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SYNTHESIS OF PHOSPHONO ANALOGUES OF 3-DEOXY-D-*arabino*-HEPT-2-ULOSONIC ACID 7-PHOSPHATE*

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ABSTRACT

3,7,8-Trideoxy-8-phosphono-D-*arabino*-octulosonic acid and 7,8-dideoxy-8-phosphono-D-*gluco*-octulosonic acid were synthesised in six steps from known, protected derivatives of 2-deoxy-D-*arabino*-hexose and D-glucose. The protected 6-*aldehydo*-hexodialdoses were condensed with tetraethyl methylenediphosphonate, and hydrolysis of vinylphosphonates was effected indirectly by transesterification with bromotrimethylsilane. Cyanide addition to deprotected heptose phosphonates was followed by chlorate–vanadate oxidation to octulosonic acid derivatives. The corresponding 3,7-dideoxy-7-phosphono-D-*arabino*-heptulosonic acid and 7-deoxy-7-phosphono-D-*gluco*-heptulosonic acid were obtained by reaction of protected 6-bromo-6-deoxyhexoses with triethyl phosphite, followed by treatment with bromotrimethylsilane, hydrolysis with water, cyanide homologation, and chlorate–vanadate oxidation. All four, final phosphono compounds are competitive inhibitors of 3-dehydroquinate synthetase.

INTRODUCTION

In the aromatic biosynthetic pathway, 3-dehydroquinate synthetase catalyses, in a reaction dependent on NAD^+ and Co^{2+}, the cyclisation step leading from 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) to 3-dehydroquinate[1] (3-DHQ). Measurements of kinetic isotope effects have shown, as already suspected[2], that the first step of the cyclisation reaction is an oxidation of O-5 of DAHP[3] (Scheme 1). The terminal methylene group, resulting from the facilitated elimination of phosphate from the 5-keto intermediate, is then responsible for the condensation of C-7 with the C-2 carbonyl group, probably after reduction of the 5-keto group. This mechanism has been recently confirmed by Sprinson *et al.*[4,5].

In order to obtain direct evidence of the occurrence of an intermediate 5-keto

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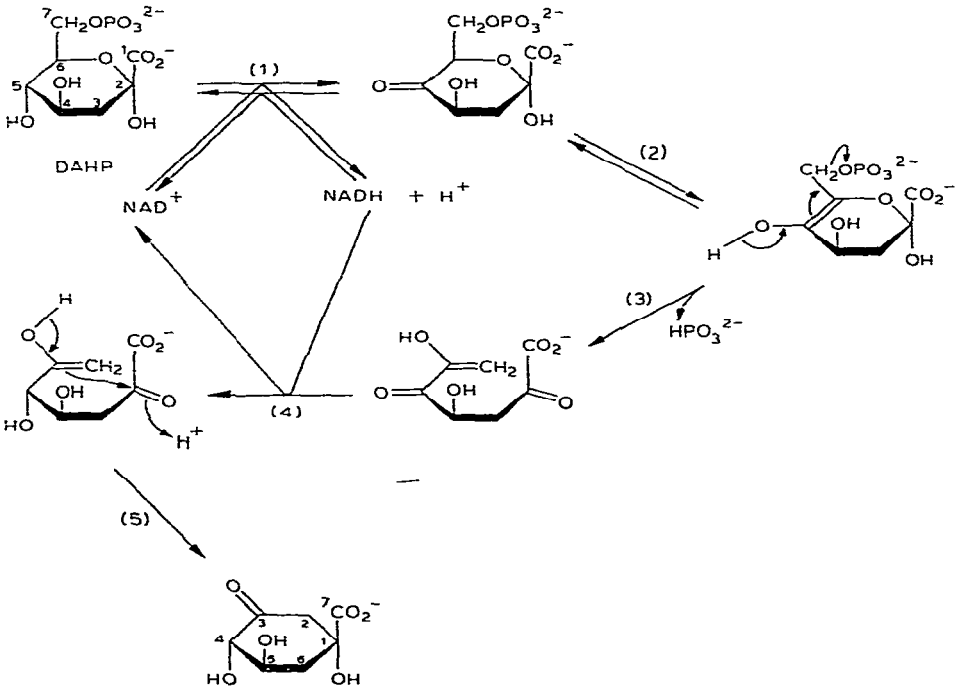


Scheme 1

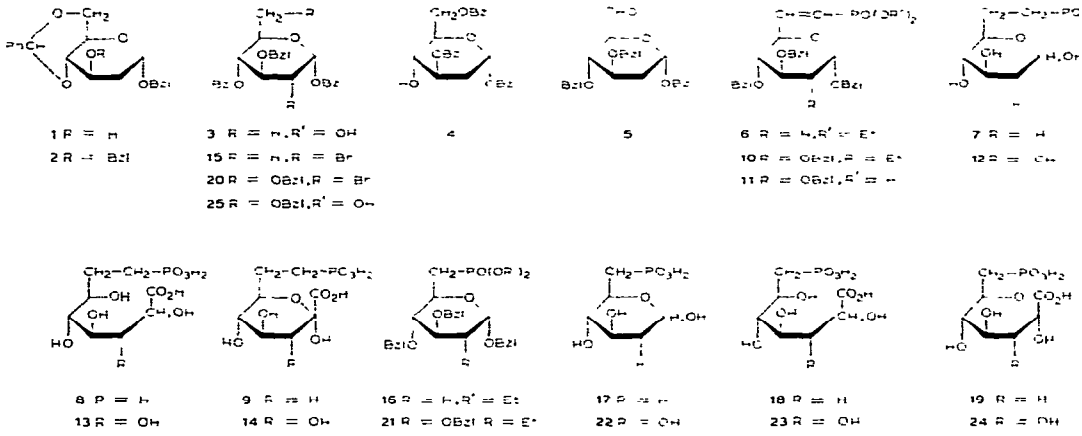
compound, we have synthesised phosphono analogues of DAHP, which would be unable to undergo the elimination of a phosphate group, but were expected to be substrates of the NAD^+-mediated oxidation, thus leading to an eventual accumulation of a 5-keto intermediate either in the free state or bound to the enzyme–NADH complex.

DISCUSSION

The synthesis of phosphono analogues of DAHP is based on that[6] for DAHP, but using the phosphono analogue of 2-deoxy-D-*arabino*-hexose 6-phosphate as substrate for cyanide addition and chlorate–vanadate oxidation.

The synthesis of 2,6,7-trideoxy-7-phosphono-D-*arabino*-heptose (**7**) constitutes a modification of the original homophosphonate synthesis of Jones and Moffatt[7]. In our procedure, the partially protected deoxyhexose **3** was oxidised[8] to the aldehydo compound **5**, which was then condensed with tetraethyl methylenediphosphonate[9] to give the vinylphosphonate **6**. The aldehydo compound could not be isolated crystalline, but the ^{1}H-n.m.r. data leave no doubt about its structure. After chromatography on silica gel, **6** was obtained in 74% yield as an oil that showed ^{1}H-n.m.r. data in agreement with the values expected for a *trans*-geometry of the vinyl group. The elimination of ethyl groups was effected by transesterification with bromotrimethylsilane[10] followed by hydrolysis of the product with water. Catalytic hydrogenation over 10%

Pd/C in methanol then yielded **7**, which was purified by ion-exchange chromatography and isolated as the barium salt in an overall yield (from **3**) of 11%. The free acid **7** can be easily recovered by exchange with a cationic resin. The ^{1}H-n.m.r. spectrum of **7** gave little information about the structure, but the specific rotation and the behavior in electrophoresis were closely similar to those of 2,6-dideoxy-6-phosphono-D-*arabino*-hexose. The amount of malonaldehyde formed in the periodate oxidation of **7**, as determined by the thiobarbituric acid reaction[11], agreed exactly with the phosphorus content.



Identical steps performed with the D-*gluco*-hexose derivative **25** gave a similar yield of the homophosphonic acid **12**, which is identical with the product described by Adams *et al.*[12] and is a substrate of D-glucose 6-phosphate dehydrogenase (K_m 0.1mM at pH 7.6).

The most critical step of the procedure used for the synthesis of **7** and **12** was the bromotrimethylsilane transesterification of the homophosphonate. Chlorotrimethylsilane (70°, several days) effected only monosilylation of the vinylphosphonic diester, and the monoethyl ester resulting from hydrolysis was isolated and identified by ^{1}H-n.m.r. spectroscopy. Only a small proportion of monosilylated compound was formed in the bromotrimethylsilane treatment (25°, 1.5 h), but an extension of the reaction time led to undesirable degradation products. The dimethyl vinylphosphonic esters, which were obtained by the reaction of tetramethyl methylenediphosphonate, were more easily transesterified[10] (no monoester detectable), but the reagent itself had to be prepared from the tetraethyl phosphono derivative.

Compound **7** and **12** were converted respectively into the 2-deoxy-D-*arabino*- and D-*gluco*-octulosonic acids **9** and **14**, in the usual way, by cyanhydrin formation, alkaline hydrolysis, and chlorate–vanadate oxidation, which were performed as described[6] for the synthesis of DAHP and gave the expected products with similar yields.

Compound **9** reacted in the periodate–thiobarbiturate test exactly as[11] DAHP,

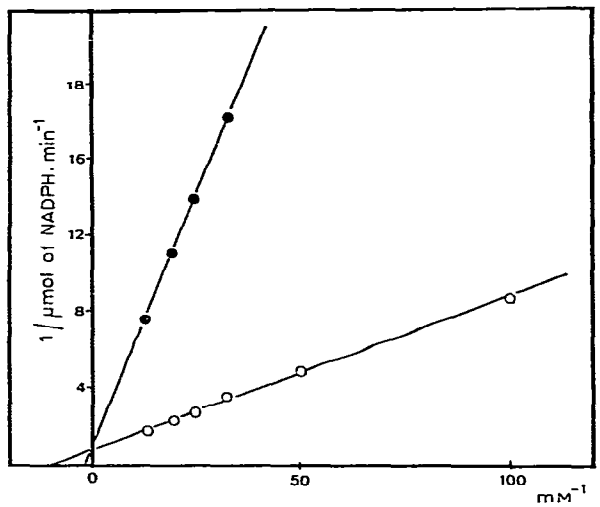


Fig. 1. Double reciprocal plot of the initial rate of D-glucose 6-phosphate dehydrogenase in the absence (—○—) or presence (—●—) of 6-deoxy-6-phosphono-D-glucose (2mM). The reaction mixture contained, in a final volume of 1 ml, triethanolamine buffer (pH 7.6, 0.1 mmol), $MgCl_2$ (10 µmol), NADP (0.5 µmol), and D-glucose 6-phosphate dehydrogenase (0.3 u).

and its electrophoretic and chromatographic migrations were similar. Moreover, **9** was identical with the product obtained in the enzymic condensation[13] of D-erythrose 4-homophosphonate with phosphoenolpyruvate in the presence of DAHP synthetase from *E. coli*.

Compound **12** could not be enzymically synthesised and did not react in the periodate–thiobarbiturate test, as expected for a *gluco* derivative. However, it migrated very similarly to the corresponding D-*gluco*-heptulosonate 7-phosphate[14] in all chromatographic and electrophoretic systems used. Both **9** and **12** behaved as weak, competitive inhibitors towards DAHP for 3-DHQ synthetase with respective K_i values[15] of 0.26 and 1.2mM.

The synthesis of the hexose phosphonates **17** and **22** was easily effected in 20% yield by the Michaelis–Arbuzov reaction[16,17] of the protected 6-bromo-6-deoxy-hexoses **15** and **20** with triethyl phosphite, followed by bromotrimethylsilane transesterification and hydrolysis. 2,6-Dideoxy-6-phosphono-D-*arabino*-hexose (**17**) was characterised by the usual periodate–thiobarbiturate reaction. 6-Deoxy-6-phosphono-D-glucose (**22**), in contrast to the homophosphonate derivative, behaved as a competitive inhibitor of D-glucose 6-phosphate dehydrogenase (K_i 0.4mM) (Fig. 1).

Application of cyanide addition and chlorate–vanadate oxidation to **17** and **22** yielded the heptulosonic acids **19** and **24**, characterised as described for the homophosphonic acids **9** and **14**. Compound **19** was identical with the product of the enzymic condensation of 4-deoxy-4-phosphono-D-erythrose with phosphoenolpyruvate in the presence of DAHP synthetase[13]. Both **19** and **24** were strong, competitive inhibitors of 3-dehydroquinate synthetase[15] with respective K_i values of 2.5 and 5µM.

EXPERIMENTAL

General — Melting points were determined on a Büchi apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. Elemental analyses were performed by the Service Central de Microanalyse du C.N.R.S., Gif-sur-Yvette. ^{1}H-N.m.r. spectra (internal Me_4Si) were recorded with a Perkin–Elmer R-32 spectrometer. Mass measurements were obtained with an AEI MS-50 mass spectrometer operating at 70 eV. T.l.c. was performed on Silica gel 60 F-254 or Cellulose F-254 (Merck). Paper electrophoresis was performed with a Gilson model D instrument, Whatman No. 3 paper, and a pyridinium acetate buffer (pH 4.0) (90 V/cm). Electrophoretic mobilities (M_{Pi}) were determined relative to that of inorganic phosphate. Detection on silica gel was effected by u.v. light and/or charring with sulfuric acid; phosphonates and phosphonic monoesters on cellulose plates were detected with the ferric chloride–sulfosalicylic acid reagent[6]. Phosphorus in phosphono compounds was determined[7] after heating with a $HClO_4$–H_2SO_4 (4:1) mixture for 5 h at 250°. K^{14}CN (45 mCi/mmol) was obtained from CEA, Saclay. D-Glucose 6-phosphate dehydrogenase Type VII was obtained from Sigma.

*Benzyl 4,6-O-benzylidene-2-deoxy-α-*D-arabino-*hexopyranoside* (**1**). — Benzyl 2-deoxy-α-D-*arabino*-hexopyranoside[8] (9 g) was dissolved in dry *N*,*N*-dimethylformamide (30 ml), and α,α-dimethoxytoluene[22] (5.2 g) and toluene-*p*-sulfonic acid (15 mg) were added. After heating (60°) for 90 min, the mixture was concentrated under diminished pressure. The residue was triturated with 0.1M sodium hydrogencarbonate (100 ml) at 100° for 5 min, and then cooled, collected, and crystallised from 1-propanol, to give **1** (8.6 g, 70%), m.p. 145–146°, $[\alpha]_D^{20}$ +90° (*c* 1, chloroform), R_F 0.16, (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.37 (ddd, 1 H, $J_{2a,1}$ ~3, $J_{2a,2e}$ ~13, $J_{2a,3}$ ~10 Hz, H-2*a*), 2.2 (ddd, 1 H, $J_{2e,3}$ ~5, $J_{2e,1}$ ~1.5 Hz, H-2*e*), 4.97 (ddd, 1 H, H-1), 5.53 (s, 1 H, PhC*H*), and 7.35 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{20}H_{22}O_5$: C, 70.17; H, 6.43. Found: C, 69.42; H, 6.39.

*Benzyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-*D-arabino-*hexopyranoside* (**2**). — A mixture of **1** (8 g) and NaH (2.2 g of a 50% dispersion in mineral oil) in dry *N*,*N*-dimethylformamide (35 ml) was stirred at 0° and a solution of benzyl bromide (3.8 g) in *N*,*N*-dimethylformamide (10 ml) was added during 30 min. After 6 h at room temperature, the excess of NaH was destroyed with ice–water (300 ml), and the precipitate was collected, washed with cold water, and crystallised from 2-propyl ether, to give **2** (8.4 g, 83%), m.p. 131°, $[\alpha]_D^{20}$ +102° (*c* 1, chloroform), R_F 0.74 (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.75 (ddd, 1 H, $J_{2a,1}$ ~2.5, $J_{2a,2e}$ ~13, $J_{2a,3}$ ~9 Hz, H-2*a*), 2.22 (ddd, 1 H, $J_{2e,1}$ ~1.5, $J_{2e,3}$ ~5 Hz, H-2*e*), 5.00 (dd, 1 H, H-1), 5.54 (s, 1 H, PhC*H*), and 7.35 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{27}H_{28}O_5$: C, 75.00; H, 6.48. Found: C, 74.81; H, 6.47.

*Benzyl 3,4-di-O-benzyl-2-deoxy-α-*D-arabino-*hexopyranoside* (**3**). — $LiAlH_4$ (2.7 g) was added in portions to a solution of **2** (8 g) in ether (80 ml) and dichloromethane (80 ml). $AlCl_3$ (8 g) in ether (80 ml) was added and the mixture was boiled under reflux for 90 min. The excess of hydride in the cooled reaction mixture was

decomposed with ethyl acetate (25 ml) and water (30 ml), and the resulting precipitate was removed. The filtrate was concentrated, leaving a syrup that was eluted from a column (40 × 2.5 cm) of silica gel (Merck H 60) with ethyl acetate–hexane (1:4). Benzyl 3,6-di-*O*-benzyl-2-deoxy-α-D-*arabino*-hexopyranoside (**4**, 750 mg) was first eluted and had m.p. 71°, R_F 0.44 (t.l.c., silica gel, chloroform). Compound **3** was eluted next, and isolated as a colorless syrup (6.25 g, 78%), $[\alpha]_D^{20}$ +53° (*c* 1.5, chloroform), R_F 0.5 (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.65 (ddd, 1 H, $J_{2a,1}$ ~3.5, $J_{2a,3}$ ~11.5, $J_{2a,2e}$ ~13.5 Hz, H-2*a*), 2.29 (ddd, 1 H, $J_{2e,1}$ ~1.5, $J_{2e,3}$ ~4.5 Hz, H-2*e*), 5.00 (dd, 1 H, H-1), and 7.20 (m, 15 H, 3 Ph).

*Benzyl 3,4-di-*O*-benzyl-2-deoxy-α-*D*-*arabino*-hexodialdo-1,5-pyranoside* (**5**). — A mixture of **3** (6 g), pyridine (1.5 ml), 85% phosphoric acid (0.85 ml), and dicyclohexylcarbodi-imide (15 g) in dimethyl sulfoxide (30 ml) was stirred at room temperature. After 6 h, the mixture was poured into a solution of oxalic acid (15 g) in methanol (35 ml) and stirred for 45 min. Sufficient ether was added to precipitate salts, which were then removed. The filtrate was concentrated, poured into water, and extracted with ether, to give **5** as a slightly yellow syrup (5.6 g, 93%), R_F 0.55 (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.98 (ddd, 1 H, $J_{2a,2e}$ ~14, $J_{2a,3}$ ~10, $J_{2a,1}$ ~3 Hz, H-2*a*), 2.56 (ddd, 1 H, $J_{2e,3}$ ~6, $J_{2e,1}$ ~2.5 Hz, H-2*e*), 5.2 (dd, 1 H, H-1), 7.24 (m, 15 H, 3 Ph), and 9.90 (s, 1 H, -C*H*=O).

*Benzyl 3,4-di-*O*-benzyl-2,6,7-trideoxy-7-diethoxyphosphinoyl-α-*D*-arabino-hept-6-trans-enopyranoside* (**6**). — NaH (0.54 g) was added to a cold solution of tetraethyl methylenediphosphonate[9] (3.2 g) in dry tetrahydrofuran. After stirring for 2 h, the mixture was added dropwise to a solution of crude **5** (5 g) in dry tetrahydrofuran (10 ml). After 4 h, the mixture was extracted with ether–water. The organic phase was dried (Na_2SO_4) and concentrated, and the yellow, syrupy residue was eluted from a column (40 × 2.5 cm) of silica gel (Merck H 60) with ethyl acetate–hexane (2:3), to give **6** as a clear syrup (4.8 g, 74%), $[\alpha]_D^{20}$ +56° (*c* 1.8, chloroform), R_F 0.22 (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.38 (t, 6 H, $J_{H,H}$ ~7 Hz, O-CH_2-CH_3), 1.74 (ddd, 1 H, $J_{2a,1}$ ~3, $J_{2a,3}$ ~11, $J_{2a,2e}$ ~13 Hz, H-2*a*), 2.40 (ddd, 1 H, $J_{2e,1}$ ~2, $J_{2e,3}$ ~4.5 Hz, H-2*e*), 4.14 (quin, 4 H, $J_{H,H}$ ~7, $J_{H,P}$ ~7 Hz, PO-CH_2-CH_3), 5.02 (dd, 1 H, H-1), 6.14 (ddd, 1 H, $J_{7,P}$ ~20.5, $J_{7,6}$ ~17, $J_{7,5}$ ~1.5 Hz, H-7), 6.94 (ddd, 1 H, $J_{6,P}$ ~22, $J_{6,5}$ ~6.5 Hz, H-6), and 7.34 (m, 15 H, 3 Ph). Mass spectrum: *m/z* 566 (M^+) and 91.

Anal. Calc. for $C_{32}H_{38}O_7P$: C, 67.80; H, 6.89; P, 5.48. Found: C, 66.08; H, 6.79; P, 5.72.

*2,6,7-Trideoxy-7-phosphono-*D*-arabino-heptose* (**7**). — Bromotrimethylsilane (1.3 ml) was stirred with **6** (1 g) with cooling and exclusion of moisture. The mixture was stored for 90 min at room temperature and concentrated to a viscous oil, which was treated with water (50 ml) at room temperature overnight. After concentration, the resulting vinylphosphonic acid was dissolved in methanol (20 ml) and hydrogenolysed over 10% Pd/C at room temperature and atmospheric pressure. The solution was filtered and concentrated, and a solution of the colorless, syrupy residue in water was adjusted to pH 8 with lithium hydroxide and applied to a column (20 ×

1.5 cm) of Dowex 1-X8 (Cl^-) resin (100–200 mesh). The column was washed with water and eluted with a linear gradient of HCl (0→0.02M, 1 litre). Elution of **7** was monitored by the periodate–thiobarbiturate assay[11]. The monoethyl ester (traces) was eluted with 5mM HCl and **7** with 12mM HCl. The fractions containing **7** were combined, concentrated to dryness, and stored *in vacuo* in the presence of solid sodium hydroxide to remove residual HCl. Addition of saturated barium hydroxide and cold ethanol gave the barium salt of **7** (0.61 g, 85%), $[\alpha]_D^{20}$ +58° (*c* 0.5, water; acidic form), R_F 0.3 (t.l.c.; cellulose; 1-propanol–ammonia–water, 6:3:1), M_{Pi} 0.65.

Anal. Calc. for $C_7H_{13}BaO_7P \cdot 1.5\ H_2O$: C, 20.79; H, 3.96; P, 7.67. Found: C, 20.97; H, 4.12; P, 8.00.

3,7,8-Trideoxy-8-phosphono-octonic acid (**8**). — A solution of $K^{14}CN$ (0.1 mCi) in water (0.5 ml) was added to **7** (0.19 g, potassium salt). After 1 h, KCN (65 mg) was added. The mixture was left for 40 h at 4° and then applied to a column of Amberlite IR-120 (H^+) resin (100–200 mesh). The filtrate was adjusted to pH 8.5 with barium hydroxide, heated for 15 min at 100°, and concentrated to 2 ml, and the barium salt of **8** (0.2 g, 74%) was precipitated by the addition of ethanol (10 ml). It had $[\alpha]_D^{20}$ +4° (*c* 0.5, water; acidic form), R_F 0.25 (t.l.c.; cellulose; 1-propanol–ammonia–water, 6:3:1), M_{Pi} 0.75.

Anal. Calc. for $C_8H_{14}BaO_9P \cdot 4.5\ H_2O$: C, 16.79; H, 4.00; P, 5.40. Found: C, 16.76; H, 3.73; P, 5.40.

3,7,8-Trideoxy-8-phosphono-D-arabino-*oct-2-ulosonic acid* (**9**). — A mixture of vanadium oxide (2.4 mg), sodium chlorate (28 mg), water (0.5 ml), and 85% phosphoric acid (0.035 ml) was stirred with the potassium salt of **8** [obtained by treatment of the barium salt (0.2 g) with potassium sulfate] for 3 days. The mixture was filtered through Amberlite IR-120 (H^+) resin (100–200 mesh), neutralised with lithium hydroxide, and applied to a column (20 × 1.5 cm) of Dowex 1-X8 (Cl^-) resin (100–200 mesh). The column was washed with water and then eluted with a linear gradient of 0→0.03M HCl (1 litre). Elution of **9** was monitored on the basis of the periodate–thiobarbiturate assay and radioactivity. The fractions containing **9** were neutralised with sodium hydroxide, applied to a similar column, and eluted, after washing with water, with a linear gradient of 0→0.5M ammonium bromide (600 ml). The fractions containing **9** were combined, concentrated to a small volume, and treated with barium bromide (5 equiv.) and ethanol (5 vol.), to give the barium salt of **9** (0.1 g, 50%), $[\alpha]_D^{20}$ +8° (*c* 0.5, water; acidic form), R_F 0.19 (t.l.c.; cellulose; 1-propanol–ammonia–water, 6:3:1), M_{Pi} 0.95.

Anal. Calc. for $C_8H_{12}BaO_9P \cdot 2\ H_2O$: C, 18.28; H, 3.05; P, 5.90. Found: C, 18.28; H, 3.01; P, 6.18.

*Benzyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy-7-diethoxyphosphinoyl-α-*D*-gluco-hept-6-trans-enopyranoside* (**10**). — Compound **10** was prepared (55%) from benzyl 2,3,4-tri-*O*-benzyl-α-D-*gluco*-hexodialdo-1,5-pyranoside[8,12], as described for **7**, and had $[\alpha]_D^{20}$ +75° (*c* 2, chloroform), R_F 0.5 (t.l.c., silica gel, chloroform). N.m.r. data ($CDCl_3$): δ 1.42 (t, 6 H, $J_{H,H}$ ~7 Hz, $POCH_2$-CH_3), 3.25 (dd, 1 H, $J_{3,4}$ ~9, $J_{3,2}$ ~9.5 Hz, H-3), 3.51 (dd, 1 H, $J_{4,5}$ ~3.5 Hz, H-4), 4.05 (quin, 4 H, $J_{H,H}$ ~7, $J_{H,P}$

~7 Hz, PO-CH_2-CH_3), 5.96 (ddd, 1 H, $J_{7,5}$ ~2, $J_{7,6}$ ~17, $J_{7,P}$ ~20 Hz, H-7), 6.85 (ddd, 1 H, $J_{6,5}$ ~4.5, $J_{6,P}$ ~24 Hz, H-6), and 7.31 (m, 20 H, 4 Ph). Mass spectrum: *m/z* 672 (M^+), 581 (M — 91), 565 (M — 107), and 91.

Anal. Calc. for $C_{39}H_{45}O_8P$: C, 69.64; H, 6.69. Found: C, 69.43; H, 6.51.

*Benzyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy-7-phosphono-α-*D-gluco*-hept-6-*trans*-eno-pyranoside* (**11**). — Compound **11**, prepared (90%) from **10** by treatment with bromotrimethylsilane and crystallised from benzene, had m.p. 173°, $[\alpha]_D^{20}$ +70° (*c* 0.5, chloroform).

Anal. Calc. for $C_{35}H_{37}O_8P$: C, 68.18; H, 6.00; P, 5.03. Found: C, 67.53; H, 6.17; P, 5.43.

*6,7-Dideoxy-7-phosphono-α-*D-gluco*-heptose* (**12**). — Compound **12**, prepared from **11** and precipitated as the barium salt in 90% yield, had $[\alpha]_D^{20}$ +61° (*c* 2, methanol; acidic form). M_{Pi} 0.65; lit.[12] $[\alpha]_D^{20}$ +61.9°.

Anal. Calc. for $C_7H_{13}BaO_8P \cdot 3\ H_2O$: C, 19.39; H, 4.38; P, 7.16. Found: C, 19.39; H, 4.37; P, 7.80.

7,8-Dideoxy-8-phosphono-octonic acid (**13**). — Compound **13**, prepared from **12** and precipitated as the barium salt in 95% yield, had M_{Pi} 0.75.

Anal. Calc. for $C_8H_{14}Ba_{1.5}O_{10}P \cdot 2\ H_2O$: C, 17.70; H, 3.30; P, 5.72. Found: C, 17.70; H, 3.77; P, 5.89.

*7,8-Dideoxy-8-phosphono-*D-gluco*-oct-2-ulosonic acid* (**14**). — Compound **14**, prepared from **13** and precipitated as the barium salt in 40% yield, had $[\alpha]_D^{20}$ +60° (*c* 0.5, water; acidic form), R_F 0.1 (t.l.c.; 1-propanol–ammonia–water, 6:3:1), M_{Pi} 0.95.

Anal. Calc. for $C_8H_{12}Ba_{1.5}O_{10}P \cdot 3\ H_2O$: C, 17.18; H, 3.22; P, 5.55. Found: C, 17.39; H, 3.91; P, 5.92.

*Benzyl 3,4-di-*O*-benzyl-6-bromo-2,6-dideoxy-α-*D-arabino*-hexopyranoside* (**15**). — To a mixture of **3** (4.5 g) and *N*-bromosuccinimide (3.6 g) in dichloromethane (100 ml) was added triphenyl phosphine (5.24 g) with stirring at 0°[21]. After boiling under reflux at 45° for 1 h, the mixture was filtered and concentrated, and the residue was extracted with water–ether. Concentration of the organic phase gave **15** as a pale-yellow syrup (4 g, 80%), $[\alpha]_D^{20}$ +161° (*c* 1, chloroform), R_F 0.6 (t.l.c.; silica gel; chloroform–methanol, 1:4).

Anal. Calc. for $C_{27}H_{29}BrO_4$: C, 65.19; H, 5.83. Found: C, 64.84; H, 5.79.

*Benzyl 3,4-di-*O*-benzyl-2,6-dideoxy-6-diethoxyphosphinoyl-α-*D-arabino*-hexopyranoside* (**16**). — A solution of **15** (4 g) in triethyl phosphite (25 ml) was boiled under reflux in a nitrogen atmosphere. The excess of triethyl phosphite was evaporated under diminished pressure at 90°, and the remaining oil was dissolved in benzene, applied to a column (40 × 2.5 cm) of silica gel, and eluted with benzene–chloroform (98:2), to give **16** as a pale-yellow oil (3 g, 67%), $[\alpha]_D^{20}$ +56° (*c* 1, chloroform), R_F 0.2 (t.l.c.; silica gel; benzene–chloroform, 98:2). N.m.r. data ($CDCl_3$): δ 1.27 (t, 6 H, $J_{H,H}$ ~7 Hz, POCH$_2$CH_3), 1.82 (ddd, 1 H, $J_{2a,1}$ ~3.5, $J_{2a,3}$ ~11, $J_{2a,2e}$ ~13 Hz, H-2*a*), 2.35 (ddd, 1 H, $J_{2e,1}$ ~2, $J_{2e,3}$ ~4.5 Hz, H-2*e*), 3.2 (t, 1 H, $J_{3,2}$

~8.5, $J_{3,4}$ ~8.5 Hz, H-3), 4.15 (quin, 4 H, $J_{H,H}$ ~7, $J_{H,P}$ ~7 Hz, PO-CH_2-CH$_3$), 5.0 (dd, 1 H, H-1), and 7.3 (s, 15 H, 3 Ph).

Anal. Calc. for $C_{31}H_{39}O_7P$: C, 67.15; H, 7.04; P, 5.59. Found: C, 67.22: H, 7.24; P, 5.58.

2,6-Dideoxy-6-phosphono-D-arabino-*hexopyranoside* (**17**). — Compound **16** (3 g) was treated with bromotrimethylsilane (4 ml) as described for **7**. The resulting phosphonic acid was dissolved in methanol and hydrogenolysed over 10% Pd/C at atmospheric pressure to give **17**, which was purified as described for **7** and converted into the barium salt (0.88 g, 40%), $[\alpha]_D^{20}$ +31° (*c* 0.5, *water; acidic form*), M_{Pi} 0.65.

Anal. Calc. for $C_6H_{11}BaO_7P \cdot 3\ H_2O$: C, 17.39; H, 4.17; P, 7.50. Found: C, 17.26; H, 4.07; P, 7.43.

3,7-Dideoxy-7-phosphono-heptonic acid (**18**). — Treatment of **17** (0.8 g, barium salt) with $K^{14}CN$ (0.1 mCi), as described for **8**, gave **18**, isolated as the barium salt (0.8 g, 80%), $[\alpha]_D^{20}$ +2° (*c* 0.5, water; acidic form), M_{Pi} 0.75.

Anal. Calc. for $C_7H_{12}Ba_{1.5}O_9P \cdot 3\ H_2O$: C, 15.83; H, 2.26; P, 5.84; Found: C, 15.80; H, 2.42; P, 6.00.

3,7-Dideoxy-7-phosphono-D-arabino-*hept-2-ulosonic acid* (**19**). — Selective oxidation of **18** (0.8 g) with chlorate–vanadate and purification, as described for **9**, gave **19**, which was precipitated as the barium salt (0.35 g, 40%), $[\alpha]_D^{20}$ +52° (*c* 0.5, water; acidic form), R_F 0.2 (t.l.c.; cellulose; 1-propanol–ammonia–water, 6:3:1), M_{Pi} 0.90.

Anal. Calc. for $C_7H_{10}Ba_{1.5}O_9P \cdot 4\ H_2O$: C, 15.37: H, 3.29; P, 5.67. Found: C, 15.50; H, 3.32; P, 5.90.

*Benzyl 2,3,4-tri-*O*-benzyl-6-bromo-6-deoxy-α-*D*-glucopyranoside*[21] (**20**). — Compound **20**, prepared from benzyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside as described[21] for **15**, had $[\alpha]_D^{20}$ +69° (*c* 1, chloroform).

Anal. Calc. for $C_{34}H_{35}BrO_5$: C, 67.66; H, 5.80. Found: C, 67.78; H, 5.89.

*Benzyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-diethoxyphosphinoyl-α-*D*-glucopyranoside* (**21**). — Prepared from **20** as described for **16**, **21** (50%) had m.p. 50°, $[\alpha]_D^{20}$ +65° (*c* 1, chloroform).

Anal. Calc. for $C_{38}H_{45}O_8P$: C, 69.09; H, 6.82; P, 4.69. Found: C, 69.33; H, 6.95; P, 5.17.

6-Deoxy-6-phosphono-D-*glucopyranose* (**22**). — Prepared from **21** as described for **17**, **22** (45%), after precipitation as the barium salt, had $[\alpha]_D^{20}$ +28° (*c* 0.5, water; acidic form), M_{Pi} 0.65.

Anal. Calc. for $C_6H_{11}BaO_8P$: C, 18.99; H, 2.90; P, 8.18. Found: C, 19.48: H, 2.63; P, 8.17.

7-Deoxy-7-phosphonoheptonic acid (**23**). — Prepared from **22** as described for **18** and precipitated as the barium salt (90%), **23** had $[\alpha]_D^{20}$ +9° (*c* 0.5, water; acidic form), M_{Pi} 0.75.

Anal. Calc. for $C_7H_{12}Ba_{1.5}O_{10}P \cdot 2\ H_2O$: C, 15.89; H, 3.02; P, 5.86. Found: C, 15.75; H, 3.13; P, 5.92.

7-Deoxy-7-phosphono-D-gluco-*hept-2-ulosonic acid* (**24**). — Prepared from **23** as described for **19** and precipitated as the barium salt (45%), **24** had $[\alpha]_D^{20}$ +11° (*c* 0.5, water; acidic form), R_F 0.12 (t.l.c.; cellulose; 1-propanol–ammonia–water, 6:3:1), M_{P_i} 0.92.

Anal. Calc. for $C_7H_{10}Ba_{1.5}O_{10}P \cdot H_2O$: C, 16.52; H, 2.36; P, 6.13. Found: C, 16.63; H, 2.62; P, 6.22.

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