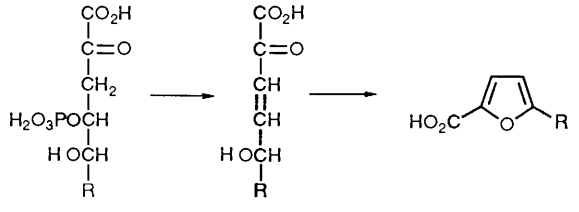
# Phosphorylated Sugars. Part 27.[1] Synthesis and Reactions, in Acid Medium, of 5-*O*-Substituted Methyl 3-Deoxy-α-D-*manno*-oct-2-ulopyranosidonic Acid 4-Phosphates

**France-Isabelle Auzanneau, Daniel Charon and Ladislas Szabó***
*Equipe 'Endotoxines' (URA 1116) du Centre National de la Recherche Scientifique, Centre d'Etudes Pharmaceutiques, Université de Paris Sud, 92290 Châtenay-Malabry, France*

Synthesis of the α-methyl glycosides of 5-*O*-methyl-, 5-*O*-benzyl-8-*O*-methyl-, and 5,7,8-tri-*O*-methyl-3-deoxy-D-*manno*-oct-2-ulosonic acid 4-phosphates are described. The corresponding 'reducing' glycose, formed upon hydrolysis at pH 4 and 100 °C, immediately eliminates the phosphate group. The olefinic acids thus produced from 5-*O*-methyl-, and from 5-*O*-benzyl-8-*O*-methyl 4-phosphates are not stable under these conditions: they are transformed into 4,7- and 4,8-anhydro-derivatives which have no ethylenic protons and show negligible absorption in the region 220–300 nm. The olefinic acid produced from the 5,7,8-tri-*O*-methyl derivative is unable to form such an anhydro-derivative: two ethylenic protons are present in its $^1$H NMR spectrum and, like other conjugated olefinic α-keto acids, it has a strong UV absorption band ($\lambda_{max}$ 235 nm, ε 8000).

The hydrophobic ('Lipid A') and hydrophilic (polysaccharide chain) domains of endotoxic lipopolysaccharides that are major, antigenic components of the outer membrane of gram negative bacteria, are joined by one or several units of 3-deoxy-D-*manno*-oct-2-ulosonic acid ('KDO'). As the glycosidic bond of this glycose is very labile to acid,[2] it is general practice to treat lipopolysaccharides with mild acid (mmol dm$^{-3}$ acetic acid at 100 °C), and thus to sever the macromolecule at that point. Under these conditions KDO units that are not substituted by a glycose unit of the polysaccharide chain are released as monosaccharides, unless they carry a phosphate substituent in position 4.[3]

Phosphorylated KDO units have been isolated[4] from, or detected[5] in, a number of endotoxin preparations. KDO derivatives phosphorylated in position 4 behave differently from the other positional isomers inasmuch as, at 100 °C, they eliminate their phosphate group with extreme facility in acidic, neutral or alkaline environment.[3] The olefinic acid, presumably formed as the first product, eventually leads to a derivative of furoic acid (Scheme 1) by ring-closure involving (C-2)–(O-5)–(C-5). This



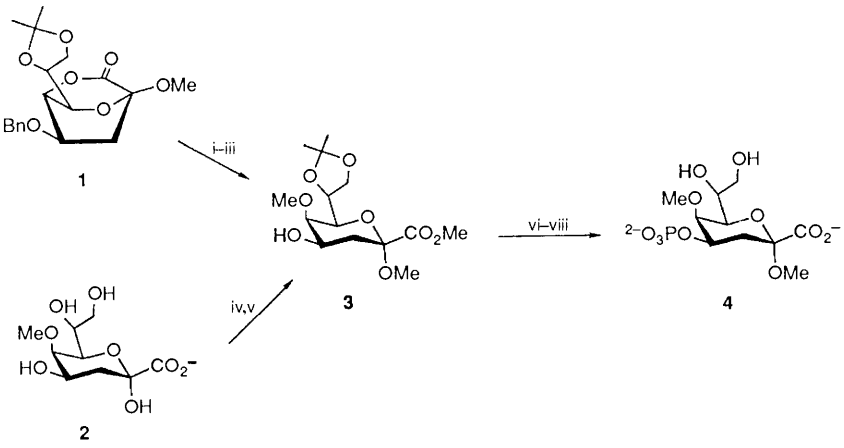
**Scheme 1**

reaction is general: it was observed for 3-deoxy-hex-2-ulosonic, -hept-2-ulosonic, and -oct-2-ulosonic acid 4-phosphates.[3] In Lipopolysaccharide-2 of the *Bordetella pertussis* endotoxin, the KDO unit which is substituted on O-5 by the polysaccharide chain also carries a phosphate substituent at position 4.[6] Following hydrolysis of the glycosidic bond of the KDO unit, that phosphate group was eliminated[6] but, O-5 being substituted, a furoic acid derivative was not produced, and the product formed was still attached to the polysaccharide chain and could not be identified. In order to facilitate the identification of the KDO derivatives formed from the natural product, derivatives of 5-*O*-substituted methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosidonic acid 4-phosphate were synthesized that carried 5-*O*-substituents not removed under the conditions in which the glycosidic bond of the glycose unit was cleaved, and the structure of the products formed upon mild acid hydrolysis was determined.

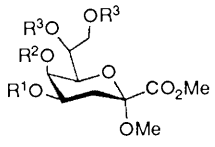
## Results and Discussion

*Syntheses.*—5-*O*-Methyl-KDO 4-phosphate was prepared starting from either the fully protected 1,5-lactone **1**[7] or from 5-*O*-methyl-KDO[8] **2**. In the first synthesis (Scheme 2) treatment of the lactone **1** with sodium methoxide in methanol gave the benzylated methyl ester **5**. This was not isolated, but methylated with iodomethane–$Ag_2O$ to give, after chromatographic purification, the 5-*O*-methyl ether of the fully protected glycoside, compound **6**, in 90% yield, from which the benzyl group was removed by hydrogenolysis. The resulting alcohol **3** gave, upon treatment with diphenyl phosphorochloridate–imidazole in tetrahydrofuran (THF), a nearly quantitative yield of the phosphotriester **7**, which was transformed into the corresponding phosphomonoester by hydrogenolytic removal of the phenyl groups. The acidity developed during this step was sufficient to remove, *in situ*, the exocyclic acetal; the phosphorylated ester **8** produced was isolated as its monoammonium salt. Saponification of the methoxycarbonyl function with sodium hydroxide gave the phosphorylated carboxylic acid **4** that was isolated as its Ca-salt. In the second synthesis 3-deoxy-5-*O*-methyl-D-*manno*-oct-2-ulosonic acid **2**[8] was first converted into its methyl ester α-methyl glycopyranoside **9** by successive treatments with diazomethane and acidified methanol, and the product was transformed into the isopropylidene acetal **3** by treatment with 2-methoxypropene. The acetal was then phosporylated as described above.

The starting material for 5-*O*-benzyl-8-*O*-methyl-KDO 4-phosphate **18** was methyl (methyl 5-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate[9] **10** (Scheme 3). Benzoylation of the alcoholic function of this compound with benzoic anhydride–pyridine–4-dimethylaminopyridine (DMAP) afforded the benzoate **11** from which the isopropylidene acetal protecting 7-OH and 8-OH was removed in 90% yield by treatment with trifluoroacetic acid (TFA) adsorbed on silica gel.[10] The primary alcohol of the diol **12** thus obtained was selectively methylated by transformation first into the 7,8-dibutylstannylene acetal, which upon treatment with iodomethane under catalysis with tetrabutylammonium bromide gave the expected[11] 8-*O*-methyl ether **13**. The

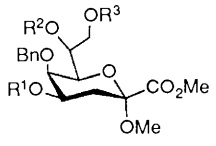


**Scheme 2** *Reagents:* i, $MeO^-$; ii, MeI, $Ag_2O$; iii, $H_2$, Pd/C; iv, $CH_2N_2$; then $CH_3OH$, $H^+$; v, $CH_2$=C(OMe)Me, PTSA; vi, $(PhO)_2POCl$, imidazole; vii, $H_2/PtO_2$; viii, NaOH



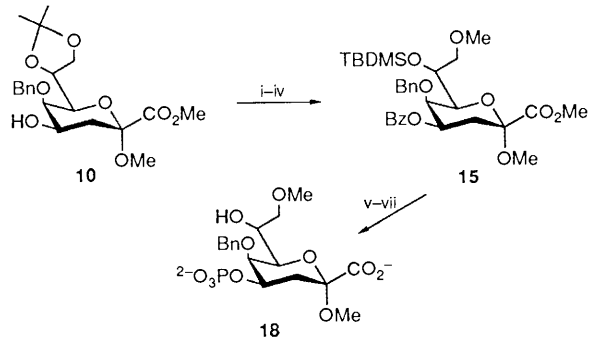
**5** $R^1$ = Bn, $R^2$ = H, $R^3$ $R^3$ = $CMe_2$
**6** $R^1$ = Bn, $R^2$ = Me, $R^3$ $R^3$ = $CMe_2$
**7** $R^1$ = $PO(OPh)_2$, $R^2$ = Me, $R^3$ $R^3$ = $CMe_2$
**8** $R^1$ = $PO_3^{2-}$, $R^2$ = Me, $R^3$ = H
**9** $R^1$ = $R^3$ = H, $R^2$ = Me

TBDMS = $SiMe_2Bu^t$



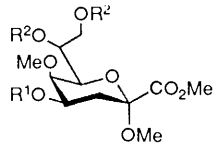
**10** $R^1$ = H, $R^2$ $R^3$ = $CMe_2$
**11** $R^1$ = Bz, $R^2$ $R^3$ = $CMe_2$
**12** $R^1$ = Bz, $R^2$ = $R^3$ =H
**13** $R^1$ = Bz, $R^2$ = H, $R^3$ = Me
**14** $R^1$ = Bz, $R^2$ = Ac, $R^3$ = Me
**15** $R^1$ = Bz, $R^2$ = TBDMS, $R^3$ = Me
**16** $R^1$ = H, $R^2$ = TBDMS, $R^3$ = Me
**17** $R^1$ = $PO(OCH_2CCl_3)_2$, $R^2$ = TBDMS, $R^3$ = Me

TBDMS = $SiMe_2Bu^t$



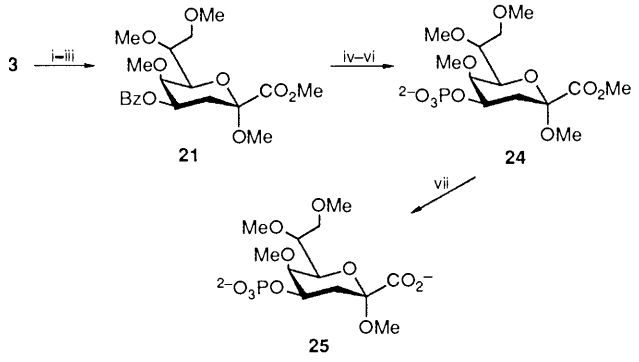
**Scheme 3** *Reagents:* i, Benzoic anhydride, DMAP; ii, $H^+$; iii, $Bu_2SnO$, MeI; iv, $Bu^tMe_2SiCl$, *N*-methylimidazole; v, $MeO^-$; vi, $ClPO(OCH_2CCl_3)_2$, *N*-methylimidazole; vii, Zn/Ag, Dowex AG50WX8 ($H^+$), $HO^-$

structure of the latter was confirmed by the $^1H$ NMR spectrum of its 7-*O*-acetyl derivative **14**. The free alcoholic function of compound **13** was then transformed into the t-butyldimethylsilyl (TBDMS) ether **15**. This reaction, when promoted by *N*-methylimidazole, is slow (48 h at 60 °C) but the yield, 90% after purification by chromatography, is rewarding. Removal of the benzoate by transesterification with NaOMe–MeOH set free the alcoholic function on C-4 (compound **16**), which was then phosphorylated with bis-(2,2,2-trichloroethyl) phosphorochloridate.[12] Despite several attempts, and for reasons not yet determined, no satisfactory elemental analysis could be obtained for the phosphotriester **17**, but its structure was ascertained from its $^1H$ NMR spectrum. Sequential deprotection of the phosphotriester **17** was accomplished by, first, removal of the trichloroethyl groups with Zn activated by Ag,[13] followed by acid-catalysed hydrolysis of the TBDMS group. Saponification of the methoxycarbonyl group then gave the phosphorylated KDO-derivative **18** in 56% yield, based on the phosphotriester **17**.

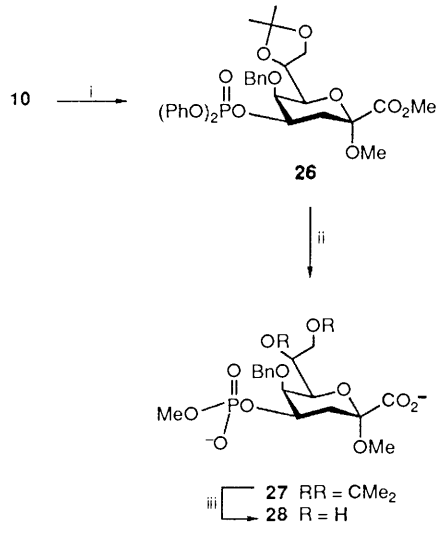


**19** $R^1$ = Bz, $R^2$ = $CMe_2$
**20** $R^1$ = Bz, $R^2$ = H
**21** $R^1$ = Bz, $R^2$ = Me
**22** $R^1$ = H, $R^2$ = Me
**23** $R^1$ = $PO(OPh)_2$, $R^2$ = Me
**24** $R^1$ = $PO_3^{2-}$, $R^2$ = Me

To produce the fully substituted (methyl-3-deoxy-5,7,8-tri-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onic acid 4-phosphate **25** (Scheme 4), the alcohol **3** was first benzoylated to give the benzoate **19**. The isopropylidene group was then removed (hydrolysis), and the diol **20** thus formed was methylated (iodomethane–$Ag_2O$) to yield the tri-*O*-methyl derivative **21**. Upon removal, by transesterification (NaOMe–MeOH), of the benzoyl group from compound **21** the alcohol **22** was obtained which was then phosphorylated with diphenyl phosphorochloridate–*N*-methylimidazole in THF. The phenyl groups were removed by hydrogenolysis from the diphenyl phosphate **23** thus formed to produce the phosphomonoester **24** which was isolated as its monoammonium salt. Saponification of the methoxycarbonyl group then gave the required phosphorylated KDO-derivative **25**, isolated as its neutral Ca-salt.




**Scheme 4** *Reagents:* i, Benzoic anhydride, DMAP; ii, $H^+$; iii, MeI, $Ag_2O$; iv, $MeO^-$; v, $(PhO)_2POCl$, *N*-methylimidazole; vi, $H_2/PtO_2$; vii, $HO^-$




**Scheme 5** *Reagents:* i, $(PhO)_2POCl$, *N*-methylimidazole; ii, NaOH, aq. MeOH; iii, $H^-$

Phosphorylation of methyl (methyl-5-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate **10** (Scheme 5) with diphenyl phosphorochloridate gave the known[9] phosphotriester **26**. Upon attempted removal of one phenyl group[14] in a mixture of methanol and 5 mol $dm^{-3}$ NaOH at 30 °C, compound **26** was transformed into the methyl phosphate **27**, from which the isopropylidene group could be removed without affecting the methyl phosphate group to afford the phosphodiester **28**.

*Reactions.*—When a solution of methyl 3-deoxy-5-*O*-methyl-α-D-*manno*-oct-2-ulopyranosidonate 4-phosphate **4** in 0.2 mol $dm^{-3}$ acetate buffer of pH 4 was kept at 100 °C, the glycosidic bond was cleaved rapidly ($t_{\frac{1}{2}}$ 12–15 min) as determined by the reaction with semicarbazide under the conditions used for the estimation of α-keto acids[15] (Fig. 1). The absorption band of the product formed with semicarbazide was centred at 250 nm, and the molar absorbancy (ε 10 200) equalled that of non-conjugated α-keto acids.[16] Simultaneously, but somewhat more slowly, the ester-bound phosphate of the sample was released as inorganic phosphate (Fig. 1). By both TLC ($R_f$ 0.58, charring) and paper electrophoresis (alkaline $AgNO_3$, pH 6), only one, phosphate-free, product was detected. The material did not absorb UV light either *in situ*, as obtained by treatment with the buffer, or as isolated by TLC or paper electrophoresis.

The reaction was next examined by $^1H$ NMR spectroscopy: a solution of the phosphate in $D_2O$ was decationised with Dowex 50W × 8 ($H^+$) resin. At room temperature this treatment did not alter the structure of the compound as determined from its

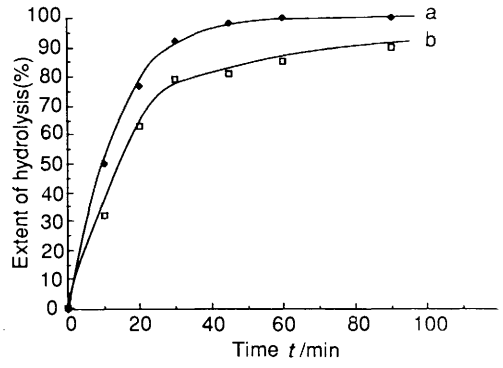



**Fig. 1** Hydrolysis of compound **4** at pH 4 and 100 °C: cleavage of the glycosidic bond as measured by the appearance of α-keto acid (a), and release of inorganic phosphate expressed as a percentage of total phosphate present (b)

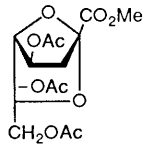
**Table 1** Chemical shifts (δ) and coupling constants (*J*/Hz) measured for compounds **A** (9 mg) and **B** (15 mg) ($CDCl_3$; 250 MHz)

| (i) δ | 3-H | 3-H′ | 4-H | 5-H | 6-H | 7-H | 8-H | 8-H′ |
|---|---|---|---|---|---|---|---|---|
| **A** | 2.42 | | 4.01 | 3.57 | 5.03 | 3.9–4.0 | 4.11 | 4.24 |
| **B** | 2.24 | 2.45 | 4.07 | 3.72 | 5.03 | ≈4.0 | 4.1 | 4.3 |

| (ii) *J*/Hz | 3,3′ | 3,4 | 3′,4 | 4,5 | 5,6 | 6,7 | 7,8 | 7,8′ | 8.8′ |
|---|---|---|---|---|---|---|---|---|---|
| **A** | | 5.5 | 5.5 | 3.75 | ≈1 | 2.5 | 6.25 | 5.25 | 11.25 |
| **B** | 15.0 | 8.0 | 5.0 | 5.0 | 2.5 | 2.5 | ≈6 | ≈6 | |

$^1H$ NMR spectrum. When the acid solution was kept at 100 °C for 30 min, the product formed appeared (TLC, paper electrophoresis, optical properties) to be the same as that produced by treatment with the buffer. However, its $^1H$ NMR spectrum revealed that it consisted of a mixture of closely related products. Because of its complexity, the 200 MHz spectrum could not be interpreted, but it clearly established that no olefinic protons (δ >4) were present. Moreover, no signals were visible in the range δ 1.7–2.4, where those of 3-$H_2$ of derivatives of 3-deoxyald-2-ulosonic acids usually appear, suggesting that these were enolisable and underwent fast $^1H$–$^2H$ exchange, while exchange of 3-H and 3-H′ of 3-deoxy-D-*manno*-oct-2-ulosonic acid proceeds very slowly. On the other hand eight >C(H)O*Me* signals of different intensity were present in the spectrum. The reaction was then carried out on a preparative scale (500 mg). After removal of the inorganic phosphate, the product was treated with diazomethane and then acetylated (acetic anhydride–pyridine; 20 °C; 15 h). Three closely spaced products, two (**A** and **B**) of which appeared to be preponderant, could be detected by TLC. Although complete separation could not be achieved, preparations containing mainly **A** (9 mg) and **B** (15 mg) were obtained by repeated column chromatography. The $^1H$ NMR spectra of **A** and **B** were very similar and established the presence of two acetate groups (**A**: δ 2.07, 2.08; **B**: δ 2.08, 2.10), one COMe (**A**: δ 3.42; **B**: δ 3.39) and one $CO_2Me$ (**A**: δ 3.76; **B**: δ 3.75) in both products. Protons of the methylene group (3-$H_2$), characteristic of KDO derivatives were also present (Table 1) and served as the starting point for the identification of all other protons by selective decoupling. Under the conditions used, elimination of a phosphate group from position 4 of otherwise unsubstituted 3-deoxyald-2-ulosonic acids is not accompanied by rearrangement, or by loss of carbon of the glycose unit.[3] The presence of the funtions $CO_2Me$, >CO, $CH_2$ and OMe, and of only two acetate groups in both products **A** and **B** suggested that one of the three OH functions originally present in the phosphorylated

KDO derivative was substituted in a different manner, possibly in forming a ring. Compared with those observed for the starting material **4** the signals for 8-H and 8-H′ of both compounds (**A**: δ 4.11, 4.24; **B**: 4.1–4.3, no resolved) appeared at lower field, in a region where 8-H and 8-H′ of the α- and β-peracetates of KDO furanose (α: δ 4.20 and 4.35; β: δ 4.20 and 4.39) have been observed,[17] as were those[18] of 8-H and 8-H′ (both at δ 4.18) of methyl (4,6,8-tri-*O*-acetyl-2,7-anhydro-3-deoxy)-α-D-*manno*-oct-2-ulofuranos)onate **29**. A similar

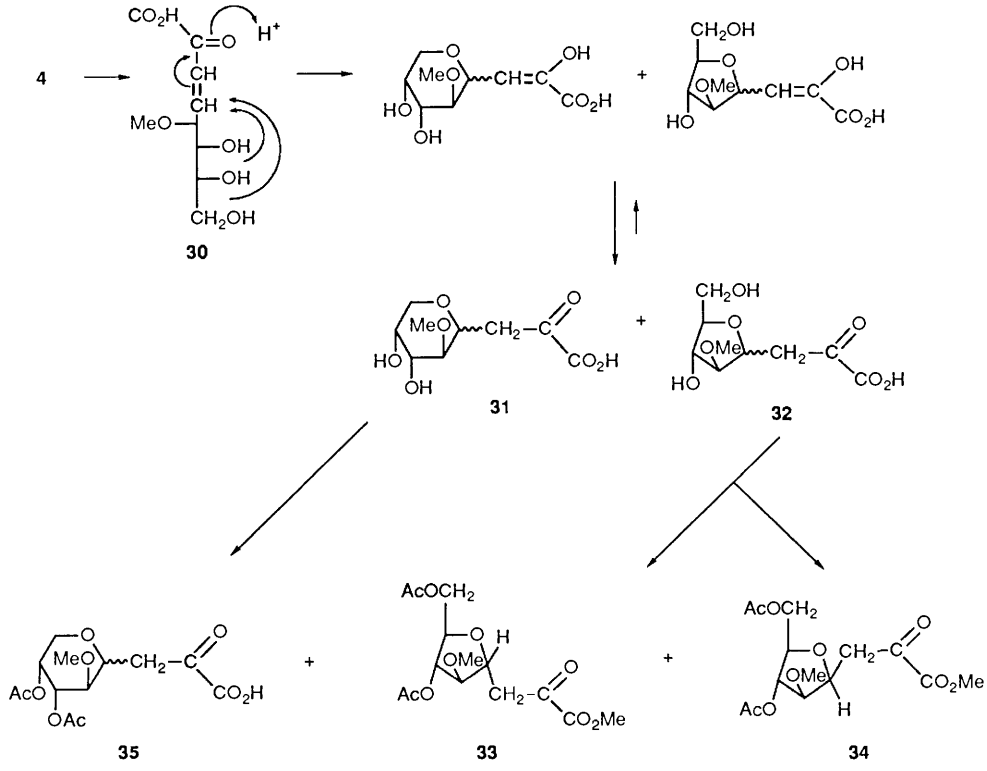


**29**

downfield shift of the resonances of 6-H in both products **A** and **B** indicated that 6-OH carried the second acetyl group. It was concluded (Scheme 6) from these data that the olefinic acid **30**, initially formed as the result of elimination of the phosphate group, underwent intramolecular addition of the alcoholic function 7-OH or 8-OH to the olefinic bond to produce enantiomeric pairs of 4,7- and 4,8-anhydro-derivatives of the enolisable α-keto acids **31** and **32**, and that compounds **A** and **B** represented the 6,8-di-*O*-acetyl derivatives **33** and **34** of the enantiomeric furanoid structures that seem to be formed preferentially. It is very probable that the minor compound was the corresponding pyranoid structure **35**. The presence of signals for eight >C(H)O*Me* groups in the $^1$H NMR spectrum of the mixture of products formed upon hydrolysis at 100 °C of decationised methyl (3-deoxy-5-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onic acid 4-phosphate **4** is in complete agreement with this interpretation.

To verify the hypothesis, (methyl 5-*O*-benzyl-3-deoxy-8-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onate **18**—from which no pyranoid derivative can be formed—was treated under conditions identical with those used for the degradation of the 5-*O*-methyl-KDO 4-phosphate **4**. Hydrolysis of the glycosidic bond and release of inorganic phosphate occurred at rates almost identical with those observed for the 5-*O*-methyl derivative **4**, and by TLC the dephosphorylated product appeared as a single spot. The product was identified as an α-keto acid by its reaction with semicarbazide ($\lambda_{max}$ 250 nm, ε 10 000), and exchange with $^2H_2O$ indicated that 3-H and 3-H′ were rapidly enolisable. The $^1$H NMR spectrum of the material was, besides the additional signals due to the benzyl group, very similar to, but somewhat simpler than, that of the mixture formed upon degradation of the 5-*O*-methyl ether **4**.

As expected, when (methyl 3-deoxy-5,7,8-tri-*O*-methyl-α-D-*manno*-oct-2-ulopyranosid)onate 4-phosphate **25** was treated with the same acetate buffer at 100 °C, phosphate was released at a rate similar to that measured for the 4-phosphates of the monomethyl ether **4** and the 5,8-diether **18**, but the optical phenomena observed differed from those observed during their degradation. Concomitantly with the release of phosphate, an intense absorption band, centred at 235 nm, was seen (Fig. 2A, insert); its rate of appearance (Fig. 2A) was of apparent first-order (Fig. 2B) and simultaneous with the development of a chromogen (Fig. 2C) that reacted with semicarbazide under the conditions[15] used for the determination of α-keto acids ($\lambda_{max}$ 250 nm, ε 10 000, calculated for the starting material). When the degradation was carried out in $^2H_2O$ in an NMR tube under conditions identical with those used for the 5-*O*-methyl 4-phosphate **4**, one, by far major, and two minor products appeared, each of which had two ethylenic protons (Fig. 3). Upon hydrogenation (Pd–C) these disappeared and simultaneously complex signals of higher order emerged in the region δ 1.3–2.3. Signals and coupling constants of protons 3-, 4-, 5- and 6-H of the major product were easily identified by selective saturation (Table 2). These data, in particular the large coupling constant for 3-H/4-H (16.25 Hz), defined the major product formed by mild acid treatment of the fully substituted KDO derivative as 3,4-dideoxy-5,7,8-tri-*O*-methyl-D-*arabino*-oct-3-en-2-ulosonic acid **36**, the two minor compounds being very probably (5,7,8-tri-*O*-methyl-α- and -β-D-*arabino*-oct-3-en-2-ulopyranos)onic acids.



**30** **31** **32** **35** **33** **34**

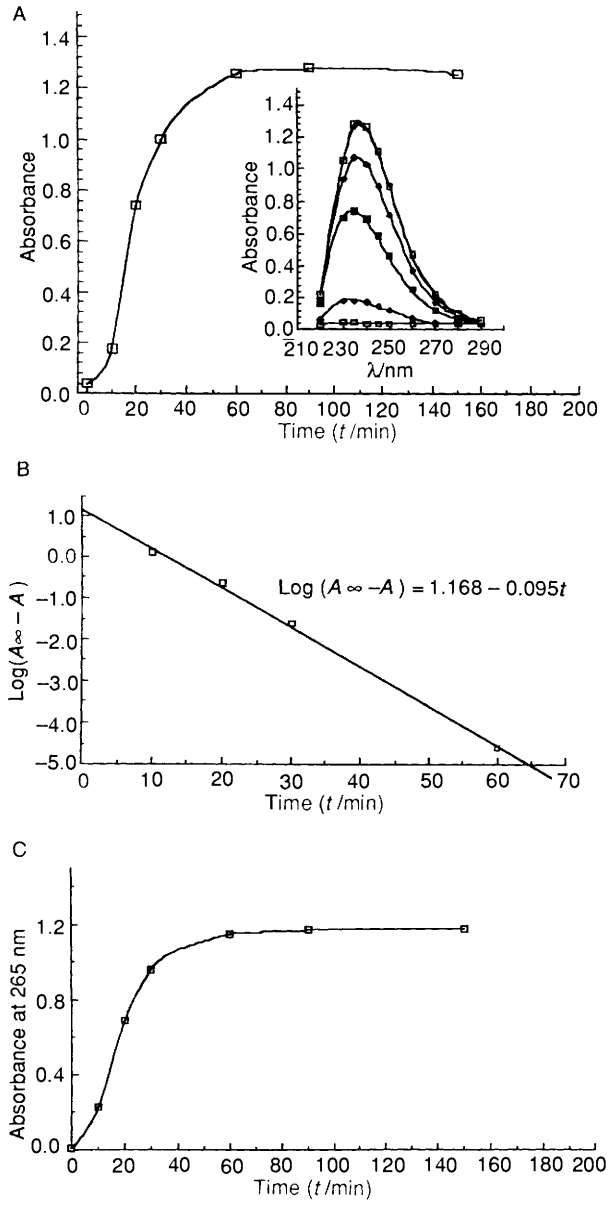
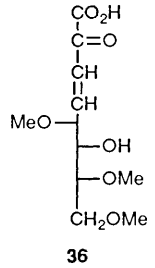



**Fig. 2** A, Evolution of the absorption band appearing at 235 nm upon exposure of compound **25** to pH 4 at 100 °C, and absorption spectra after 0, 10, 20, 30 and 60 min of treatment (inert). B, Kinetics of the evolution of the absorption band. C, Appearance of α-keto acid as measured with semicarbazide upon exposure of compound **25** to pH 4 at 100 °C.




**36**

It follows from these experiments that in endotoxins in which the KDO unit that carries the polysaccharide chain attached to O-5 is also phosphorylated in position 4—as is the case for Lipopolysaccharide 2 of the *Bordetella pertussis* endotoxin,[6] and probably for many other 'KDO-less' of 'thiobarbiturate negative' endotoxins[19]—mild acid hydrolysis will produce either a mixture of type-specific polysaccharide chains having 4,7- or 4,8-anhydro-KDO units as 'reducing' terminal sugars or, if the anhydro-rings cannot be formed because of substitution or steric reasons, a polysaccharide chain with a terminal D-*arabino*-oct-3-en-2-ulonic acid, perhaps accompanied by small amounts of the corresponding pyranoid forms. Several years ago 4,7- and 4,8-anhydro-derivatives of KDO were been identified[20] by GLC–MS amongst the product formed upon acid treatment of a 'thiobarbiturate negative' *Xanthomonas sinensis* endotoxin preparation from which only trace amounts of KDO were released.

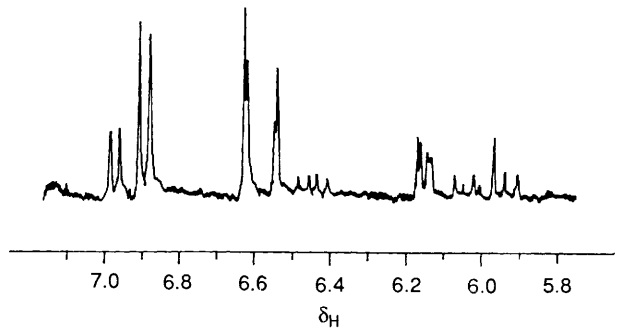



**Fig. 3** Ethylenic protons appearing in the $^1$H NMR spectrum ($D_2O$; 200 MHz) of compound **25** exposed to pH 4 at 100 °C for 30 min

**Table 2** Chemical shifts (δ) and coupling constants (*J*/Hz) measured for compound **36** (250 MHz; $D_2O$)

| | | 3-H | 4-H | 5-H | 6-H | 7-H | 8-$H_2$ |
|---|---|---|---|---|---|---|---|
| (i) | δ | 6.59 | 6.93 | 4.10 | 3.71 | 3.25–3.6 | |
| (ii) | *J*/Hz | 3,4 | 3,5 | 4,5 | 5,6 | 6,7 | |
| | | 16.25 | 1.25 | 5.3 | 2.5 | 6.5 | |

Upon treatment of (methyl 5-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate 4-methyl phosphate **28** at pH 4 and 100 °C no release of inorganic phosphate was observed, whereas the $^1$H NMR spectrum of the elimination product and its reaction with semicarbazide were analogous to those obtained for the 5-*O*-methyl 4-(dihydrogen phosphate) **4**. This indicated that, as regards the dephosphorylated derivative formed, 4-(alkyl phosphates) of KDO undergo the same type of reaction as 4-*O*-(dihydrogen phosphates), an observation of some interest in the structural analysis of natural substances containing KDO. The presence of extracatenary KDO units has been postulated in some endotoxins.[21,22] If phosphorylated in position 4, such KDO derivatives will, upon cleavage of their glycosidic bond by mild acid at 100 °C, undergo the same type of reaction, and the product, a small fragment now detached from the macromolecule, will be difficult to recover and identify.

## Experimental

*General Methods.*—These were identical with those described in ref. 1. Tetramethylsilane was used as internal standard for $^1$H NMR data; spectra were recorded at room temperature (18–25 °C), and *J*-values are given in Hz.

*Methyl (Methyl* 4-O-*Benzyl*-3-*deoxy*-7,8-O-*isopropylidene*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **6**.—1 mol $dm^{-3}$ NaOMe (200 $mm^3$) was added to a solution of methyl 4-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyrano-sidono-1,5-lactone[7] (996 mg, 2.7 mmol) in a mixture (1:1) of anhydrous methanol and chloroform (30 $cm^3$).

After one hour, cations were removed with Dowex AG50WX8 ($H^+$) resin, the mixture was filtered, and the solvents were removed. Iodomethane (6 $cm^3$), silver(I) oxide (300 mg), and anhydrous calcium sulphate (500 mg) were added to the residual oil and the mixture was stirred for 24 h at 35 °C. Solids were filtered off and rinsed with dichloromethane (15 $cm^3$). The oily product remaining after removal of the solvents was methylated for eight hours under the same conditions. The still incompletely methylated product was methylated a third time for eight hours at 40 °C with $Ag_2O$ (750 mg) and $CaSO_4$ (950 mg). Column (19 × 2.5 cm) chromatography [ethyl acetate–cyclohexane (3:7)] of the residual material gave the *title product* (1 g, 91%) as an oil (Found: C, 61.3; H, 7.2. $C_{21}H_{30}O_8$ requires C, 61.5; H, 7.3%); $[\alpha]_D$ +36.9° (*c* 2.2, $CHCl_3$); $\delta_H$($CDCl_3$; 200 MHz) 1.38 and 1.44 (2 × 3 H, 2 s, $CMe_2$), 2.05–2.38 (2 H, m, 3-$H^a$, 3-$H^e$), 3.21 (3 H, s, 2-OMe), 3.46 (1 H, ≈d, $J_{6,7}$ 8, 6-H), 3.63 (3 H, s, 5-OMe), 3.75 (1 H, m, $J_{5,4}$ 2.8, 5-H), 3.79 (3 H, s, $CO_2Me$), 3.92 (1 H, m, $J_{4,3e}$ 6, $J_{4,3a}$ 11.5, 4-H), 3.96 (1 H, dd, $J_{8,8'}$ 12.5, $J_{8,7}$ 5, 8-H), 4.18 (1 H, dd, $J_{8',7}$ 6, 8-H′), 4.41 (1 H, m, 7-H), 4.63 (2 H, s, $CH_2Ph$) and 7.35 (5 H, m, Ph).

*Methyl* (*Methyl* 3-*Deoxy*-7,8-O-*isopropylidene*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **3**.—(a) *From the benzyl ether* **6**. Pd–C (10% Pd; 100 mg) was added to a solution of the benzyl ether **6** (900 mg, 2.2 mmol) in ethyl acetate (14 $cm^3$) containing 1% ammonia in methanol (one drop). The mixture was stirred under $H_2$ for one hour. The catalyst was filtered off and rinsed with ethyl acetate (6 $cm^3$); a second quantity (100 mg) of the same catalyst was added to the combined filtrate and washings, and hydrogenation was resumed. Removal of the benzyl group was complete within 30 min [TLC: ethyl acetate–cyclohexane (1:1); $R_f$ 0.33]. Column (21.5 × 2.5 cm) chromatography (same solvent as TLC) of the residue, recovered after removal of the catalyst and the solvent, afforded the oily *title product* (647 mg, 92%) (Found: C, 52.3; H, 7.3. $C_{14}H_{24}O_8$ requires C, 52.5; H, 7.5%); $[\alpha]_D$ +63.6° (*c* 1.3, $CHCl_3$); $\delta_H$($CDCl_3$; 250 MHz) 1.39 and 1.44 (2 × 3 H, 2 s, $CMe_2$), 1.83 (1 H, dd, $J_{3a,3e}$ 12.5, $J_{3a,4}$ 11.5, 3-$H^a$), 2.16 (1 H, dd, $J_{3e,4}$ 5, 3-$H^e$), 3.22 (3 H, s, 2-OMe), 3.49 (1 H, dd, $J_{6,5}$ 1, $J_{6,7}$ 9, 6-H), 3.63 (1 H, ≈d, $J_{5,4}$ 3.5, 5-H), 3.65 (3 H, s, 5-OMe), 3.78 (3 H, s, Ac), 3.96 (1 H, dd, $J_{8,8'}$ 9, $J_{8,7}$ 5 Hz, 8-H), 4.04 (1 H, m, 4-H), 4.19 (1 H, dd, $J_{8',7}$ 6, 8-H′) and 4.36 (1 H, m, 7-H).

(b) *From the* 5-O-*methyl ether* **9**. 2-Methoxypropene (200 $mm^3$, 1.5 mol equiv.) was added to a cold (+4 °C), stirred solution of the 5-*O*-methyl ether **9** (380 mg, 1.36 mmol) (see below) in dioxane containing toluene-*p*-sulphonic acid (PTSA) (100 mg). After *ca.* one hour, during which the solution reached room temperature, the acid was neutralised with a few drops of $CCl_4$ equilibrated with conc. aq. ammonia and then deposited on a layer (3 × 6.5 cm) of silica gel into which $NaHCO_3$ (1 g) has been incorporated. The silica layer was eluted with a mixture of ethyl acetate–cyclohexane (1:1; 400 $cm^3$). Evaporation of the solvent from the pooled fractions containing the title product (TLC: same solvent; $R_f$ 0.33) afforded compound **3** as an oil (398 mg, 91%) that had the same characteristics as the product prepared by route (a).

*Methyl* (*Methyl* 3-*Deoxy*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **9**.—Cations were removed from a solution of ammonium 3-deoxy-5-*O*-methyl-D-*manno*-oct-2-ulosonate[8] (500 mg, 1.63 mmol) in methanol (30 $cm^3$) with Dowex AG50WX8 ($H^+$) resin. Ethereal diazomethane was added to the solution decanted from the resin until neutrality was obtained (pH paper). Solvents were removed, and anhydrous toluene was added to and evaporated from (3 × 10 $cm^3$) the residual oily material, which was then treated with methanolic HCl (0.6 mol $dm^{-3}$; 15 $cm^3$) for three hours at 60 °C. The solution was neutralised with Amberlite IR45 ($OH^-$) resin, filtered, and the solvents were removed. Column (15 × 2 cm) chromatography [chloroform–methanol (9:1)] gave the *title product* as an oil (320 mg, 80%) that crystallised rapidly. When recrystallised from diethyl ether the product crystallised with 0.5 mol equiv. of MeOH, the presence of MeOH being confirmed by the $^1H$ NMR spectrum; m.p. 153–155 °C (from $Et_2O$); $[\alpha]_D$ +82.9° (*c* 1, MeOH) (Found: C, 46.6; H, 7.5. $C_{11}H_{20}O_8$·0.5MeOH requires C, 46.6; H, 7.4%); $\delta_H$($CDCl_3$; 250 MHz) 1.88 (1 H, t, $J_{3a,3e}$ ≈ $J_{3a,4}$ ≈12, 3-$H^a$), 2.11 (1 H, dd, $J_{3e,4}$ 5, 3-$H^e$), 3.20 (3 H, s, 2-OMe), 3.58 (1 H, dd, $J_{6,5}$ 1, $J_{6,7}$ 9, 6-H), 3.64 (3 H, s, 5-OMe), 3.69 (1 H, dd, $J_{8,8'}$ 11, $J_{8,7}$ 5, 8-H), 3.72 (1 H, m, $J_{5,4}$ ≈3, 5-H), 3.80 (3 H, s, Ac), 3.87 (1 H, dd, $J_{8',7}$ 3.5, 8-H′), 3.95 (1 H, m, 7-H) and 4.05 (1 H, m, 4-H).

*Methyl*(*Methyl*3-*Deoxy*-7,8,-O-*isopropylidene*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 4-(*Diphenylphosphate*) **7**.—Imidazole (450 mg, 4 mol equiv.) and diphenyl phosphorochloridate (760 $mm^3$, 3 mol equiv.) were added to a solution of the alcohol **3** (588 mg, 1.84 mmol) in anhydrous THF (20 $cm^3$). The stirred mixture was kept at 60 °C for 17 h, cooled, diluted with diethyl ether (50 $cm^3$), and washed with water (3 × 30 $cm^3$), the aqueous phases being re-extracted with diethyl ether (2 × 25 $cm^3$). The ethereal solutions were pooled, dried and concentrated. Column (10 × 4.5 cm) chromatography [ethyl acetate–cyclohexane (2:3)] of the residue afforded the *phosphotriester* **7** (1 g, 99%) as an oil (Found: C, 56.7; H, 5.7. $C_{26}H_{33}O_{11}P$ requires C, 56.5; H, 6.0%); $[\alpha]_D$ +42.8° (*c* 1.1, $CHCl_3$); $\delta_H$($CDCl_3$; 250 MHz) 1.35 and 1.43 (2 × 3 H, 2 s, $CMe_2$), 2.27 (2 H, m, 3-$H^a$, 3-$H^e$), 3.22 (3 H, s, OMe), 3.47 (4 H, m, 5-OMe and 6-H), 3.77 (4 H, m, Ac and 5-H), 3.95 (1 H, dd, $J_{8,8'}$ 8.5, $J_{8,7}$ 5, 8-H), 4.14 (1 H, dd, $J_{8',7}$ 6, 8-H′), 4.33 (1 H, m, $J_{7,6}$ 8.5, 7-H), 5.04 (1 H, ddd, $J_{4,P}$ 16, $J_{4,3a}$ 8.5, $J_{4,3e}$ ≈ 3, $J_{4,5}$ ≈ 1, 4-H) and 7.27 (10 H, m, Ph).

*Methyl* (*Methyl* 3-*Deoxy*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 4-(*Ammonium Hydrogen Phosphate*) **8**.—Adams' catalyst ($PtO_2$) was added in three portions (3 × 100 mg) to a stirred solution of the phosphotriester **7** (1 g, 1.81 mmol) in methanol (30 $cm^3$) under $H_2$. When removal of the phenyl groups was complete (~six hours) the acid solution was neutralised with aq. ammonia (2%) in methanol. The catalyst was removed by centrifugation and washed with methanol. Evaporation of the pooled filtrate and washings gave the *title compound* (700 mg, 98%) as an oil (Found: C, 33.4; H, 6.5; N, 3.8. $C_{11}H_{24}NO_{11}P$·$H_2O$ requires C, 33.4; H, 6.6; N, 3.5%); $[\alpha]_D$ +51.7° (*c* 1.7, MeOH); $\delta_H$($CD_3OD$; 250 MHz) 2.04 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 12, 3-$H^a$), 2.24 (1 H, dd, $J_{3e,4}$ 5, 3-$H^e$), 3.18 (3 H, s, 2-OMe), 3.60 (1 H, dd, $J_{6,5}$ ≈ 1, $J_{6,7}$ 9, 6-H), 3.62 (1 H, m, 8-H), 3.65 (3 H, s, 5-OMe), 3.73–3.89 (2 H, m, 7-H and 8-H′), 3.76 (3 H, s, Ac), 3.93 (1 H, ≈d, $J_{5,4}$ 2, 5-H) and 4.60 (1 H, m, $J_{4,P}$ 11, 4-H).

(*Methyl* 3-*Deoxy*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onic Acid* 4-(*Calcium Phosphate*) **4**.—A solution of the ammonium phosphate **8** (690 mg, 1.8 mmol) in 0.5 mol $dm^{-3}$ NaOH (25 $cm^3$) was kept at room temperature for two hours. After addition of water (25 $cm^3$) the mixture was cooled to 0 °C and cations were removed by addition of Dowex AGWX8 ($H^+$) resin. Solids were filtered off in the cold, and the pH of the solution was brought to 7.5 with saturated, aq. calcium hydroxide. The syrup remaining after removal of the solvent was triturated first with ethanol, then with acetone, to yield the *title product* as a solid (760 mg, 99%) (Found: C, 27.2; H, 4.4. $C_{10}H_{16}Ca_{1.5}O_{11}P$·$2H_2O$ requires C, 27.3; H, 4.5%); $[\alpha]_D$ +48.0° (*c* 0.83, water); $\delta_H$($D_2O$; 250 MHz) 1.93 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 11.5, 3-$H^a$), 2.20 (1 H, dd, $J_{3e,4}$ 5, 3-$H^e$), 3.20 (3 H, s, 5-Me), 3.65–3.77 and 3.93–4.02 (2 H, and 3 H, 2 m, 5-, 6-, 7-H and 8-$H_2$), 3.71 (3 H, s, Ac) and 4.49 (1 H, m, 4-H).

*Methyl (Methyl* 4-O-*Benzoyl*-5-O-*benzyl*-3-*deoxy*-7,8-O-*isopropylidene*-α-D-manno-*oct*-2-*ulopyranosid)onate* **11**.—A stirred solution of methyl (methyl 5-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate[9] **10** (2.1 g, 5 mmol) in anhydrous pyridine (20 $cm^3$) containing benzoic anhydride (1.7 g, 1.5 mol equiv.) and DMAP (100 mg) was kept at 35 °C for 15 h. Methanol (1 $cm^3$) was added, a few minutes later the solvents were removed, and toluene (25 $cm^3$) was added to and evaporated from the residue. The residual oily product was taken up in dichloromethane (200 $cm^3$), the solution was washed successively with cold, dil. (5%) sulphuric acid (80 $cm^3$), saturated, aq. sodium hydrogen carbonate (2 × 80 $cm^3$) and saturated, aq. NaCl. All aqueous phases were re-extracted with dichloromethane (100 $cm^3$). The organic phases were united, then dried, and the solvents were removed. Column (17 × 3.5 cm) chromatography [ethyl acetate–cyclohexane (15:85) 250 $cm^3$; (20:80) 400 $cm^3$; (25:85) 800 $cm^3$] afforded the *title compound* as a crystalline solid (1.6 g, 61%); m.p. 105–108 °C (from diethyl ether–cyclohexane) (Found: C, 64.9; H, 6.4. $C_{27}H_{32}O_9$ requires C, 64.8; H, 6.4%); $[\alpha]_D$ +64.1° (*c* 1, $CDCl_3$); $\delta_H$($CDCl_3$; 200 MHz) 1.35 and 1.44 (2 × 3 H, 2 s, $CMe_2$), 2.26 (1 H, dd, $J_{3a,3e}$ 12, $J_{3e,4}$ 5, $J_{3e,5}$ 1, 3-H$^e$), 2.43 (1 H, t, $J_{3a,4}$ 12, 3-H$^a$), 3.28 (3 H, s, OMe), 3.67 (1 H, dd, $J_{6,5} \approx 1$, $J_{6,7}$ 9, 6-H), 3.80 (3 H, s, $CO_2Me$), 3.99 (1 H, dd, $J_{8,8'}$ 9, $J_{8,7}$ 5, 8-H), 4.17 (1 H, dd, $J_{8',7}$ 6, 8-H′), 4.18 (1 H, ≈s, $J_{5,4}$ 2, 5-H), 4.41 (1 H, ddd, 7-H), 4.71 (2 H, 2 d, $OCH_2Ph$), 5.51 (1 H, dq, 4-H) and 7.21–7.63 and 7.98 (10 H, m, Ph).

*Methyl (Methyl* 4-O-*Benzoyl*-5-O-*benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid)onate* **12**.—Aq. TFA (1.5 mol $dm^{-3}$; 700 $mm^3$) and silica gel (6 g) were added to a stirred solution of the acetal **11** (1.5 g, 3 mmol) in dichloromethane (3 $cm^3$) and the mixture was stirred at room temperature for five hours, whereafter some solid $NaHCO_3$ was added. The mixture was shaken vigorously, deposited on a dry column (7 × 4.5 cm), and eluted successively with ethyl acetate–cyclohexane (1:1; 150 $cm^3$) and ethyl acetate until elution of the diol was complete. Solvents were removed from the pooled fractions containing the diol **12** [TLC: ethyl acetate–cyclohexane (1:1); $R_f$ 0.2] and the *title product* (1.2 g, 90%) crystallised slowly, m.p. 89–91 °C (Found: C, 62.6; H, 6.1. $C_{24}H_{28}O_9$ requires C, 62.6; H, 6.1%); $[\alpha]_D$ + 53.8° (*c* 0.96, $CHCl_3$); $\delta_H$($CDCl_3$; 200 MHz) 2.24 (1 H, dd, $J_{3a,3e}$ 12.5, $J_{3e,4}$ 5, 3-H$^e$), 2.38 (1 H, ≈t, $J_{3a,4}$ 12, 3-H$^a$), 3.20 (3 H, s, OMe), 3.46–3.93 (4 H, m, 6-, 7-H and 8-$H_2$), 3.74 (3 H, s, $CO_2Me$), 4.13 (1 H, ≈s, $J_{5,4} \approx 2.5$, $J_{5,6} \approx 0.1$, 5-H), 4.57 and 4.80 (2 H, 2 d, $OCH_2Ph$), 5.48 (1 H, dq, 4-H) and 7.18–8.04 (10 H, m, Ph).

*Methyl (Methyl* 4-O-*Benzoyl*-5-O-*benzyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid)onate* **13**.—A mixture of the diol **12** (200 mg, 0.43 mmol) and dibutyltin oxide (200 mg, 1.8 mol equiv.) in benzene (40 $cm^3$) was heated to reflux for two hours in a flask equipped with a Dean–Stark water separator. After removal of the solvent the residual oil was taken up in anhydrous benzene (40 $cm^3$), dry tetrabutylammonium bromide (270 mg, 2 mol equiv.) and iodomethane (1 $cm^3$) were added, and the stirred mixture was kept at 100 °C in a sealed vessel for 16 h. Solvents were removed from the cold mixture and the *title compound* was recovered from the residue by column (23.5 × 2.2 cm) chromatography [ethyl acetate–cyclohexane (1:1)] as an oil (150 mg, 73%) (Found: C, 63.1; H, 6.5. $C_{25}H_{30}O_9$ requires C, 63.3; H, 6.3%); $[\alpha]_D$ +74.9° (*c* 0.87, $CHCl_3$); $\delta_H$($CDCl_3$; 200 MHz) 2.21 (1 H, d, $J_{OH,7}$ 7, 7-OH), 2.29 (1 H, dd, $J_{3a,3e}$ 12.5, $J_{3e,4}$ 5, 3-H$^e$), 2.46 (1 H, ≈t, $J_{3a,4}$ 12, 3-H$^a$), 3.26 (3 H, s, 2-OMe), 3.40 (3 H, s, 8-OMe), 3.67 (2 H, m, 8-$H_2$), 3.77 (1 H, ≈d, $J_{6,5} \approx 1$, $J_{6,7} \approx 9$, 6-H), 3.81 (3 H, s, $CO_2Me$), 4.08 (1 H, m, 7-H), 4.17 (1 H, ≈s, $J_{5,4} \approx 2.5$, 5-H), 4.77 (2 H, m, $OCH_2Ph$), 5.43 (1 H, m, 4-H) and 7.18–7.98 (10 H, m, Ph).

*Methyl (Methyl* 7-O-*Acetyl*-4-O-*benzoyl*-5-O-*benzyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid)onate* **14**.—Acetylation of the alcohol **13** (32 mg, 0.07 mmol) with a mixture of acetic anhydride–pyridine (1:1; 800 $mm^3$) for 12 h at room temperature, followed by removal of the reagent by co-evaporation with toluene, and purification by column (5 × 0.7 cm) chromatography [ethyl acetate–cyclohexane (1:1)] afforded the acetate **14** as an oil (29 mg, 80%), characterised by its $^1H$ NMR spectrum: $\delta_H$($CDCl_3$; 200 MHz) 2.00 (3 H, s, Ac), 2.23 (1 H, dd, $J_{3e,3a}$ 12.5, $J_{3e,4}$ 5, 3-H$^e$), 2.40 (1 H, ≈t, $J_{3a,4}$ 12, 3-H$^a$), 3.26 and 3.29 (2 × 3 H, 2 s, 2 × OMe), 3.69 (2 H, m, 8-$H_2$), 3.74 (3 H, s, $CO_2Me$), 3.95 (1 H, ≈s, $J_{5,4} \approx 2.5$, $J_{5,6} \approx 1$, 5-H), 4.10 (1 H, ≈d, $J_{6,7} \approx 9$, 6-H), 4.41 and 4.63 (2 H, 2 d, $OCH_2Ph$), 5.26 (1 H, ≈dt, $J_{7,8} \approx J_{7,8'} \approx 3$, 7-H), 5.52 (1 H, dq, 4-H) and 7.11–8.01 (10 H, m, Ph).

*Methyl (Methyl* 4-O-*Benzoyl*-5-O-*benzyl*-7-O-t-*butyldimethylsilyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid)onate* **15**.—*N*-Methylimidazole (450 $mm^3$, 9 mol equiv.) and t-butyl(chloro)dimethylsilane (470 mg, 5 mol equiv.) were added to a stirred solution of the alcohol **13** in anhydrous THF (15 $cm^3$) and the mixture was kept at 60 °C for 24 h. Further *N*-methylimidazole (160 $mm^3$, 3 mol equiv.) and t-butyl(chloro)dimethylsilane (260 mg, 2.7 mol equiv.) were then added and the stirred mixture was kept for another 24 h at 60 °C. The cooled reaction mixture was then deposited on a layer (2 × 4.5 cm) of silica gel and eluted with diethyl ether (200 $cm^3$). Fractions (25 $cm^3$) containing the silyl ether **15** [TLC; ethyl acetate–cyclohexane (3:7); $R_f$ 0.65] were pooled, and purified by column (10 × 2.2 cm) chromatography [ethyl acetate–cyclohexane (1:9)] to afford the *title compound* (350 mg, 94%) as an oil (Found: C, 63.3; H, 7.5. $C_{31}H_{44}O_9Si$ requires C, 63.4; H, 7.6%); $[\alpha]_D$ +68.2° (*c* 0.9, $CHCl_3$); $\delta_H$($CDCl_3$; 250 MHz) 0.09 and 0.04 (2 × 3 H, 2 s, $Me_2Si$), 0.94 (9 H, s, $Me_3CSi$), 2.33 (1 H, ddd, $J_{3e,3a}$ 12, $J_{3e,4}$ 5, $J_{3e,5}$ 1, 3-H$^e$), 2.47 (1 H, ≈t, $J_{3a,4}$ 11.5, 3-H$^a$), 3.33 and 3.40 (2 × 3 H, 2 s, 2 × OMe), 3.67 (2 H, m, 8-$H_2$), 3.83 (3 H, s, $CO_2Me$), 3.94 (1 H, dd, $J_{6,5}$ 1, $J_{6,7} \approx 9$, 6-H), 4.26 (1 H, ≈s, $J_{5,4} \approx 2.5$, 5-H), 4.29 (1 H, m, 7-H), 4.71 and 4.94 (2 H, 2 d, $OCH_2Ph$), 5.61 (1 H, m, 4-H) 7.22–7.63 and 7.94–8.04 (10 H, m, Ph).

*Methyl (Methyl* 5-O-*Benzyl*-7-O-*t-butyldimethylsilyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid)onate* **16**.—A solution of the benzoate **15** (300 mg, 0.5 mmol) in 0.1 mol $dm^{-3}$ sodium methoxide in methanol (16.5 $cm^3$) was stirred for two hours at room temperature, decationised with Dowex AG50WX8 ($H^+$) resin and filtered. Column (12 × 1.3 cm) chromatography [ethyl acetate–cyclohexane (3:7)] of the residue remaining after removal of the solvent afforded the *alcohol* **16** (225 mg, 90%) as an oil (Found: C, 59.0; H, 8.2. $C_{24}H_{40}O_8Si$ requires C, 59.5; H, 8.3%); $[\alpha]_D$ +57.8° (*c* 1.2, $CHCl_3$); $\delta_H$($CDCl_3$; 200 MHz) 0.10 and 0.12 (2 × 3 H, 2 s, $Me_2Si$), 0.95 (9 H, s, $Me_3CSi$), 1.92 (1 H, ≈t, $J_{3e,3a}$ 12, $J_{3a,4}$ 11.5, 3-H$^a$), 2.13 (1 H, dd, $J_{3e,4}$ 5, 3-H$^e$), 3.24 and 3.32 (2 × 3 H, 2 s, 2 × OMe), 3.65 (2 H, m, 8-$H_2$), 3.75 (1 H, ≈d, $J_{6,5} \approx 1$, $J_{6,7}$ 9, 6-H), 3.80 (3 H, s, $CO_2Me$), 4.01 (1 H, m, 5-H), 4.07 (1 H, m, 4-H), 4.24 (1 H, dt, $J_{7,8} \approx J_{7,8'} \approx 2.5$, 7-H), 4.73 and 4.94 (2 H, 2 d, $OCH_2Ph$) and 7.36 (5 H, m, Ph).

*Methyl (Methyl* 5-O-*Benzyl*-7-O-*t-butyldimethylsilyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid)onate* 4-[*bis*-(2,2,2-*trichloroethyl*)*Phosphate*] **17**.—*N*-Methylimidazole (220 $mm^3$, 4 mol equiv.) and bis-(2,2,2-trichloroethyl) phosphorochloridate (540 mg, 2 mol equiv.) were added to a solution of the alcohol **16** (340 mg, 0.7 mmol) in anhydrous THF (15 $cm^3$). The stirred mixture was kept at 40 °C for 4 h, cooled, and deposited on a bed (2 × 4 cm) of silica gel wetted with ethyl acetate–cyclohexane (4:6; 100 $cm^3$) and then eluted with the

same solvent. Fractions (30 $cm^3$) containing the phosphorylated product [TLC: ethyl acetate–cyclohexane (3:7); $R_f$ 0.6] were pooled and concentrated. The crude product (560 mg, 95%) was purified by column (11 × 2.2 cm) chromatography [ethyl acetate–cyclohexane (2:8) 50 $cm^3$; (3:7) 100 $cm^3$], but no satisfactory elemental analysis could be obtained (Found: C, 38.6; H, 5.7. $C_{28}H_{43}Cl_6O_{11}PSi$ requires C, 40.6; H, 5.2%); $[\alpha]_D$ +42.2° (*c* 1.2, $CHCl_3$). The identity of the compound was confirmed by its $^1H$ NMR spectrum: $\delta_H(CDCl_3$; 200 MHz) 0.08 and 0.11 (2 × 3 H, 2 s, $Me_2Si$), 0.90 (9 H, s, $Me_3CSi$), 2.41 (2 H, m, 3-$H_2$), 3.22 and 3.31 (2 × 3 H, 2 s, 2 × OMe), 3.60 (2 H, m, 8-$H_2$), 3.80 (4 H, ≈s, 6-H, $CO_2Me$), 4.20 (1 H, m, 5-H), 4.21 (1 H, m, 7-H), 4.59 (4 H, m, $OCH_2CCl_3$), 4.76 and 4.96 (2 H, 2 d, $OCH_2Ph$), 5.09 (1 H, m, 4-H) and 7.22–7.39 (5 H, m, Ph).

(*Methyl* 5-O-*Benzyl*-3-*deoxy*-8-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onic Acid* 4-(*Calcium Phosphate*) **18**.—Activated Zn (500 mg) and $Ag_2CO_3$ (20 mg) were added to a stirred solution (9 $cm^3$) of the phosphotriester **17** (512 mg, 0.62 mmol) in pyridine containing 10% of acetic acid, and the mixture was stirred for 18 h. Solids were filtered off and washed with methanol (4 $cm^3$). The combined filtrate and washings were freed of solvent, and the residue was dissolved in a mixture of dichloromethane–methanol–ethyl acetate–water–conc. ammonia (*d* 0.91) (650:300:200:10:5) and passed through a column (100 × 2.5 cm) of Sephadex LH20 gel (Pharmacia Fine Chemicals) equilibrated with the same solvent. Appropriate [TLC: propan-2-ol–conc. ammonia (*d* 0.91)–water (8:1:1); $R_f$ 0.23] fractions (8 $cm^3$) were pooled and concentrated to leave an oil (420 mg), which was dissolved in MeOH (15 $cm^3$), Dowex AG50WX8 ($H^+$) resin was added, and the stirred mixture was kept at 50 °C for 15 h. The resin was filtered off, the filtrate was neutralised with a few drops of dil. ammonia, the solvents were removed and the residue was taken up in 0.5 mol $dm^{-3}$ NaOH (10 $cm^3$) and kept for two hours at room temperature. The cooled (0 °C) solution was decationised with Dowex AG50WX8 ($H^+$) resin which was then filtered off; the pH of the filtrate was then adjusted to 7.5 with saturated, aq. $Ca(OH)_2$. The solution was concentrated to *ca.* 0.5 $cm^3$ and acetone was added to precipitate the *title compound* (180 mg, 56%), which was collected by centrifugation, washed with acetone (2 × 2 $cm^3$) and dried (Found: C, 39.0; H, 5.3. $C_{17}H_{23}CaO_{11}P{\cdot}2.5H_2O$ requires C, 39.3; H, 5.4%); $[\alpha]_D$ +65.8° (*c* 1.3, water); $\delta_H(D_2O$; 200 MHz) 1.86 (1 H, ≈t, $J_{3a,3e}$ 13, $J_{3a,4}$ 11.5, 3-H$^a$), 2.11 (1 H, dd, $J_{3e,4}$ 4.5, 3-H$^e$), 3.10 and 3.35 (2 × 3 H, 2 s, 2 × OMe), 3.55 (1 H, dd, $J_{8,8'}$ 11, $J_{8,7}$ 7, 8-H), 3.60 (1 H, ≈d, $J_{6,5}$ ≈ 1, $J_{6,7}$ 9, 6-H), 3.71 (1 H, dd, $J_{8',7}$ 2.5, 8-H′), 3.99 (1 H, m, 7-H), 4.21 (1 H, m, $J_{5,4}$ ≈ 2, 5-H), 4.46 (1 H, m, 4-H), 4.67 and 5.05 (2 H, 2 d, $OCH_2Ph$), 4.80 (s, HDO) and 7.31–7.52 (5 H, m, Ph).

*Methyl* (*Methyl* 4-O-*Benzoyl*-3-*deoxy*-7,8-O-*isopropylidene*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **19**.—The alcohol **3** (130 mg, 0.4 mmol) was benzoylated to yield the oily *title product* (126 mg, 73%) by the procedure described above for the preparation of compound **11**; $[\alpha]_D$ +32.3° (*c* 0.8, $CHCl_3$) (Found: C, 59.0; H, 6.6. $C_{21}H_{28}O_9$ requires C, 59.4; H, 6.6%); $\delta_H(CDCl_3$; 250 MHz) 1.29 and 1.48 (2 × 3 H, 2 s, $CMe_2$), 2.18 (1 H, dd, $J_{3e,3a}$ 12.5, $J_{3e,4}$ 5, 3-H$^e$), 2.27 (1 H, dd, $J_{3a,4}$ 11.5, 3-H$^a$), 3.21 (3 H, s, 2-OMe), 3.50 (3 H, s, 5-OMe), 3.56 (1 H, dd, $J_{6,5}$ ≈ 0.9, $J_{6,7}$ 8.5, 6-H), 3.72 (3 H, s, $CO_2Me$), 3.85 (1 H, m, 5-H), 3.93 (1 H, dd, $J_{8,8'}$ 9, $J_{8,7}$ 5, 8-H), 4.09 (1 H, dd, $J_{8',7}$ 6, 8′-H), 4.31 (1 H, m, 7-H), 5.42 (1 H, m, $J_{4,5}$ 2.5, 4-H), 7.35–7.60 and 7.97–8.09 (3 H and 2 H, m, Ph).

*Methyl* (*Methyl* 4-O-*Benzoyl*-3-*deoxy*-5-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **20**.—The isopropylidene group was removed from the acetal **19** (1.7 g, 4 mmol) under the conditions described for the preparation of the diol **12**. After column (7 × 4 cm) chromatography [ethyl acetate–cyclohexane (7:3)] and removal of the solvent the *title diol* (1.25 g, 81%) crystallised spontaneously (Found: C, 56.1; H, 6.4. $C_{18}H_{24}O_9$ requires C, 56.2; H, 6.2%); m.p. 82–85 °C (from diethyl ether–hexane); $[\alpha]_D$ +48.3° (*c* 1.4, $CHCl_3$); $\delta_H(CDCl_3$; 250 MHz) 2.29 (1 H, dd, $J_{3e,3a}$ 12, $J_{3e,4}$ 5.5, 3-H$^e$), 2.37 (1 H, ≈t, $J_{3a,4}$ 11, 3-H$^a$), 3.27 (3 H, s, 2-OMe), 3.59 (3 H, s, 5-OMe), 3.79 (3 H, s, $CO_2Me$), 3.81 (1 H, dd, $J_{6,5}$ 1, $J_{6,7}$ 7.5, 6-H), 3.86–3.92 (2 H, m, 8-$H_2$), 3.99 (1 H, m, 5-H), 4.04 (1 H, m, 7-H), 5.50 (1 H, ddd, $J_{4,5}$ 2.5, 4-H) and 7.43, 7.58 and 8.05 (2 H, 1 H and 2 H, 3 m, Ph).

*Methyl* (*Methyl* 4-O-*Benzoyl*-3-*deoxy*-5,7,8-*tri*-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **21**.—Silver(I) oxide (500 mg) was added portionwise during three hours to a stirred solution, kept at 35 °C, of the benzoate **20** (107 mg, 0.28 mmol) in iodomethane (4 $cm^3$) containing anhydrous $CaSO_4$ (600 mg). After 12 h the solution was filtered, solids were washed with dichloromethane (5 $cm^3$) and the solvents were removed from the combined filtrate and washings. Dry toluene (5 $cm^3$) was added to and evaporated from the residue, which was then remethylated by the same procedure. The residue remaining after removal of the solvents was purified by column (6.5 × 2 cm) chromatography [ethyl acetate–cyclohexane (3:7) 50 $cm^3$; (4:6) 60 $cm^3$]. Appropriate fractions of the eluate were pooled and concentrated, and the *trimethyl ether* **21** (90 mg, 80%) was crystallised from hexane (Found: C, 58.2; H, 6.7. $C_{20}H_{28}O_9$ requires C, 58.2; 6.8%); $[\alpha]_D$ +41.2° (*c* 1.4, $CHCl_3$); $\delta_H(CDCl_3$; 200 MHz) 2.21–2.36 (2 H, m, 3-$H_2$), 3.27, 3.39, 3.47 and 3.58 (4 × 3 H, 4 s, 2-, 5-, 7- and 8-OMe), 3.51–3.97 (5 H, m, 5-, 6-, 7-H and 8-$H_2$), 3.80 (3 H, s, $CO_2Me$), 5.50 (1 H, m, 4-H) and 7.37–7.63 and 8.0–8.1 (3 H, 2 H, 2 m, Ph).

*Methyl* (*Methyl* 3-*Deoxy*-5,7,8-*tri*-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* **22**.The benzoate group was removed from the trimethyl ether **21** (90 mg, 0.22 mmol) under the conditions used to prepare the alcohol **16**. After column (5 × 1.4 cm) chromatography [ethyl acetate–cyclohexane (1:1)] the *title product* (57 mg) crystallised out (Found: C, 50.7; H, 7.8. $C_{13}H_{24}O_8$ requires C, 50.6; H, 7.8%); m.p. 73–75 °C (from diethyl ether–hexane); $[\alpha]_D$ +74.0° (*c* 1.1, $CHCl_3$); $\delta_H(CDCl_3$; 200 MHz) 1.82 (1 H, ≈t, $J_{3a,3e}$ ≈ $J_{3a,4}$ ≈ 13, 3-H$^a$), 2.11 (1 H, dd, $J_{3e,4}$ 5, 3-H$^e$), 3.20, 3.35, 3.43 and 3.58 (4 × 3 H, 4 s, 2-, 5-, 7- and 8-OMe), 3.51–3.83 (5 H, m, 5-, 6-, 7-H and 8-$H_2$), 3.75 (3 H, s, $CO_2Me$) and 4.03 (1 H, m, 4-H).

*Methyl* (*Methyl* 3-*Deoxy*-5,7,8-*tri*-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 4-(*Diphenyl Phosphate*) **23**.—*N*-Methylimidazole (70 $mm^3$, 4 mol equiv.) and diphenyl phosphorochloridate (90 $mm^3$, 2 mol equiv.) were added to a stirred solution of the trismethyl ether **22** (50 mg, 0.16 mmol) in anhydrous THF (2 $cm^3$). The mixture was kept at 40 °C for one hour, then deposited on a column (3.5 × 2 cm) of silica gel and the product was eluted with ethyl acetate–cyclohexane (5:1). Fractions (5 $cm^3$) containing the product [TLC: ethyl acetate–cyclohexane (4:6); $R_f$ 0.26] were pooled, brought to dryness and purified by column (6 × 2 cm) chromatography (same solvent) to afford the *diphenyl phosphate* **23** (60 mg, 68%) as an oil (Found: C, 55.4; H, 6.2. $C_{25}H_{33}O_{11}P$ requires C, 55.5; H, 6.1%); $[\alpha]_D$ +48.6° (*c* 0.8, $CHCl_3$); $\delta_H(CDCl_3$; 250 MHz), 2.26–2.35 (2 H, m, 3-$H_2$), 3.24 (3 H, s, 2-OMe), 3.38, 3.47 and 3.50 (3 × 3 H, 3 s, 5-, 7- and 8-OMe), 3.6–3.9 (5 H, m, 5-, 6-, 7-H and 8-$H_2$), 3.78 (3 H, s, $CO_2Me$), 5.05 (1 H, m, 4-H) and 7.15–7.40 (10 H, m, Ph).

*Methyl* (*Methyl* 3-*Deoxy*-5,7,8-*tri*-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 4-(*Ammonium Hydrogen Phosphate*) **24**.—Adams' catalyst ($PtO_2$) (50 mg) was added to a solution of

the diphenyl phosphate **23** (61 mg, 0.11 mmol) in methanol (2 $cm^3$) and the mixture was stirred under $H_2$. Progress of the hydrogenolysis, as observed by TLC [propan-2-ol–conc. ammonia–water (7:2:1)] being slow, at hourly intervals more $PtO_2$ (3 × 50 mg) was added and the reaction was allowed to proceed overnight: it then appeared to be complete. The catalyst was filtered off and washed with methanol (3 $cm^3$), and the pooled filtrate and washings were neutralised with aq. ammonia (2% in methanol) and concentrated to give the *title compound* as an oil (46 mg, 99%) (Found: C, 37.1; H, 7.0; N, 2.9. $C_{13}H_{28}NO_{11}P{\cdot}H_2O$ requires C, 36.9; H, 7.1; N, 3.3%); $[\alpha]_D$ +51.1° (*c* 0.83, $CHCl_3$); $\delta_H(CD_3OD$; 200 MHz) 1.99 (1 H, dd, $J_{3a,3e}$ 12.5, $J_{3a,4}$ 12, 3-H$^a$), 2.20 (1 H, dd, $J_{3e,4}$ 5, 3-H$^e$), 3.13, 3.26 and 3.41 (3 × 3 H, 3 s, 3 × OMe), 3.4–3.8 (4 H, m, 6- and 7-H and 8-$H_2$), 3.57 (3 H, s, OMe), 3.73 (3 H, s, $CO_2Me$), 3.82 (1 H, m, 5-H) and 4.53 (1 H, m, 4-H).

(*Methyl* 3-*Deoxy*-5,7,8-*tri*-O-*methyl*-α-D-manno-*oct*-2-*ulopyranosid*)*onic Acid* 4-(*Calcium Phosphate*) **25**.—Saponification of the phosphorylated methyl ester **24** (45 mg, 0.11 mmol) was accomplished as described for the ester **8**. The *Ca salt* **25** (52 mg, 94%) was precipitated and washed with acetone (Found: C, 27.8; H, 5.3. $C_{12}H_{20}Ca_{1.5}O_{11}P{\cdot}5H_2O$ requires C, 27.6; H, 5.7%); $[\alpha]_D$ +38.7° (*c* 1, water); $\delta_H(D_2O$; 250 MHz) 1.89 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 12, 3-H$^a$), 2.16 (1 H, dd, $J_{3e,4}$ 5, 3-H$^e$), 3.17, 3.43 and 3.54 (3 × 3 H, 3 s, 3 × OMe), 3.63–3.99 (5 H, m, 5-, 6-, 7-H and 8-$H_2$), 3.68 (3 H, s, OMe) and 4.45 (1 H, m, 4-H).

(*Methyl* 5-O-*Benzyl*-3-*deoxy*-7,8-O-*isopropylidene*-α-D-manno-*oct*-2-*ulopyranosid*)*onic Acid* 4-(*Methyl Hydrogen Phosphate*) **27**.—Sodium hydroxide (5 mol $dm^{-3}$, 3 $cm^3$) was added to a solution of methyl (methyl 5-*O*-benzyl-3-deoxy-7,8-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate 4-(diphenyl phosphate)[9] **26** (230 mg, 0.36 mmol) in methanol (10 $cm^3$). The stirred solution was kept at 30 °C for 17 h, then at room temperature for another 24 h before being decationised with Amberlite IRN 77 ($H^+$) resin, which was then filtered off and washed. The filtrate was neutralised with triethylamine and brought to dryness. Following column (8 × 2 cm) chromatography [propan-2-ol–conc. ammonia–water (7:1:1)] the mixed triethylammonium–ammonium salt of the *title compound* (183 mg) were recovered from the concentrated effluent by lyophilisation (Found: C, 51.4; H, 8.0; N, 4.4. $C_{26}H_{47}N_2O_{11}P{\cdot}H_2O$ requires C, 51.0; H, 7.7; N, 4.6%); $[\alpha]_D$ +39.1° (*c* 0.7, MeOH); $\delta_H(D_2O/CDCl_3$; 250 MHz) 1.25 (9 H, t, 3 × *Me*$CH_2$N), 1.35 and 1.40 (2 × 3 H, 2 s, $CMe_2$), 2.17 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 11.5, 3-H$^a$), 2.37 (1 H, dd, $J_{3e,4}$ 4.5, 3-H$^e$), 3.02 (6 H, q, 3 × NC$H_2$Me), 3.24 (3 H, s, OMe), 3.57 (1 H, ≈d, $J_{6,5}$ ≈ 0.1, $J_{6,7}$ 7, 6-H), 3.65 (3 H, d, $J_{CH,P}$ 10.5, POMe), 3.93 (1 H, dd, $J_{8,8'}$ 8.5, $J_{8,7}$ 5, 8-H), 4.07 (1 H, m, 5-H), 4.10 (1 H, dd, $J_{8',7}$ 6, 8-H′), 4.35 (1 H, m, 7-H), 4.70 (1 H, d, C*H*HPh), 4.73 (1 H, m, 4-H), 5.01 (1 H, d, CH*H*Ph) and 7.20–7.45 (5 H, m, Ph).

(*Methyl* 5-O-*Benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onic Acid* 4-(*Calcium Methyl Phosphate*) **28**.—Dowex AG50WX8 ($H^+$) resin (1 $cm^3$) was added to a solution of the phosphodiester **27** (142 mg, 0.22 mmol) in methanol (5 $cm^3$) and the mixture was stirred for 210 min. The solid was filtered off and washed with methanol (10 $cm^3$), and the combined filtrate and washings were neutralised with saturated, aq. $Ca(OH)_2$ and the volume was reduced to *ca.* 0.5 $cm^3$. Upon addition of ethanol (5 $cm^3$) the *title compound* was obtained as a precipitate; it was collected, washed with acetone (3 $cm^3$) and dried (Found: C, 39.3; H, 5.4. $C_{17}H_{23}CaO_{11}P{\cdot}2.5H_2O$ requires C, 39.3; H, 5.1%); $[\alpha]_D$ +64.1° (*c* 1.3, $D_2O$); $\delta_H(D_2O$; 250 MHz) 2.05 (1 H, dd, $J_{3a,3e}$ 13, $J_{3a,4}$ 12, 3-H$^a$), 2.22 (1 H, dd, $J_{3e,4}$ 5, 3-H$^e$), 3.20 (3 H, s, OMe), 3.65–3.75 (2 H, m, 8-H and 6-H or 8-H′), 3.70 (3 H, d, $J_{CH,P}$ 11, POMe), 3.90–4.05 (2 H, m, 7-H and 8-H′ or 6-H), 4.30 (1 H, ≈s, 5-H), 4.65 (1 H, m, 4-H), 4.75–5.10 (m, C$H_2$Ph and HDO) and 7.4–7.65 (5 H, m, Ph).

Treatment of the mother liquors containing a compound less polar than the title compound [presumably its (carboxy) methyl ester] with 0.5 mol $dm^{-3}$ NaOH for two hours at room temperature followed by work-up gave a further amount (21 mg, 19%) of the same compound **28** as judged from its $^1H$ NMR spectrum.

## References


1 Part 26, F.-I. Auzanneau, D. Charon and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2831.
2 M. J. Osborn, *Proc. Natl. Acad. Sci. USA*, 1963, **50**, 499.
3 A. Danan, M. Mondange, S. R. Sarfati and P. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1982, 1275.
4 R. Chaby and L. Szabó, *Eur. J. Biochem.*, 1975, **59**, 277.
5 H. Brade, *J. Bacteriol.*, 1985, **161**, 795.
6 M. Caroff, S. Lebbar and L. Szabó, *Carbohydr. Res.*, 1987, **161**, C4.
7 F.-I. Auzanneau, D. Charon, L. Szabó and C. Mérienne, *Carbohydr. Res.*, 1988, **179**, 125.
8 D. Charon and L. Szabó, *Eur. J. Biochem.*, 1972, **29**, 184.
9 S. R. Sarfati, A. Le Dur and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1988, 707.
10 F. Huet, A. Lechevallier, M. Pellet and J. M. Conia, *Synthesis*, 1978, 63.
11 S. David and S. Hanessian, *Tetrahedron*, 1985, **41**, 643.
12 J. G. Lammers and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 243.
13 O. Hindsgaul, T. Norberg, J. Le Pendu and R. U. Lemieux, *Carbohydr. Res.*, 1982, **109**, 109.
14 J. R. Cox and O. B. Ramsay, *Chem. Rev.*, 1964, **64**, 317.
15 J. MacGee and M. Doudoroff, *J. Biol. Chem.*, 1954, **210**, 617.
16 J. A. Olson, *Arch. Biochem. Biophys.*, 1959, **85**, 225.
17 P. A. McNicholas, M. Batley and J. W. Redmond, *Carbohydr. Res.*, 1986, **146**, 219.
18 F.-I. Auzanneau, D. Charon and L. Szabó, *Carbohydr. Res.*, 1990, **201**, 337.
19 M. Caroff, S. Lebbar and L. Szabó, *Biochem. Biophys. Res. Commun.*, 1987, **143**, 845.
20 W. A. Volk, N. L. Salomonsky and D. Hunt, *J. Biol. Chem.*, 1972, **247**, 3381.
21 W. Dröge, V. Lehmann, O. Lüderitz and O. Westphal, *Eur. J. Biochem.*, 1970, **14**, 175.
22 A. Tacken, E. Th. Rietschel and H. Brade, *Carbohydr. Res.*, 1986, **149**, 279.


*Paper* 0/04264A
*Received* 19*th September* 1990
*Accepted* 8*th October* 1990