Phosphorylated Sugars. Part 26.[1] Synthesis of 3-Deoxy-D-*manno*-oct-2-ulosonic Acid 7-Phosphate

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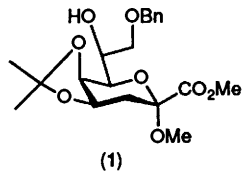
Of several phosphorylation methods tried, only that with bis(trichloroethyl) phosphorochloridate was satisfactory in producing the title compound starting either from methyl (methyl 8-*O*-benzyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate or from methyl 4,8-di-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosidono-1,5-lactone. The synthesis of the phosphodiester methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate 7-(acetamidoethyl ammonium phosphate) is also described.

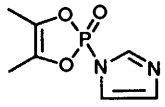
3-Deoxy-D-*manno*-oct-2-ulosonic acid (KDO) is an obligatory[2] component of the endotoxic lipopolysaccharides that are major constituents of the outer membrane of gram-negative bacteria. Its 8-phosphate is an intermediate in the biosynthesis[3] of 3-deoxy-D-*manno*-oct-2-ulosonic acid. Other phosphorylated derivatives of this acid have been identified as constituents of various endotoxin preparations. Thus KDO 5-phosphate has been isolated from *Bordetella pertussis*[4] and identified in *Vibrio cholerae*,[5] the 7-(2-aminoethyl phosphate) has been detected in the rough mutants 595, mR3, Rd_2P^-, and Rd_1P^+ of *Salmonella minnesota*,[6,7] and KDO phosphorylated in position 4 and substituted by the polysaccharide chain in position 5 has been postulated[8] to be present in Lipopolysaccharide-2 of the *Bordetella pertussis* endotoxin. Derivatives of KDO phosphorylated in positions 4,[9] 5,[10] and 8[11] have been synthesized. Syntheses of the α-methyl glycosides of 3-deoxy-D-*manno*-oct-2-ulosonic acid 7-phosphate (**7**), the corresponding methyl ester (**6**), and the 7-(2-acetamidoethyl phosphate) (**18**) are described in this paper. The phosphodiester containing acetylated ethanolamine has not been detected in endotoxins so far. It was required for studies concerning this type of phosphodiester.

Phosphorylation of methyl (methyl 8-*O*-benzyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosid)onate[12] (**1**) with an excess of 'acetoin enediol cyclophosphoimidazole'[13,14] (CEP-Im) (**2**) was attempted first, but was found not to be expedient. The cyclic phosphotriester (**3**) that was formed in the first step was not isolated but was treated, *in situ*, with benzyl alcohol to produce a mixture of the diastereoisomeric phosphotriesters (**4**). Large amounts of the acyclic dibenzyl phosphotriester (**5**) were formed simultaneously. A difficult chromatographic separation then gave a 36% yield of compounds (**4**). Deprotection and isolation of the product proved to be tedious: the 'acetoinyl' protecting group was removed first by treatment with triethylamine (2 mol equiv.) in aq. pyridine, then both benzyl groups by hydrogenolysis. Finally the isopropylidene group was hydrolysed upon treatment of the solution with Dowex 50 (H^+) resin; the acid solution was then neutralized with ammonia. Two successive chromatographic steps were necessary to purify the resulting 7-phosphate of the methyl ester, compound (**6**), that was saponified; the acid (**7**) produced was then isolated as its Ca salt in very poor overall yield (25% from the phosphotriester). In view of the difficulties encountered during the deprotection steps, no attempt was made to improve the method.

The synthesis of the 7-phosphate (**7**) was then attempted starting from methyl 4,8-di-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosidono-1,5-lactone[12] (**8**). This, when treated with diphenyl phosphorochloridate and *N*-methylimidazole in tetrahydrofuran (THF), gave a 70% yield of the phosphotriester (**9**). However, following hydrogenolytic removal of the protecting benzyl and phenyl groups (Pd-on-carbon, and Adams' Pt catalysts) two phosphate esters were detected upon paper electrophoresis (pH 6). After treatment with base, the rate of electrophoretic migration of both phosphate esters increased; this established that the lactone structure was still present in both compounds produced upon hydrogenolysis. During removal of the phenyl groups the medium turned acid; it was therefore reasonable to suppose that either the debenzylated phosphotriester or the intermediate phosphodiester underwent acid-catalysed phosphate migration,[15,16] and gave rise to the two isomeric phosphate esters (**10**) and (**11**).



(1)



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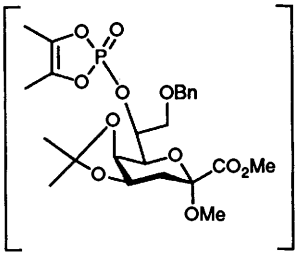

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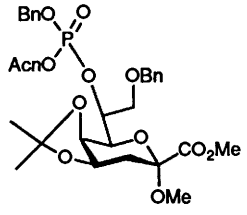
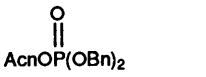
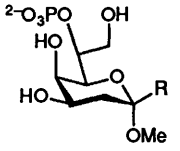


(4)



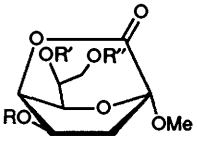
(4), (5) Acn = CH(Me)COMe



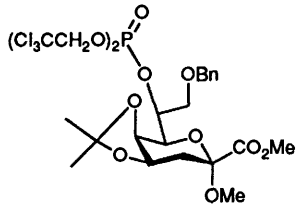
(6) R = CO_2Me
(7) R = CO_2^-

The alcoholic function of the protected ester/glycoside (**1**) was next phosphorylated with bis-(2,2,2-trichloroethyl) phosphorochloridate[17] in THF in the presence of *N*-methylimidazole; after chromatography, the phosphotriester (**12**) was obtained in 75% yield. Treatment of the latter with a mixture of zinc[17] and silver carbonate[18] in pyridine containing 10% of its volume of acetic acid, removed the trichloroethyl groups smoothly. The isopropylidene group was split off from the phosphorylated acetal (**13**) by short treatment with Dowex 50 (H^+) resin at room temperature, and the benzyl group by hydrogenolysis. The acid solution thus obtained was neutralized with aq. calcium hydroxide, and methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate 7-phosphate (**6**) was isolated as its Ca salt (60% from the phosphotriester).

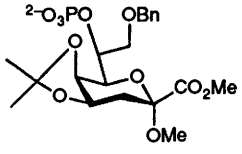
Phosphorylation of methyl 4,8-di-*O*-benzyl-3-deoxy-α-D-*manno*-oct-2-ulopyranosidono-1,5-lactone (**8**) with bis-(2,2,2-trichloroethyl) phosphorochloridate gave, after chromatographic purification, an 80% yield of the crystalline phosphotriester (**14**). Removal of the trichloroethyl groups under conditions similar to those used for the phosphotriester (**12**), including the chromatographic step, saponification of the lactone, and hydrogenolytic removal of the benzyl groups in an uninterrupted sequence gave a 75% yield of the phosphorylated acid (**7**) isolated as its Ca salt.



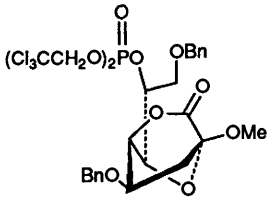
(**8**) R = R″ = Bn, R′ = H
(**9**) R = R″ = Bn, R′ = P(O)(OPh)$_2$
(**10**) R = R″ = H, R′ = PO_3H_2
(**11**) R = R′ = H, R″ = PO_3H_2
(**14**) R = R″ = Bn, R′ = P(O)(OCH$_2$CCl$_3$)$_2$



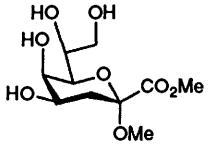
(**12**)



(**13**)



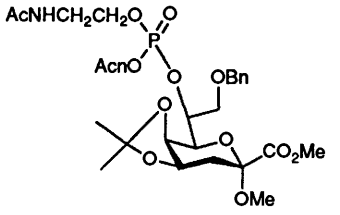
(**14**)



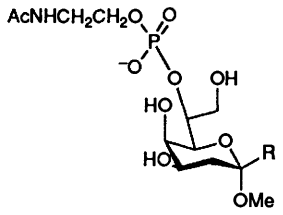
(**15**)

The most readily accessible starting material for the synthesis of KDO-pyranosides is the methyl ester of its α-methyl pyranoside, compound (**15**).[19–21] When calculated for this, the yields of the phosphorylated ester (**6**), *via* the 8-*O*-benzyl-4,5-*O*-isopropylidene derivative (**1**), in 7 steps, is 13%; that of the phosphorylated acid (**7**), *via* the lactone (**8**), in 5 steps, is 10%.

The phosphodiester (**18**) was prepared in three steps from the protected methyl ester methyl glycoside (**1**). This, when treated in succession, first with CEP-Im[14] and then with 2- acetamidoethanol, gave a mixture of the diastereoisomeric phosphotriesters (**16**) in only 29% yield. These were sufficiently stable to be isolated by column chromatography, but could not be freed of solvent as they decomposed above room temperature. The proposed structure is supported by the ^{1}H NMR spectra. The 'acetoinyl' group was then removed from the mixed phosphotriesters with triethylamine, the isopropylidene group by mild treatment with dil. trifluoroacetic acid (TFA), and the benzyl group by hydrogenolysis. The methyl ester (**17**) was then isolated and purified by chromatography (68%). Following saponification of the methyl ester group the unprotected phosphodiester (**18**) was isolated as its amorphous ammonium salt.



(**16**) Acn = CH(Me)COMe



(**17**) R = CO_2Me
(**18**) R = CO_2^-

Experimental

Organic solutions were dried with Na_2SO_4, and solvents were removed under reduced pressure at <45 °C. Column dimensions (height by diameter) are given in cm. Unless otherwise stated, column chromatography was carried out on silica gel (Merck 60, 70–230 mesh) TLC on Merck 60 F_{254} silica gel plates. Compounds were detected with sulphuric acid (10% in EtOH) and charring. M.p.s were determined on a Kofler block and are uncorrected. Solvent proportions are given v/v. To 'activate' Zn, commercial Zn powder was washed successively and rapidly with M-HCl and water, and was dried at 120 °C for a few hours. $[\alpha]_D$-Values were measured at 20 °C.

Methyl (*Methyl* 8-O-*Benzyl*-3-*deoxy*-4,5-O-*isopropylidene*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-(*benzyl* 1-(*Methyl*-2-*isopropyl Phosphate*) (**4**).—A solution of the alcohol[12] (**1**) (428 mg, 1.08 mmol) in anhydrous acetonitrile (1 ml) was added dropwise to a stirred solution of CEP-Im[13] (375 mg, 1.7 mol equiv.) in anhydrous acetonitrile (1 ml) under Ar at room temperature. Benzyl alcohol (4 ml) was added after 1 h and the mixture was stirred under Ar for 24 h. The product was extracted into diethyl ether (3 × 10 ml) (insoluble solids were discarded), and solvents were removed. Column (17 × 2.5) chromatography (diethyl ether–cyclohexane, 7:1) of the residue gave:

(i) 'acetoinyl dibenzyl phosphate' (**5**) (400 mg) identified by its ^{1}H NMR spectrum, δ_H($CDCl_3$; 90 MHz) 1.29 (3 H, d, *J* 6 Hz, *Me*CH), 2.0 (3 H, s, Ac), 4.59 (1 H, m, $J_{H,P}$ ~ 9 Hz), 4.94 (4 H, m, 2 × OC*H*$_2$Ph), and 7.20 (10 H, s, Ph).

(ii) a mixture (190 mg) of (**5**) (43% by NMR) of the diastereoisomeric phosphotriesters (**4**) (57%), and

(iii) an uncontaminated mixture of the diastereoisomeric phosphotriesters (**4**) (250 mg, 36%) characterized by their ^{1}H NMR spectrum, δ_H($CDCl_3$; 90 MHz) 1.2–1.5 (9 H, m, Me_2C, *Me*CH), 2.15 (5 H, m, Ac and 3-H$_2$), 3.1 (3 H, s, OMe), 3.72 (3 H, s, CO_2Me), 3.8–5.2 (11 H, m, 4-, 5-, 6-, and 7-H, 8-H$_2$, 2 × C*H*$_2$Ph, CHOP), and 7.25 (10 H, m, Ph).

(*Methyl* 3-*Deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-(*Calcium Hydrogen Phosphate*) (**7**).—(a) *From compound* (**4**). Triethylamine (825 μl) was added to a stirred solution of the mixed phosphotriesters (**4**) (0.59 mmol; determined by ^{1}H NMR spectroscopy) in a mixture of pyridine and water (1:1; 12

ml) kept at 35 °C for 4 h. The mixture was left at room temperature overnight. Solvents were removed, the residue was taken up in methanol (20 ml), Pd-on-C (10% Pd; 70 mg) was added, and the mixture was treated with hydrogen and stirred for 2 h. Solids were filtered off, and the filtrate was neutralized with triethylamine. The residue remaining after removal of volatile material was again taken up in methanol and treated with hydrogen and Pd-on-C (10%) overnight. The mixture was filtered, Dowex 50 (H^+) was added to, and removed from, the filtrate, and the acid mixture was kept at 40 °C: within 4 h a major product (R_F 0.23) was formed [TLC: propan-2-ol–aq. ammonia) (*d* 0.91)–water, 7:2:1]. The solution was neutralized with triethylamine (1%) in methanol–water (3:2), then concentrated, and the residue was purified by column (99 × 2.5) chromatography (Sephadex G10; 40–120 μ; prepared in MeOH–water, 2:1; eluted with MeOH–water–triethylamine, 40:20:0.1). A mixture of three products (200 mg), that having R_F 0.1 (TLC solvent as above) being quite predominant, was recovered. Column (12 × 1.5) chromatography (solvent as above) of the residue afforded the 7-phosphate of methyl (methyl 3-deoxy-α-D-*manno*-oct-2-ulopyranosid)onate 7-phosphate (**6**) was an oil (70 mg), characterized by its ^1H NMR spectrum, which was identical with that described below. The oil was dissolved in aq. NaOH (0.5M; 1.5 ml); after 2 h the solution was cooled (0 °C), decationized by cautious addition of Dowex AG 50Wx8 (H^+) resin, and filtered; its pH was then adjusted to 7.5 with saturated aq. $Ca(OH)_2$. The volume was reduced to *ca.* 0.2 ml. by evaporation, and the *Ca salt* (57 mg, 25%) was precipitated by addition of acetone, and washed with acetone (2 × 3 ml) (Found: C, 25.0; H, 4.4. $C_9H_{14}Ca_{1.5}O_{11}P\cdot 2.5H_2O$ requires C, 24.9; H, 4.4%); $[\alpha]_D^{20}$ + 39.9° (*c* 0.8, water); $\delta_H(D_2O$; 250 MHz) 1.81 (1 H, dd, J_{gem} 13, $J_{3a,4}$ 10.5 Hz, 3-H^a), 2.04 (1 H, dd, $J_{3e,4}$ 5 Hz, 3-H^e), 3.17 (3 H, s, OMe), 3.70 (1 H, ≈d, $J_{6,5} \approx 1$, $J_{6,7}$ 9.5 Hz, 6-H), 3.82 (1 H, dd, J_{gem} 12, $J_{8,7}$ 4 Hz, 8-H), 3.98 (1 H, dd, $J_{8',7}$ 2.5 Hz, 8′-H), and 4.09–4.29 (3 H, m, 4-, 5-, and 7-H).

(b) *From methyl*(4,6-*di*-O-*benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*ono*-1,5-*lactone* 7-*bis*(*trichloroethyl*) *phosphate* (**14**). Activated Zn powder (150 mg) and Ag_2CO_3 (5 mg) were added to a stirred solution of the bis(trichloroethyl) phosphate (**14**) (157 mg, 0.21 mmol) in pyridine containing 10% acetic acid (3 ml) and the reaction was allowed to proceed for 18 h at room temperature. Solids were filtered off and the solvents were removed from the pooled filtrate and washings. Column (120 × 2.5) chromatography [dichloromethane–methanol–ethyl acetate–water–conc. ammonia (*d* 0.91), 65:30:20:1:0.5] on Sephadex LH-20 gel gave an oil (170 mg), which was dissolved in MeOH (3 ml). M-NaOH (3 ml) was added and the mixture was stirred for 1 h; cations were then removed by addition of Dower AG50Wx8 (H^+) resin, and the solid was filtered off. Methanol (10 ml) and Pd-on-C (10% Pd; 60 mg) was added to the filtrate, which was then treated with H_2 until hydrogenation was complete (*ca.* 1 h). Solids were filtered off and washed with methanol, the pH of the filtrate was brought to 7.5 with saturated aq. $Ca(OH)_2$, and the volume was reduced to *ca.* 0.5 ml. Upon addition of acetone (3 ml) the solid title compound (60 mg, 75%) was formed, and was collected by centrifugation, washed with acetone (2 × 3 ml), and dried. The ^1H NMR spectrum was identical with that described under (a).

(*Methyl* 4,8-*Di*-O-*benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*ono*-1,5-*lactone* 7-(*Diphenyl Phosphate*) (**9**).—Imidazole (180 mg, 4.5 mol equiv.) and diphenyl phosphorochloridate (366 μl, 3 mol equiv.) were added to a stirred solution of the lactone (**8**) (240 mg, 0.6 mmol) in anhydrous THF (4 ml). The mixture was kept at 60 °C for 17 h, then cooled and diluted with diethyl ether (10 ml). Insoluble material was filtered off and washed with diethyl ether (15 ml). Filtrate and washings were pooled and washed with water (2 × 20 ml) and the aq. phases were back-extracted with diethyl ether (20 ml). Ethereal solutions were pooled, then dried, and the solvent was evaporated off. Column (7.3 × 2.1) chromatography (ethyl acetate–cyclohexane, 15:85) of the residue afforded the *phosphotriester* (**9**) (250 mg, 68%) as an oil (Found: C, 65.0; H, 5.5. $C_{35}H_{35}O_{10}P$ requires C, 65.0; H, 5.4%); $[\alpha]_D^{20}$ −6.9° (*c* 1.2, $CHCl_3$); $\delta_H(CDCl_3$; 200 MHz) 1.98 (1 H, dd, J_{gem} 15, $J_{3,4}$ 1.5 Hz, 3-H), 2.56 (1 H, dd, $J_{3',4}$ 9 Hz, 3-H′). 3.51 (3 H, s, OMe), 3.79 (1 H, dd, J_{gem} 11.5, $J_{8,7}$ 3 Hz, 8-H), 3.88 (2 H, m, 4-H and 8-H′), 4.21 (1 H, ≈d, $J_{6,5} \approx 0$–1, $J_{6,7}$ 9.5 Hz, 6-H), 4.32, 4.45, and 4.56 (4 H, 3 d, 2 × OCH_2Ph), 4.70 (1 H, m, 7-H), 4.85 (1 H, m, 5-H), and 7.31 (20 H, m, Ph).

Methyl (*Methyl* 8-O-*Benzyl*-3-*deoxy*-4,5-O-*isopropylidene*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-[*Bis*-(2,2,2-*trichloroethyl*) *Phosphate*] (**12**).—N-Methylimidazole (400 μl, 4 mol equiv.) and bis-(2,2,2-trichloroethyl) phosphorochloridate (960 mg, 2 mol equiv.) were added to a stirred solution of the alcohol (**1**) (430 mg, 1.09 mmol) in THF (25 ml) at 40 °C. After 1 h the mixture was cooled to room temperature, and deposited on a layer (3 × 5) of silica gel wetted with diethyl ether, which was then eluted with diethyl ether (500 ml). Fractions (30 ml) were collected. Those containing the phosphotriester (TLC: ethyl acetate–cyclohexane, 1:1; R_f 0.69) were pooled, and the solvent was removed. The crude product (650 mg) was purified by column (25 × 2.2) chromatography (ethyl acetate–cyclohexane, 3:7) to yield the *title compound* (600 mg) as an oil (Found: C, 39.3; H, 4.5. $C_{24}H_{31}Cl_6O_{11}P$ requires C, 39.0; H, 4.2%); $[\alpha]_D$ +16.7° (*c* 2, $CHCl_3$); $\delta_H(CDCl_3$; 200 MHz) 1.31 and 1.43 [2 × 3 H, 2 s, CMe_2], 1.91 (1 H, dd, J_{gem} 15, $J_{3,4}$ 2 Hz, 3-H), 2.78 (1 H, dd, $J_{3',4}$ 4 Hz, 3-H′), 3.17 (3 H, s, OMe), 3.80 (3 H, s, CO_2Me), 3.92 (1 H, dd, J_{gem} 11, $J_{8,7}$ 4 Hz, 8-H), 4.04 (1 H, dd, $J_{6,5}$ 2, $J_{6,7}$ 9 Hz, 6-H), 4.09 (1 H, dd, $J_{8',7} \approx 2.5$ Hz, 8-H′), 4.36 (1 H, dd, $J_{5,4}$ 8 Hz, 5-H), 4.47–4.74 (7 H, m, CH_2Ph, 2 × CH_2CCl_3, and 4-H), 4.99 (1 H, m, 7-H), and 7.29–7.42 (5 H, m, Ph).

Methyl (*Methyl* 3-*Deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)-*onate* 7-(*Calcium Hydrogen Phosphate*) (**6**).—Activated zinc dust (500 mg) and silver carbonate (50 mg) were added to a stirred solution of the phosphotriester (**12**) (500 mg, 0.68 mmol) in pyridine containing 10% acetic acid (10 ml). The mixture was stirred during 18 h at room temperature; it was then filtered and solvents were removed from the filtrate. Upon column (100 × 2.5) chromatography [dichloromethane–methanol–ethyl acetate–water–conc. ammonia (*d* 0.91) 650:300:200:10:5] of the residue on Sephadex LH-20 (Pharmacia Fine Chemicals), crude material (500 mg) having R_f 0.2 [TLC: propan-2-ol–aq. ammonia (*d* 0.91)–water, 8:1:1] was recovered from the pooled fractions (8 ml fraction^{-1}). This was dissolved in methanol (15 ml), IR 77 (H^+) resin was added, and the mixture was stirred at 40 °C for 1 h. The solid was filtered off, Pd-on-C (10% Pd) (50 mg) was added, and the mixture was treated with H_2 and stirred for 1 h. The catalyst was filtered off (sintered glass) and washed with MeOH. The pH of the methanolic solution was adjusted to 7.5 with saturated aq. $Ca(OH)_2$ and its volume was reduced to *ca.* 0.5 ml. Upon addition of acetone (5 ml) the *Ca salt* (**6**) precipitated; it was washed with acetone (2 × 5 ml) and dried (163 mg, 60%) (Found: C, 29.8; H, 5.5. $C_{10}H_{18}Ca_{0.5}O_{11}P\cdot 2H_2O$ requires C, 29.9; H, 5.5%); $[\alpha]_D$ +39.5° (*c* 1.1, water); $\delta_H(D_2O$; 250 MHz) 1.82 (1 H, dd, J_{gem} 13, $J_{3,4}$ 12 Hz, 3-H^a), 1.98 (1 H, dd, $J_{3e,4}$ 5 Hz, 3-H^e), 3.18 (3 H, s, OMe), 3.79 (3 H, s, CO_2Me), 3.79 (1 H, dd, J_{gem} 12, $J_{8,7}$ 3 Hz, 8-H), 3.81 (1 H, d, $J_{6,7}$ 9 Hz, 6-H), 3.92 (1 H, dd, $J_{8',7}$ 2.5 Hz, 8-H′), 4.01 (1 H, d, $J_{5,4}$ 3 Hz, 5-H), 4.08 (1 H, ddd, 4-H), and 4.22 (1 H, tt, $J_{7,P} \approx 10$ Hz, 7-H).

(*Methyl* 4,8-*Di*-O-*benzyl*-3-*deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*ono*-1,5-*lactone* 7-[*Bis*(*trichloroethyl*) *Phosphate*] (**14**).—The lactone (**8**) (130 mg, 0.31 mmol) was phosphorylated under the same conditions as those used to prepare the phosphotriester (**12**). The crude product gave upon column (6.5 × 2.5) chromatography (ethyl acetate–cyclohexane, 2:8) of the crystalline *title compound* (190 mg, 80%), m.p. 120–123 °C (from Et_2O) (Found: C, 43.0; H, 4.0. $C_{27}H_{29}Cl_6O_{10}P$ requires C, 42.8; H, 3.8%); $[\alpha]_D$ −0.55° (*c* 0.7, $CHCl_3$); δ_H($CDCl_3$; 200 MHz) 2.04 (1 H, ≈d, J_{gem} 15, $J_{3,4}$ 2 Hz, 3-H), 2.59 (1 H, dd, $J_{3',4}$ 8 Hz, 3-H'), 3.54 (3 H, s, OMe), 3.77 (1 H, dd, J_{gem} 11.5, $J_{8,7}$ 4.5 Hz, 8-H), 3.92 (1 H, dd, $J_{8',7}$ 2 Hz, 8-H'), 3.96 (1 H, m, 4-H), 4.14 (1 H, br d, $J_{6,5}$ ≈ 0.1, $J_{6,7}$ 9 Hz, 6-H), 4.48–4.72 (9 H, m, 2 × OCH_2Ph, 2 × CH_2CCl_3, and 7-H), 5.11 (1 H, ≈s, $J_{5,4}$ ≈ 3.5 Hz, 5-H), and 7.34 (10 H, m, Ph).

Methyl (*Methyl* 8-O-*benzoyl*-3-*deoxy*-4,5-O-*isopropylidene*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-(2-*Acetamidoethyl* 1-*Methyl*-2-*oxopropyl Phosphate*) (**16**).—A solution of the benzyl ether (**1**) (813 mg, 2.05 mmol) in dry acetonitrile (1 ml) was added dropwise to a stirred solution of CEP-Im (464 mg, 1.1 mol equiv.) in dry acetonitrile (1 ml) under Ar. The stirred mixture was kept under Ar for 1 h, then 2-acetamidoethanol (215 μl, 1.13 mol equiv.) was added and the reaction mixture was kept at room temperature while being stirred for 42 h. The mixture was then extracted with diethyl ether (6 × 10 ml), the extract was evaporated, and the dry residue (900 mg) was submitted to column (12 × 2.5) chromatography [diethyl ether–cyclohexane, 3:1 (60 ml); diethyl ether (300 ml); diethyl ether–ethyl acetate, 5:1 (60 ml); diethyl ether–ethyl acetate, 5:2 (140 ml)]. The main product (TLC: $CHCl_3$–MeOH, 10:1; R_f 0.8) (397 mg, 29%) eluted with diethyl ether–ethyl acetate (5:1) was a mixture of diastereoisomers of the *title compound*. The material cannot be freed from solvent of crystallization as it decomposes upon heating; it can be conserved for some time at +4 °C (Found: C, 50.1; H, 6.9; N, 2.1. $C_{28}H_{42}NO_{13}P{\cdot}2H_2O$ requires C, 50.4, H, 6.9, N, 2.1%); δ_H($CDCl_3$; 250 MHz) 1.21–1.49 (9 H, m, Me_2C, *Me*CH), 1.81–2.23 (8 H, m, $MeCO_2$, MeCON, and 3-H_2), 3.08–3.2 (3 H, m, OMe), 3.41 (2 H, m, CH_2N), 3.75 (3 H, br s, CO_2Me), 3.80–4.68 (9 H, m, CH_2OP, C*H*$_2$Ph, 4-, 5-, and 6-H, and 8-H_2), 4.80 (2 H, m, CHOP and 7-H), and 7.23 (5 H, m, Ph).

Methyl (*Methyl* 3-*Deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-(2-*Acetamidoethyl Ammonium Phosphate*) (**17**).—Triethylamine (100 mg, 2 mol equiv.) was added to a stirred solution of the phosphotriester (**16**) (300 mg, 0.45 mmol) in a mixture of pyridine–water (1:1; 4 ml) at room temperature, and the mixture was stirred for 2 h. When hydrolysis of the 'acetoinyl' group appeared to be complete [TLC: $CHCl_3$–MeOH–aq. ammonia (*d* 0.91), 65:25:4; R_f of the main product 0.73], solvents were moved, and water (5 ml) was evaporated from the residual oil, which was then dissolved in methanol (2 ml). TFA (99%; 2 ml) was added and the mixture was stirred for 1 h at room temperature. Solvents were removed, and a mixture of CCl_4–MeOH (10:1) (3 × 10 ml) was added to and evaporated from the residue, which was then dissolved in methanol (5 ml). A few drops of dil. aq. ammonia (0.05M) were added to neutralize any residual TFA, then Pd-on-C catalyst (10% Pd; 50 mg) and the mixture was stirred under H_2 for 1 h. The catalyst was then filtered off, washed with methanol (3 × 2 ml), fresh catalyst (50 mg) was added, and hydrogenation was continued until hydrogenolysis of the benzyl group appeared [TLC: $CHCl_3$–MeOH–aq. ammonia (*d* 0.91), 65:25:4; R_f 0.2] to be complete (*ca.* 1.5 h). The catalyst was filtered off and washed, and the filtrate and washings were concentrated. Column (6.8 × 2) chromatography [$CHCl_3$–MeOH–aq. ammonia (*d* 0.91), 65:25:4 (100 ml); 60:30:4 (100 ml)] of the dry residue gave the *title compound* as an oil (130 mg, 68%) (Found: C, 38.0; H, 6.7; N, 5.9. $C_{14}H_{29}N_2O_{12}P$ requires C, 37.5; H, 6.5; N, 6.2%); $[\alpha]_D$ +62.2° (*c* 0.81, water); δ_H(D_2O; 250 MHz) 1.88 (1 H, ≈t, J_{gem} 13, $J_{3a,4}$ 12 Hz, 3-H^a), 2.00 (3 H, s, Ac), 2.04 (1 H, dd, J_{gem} 5 Hz, 3-H^e), 3.23 (3 H, s, OMe), 3.42 (2 H, m, CH_2N), 3.82 (3 H, s, CO_2Me), 3.78–4.06 (6 H, m, CH_2OP, 5- and 6-H, and 8-H_2), 4.12 (1 H, ddd, $J_{4,5}$ 3 Hz, 4-H), and 4.32 (1 H, m, 7-H).

(*Methyl* 3-*Deoxy*-α-D-manno-*oct*-2-*ulopyranosid*)*onate* 7-(2-*Acetamidoethyl Ammonium Phosphate*) (**18**).—Aq. NaOH (1M; 2.5 ml) was added to a stirred solution of the methyl ester (**17**) (130 mg, 0.29 mmol) in methanol (2.5 ml) and the mixture was stirred for 30 min. Dowex AG 50Wx8 (H^+) resin was added to the mixture, previously diluted with methanol–water (1:1; 10 ml), to remove cations. The resin was then filtered off and the filtrate was neutralized with M-aq. ammonia. Solvents were removed, and the residue was triturated with ethanol–acetone to produce a white solid, which was collected by centrifugation, washed with acetone (2 × 2 ml), and dried (131 mg, 91%) (Found: C, 31.4; H, 7.1; N, 8.4. $C_{13}H_{30}N_3O_{12}P{\cdot}2.5H_2O$ requires C, 31.4; H, 7.0; N, 8.5%); $[\alpha]_D$ +62.8° (*c* 0.65, water); δ_H(D_2O; 250 MHz) 1.76 (1 H, ≈t, J_{gem} 13, $J_{3a,4}$ 12 Hz, 3-H^a), 2.01 (3 H, s, Ac), 2.02 (1 H, dd, $J_{3e,4}$ 5 Hz, 3-H^e), 3.17 (3 H, s, OMe), 3.43 (2 H, m, CH_2N), 3.72–4.14 (7 H, m, CH_2OP, 4-, 5-, and 6-H, and 8-H_2), and 4.32 (1 H, m, 7-H).

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Paper 0/00915F
Received 27*th February* 1990
Accepted 31*st May* 1990