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**Preliminary communication**

# Cyclic and linear oligo(mannosyl phosphates) from partially protected α-D-mannopyranosyl hydrogenphosphonate

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Poly(glycosyl phosphates) are immunologically active components of the cell wall or capsule of several micro-organisms[1]. These regular biopolymers are composed of mono- or oligo-saccharide units connected by phosphoric diester linkages between the hemiacetal and alcoholic hydroxyl groups of the neighbouring units. Chemical synthesis of these polymers has not been achieved hitherto, although several syntheses of the dimeric fragments have been reported[2–10]. Preparation of phosphoric diesters using the hydrogenphosphonate approach, initially suggested for oligonucleotides[11,12], can be used for the synthesis of glycosyl phosphosugars[10,13]. The polycondensation of partially protected glycosyl hydrogenphosphonates may be a promising synthetic approach to poly(glycosyl phosphates).

The key intermediate, 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl hydrogenphosphonate (**1**), $[\alpha]_D$ −110° (chloroform), was prepared from D-mannose in five steps (all intermediates were characterised) as shown in Scheme 1, with an overall yield of 57%.The structure of **1** was confirmed by the n.m.r. data. The low-field position of the signals for H-2,3,4 at 5.73, 6.04, and 5.83 p.p.m., respectively, indicated the positions of the acyl groups. Signals characteristic for the hydrogenphosphonate group [$\delta_P$ 1.63, $\delta_{HP}$ 7.1 ($J_{H,P}$ 640 Hz), $\delta_{H\text{-}1}$ 5.86 ($J_{1,2}$ 2, $J_{1,P}$ 8.5 Hz)] were present in the $^1$H- and $^{31}$P-n.m.r. spectra. The α configuration followed from the value (171 Hz) of $J_{C\text{-}1,H\text{-}1}$ for the signal of C-1 [δ 92.9 ($J_{C,P}$ 4.9 Hz)].

Treatment of a 0.1M solution of **1** in pyridine with 2.5 equiv. of trimethylacetyl chloride (20 min, 20°) gave a mixture of oligomeric products. A series of signals characteristic for hydrogenphosphonic diesters [$\delta_P$ 7.3–10.6 ($J_{P,H}$ ~700 Hz); *cf.* ref. 16] was present in the $^{31}$P-n.m.r. spectrum of the mixture of products. Treatment of the mixture with iodine (4 equiv.) in aqueous pyridine in the presence of triethylamine (15 min, 20°) effected conversion into the diesters of phosphoric acid as shown by the appearance of signals between −2.5 and −3.7 p.p.m. in the $^{31}$P-n.m.r. spectrum.

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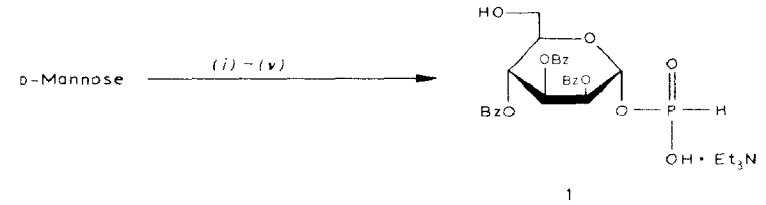


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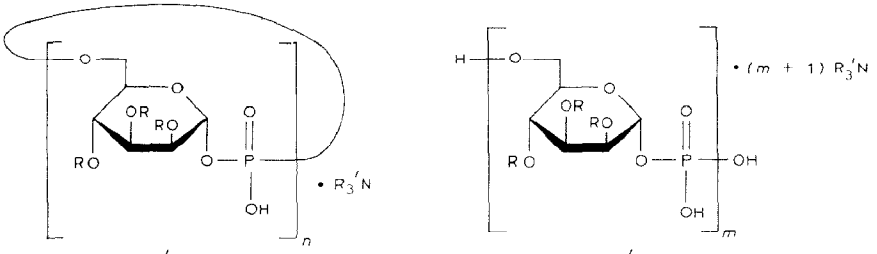
*(i)* (4-$MeOC_6H_4)_2$PhCCl–pyridine, *(ii)* PhCOCl–pyridine, *(iii)* $Me_2NH$–MeCN (refs. 10 and 14), *(iv)* $PCl_3$–imidazole–$Et_3N$–MeCN followed by M $Et_3NHHCO_3$ (pH 8) (refs. 11 and 13), *(v)* $C_5H_5NHClO_4$–MeOH–$MeNO_2$ (ref. 15).




**2a** R = Bz, R' = Et, *n* = 2
**2b** R = Bz, R' = Et, *n* = 3
**3a** R = R' = H, *n* = 2
**3b** R = R' = H, *n* = 3

**4** R = Bz, R' = Et
**5** R = R' = H

Scheme 1.

After dilution of the mixture with water, the products were extracted with chloroform and subjected to chromatography on silica gel (MeOH–$CH_2Cl_2$ containing 1% of $Et_3N$) to give three main fractions (A–C, Table I). These fractions were debenzoylated with 0.1M sodium methoxide in MeOH–1,4-dioxane (1:1, 1 h, 20°), and the products were purified by ion-exchange chromatography on DEAE-TSK ($HCO_3^-$-form) by elution with 0→0.5M $NH_4HCO_3$. Gel filtration on a column (64 × 1.5 cm) of Sephadex G-25 was used for determination of the d.p. of the products. The column was eluted with 0.5M $NH_4HCO_3$ at 1 mL/min*.

The main product in fraction *A* was identified as a cyclic dimer **2a** that was converted into **3a** on debenzoylation. Gel filtration indicated a d.p. of 2 for **3a**, but only six signals were present in the $^{13}$C-n.m.r. spectra of **2a** and **3a** (Table II), which is possible only for a cyclic product. The $^{13}$C-n.m.r. spectra clearly showed the presence of a phosphoryl group at C-1 and C-6 of the α-D-mannopyranose residue since the signals of C-1,2,5,6 each appeared as a doublet due to coupling with P. This conclusion was supported by the $^1$H-n.m.r. spectrum of **3a**. The signals of

*The column was calibrated with D-mannose, α-D-glucopyranosyl phosphate, methyl α-D-mannopyranoside 6-(α-D-mannopyranosyl phosphate)[13], and several samples of glycerol and ribitol teichoic acids kindly provided by Dr. I. B. Naumova and Dr. F. M. Streshinskaya (Lomonosov Moscow State University).

TABLE I

RESULTS OF THE FRACTIONATION AND SOME PROPERTIES OF THE REACTION PRODUCTS

| | *Fractions* | | |
|---|---|---|---|
| | A | B | C |
| Yield (%) | 50 | 15 | 21 |
| $R_F$ (t.l.c., in $CH_2Cl_2$–MeOH, 85:15) | 0.37 | 0.18 | ~0 |
| Structure of the product | **2a** | **2b** | **4** |
| $\delta_P$ ($CDCl_3$) | −2.1 | −3.5 | −3.2[a] |
| Structure of the debenzoylated product | **3a** | **3b** | **5** |
| $\delta_P$ ($D_2O$) | −0.4 | −1.1 | −1.05[a] |
| $[\alpha]_D$ (water) (degrees) | +34 | +18 | +35 |

[a]The main signal.

H-1,6a,6b were identified readily and an additional splitting due to H–P interaction was observed: $\delta$ 5.42 (dd, $J_{1,2}$ 2.0, $J_{1,P}$ 6.5 Hz, H-1), 4.30 (ddd, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 10.0, $J_{6a,P}$ 5.5 Hz, H-6a), 3.91 (ddd, $J_{5,6b}$ 9.0, $J_{6b,P}$ 4.5 Hz, H-6b). There was a single signal in the phosphoric diester region of the $^{31}$P-n.m.r. spectra of **2a** and **3a**. As expected for a cyclic product, mannose 6-phosphate was identified as the single product of mild acid hydrolysis (0.1M HCl, 20 min, 100°) of **3a**.

The cyclic trimer **2b** was identified as a main component of fraction *B* (Table I). After debenzoylation and ion-exchange chromatography, **3b** was isolated (7% from **1**). Its d.p. was shown to be 3 by gel filtration. The $^{13}$C- and $^{31}$P-n.m.r. spectra of **2b** and **3b** (Tables I and II) accord with a cyclic structure composed of $\alpha$-D-mannopyranose residues linked through O-1 and O-6 by phosphoric diester groups. The $^1$H-n.m.r. spectrum of **3b** [$\delta$ 5.41 (dd, $J_{1,2}$ 1.7, $J_{1,P}$ 8.7 Hz, H-1), 4.26 (ddd, $J_{5,6a}$

TABLE II

$^{13}$C-N.M.R. DATA FOR CYCLIC OLIGO(GLYCOSYL PHOSPHATES)

| *Atoms* | **2a**[a] | **2b**[a] | **3a**[b] | **3b**[b] |
|---|---|---|---|---|
| $\delta$ *(p.p.m.)* | | | | |
| C-1 | 93.9d | 94.2br | 97.4d | 97.5d |
| C-2 | 70.8d | 70.8d | 71.4d | 71.8d |
| C-3 | 70.2s | 70.8s | 71.0s | 70.9s |
| C-4 | 68.2s | 66.9s | 68.5s | 66.4s |
| C-5 | 70.8d | 71.2d | 74.1d | 73.8d |
| C-6 | 65.4d | 64.2d | 66.5d | 65.2d |
| J *(Hz)* | | | | |
| 1,P | ~5 | | ~7 | ~5 |
| 2,P | ~11 | ~7 | ~11 | ~7 |
| 5,P | ~11 | ~9 | ~9 | ~8 |
| 6,P | ~5 | ~5 | ~5 | ~4 |

[a]In $CDCl_3$. [b]In $D_2O$.

~1, $J_{6a,6b}$ 11.0, $J_{6a,P}$ 7.5 Hz, H-6a)] also accords with the structure. Some differences in the $^{13}C$- and $^{31}P$-n.m.r. spectra of the dimers and trimers probably reflect restriction of rotation in the C-1–O–P–O–C-6 ystem for the smaller cyclic compounds.

In addition to cyclic di- and tri-mers, linear oligomers of α-D-mannopyranosyl phosphate **5** ($m$ = 3–7) were isolated. Their benzoates (**4**) were present in fraction *C* (Table I). After debenzoylation and ion-exchange chromatography, the main portion of the oligomers ($m$ = 5–6 as determined by gel chromatography) was isolated. In accordance with structure **5**, the $^{13}C$-n.m.r. spectrum of the product contained the main sequence of signals corresponding to 1,6-substituted internal mannopyranosyl residues: δ 97.6 (d, $J_{C,P}$ ~4 Hz, C-1), 71.7 (d, $J_{C,P}$ ~7 Hz, C-2), 71.1 (s, C-3), 73.9 (bs, C-5). The signals of C-4 and C-6 seemed to be sensitive to the position of the mannosyl residue in the chain: at least three singlets (C-4) were present between 67.0 and 67.6 p.p.m., and three doublets (C-6, $J_{C,P}$ ~4 Hz) were observed between 65.5 and 66.2 p.p.m. A singlet for unsubstituted C-6 at 62.0 p.p.m. for the terminal mannopyranosyl unit was also identified. In the $^{31}P$-n.m.r. spectrum, in addition to the major signal for phosphoric diester at δ −1.05, a minor signal was present at δ 2.0 which corresponded to a mannosyl phosphate terminal residue.

Several minor signals were observed in the $^{13}C$- and $^{31}P$-n.m.r. spectra for the linear oligomeric fraction, showing the presence of some minor species in addition to **5**. In particular, evidence was obtained for the presence of some chains bearing terminal mannose residues with a free hemiacetal group and a mannosyl 6-phosphate unit. The components of the linear oligomer fractions were not converted completely into D-mannose 6-phosphate on mild acid hydrolysis. A small quantity (8% of the total phosphate) of an acid-stable phosphoric diester ($E_{Man\text{-}6\text{-}P}$ 0.5 at pH 7.5; $\delta_P$ 1.8) was isolated which was apparently D-mannose 6-(D-mannose 6-phosphate). The 6,6′-phosphoric diester fragment might be formed as a result of cleavage of glycosyl hydrogenphosphonate diester bonds in the growing oligomer chains followed by reaction of the 6-hydrogenphosphonate formed with the HO-6 group of **1**.

Thus, the unusual cyclic dimer **3a** was the main product formed from partially protected α-D-mannopyranosyl hydrogenphosphonate during attempted polycondensation. The previously inaccessible cyclic trimer **3b** and the linear oligo(glycosyl phosphates) **5** were also isolated.

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