JC,P =* 5.6 Hz, C1), 82.4 (*JC,F* = 170.0 Hz, C6), 71.5 (C2), 71.1 (C3), 69.7 (C4), 65.5 (*JC,F* = 20.0 Hz, C5); **31P {1H} NMR** (162 MHz, D2O) δ -1.81; **19F {1H} NMR** (282 MHz, D2O) δ -122.8; **HRMS** *m/z* (ESI-) Found: (M-H)- 261.0178, C6H10FO8P requires (M-H)- 261.0181.

*1,2,3,4-Tetra-O-acetyl-6-deoxy-6,6-difluoro-β-D-mannopyranose* **16**

Dess-Martin periodinane (749 mg, 1.8 mmol) was added to a solution of 1,2,3,4-tetra-*O*-acetyl-β-D-mannose[30] (513 mg, 1.5 mmol) in dichloromethane (5.0 mL) and the reaction mixture was stirred at room temperature for 18 h. Next, the reaction mixture was quenched with a saturated aqueous solution of Na2S2O3 (5.0 mL) and the aqueous layer was extracted with CH2Cl2 (3 × 10 mL). The combined organic phases were dried over MgSO4 and evaporated *in vacuo*. The crude aldehyde was then dissolved in dichloromethane (10.0 mL) and cooled to 0 °C. DAST (0.58 mL, 4.4 mmol) was added dropwise, after which the reaction mixture was allowed to warm to room temperature. After 18 h, the reaction mixture was diluted with dichloromethane (10 mL) and quenched with a saturated aqueous solution of NaHCO3 (10.0 mL). The aqueous layer was extracted with dichloromethane (3 × 10.0 mL). The combined organic phases were dried over MgSO4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography to yield **16** (45 mg, 8%). [α]$\begin{matrix}26\\D\end{matrix}$ -19.5 (*c* 1.1, CHCl3); **1H NMR** (400 MHz, CDCl3) δ 5.92 (1H, d, *J* = 1.2 Hz, H1), 5.89 (1H, td, *JH,F* = 54.3 Hz, *JH6,H5* = 3.4 Hz, H6), 5.50-5.42 (m, 2H), 5.18 (dd, *J* = 9.6, 3.3 Hz, 1H, H3), 3.90-3.81 (m, 1H), 2.20 (3H, s, C(O)OC*H*3), 2.11 (3H, s, C(O)OC*H*3), 2.06 (3H, s, C(O)OC*H*3), 2.02 (3H, s, C(O)OC*H*3); **13C NMR** (100 MHz, CDCl3) δ 170.2 (C=O), 169.8 (C=O), 169.5 (C=O), 168.4 (C=O), 113.3 (t, *JC,F* = 246.0 Hz, C6), 90.1 (C1), 73.2 (t, *JC,F* = 24.7 Hz, C5), 70.1 (C2 or C3), 67.7 (C2 or C3), 64.1 (t, *JC,F* = 1.2 Hz, C4), 20.84 (3H, s, C(O)OC*H*3), 20.79 (3H, s, C(O)OC*H*3), 20.71 (3H, s, C(O)OC*H*3), 20.65 (3H, s, C(O)OC*H*3); **19F NMR** (376 MHz, CDCl3) δ -126.6 (ddd, *J* = 296.6, 54.4, 9.0 Hz, 1F), -130.2 (ddd, *J* = 296.8, 54.6, 10.6 Hz, 1F); **19F {1H} NMR** (376 MHz, CDCl3) δ -126.6 (d, *J* = 296.4 Hz, 1F), -130.2 (d, *J* = 296.4 Hz, 1F); **HRMS** *m/z*(ESI+) Found: [M+Na]+ 391.0820, C14H18F2NaO9 requires [M+Na]+ 391.0811

*6-Deoxy-6,6-difluoro-α-D-mannopyranosyl 1-phosphate bis ammonium salt* **17**

**16** (44 mg, 0.10 mmol) gave **17** (15 mg, 0.05 mmol, 42%).

**1H NMR** (600 MHz, D2O) δ 6.07 (1H, td, *JH,F* = 55.3, *JH,F* = 54.9, *J6,5* = 5.5 Hz, H6), 5.28 (1H, dd, *JP,H* = 8.5, *J1,2* = 1.9 Hz, H1), 4.62-3.93 (1H, m, H5), 3.89-3.85 (2H, m, H2, H3), 3.76 (1H, t, *J* = 9.4 Hz, H4); **13C NMR** (101 MHz; D2O) δ 114.6 (t, *JC,F* = 182.0 Hz, C6), 95.0 (d, *JC,P* = 4.4 Hz, C1), 70.7 (C2), 70.3 (C5), 69.6 (C3), 67.2 (C4); 3**1P {1H} NMR** (162 MHz, D2O) δ 1.75; **19F {1H} NMR** (376 MHz, D2O) δ -131.5 (d, *J* = 284.6 Hz, 1F), -132.2 (d, *J* = 284.2 Hz, 1F); **HRMS** *m/z* (ESI-) Found: (M-H)- 279.0086, C6H9F2O8P requires (M-H)- 279.0087.

*1,2,3,4-Tetra-O-acetyl-6-para-tolenesulfonyl-β-D-mannose*

To a stirred solution of *1,2,3,4-tetra-O-acetyl-β-D-mannose*[30](250 mg, 0.72 mmol, 1.0 equiv.) in anhydrous pyridine (4.8 mL) was added *p-*TsCl (470 mg, 2.45 mmol, 3.4 equiv.). The reaction mixture was stirred for 7 h at room temperature, where TLC analysis (petroleum ether/EtOAc, 1/2) indicated complete conversion of starting material to a higher Rf value spot. Distilled water was added to the reaction mixture (10 mL) and stirred for 10 min before extraction with chloroform (30 mL). The organic layer was washed with saturated aqueous NaHCO3 solution (2 × 20 mL), distilled water (2 × 20 mL) and brine (20 mL) then the combined aqueous layers were re-extracted with chloroform (25 mL). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure. Drying under high vacuum afforded the title compound as a colourless syrup (340 mg, 0.67 mmol, 93%). Rf 0.82 (petroleum ether/EtOAc, 1/2); [α]$\begin{matrix}26\\D\end{matrix}$ -6.70 (*c* 1.65, CHCl3); **1H NMR** (400 MHz, CDCl3) δH 7.78-7.74 (2H, m, Ar-*H*), 7.33 (2H, d, *J* = 8.0 Hz, 2 × Ar-*H*), 7.30-7.27 (1H, m, Ar-*H*), 5.79 (1H, d, *J* = 1.2 Hz, H1), 5.42 (1H, dd, *J* = 3.2, 1.2 Hz, H2), 5.20 (1H, appt, *J* = 9.8 Hz, H4), 5.08 (1H, *J* = 9.9, 3.3 Hz, H3), 4.13 (2H, app. d, *J* = 4.3 Hz, H6a+b), 3.79 (1H, dt, *J* = 9.6, 4.3 Hz, H5), 2.43 (3H, s, C*H*3), 2.16 (3H, s, C(O)OC*H*3), 2.06 (3H, s, C(O)OC*H*3), 2.00 (3H, s, C(O)OC*H*3), 1.98 (3H, s, C(O)OC*H*3); **13C NMR** (100 MHz, CDCl3) δ 170.3 (C=O), 169.9 (C=O), 169.7 (C=O), 168.3 (C=O), 149.9 (Ar-*C*), 145.2 (Ar-*C*), 136.1 (Ar-*C*), 132.6 (Ar-*C*), 130.0 (Ar-*C*), 128.2 (Ar-*C*), 90.3 (C1), 70.9 (C5), 70.5 (C4), 68.1 (C3), 67.6 (C2), 65.8 (C6), 21.8 (*C*H3), 20.8 (C(O)*C*H3), 20.7 (2 × C(O)*C*H3), 20.6 (C(O)*C*H3); **HRMS** *m/z* (ESI+) Found: (M+Na)+ 525.1073, C21H26O12SNa requires [M+Na]+*,* 525.1043. Previously reported data were for the α-anomer.[36]

*1,2,3,4-Tetra-O-acetyl-6-azido-6-deoxy-β-D-mannose* **18**

To a stirred solution of *1,2,3,4-tetra-O-acetyl-6-para-tolenesulfonyl-β-D-mannose* (200 mg, 0.39 mmol, 1.0 equiv.) in DMF (3.9 mL) was added successively 15-crown-5 ether (0.23 mL, 1.20 mmol, 3.0 equiv.) and NaN3 (78 mg, 1.20 mmol, 3.0 equiv.). The reaction mixture was heated to 60 °C and stirred for 24 h, whereby TLC analysis (toluene/EtOAc, 4/1) indicated conversion of starting material to a higher Rf value spot. The reaction mixture was cooled to room temperature, poured onto distilled water (20 mL) and extracted with EtOAc (25 mL). The organic layer was washed successively with 10% aqueous Na2S2O3 solution, distilled water and brine (20 mL each) then the aqueous layers were re-extracted with EtOAc (25 mL). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with toluene/EtOAc (5/1, 4/1) afforded **18**as a clear colourless oil (31 mg, 83 µmol, 32%). Rf 0.33 (toluene/acetone, 4/1); [α]$\begin{matrix}26\\D\end{matrix}$ -13.5 (*c* 1.55, CHCl3) **1H NMR** (400 MHz, CDCl3) δ5.86 (1H, d, *J* = 1.2 Hz, H1), 5.47 (1H, dd, *J* = 3.2, 1.1 Hz, H2), 5.25 (1H, app. t, *J* = 9.8 Hz, H4), 5.11 (1H, dd, *J* = 10.0, 3.2 Hz, H3), 3.78-.3.72 (1H, m, H5), 3.38 (2H, app. qd, *J* = 13.4, 4.5 Hz, H6a, H6b), 2.21 (3H, s, C(O)C*H*3), 2.09 (3H, s, C(O)C*H*3), 2.05 (3H, s, C(O)C*H*3), 1.99 (3H, s, C(O)C*H*3); **13C NMR** (100 MHz, CDCl3) δ 170.3 (C=O), 169.9 (C=O), 169.8 (C=O), 168.5 (C=O), 90.3 (C1), 74.5 (C5), 70.7 (C3), 68.2 (C2), 66.5 (C4), 50.8 (C6), 20.9 (C(O)*C*H3), 20.8 (2 × (C(O)*C*H3), 20.6 (C(O)*C*H3); **HRMS** *m/z* (ESI+) Found: (M+Na)+ 396.1039, C14H19N3O9Na requires [M+Na]+*,* 396.1019. Previously reported data were for the α-anomer.[36]

*6-Azido-6-deoxy-α-D-mannose 1-phosphate bis ammonium salt* **19**

**18** (100 mg, 0.27 mmol) gave **19** (25 mg, 0.14 mmol, 65%).

**1H NMR** (400 MHz, D2O) δ 5.07 (1H, dd, *JP,H* = 8.4, *J1,2* = 1.9 Hz, H1), 3.72-3.67 (3H, m, H5, H2, H4), 3.50-3.45 (1H, m, H6a), 3.42-3.37 (2H, m, H3 H6b); **13C NMR** (101 MHz, D2O) δ 94.9 (C1), 72.4, 71.1, 69.7 (C2, C4 or C5), 67.2 (C3), 51.0 (C6); **31P {1H} NMR** (162 MHz, D2O) δ 1.36; **HRMS** *m/z* (ESI-) Found: (M-H)- 284.0288, C6H11N3O8P requires [M-H]-284.0284. Data previously reported for the bis-triethylammonium salt.[37]

*1,2,3,4-Tetra-O-acetyl-6-S-acetyl-6-deoxy-β-D-mannose* **20**

To a stirred solution of *1,2,3,4-Tetra-O-acetyl-6-chloro-6-deoxy-β-D-mannose* (58 mg, 0.16 mmol, 1.0 equiv.) in anhydrous DMF (1.6 ml) was added potassium thioacetate (54 mg, 0.48 mmol, 3.0 equiv.). The reaction mixture was heated to 75 °C for 24 hours, where TLC analysis (petroleum ether/EtOAc, 2/1) indicated formation of a similar Rf value product to the starting material). The reaction mixture was cooled to room temperature then poured onto water (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed successively with saturated aqueous NaHCO3 solution, water and brine (15 mL each) then the aqueous layers were re-extracted with EtOAc (20 mL). The combined organic layers were dried (MgSO4), filtered and concentrated under reduced pressure. Purification by silica gel column chromatography, eluting with petroleum ether/EtOAc (2/1) afforded **20** as a clear orange oil (55 mg, 0.14 mmol, 88%). Rf 0.38 (petroleum ether/EtOAc, 2/1); [α]$\begin{matrix}26\\D\end{matrix}$ -7.27 (*c* 1.45, CHCl3); **1H NMR** (400 MHz, CDCl3) δ5.80 (1H, s, H1), 5.45-5.43 (1H, m, H2), 5.18 (1H, app. t, *J* = 9.8, H4), 5.07 (1H, dd, *J* = 9.9, 3.2 Hz, H3), 3.68 (1H, ddd, *J* = 9.8, 7.5, 2.7 Hz, H5), 3.28, 1H, dd, *J* = 14.3, 2.5 Hz, H6a), 3.03 (1H, dd, *J* = 14.3, 7.4 Hz, H6b), 2.32 (3H, s, SCO*C*H3), 2.19 (3H, s, CO*C*H3), 2.10 (3H, s, CO*C*H3), 2.08 (3H, s, CO*C*H3), 1.98 (3H, s, CO*C*H3); **13C NMR** (100 MHz, CDCl3) δ194.9 (C=O), 170.3 (C=O), 170.1 (C=O), 169.9 (C=O), 168.4 (C=O), 90.5 (C1), 74.7 (C5), 70.7 (C3), 68.4 (C2), 67.8 (C4), 30.5 (SCO*C*H3), 30.2 (C6), 20.9 (2C, 2 × CO*C*H3), 20.8 (CO*C*H3), 20.6 (CO*C*H3);**HRMS** *m/z* (ESI+) Found: (M+Na)+ 424.0832 C16H22O10SNa, requires [M+Na]*+*424.0832.

*6-Deoxy-6-thio-α-D-mannose 1-phosphate bis ammonium salt* **21**

**20** (100 mg, 0.25 mmol) gave **21** (30 mg, 0.10 mmol, 43%).

**1H NMR** (400 MHz, D2O) δ 5.34 (d, *JP,H* = 8.1 Hz, H1 (1-phosphate disulfide), 5.16 (d, *J1,2* = 1.3 Hz (1-phosphate-hemiacetal mixed disulfide), ratio = 2.5/1; **31P {1H} NMR** (162 MHz, D2O) δ 0.38 (1P, s, 1-phosphate disulfide); **HRMS** *m/z* (ESI-) of the disulfide: Found: (M-H)- 548.9910, C12H23O16P2S2 requires [M-H]*-* 548.9908; of the mixed disulfide: Found: (M-H)- 469.0239, C12H22O13PS2 requires [M-H]-469.0240.

***DFT calculations***

The geometry optimisations were performed with Gaussian 16[38] software using restricted density functional theory. The B3LYP[39] functional hybrid method was employed and the 6-311+G(2df, p)[40,41] with diffused basis set was used for the geometry optimisation and frequency analysis in vacuum. The normal modes revealed no imaginary frequencies indicating that they represent minima on the potential energy surface.

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