Synthesis of the Dipotassium Salts of Methyl \( \alpha \)-D-Mannopyranoside 6-Phosphorothioate and
D-Mannose 6-Phosphorothioate

Alan H. Haines,* D. James R. Massy*

School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, England
Fax +44(1603)259396
Received 20 June 1996

Dipotassium methyl \( \alpha \)-d-mannopyranoside 6-phosphorothioate (4) and dipotassium D-mannose 6-phosphorothioate (7) have been prepared from per-O-trimethylsilyl derivatives of methyl \( \alpha \)-d-mannopyranoside and D-mannopyranose, respectively, through reaction sequences involving selective cleavage in the pyranoside ether, and selective replacement in the pyranose ether, of a 6-O-trimethylsilyl group.

Phosphorothioate analogues of biochemically important phosphates are of considerable interest as inhibitors of enzymes involved in metabolism of the natural phosphates and, as such, have potential application in the blocking and alteration of biosynthetic pathways, events which may be of value in the treatment of some diseases. Mannose is incorporated into complex polysaccharides, for example the glycoconjugates, by way of GDP-mannose, which is formed from GDP and mannose 1-phosphate. Since the latter compound arises by action of a mutase on mannose 6-phosphate, a study of the biochemical properties of mannose 6-phosphorothioate and related compounds is of some interest. For such a biochemical study, we required the dipotassium salts of methyl \( \alpha \)-d-mannopyranoside 6-phosphorothioate (4) and D-mannose 6-phosphorothioate (7), and describe here convenient syntheses of these two compounds. The key intermediate for the synthesis of glycoside 4 was methyl 2,3,4-tri-O-(trimethylsilyl)-\( \alpha \)-d-mannopyranoside (1), which was prepared by selective hydrolysis of the corresponding 2,3,4,6-tetra-O-(trimethylsilyl)ether utilising a procedure already described in a synthesis of methyl \( \alpha \)-d-mannopyranoside 6-phosphate disodium salt. Reaction of 2 with bis(2-cyanoethoxy)(diisopropylamino)phosphine\(^{2,3}\) in dichloromethane catalysed by 1\( H \)-tetrazole afforded (Scheme 1) the 6-O-phosphoryl derivative 2 which, without rigorous purification, was treated with sulphur in pyridine to afford methyl \( \alpha \)-D-mannopyranoside 6-bis(2-cyanoethyl)phosphorothioate (3) as an analytically pure viscous oil. The compound was further characterised by its \( H \) and \( ^{13} C \) NMR spectra, and its \( ^{31} P \) NMR spectrum contained a resonance at \( \delta = 68.5 \), typical for this type of compound.\(^{4}\) Methanolation of 3 on treatment with potassium hydroxide/methanol afforded the dipotassium salt 4 as a crystalline solid (Scheme 1).

For the synthesis of 7 we capitalised on observations by Lehmann and co-workers\(^ {5,6} \) who reported that controlled reaction of per-O-trimethylsilylated carbohydrate derivatives with acetic anhydride in pyridine afforded derivatives acylated at the primary positions, and on the later extension\(^ {1} \) of this type of selective reaction to the preparation of mannose 6-phosphate through reaction of 1,2,3,4,6-penta-O-(trimethylsilyl)-\( \alpha \)-D-mannopyranose (5) with phosphorus oxychloride followed by hydrolysis of the so-formed 6-O-dichlorophosphonyl derivative. We

![Scheme 1](image1)

![Scheme 2](image2)
in D$_2$O shows absorptions at $\delta = 44.81$ and 44.75 for the $\beta$ and $\alpha$ isomers, respectively, values which are close to that reported$^4$ for the disodium salt of the corresponding glycolic compound. Signals from both anomers, which appear to be present in the approximate ratio of 1:2, are also apparent in the $^1$H and $^{13}$C NMR spectrum of 7.

Results of our biochemical studies will be reported separately.

TLC was performed on silica gel (Merck Nr. 5554, coated alumina sheets) and chromatograms were developed by spraying with an aq solution of 0.1 M KMnO$_4$, followed by heating at 50–100°C. Column chromatography was performed on silica gel (Matrex silica 60). Solvent ratios are given as v/v. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter and [\(\alpha\)]$_D$ units are recorded in 10$^{-1}$ deg cm$^{-1}$ g$^{-1}$. $^1$H and $^{13}$C NMR spectra were recorded at 90 and 22.6 MHz on a Jeol EX90 FT spectrometer, at 270 and 67.9 MHz on a Jeol EX270 FT NMR spectrometer, or at 400 and 100.5 MHz on a Jeol GX400 FT NMR spectrometer. TMS was used as an internal standard for samples dissolved in CDCl$_3$ or CD$_3$OD, or mixtures of these. For solutions in D$_2$O, tert-butyl alcohol ([\(\delta_H\] CH$_3$] = 1.23; [\(\delta_D\] OD] = 32.33) was used as the internal standard. Proton-decoupled $^{13}$P NMR spectra were measured at 36.1 MHz on a Jeol EX90 FT spectrometer and chemical shifts are expressed relative to H$_3$PO$_4$ (\(\delta = 0\)). signals downfield of this being positive; spectra were run on external lock, the instrument being calibrated with a solution of (MeO)$_3$PO in EtOAc ([\(\delta_H\] H$_2$O] = 140.0) immediately before each spectrum was measured. J values are given in Hz. IR spectra were obtained with a Perkin-Elmer FTIR 1720X machine. Bis(2-cyanoethoxy)dipropylaminophosphine was prepared following the method described for di-tert-butyl(4-dimethylaminophenoxy) using 3-hydroxypropanenitrile in place of tert-butyl alcohol, rather than the previously described$^3$ three-step procedure. Elemental analyses were performed at the University of East Anglia by Mr. A.W.R. Saunders.

**Methyl 2-0-Mannopyranoside 6-Bis(2-cyanoethoxy)phosphorothioate (3):**

A solution of 1$^1$ (2.05 g, 5 mmol) bis(2-cyanoethoxy)dipropylaminophosphine (2.03 g, 7.5 mmol) and 1H-tetrazole (0.71 g, 10.1 mmol) in CH$_2$Cl$_2$ (42 mL) was stirred for 18 min at 25°C and the mixture was shaken with 8%aq NaHCO$_3$ solution (50 mL). The organic phase was separated, extracted with CH$_2$Cl$_2$ (2 x 50 mL). The combined organic phases were dried and concentrated to give a chromatographically homogeneous mixture ($R_{f}$ 0.63, light petroleum/EtOAc, 1:1) viscous liquid 3.2 g identified on the basis of its IR and NMR spectra as methyl 6-O-di(2-cyanoethoxy)phosphinyl-2,3,4-tri-O-(trimethylsilyl)-D-mannopyranoside (2).

**6-N-D Mannose 6-Bis(2-cyanoethoxy)phosphorothioate (6):**

To a mixture of 5 (10.82 g, 20 mmol) and 1H-tetrazole (2.8 g, 40 mmol), was added a solution of bis(2-cyanoethoxy)dipropylaminophosphine$^{2,3}$ (2.8 g, 8 mmol) in CH$_2$Cl$_2$ (100 mL) and the mixture was stirred for 40 min whilst the solvent was passed through. The mixture was then shaken with previously cooled (5°C) 8%aq NaHCO$_3$ solution (50 mL) and the aqueous phase was back-extracted with CH$_2$Cl$_2$ (25 mL). The combined organic phases were washed with a further portion of 8%aq NaHCO$_3$ solution (50 mL), dried, and concentrated (< 35°C) to afford a yellow oil (14.57 g), which on standing separated into two phases. Addition of light petroleum (30 mL) diluted the upper phase, which was shown by TLC to contain the required product, and this phase was separated and clarified by filtration through Kieselguhr. The oil (12.1 g) obtained on concentration was dissolved in pyridine (100 mL), sulphur (2 g, 62.5 mmol) was added, and the mixture was stirred for 1 h at 25°C and concentrated. Residual pyridine was removed by co-evaporation with toluene (2 x 20 mL) and residual sulphur from the oil so obtained was removed by dilution with MeOH (10 mL) and filtration. A further amount of MeOH (90 mL) was added and the solution stored for 1 h at 23°C, after which TLC indicated the presence of a major product. Concentration of this solution gave a paste (6.88 g) which, as a solution in a mixture of MeOH (5 mL) and EtOAc (2 mL), was loaded onto a silica gel chromatography column (240 g), packed in EtOAc, and eluted successively with EtOAc (2200 mL) and EtOAc/MeOH (4:1) (900 mL). Fractions containing solely the desired product (R$_f$ 0.39, CH$_2$Cl$_2$)

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**Synthesis**

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MeOH, 5:1) were combined, concentrated under reduced pressure (0.7 mbar) to give 6 as a viscous liquid (2.64 g). A further amount of 6 was obtained by recrystallization (with CH₂Cl₂, MeOH, initially 10:1 then changing to 5:1) of the non-homogeneous fractions containing the required material; total yield of 6: 3.16 g (43%); [α]D²⁺ = +14.4 (c = 0.4, H₂O).

IR (film): ν = 3426 (br. s, OH), 2258 (m, CN), 1034 (s, P–O), 666 cm⁻¹ (m, P=O).

¹H NMR (400 MHz, CD₃OD; ratio of α:β anomers = 1:0.36): δ = 2.90 (8 H, t, J₁,₂ = J₁,₃ = J₂,₃ = 6.0, 2 × α-OCH₂CH₂CN, 2 × β-OCH₂CH₂CN), 3.39–3.46 (1 H, m, β-H), 3.49 (1 H, dd, J₁,₂ = 3.2, J₁,₃ = 9.3, 3β-H), 3.58 (1 H, dd, J₁,₂ = 9.5, 4β-H), 3.65 (1 H, d, J₁,₂ = 6.4, 4α-H), 3.77 (1 H, dd, J₁,₂ = 3.2, 3α-H), 3.81 (1 H, dd, J₁,₂ = 1.5, 2α-H), 3.83 (1 H, dd, J₁,₂ = 0.9, 2β-H), 3.91 (1 H, br dd, J₅,₆ = 4.9, J₆,₇ = < 1, 5α-H), 4.21–4.36 (10 H, complex, 6β-H₂, 2 × α-OCH₂CH₂CN, 2 × β-OCH₂CH₂CN), 4.33–4.49 (2 H, complex, 6α-H₂), 4.78 (1 H, d, J₁β-H), 5.08 (1 H, d, 1α-H).

¹³C NMR (100.5 MHz, CD₃OD): δ = 19.9 (d, J₁α,C = 8.9, 2α- and 2β-C), 64.0 (m, 1α- and 1β-C), 67.8 (β-C), 68.2 (β-C), 69.1 (d, J₁α,C = -4.4, 6β-C), 69.3 (d, J₁β,C = -4.4, 6α-C), 72.1 (α-C), 72.2 (d, J₆,C = 7.3, 5α-C), 72.6 (α-C), 72.8 (β-C), 75.1 (β-C), 75.9 (d, J₅,C = 7.3, 5β-C), 95.5 (1β-C), 95.8 (1α-C), 118.6 (3α-CN and 3β-CN), 118.7 (3β-CN).

³¹P NMR (36.1 MHz, D₂O): δ = 67.98 (β-P), 68.04 (α-P).

C₅H₁₁N₂O₅PSK₃ calc. C 37.7 H 5.0 N 7.3 (382.3) found 37.5 5.1 7.3

Dipotassium D-Manose 6-Phosphorothioate (7):
A solution of 6 (2.21 g, 5.78 mmol) in MeOH (60 mL) was added over 30 min to a refluxing solution of KOH (1.26 g, 22.4 mmol) in MeOH (140 mL), during which time solid material was deposited. The cooled solution was concentrated to approximately 100 mL, the residual slurry was filtered under argon, and the collected solid was washed with cold, anhyd MeOH (2 × 2 mL) and stored for 3 h over P₂O₅ at 50°C/0.2 mbar pressure to give, as an amorphous, hygroscopic solid, the phosphorothioate 7 (1.59 g), shown by ¹H NMR spectroscopy to contain approximately 4% w/w of MeOH. Attempts to remove this residual MeOH at 80°C led to partial decomposition. The corrected yield is 1.53 g (75%); mp 150–152°C (dec.); [α]D¹³ = +6.9 (c = 1, H₂O).

IR (Nujol): ν = 3147 (very br. OH), 1005 (P=O), 721 cm⁻¹ (P=S).

¹H NMR (400 MHz, D₂O; ratio of α:β anomers = 1:0.5): δ = 3.44 (1H, dt, J₁,₂ = 9.5, J₁,₃ = 9.5, 3β-H), 3.66 (1 H, dd, J₁,₂ = 3.3, J₁,₃ = 9.9, 3β-H), 3.75 (1 H, dd, J₆,₇ = 9.6, 4β-H), 3.80–3.94 (5 H, complex, 2α, 3α, 6α, 6β, 23-H), 3.98 (1 H, ddd, J₆,₇ = 11.9, J₅,₆ = 5.6, J₅,₆ = 1.7, 5α-H), 4.02–4.13 (3 H, complex, 6α, 6β, 6β-H), 4.89 (1 H, br, s, 1α-H), 5.17 (1 H, d, J₁,₂ = 1.5, 1α-H).

¹³C NMR (67.8 MHz, D₂O): δ = 65.8 (d, J₆,₇ = -4.9, 6β-C), 65.9 (d, J₆,₇ = -4.9, 6α-C), 68.8, 69.0, 72.6, 73.6, 74.1, 74.3 (d, J₅,₆ = 8.5, 5α-C), 75.3, 78.0 (d, J₅,₆ = 8.6, 5β-C), 96.7 (1β-C), 97.3 (1α-C).

³¹P NMR (36.1 MHz, D₂O): δ = 44.81 (β-P), 44.75 (α-P).

C₅H₁₁O₅PSK₃ [contains 4% (w/w) of MeOH] calc. C 21.1 H 3.5 (352.4) found 21.5 3.4

We thank the British Technology Group Ltd for financial support of this research.