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ENANTIOSPECIFIC SYNTHESES OF 3,4-DIDEOXY-OCT-2-ULOSONIC ACIDS

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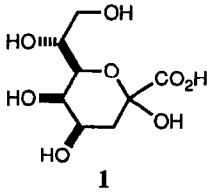
Abstract—Ethyl 5,6,7,8-tetra-*O*-acetyl-3,4-dideoxy-D-*arabino*-oct-2-ulosonate and ethyl 3,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-D-*arabino*-oct-2-ulosonate have been synthesised from peracetylated and bisacetonated *aldehydo*-D-arabinose respectively by a two stage procedure: Wittig reaction and catalytic hydrogenation. Deprotection of the blocked ethyl oct-2-ulosonate afforded the 3,4-dideoxy-D-*arabino*-oct-2-ulosonic acid (4-deoxy-KDO), isolated as its calcium salt in an overall yield of 37% from D-arabinose. Likewise reactions of D-xylose gave calcium 3,4-dideoxy-D-*xylo*-oct-2-ulosonate in an overall yield of 34%. Both calcium salts were derivatised as crystalline quinoxaline tetraacetates. Other routes attempted are also described.

3-Deoxy-D-*manno*-oct-2-ulosonic acid (KDO) **1**, initially and erroneously referred as 2-keto-3-deoxy-octonic acid which led to the abbreviated form KDO, is indispensable to the biosynthesis of the Gram-negative bacterial lipopolysaccharide (LPS).[1] Accordingly, compounds that disrupt the KDO metabolism would lead to a selective inhibition of the bacterial LPS biosynthesis and may therefore present a new class of antibacterial agents.[2] Work has already focused on the inhibition of the enzyme, cytidine 5'-monophospho-3-deoxy-D-*manno*-oct-2-ulosonic acid (CMP-KDO) synthase, which catalyses the condensation of KDO with CTP to give CMP-KDO in the LPS biosynthesis.[3] Recently, 2-deoxy-KDO has been synthesised and shown to be an excellent inhibitor of CMP-KDO synthase.[4] We would therefore like to explore the enzyme inhibitory potential of other deoxy-analogues of KDO and this paper discloses, in detail, facile and simple syntheses of calcium salts of 3,4-dideoxy-D-*arabino*-oct-2-ulosonic acid (calcium 4-deoxy-KDO) **2** and 3,4-dideoxy-D-*xylo*-oct-2-ulosonic acid (calcium 4-deoxy-*xylo*-KDO) **3**. Other routes attempted are also described. A preliminary account on part of the work has appeared.[5]



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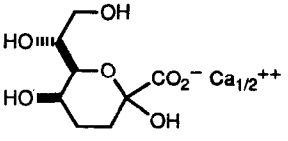

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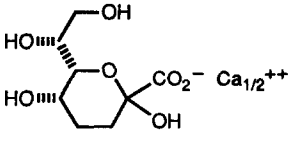
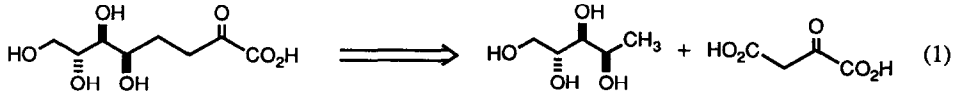


3

Most workers have synthesised analogues of KDO by a stereoselective aldol reaction[6] between oxalacetic acid or its equivalents and an appropriate D-arabinose derivative.[7] This approach, however, cannot be applied to the preparation of 4-deoxy-KDO **4** because, as shown by the retrosynthetic analysis in equation (1), there is no carbonyl group in **5** for attack by oxalacetic acid.



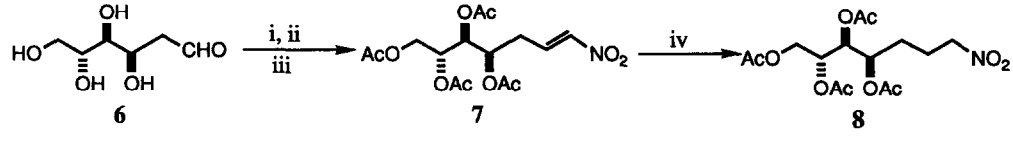
(1)

4 **5**

Route 1: attempted "6 + 1 + 1" synthesis

Since D-mannose has been chain-lengthened to KDO by sequential Fisher-Sowden nitromethane, Nef, and Kiliani reactions,[8] substitution of D-mannose by 2-deoxy-D-glucose should provide 4-deoxy-KDO *via* the same reaction sequence. Our first attempt towards 4-deoxy-KDO follows this strategy. Thus, using the well established procedure,[8] nitromethane was added to 2-deoxy-D-glucose **6** in methanol containing a stoichiometric amount of NaOMe to give, after decationisation, a syrupy mixture of epimeric deoxy-nitroalditols. These alcohols were acetylated and then heated with sodium hydrogen carbonate in benzene (Rutz reaction)[9] to give exclusively the *E*-nitroheptene **7** in 9% yield (Scheme 1). The low yield may be attributed to the high solubility of the sodium nitroalditols in the initial nitromethane reaction mixture, since only a slight precipitation of the former occurred. The nitromethane addition is known to be a reversible reaction,[10] thus in the equilibrium system containing 2-deoxy-D-glucose **6**, nitromethane, and NaOMe, a small amount of precipitation (sodium nitroalditols) would not push the equilibrium to completion and would lead to low yields. This solubility problem was reported previously when D-glucose was used as the substrate and the corresponding nitroalkene was isolated in only 5% overall yield.[11]

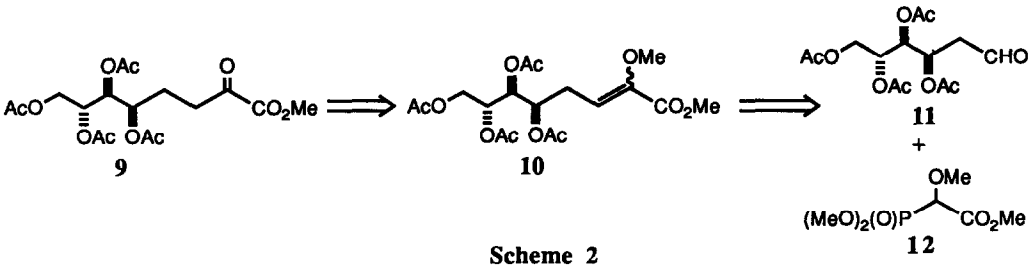
Selective hydrogenation of the double bond in **7** over palladium-on-charcoal afforded the nitroalkane **8** in 80% yield. Since **8** was obtained from **6** in an overall yield of only 7.2%, it was necessary to seek a higher yielding route to 4-deoxy-KDO and this approach was therefore abandoned.



Scheme 1. *Reagents*: i, $MeNO_2$, NaOMe, MeOH; ii, Ac_2O; iii, $NaHCO_3$, benzene; iv, H_2, Pd/C.

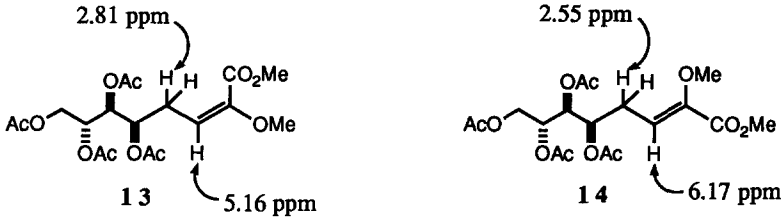
Route 2: attempted "6 + 2" synthesis

Functional group interconversion of the keto-group in the blocked 4-deoxy-KDO **9** to the enol ether **10** permits logical dissection of the bond between C-2 and C-3 to give the *aldehydo*-sugar **11**[12] and the functionalised phosphonate **12**[13] (Scheme 2). Our second attempt towards the construction of 4-deoxy-KDO follows this approach. Thus Wadsworth-Emmon-Horner alkenation of the aldehyde **11**[12] with the sodium salt of trimethyl(α-methoxy)phosphonoacetate **12** in tetrahydrofuran (THF) gave a syrupy mixture. Fractionation by chromatography afforded, after recrystallisation, *E*- and *Z*-vinyl ethers, **13** and **14**, in a ratio of 4 : 5 and in a combined yield of 72%. Phosphonoacetates substituted at the α-position were reported to induce steric interaction in the product, causing a loss of stereoselectivity which led to a mixture of geometric isomers.[14]



Scheme 2

The configuration of the vinyl ethers were assigned by an analysis of the chemical shifts of the β-olefinic and the deoxy-methylene protons. The highfield two-proton signals were assigned to the two H-4 methylene protons and the lowfield triplets in each spectrum were ascribed to the β-olefinic H-3 protons; a conclusion verified by irradiating the H-4 signals, which caused the H-3 triplets to collapse to singlets. The methylene H-4 protons in the *E*-isomer and the β-alkenic proton in the *Z*-isomer are in close proximity to the carbonyl deshielding cone and therefore would be expected to be relatively deshielded. Additionally, the *cis*-arrangement of the methoxycarbonyl group and the polyol chain in **13** probably distorts, by non-bonded steric interaction, the coplanar disposition of the conjugated π electron system and thereby reduces the deshielding caused by mesomeric effect of the carbonyl group.[15] Thus the β-alkenic proton of **13** would be expected to resonate at a higher field than that of **14**. In this way, the less polar component whose spectrum displaced a lower field β-olefinic proton and higher field β-methylene protons was assigned as the *Z*-isomer **14**.



Zemplen deacetylation[16] of the *Z*-isomer **14** gave the expected tetraol **15** in 87% yield whereas similar treatment of the *E*-isomer **13** proceeded with not only deacetylation but also with concommitant lactonisation and the product was the δ-lactone **16**. Thus the stereochemistry of the two isomers assigned earlier was confirmed.

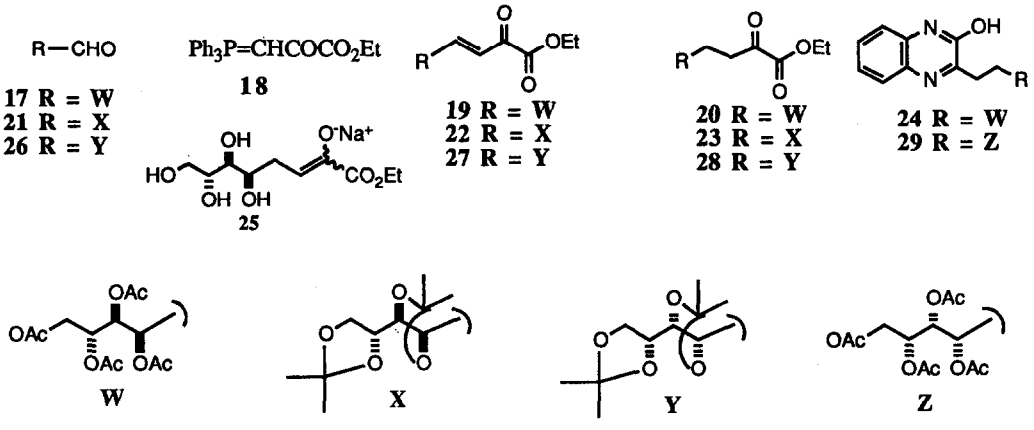
Hydrolysis of the enol ether moiety in **15** or **16** to give 4-deoxy-KDO **4**, although straightforward in principle, proved troublesome in practice. Attempted hydrolysis of either compound at room temperature in a variety of acid catalysts—H^+ ion-exchange resin, 1 N-HCl, 1 N-perchloric acid, 10% aqueous TFA or 1 N-H_2SO_4—resulted in almost quantitative recovery of

the starting material and none of the desired product was detected. Treatment of either compound at 100 °C with 2N-H_2SO_4 gave a complex mixture from which no pure compound could be isolated. Attempts to trap the 4-deoxy-KDO from the mixture as its quinoxaline derivative also failed. This route was abandoned because a simple synthesis of the target molecule was achieved using the following approach.

Route 3: successful "5 + 3" synthesis

The key step of this route was the condensation reaction between the tetra-*O*-acetyl-*aldehydo*-D-arabinose **17**[17] with the functionalised Wittig reagent, (ethoxyoxalyl)methylene-triphenylphosphorane **18**,[18] which is not commercially available. This ylid **18** was prepared previously by Zhdanov and Uzlova *via* quarternisation of triphenylphosphine with ethyl bromopyruvate in benzene, followed by neutralisation of the resulting phosphonium salt with aqueous ammonia. It was isolated as precipitate in only 26% yield with m.p. 169—171 °C.[18] By employing a modified procedure in this work, the ylid **18** could be synthesised from commercially available ethyl bromopyruvate in good yields (see experimental). After recrystallisation from aqueous ethanol, it had m.p. 182—183 °C and the yield was 75%.

Condensation of the *aldehydo*-pentose **17**,[17] readily obtainable from D-arabinose in three steps, with ylid **18** in boiling benzene solution gave the crystalline (*E*)-enone **19** as a sole product in 73% yield. The presence of an enone system was suggested by the relevant ν_{max} 1685, 1640 cm^{-1} and λ_{max} 232 nm (ε 6.5 × 10^3) absorptions. The structure was supported by a satisfactory elemental analysis and a well resolved ^{1}H-NMR spectrum. The double bond in **19** was selectively hydrogenated over palladium-on-charcoal in ethyl acetate to give the α-keto-ester **20** in practically quantitative yield. Careful monitoring of the hydrogen up-take was important, since prolonged hydrogenation led to the reduction of the keto-group to the corresponding diastereoisomeric alcohols, which could however be oxidised as a mixture with manganese dioxide in methylene chloride to the desired protected 4-deoxy-KDO **20**.



In order to find an optimised procedure for preparing 4-deoxy-KDO on the basis of good yields, ease of handling of intermediates, and the smoothness of deprotection, the reaction sequence was repeated with an arabinose derivative containing acid labile isopropylidene groups. Thus Wittig reaction of the diacetone-*aldehydo*-D-arabinose **21**,[19] readily available

from D-arabinose in three steps, with ylid **18** in boiling toluene solution afforded the *E*-enone **22** in 68% yield. The *Z*-isomer was not detected. The presence of the conjugate carbonyl system was clearly indicated by i.r. and u.v. spectral evidence. In the ^{1}H-NMR spectrum of **22**, the large *J* value (16 Hz) confirmed the *E*-geometry of the double bond.

Selective reduction of the double bond in **22** by careful monitoring of the hydrogen uptake (1 eq.) furnished the saturated ketoester **23** in quantitative yield. Deacetonation of **23** with aqueous trifluoroacetic acid afforded a syrupy product which was saponified with calcium hydroxide to give amorphous calcium 4-deoxy-KDO **2** in 83% yield. Thus the target molecule **2** was afforded in seven steps from D-arabinose in an overall yield of 37%. The calcium salt was decationised and then treated with *o*-phenylenediamine in methanol followed by standard acetylation afforded the quinoxaline tetraacetate derivative **24** in 73% yield.

On the other hand, deprotection of the peracetyl oct-2-ulosonate **20** was carried out under basic conditions with sodium ethoxide in ethanol to yield an extremely hygroscopic yellow solid, tentatively assigned as the enolate anion **25** of which the ^{1}H-NMR spectrum showed the absence of acetoxy-methyl signals. It is noteworthy that *ca.* 1.1 eq. of sodium ethoxide had to be added before the compound **25** started to precipitate from the reaction mixture (*c.f.* the normal conditions of Zemplen deacetylation in which only a catalytic amount of base would suffice). The fact the yellow solid was converted into the quinoxaline tetraacetate derivative in a yield of only 25% suggested the compound was unstable in an alkaline solution and concomitant side-reactions such as aldol condensation or Claisen condensation might have been occurred.

With the simple synthetic route to 4-deoxy-KDO developed at hand, D-xylose was also converted, *via* intermediates (**26**[19] → **27** → **28**) containing acid labile blocking groups, into the corresponding calcium 4-deoxy-D-*xylo*-KDO **3** as an amorphous solid in an overall yield of 34%. This salt **3** was also characterised as its crystalline quinoxaline tetraacetate **29**.

The present approach not only provides simple and facile entry to configurational and modified isomers of deoxy-KDO analogues, but also offers opportunities for facile syntheses of other α-keto-acids including *N*-acetylneuraminic acid derivatives in particular and 3,4-dideoxy-2-ulosonic acids in general.

Experimental

General: see T. K. M. Shing, Y.-X. Cui and Y. Tang, *Tetrahedron*, 1992, 2349.

***E*-4,5,6,7-Tetraacetoxy-D-*arabino*-1-nitro-1-heptene 7**

A solution of sodium (2.8 g) in dry methanol (65 ml) was added to a suspension of 2-deoxy-D-*arabino*-hexose (Sigma Chem. Co., 10 g) in dry methanol and nitromethane (30 ml). The mixture was shaken for 24 h and then diluted with diethyl ether (100 ml). The precipitated sodium *aci*-nitroheptitols were collected by filtration and washed with cold methanol (20 ml). The product was immediately dissolved in cold water (200 ml) and the solution quickly passed down a column of IR-120 resin (H^+, 200 ml). The effluent and washings (*ca.* 600 ml) were concentrated to a red syrup (8.7 g) which was treated with acetic anhydride (70 ml) containing three drops of concentrated sulphuric acid. When solution was complete, the mixture was heated on a steam bath for 0.5 h, cooled and poured into ice and water (1 litre). The syrupy product was extracted into chloroform (2 × 150 ml) and the combined extracts were washed with water (2 × 100 ml), dried, and concentrated. The syrupy residue (12.1 g) was dissolved in benzene (200 ml), and the solution heated under reflux with sodium hydrogen carbonate (15 g) for 4 h. The cooled mixture was filtered and the filtrate concentrated to a syrup which was dissolved in hot ethanol (75 ml) and decoloursied with activated charcoal (2 g). The filtrate on cooling to 0 °C, and on scratching with a glass rod, gave yellow crystals. Recrystallisation from

absolute ethanol afforded the nitroheptene **7** (2.1 g, 9%) as colourless needles with m.p. 108 °C, $[\alpha]_D^{19}$ + 32.2° (*c* 2.1, $CHCl_3$); R_F 0.60 [petroleum ether-diethyl ether 1:3 v/v)]; ν_{max} 1730-1760 (C=O), 1655 (C=C), 1520 $cm^{-1}(NO_2)$; λ_{max} 232 nm (ε 1.5 × 10^4); δ 2.10, 2.11, 2.13, 2.17 (4 AcO, 4 s), 2.48-2.68 (2H, m) 4.19 (1H, dd, *J* 12.5 and 4 Hz),), 4.35 (1H, dd, *J* 12.5 and 2.6 Hz),), 5.14-5.48 (3H, m), 7.08 (1H, d, *J* 13.5 Hz), 7.32 (1H, td, *J* 13.5, 7 and 7 Hz). (Found: C, 47.9; H, 5.7; N, 3.7%. $C_{15}H_{21}NO_{10}$ requires C, 48.0; H, 5.6; N, 3.7%).

4,5,6,7-Tetra-*O*-acetyl-1,2,3-trideoxy-1-nitro-D-*arabino*-heptitol 8

To a suspension of palladium-on-charcoal (200 mg, 5% w/w) in absolute EtOH (10 ml) under H_2 at atmospheric pressure was added a solution of the nitroheptene **7** (1.5 g, 0.11 mmol) in the same solvent (35 ml). The mixture was stirred for 1 h at room temp., then filtered, and the catalyst washed with absolute EtOH (5 ml). The combined filtrate and washings were concentrated to give the crystalline heptitol. Recrystallisation from ethyl acetate-hexane provided the analytical sample **8** (1.2 g, 80%) with m.p. 83—85 °C, $[\alpha]_D^{21}$ + 30.0° (*c* 1.8, $CHCl_3$); R_F 0.50 [petroleum ether-diethyl ether 1:3 v/v)]; ν_{max} 1735-1745 (C=O), 1550 $cm^{-1}(NO_2)$; δ 1.46-2.00 (4H, m, obscured by the AcO signals), 2.04 (6H, s), 2.07, 2.13 (2 s,), 4.15 (1H, dd, *J* 12.5 and 4 Hz), 4.29 (1H, *J* 12.5 and 2.2 Hz), 4.46 (1H, t, *J* 6.5 Hz), 5.05-5.25 (2H, m), 5.34 (1H, dd, *J* 8.5 and 2.7 Hz). (Found: C, 48.0; H, 6.2; N, 3.8%. $C_{15}H_{23}NO_{10}$ requires C, 47.7; H, 6.1; N, 3.7%).

Methyl *E*- and *Z*-5,6,7,8-tetra-*O*-acetyl-3,4-dideoxy-2-*O*-methyl-D-*arabino*-oct-2-enonates 13 and 14

A solution of trimethyl (α-methoxy)phosphonoacetate **12**[13] (6.3 g) in dry THF was added dropwise over a period of 1 h to a suspension of sodium hydride (80% dispersion in oil, 0.81 g) in dry THF (50 ml) at room temperature. After stirring for 30 min, a solution of the aldehyde (8.9 g) in dry THF (60 ml) was added slowly at room temperature over a period of 1 h, during which time a gummy precipitate appeared. The solution was stirred for a further 30 min and taken up in cold water (300 ml). The aqueous solution was extracted with chloroform (3 × 100 ml) and the combined dried extracts were concentrated to a syrupy mixture which was fractionated by chromatography to give firstly the less polar *Z*-isomer contaminated with the unreacted aldehyde. Recrystallisation from diethyl ether-hexane afforded the pure *Z*-isomer **14** (3.6 g, 32%) as white plates, m.p. 67—68 °C, $[\alpha]_D^{18}$ + 21.4° (*c* 0.7, $CHCl_3$); R_F 0.35 [petroleum ether-diethyl ether 1:2 v/v)]; $\nu_{max.}$ 1740-1760 (C=O), 1665, 1650 cm^{-1}(C=C); $\lambda_{max.}$ 223 nm (ε 9.7 × 10^3); δ 2.06 (9H, s), 2.17 (3H, s), 2.55 (2H, t, *J* 7.5 Hz), 3.70 (s, OMe), 3.82 (s, ester OMe), 4.14 (1H, dd, *J* 13 and 4.5 Hz), 4.34 (1H, *J* 13 and 3 Hz), 5.12-5.42 (3H, m), 6.17 (1H, t, *J* 7.5 Hz). (Found: C, 51.6; H, 6.2. $C_{18}H_{26}O_{11}$ requires C, 51.7; H, 6.3%).

Fractions containing the more polar component with R_F 0.25 [petroleum ether-diethyl ether (1:2 v/v)] were combined and concentrated to give the *E*-isomer **13** (4.5 g, 40%) with m.p. 88 °C; $[\alpha]_D^{21}$ + 19.5° (*c* 1.1, chloroform); ν_{max} 1765-1730 (C=O) and 1640 cm^{-1} (C=C); λ_{max} 237 nm (ε 5.1 × 10^3); δ 2.04 (9H, s), 2.13 (3H, s), 2.81 (2H, m), 3.61 (3H, s), 3.84 (3H, s), 4.16 (1H, dd, *J* 13 and 5 Hz), 4.33 (1H, dd, *J* 13 and 2.5 Hz), 5.16 (3H, t, *J* 8 Hz), 5.20-5.50 (3H, m). (Found: C, 51.7; H, 6.3. $C_{18}H_{26}O_{11}$ requires C, 51.7; H, 6.3%).

Methyl *Z*-3,4-dideoxy-2-*O*-methyl-D-*arabino*-oct-2-enonate 15

A solution of the *Z*-isomer **14** (0.85 g) in dry MeOH (15 ml) was treated with a solution of NaOMe in MeOH (0.3 N, 0.5 ml). After standing at room temperature overnight, the solution was neutralised by stirring with Dowex 50W-8X resin (H^+) and the mixture filtered. The filtrate was concentrated to a solid which was recrystallised from absolute ethanol to give colourless plates (0.44 g, 87%) with m.p. 117 °C; $[\alpha]_D^{23}$ + 19.3° (*c* 0.6, MeOH); R_F 0.50 [EtOAc-MeOH (3:1 v/v)]; ν_{max} 3280, 3380 (OH), 1725 (conjugated ester C=O) and 1640 cm^{-1} (C=C); λ_{max} 226 nm (ε 8.7 × 10^3); δ (d_5-pyridine-D_2O, 4:1 v/v) 2.90-3.10 (2H, m); 2.79 (3H, s), 2.86 (3H, s), 4.09 (1H, dd, *J* 7.5 and 1.8 Hz), 4.20-4.70 (4H, m), 6.85 (1H, t, *J* 7.5 Hz). (Found: C, 47.8; H, 7.2. $C_{10}H_{18}O_7$ requires C, 48.0; H, 7.3%).

***E*-3,4-Dideoxy-2-*O*-methyl-D-*arabino*-oct-2-enono-1,5-lactone 16**

A solution of the *E*-isomer **13** (0.61 g) in dry MeOH (13 ml) was deacetylated with a solution of NaOMe in MeOH (0.3 N, 0.2 ml). After standing at room temperature overnight, the product was isolated as described previously to give the crystalline lactone **16** (0.26 g, 82%) with m.p. 124-125 °C (from EtOH-EtOAc); $[\alpha]_D^{22}$ + 42.4° (*c* 0.9, MeOH); R_F 0.45 [EtOAc-MeOH (3:1 v/v)]; $\nu_{max.}$ 3260-3450 (OH), 1735, 1720 (C=O) and 1650 cm^{-1} (C=C); λ_{max} 244 nm (ε 5.3 × 10^3); δ (d_5-pyridine-

D_2O, 4:1 v/v) 2.37 (1H,ddd, *J* 18.5, 7 and 4 Hz); 3.25 (1H, ddd, *J* 18.5, 13.5 and 2.5 Hz), 3.58 (3H, s), 4.21 (1H, dd, *J* 9 and 1.8 Hz), 4.30-4.78 (3H, m), 5.46 (1H, ddd, *J* 13.5, 4 and 1.8 Hz) 5.81 (1H, dd, *J* 7 and 2.5 Hz). (Found: C, 49.5; H, 6.5. $C_9H_{14}O_6$ requires C, 49.5; H, 6.5%).

(Ethoxyoxalyl)methylenetriphenylphosphorane 18

The compound was prepared according to Zhdanov and Uzlova[18] with modification. A solution of ethyl bromopyruvate (Aldrich, purity 90%, 100 g) in dry CCl_4 (200 ml) was added dropwise over 3h to a stirred and cooled (0 °C) solution of $(Ph)_3P$ (135 g) in the same solvent (1.5 l). After 36h at room temperature, the supernatant was decanted from the yellow hygroscopic crystals which were then washed with anhydrous ether (3 × 400 ml) by trituration and decantation. The dried sticky solid was dissolved in methanol (600 ml) and the solution cooled to 0 °C. The pH was adjusted to 10 by gradual addition of iced aqueous sodium carbonate (1 N) and the solution made up to 4 litres with ice-water. The solution was then stirred for 1h at 0 °C and the precipitates collected and washed with cold water. The solids were dissolved in a minimum amount of hot ethanol, the resulting solution filtered and water added slowly until crystallisation began. The crystals separated out as plates (120 g). A second crop (11 g) was obtained in the same manner. The total yield of **18** was 131 g, 75%; m.p. 182—183 °C (lit[18]., m.p. 169—171 °C); δ 1.26 (3H, t, *J* 7.2 Hz); 4.15 (2H, q, *J* 7.2 Hz), 4.79 (1H, d, $^2J_{PH}$ 24 Hz); 8 Hz), 7.25-7.78 (15H, m). (Found: C, 73.4; H, 5.6; P, 8.2. $C_{23}H_{21}O_3P$ requires C, 73.4; H, 5.6; P, 8.2%).

Ethyl *E*-5,6,7,8-tetra-*O*-acetyl-3,4-dideoxy-D-*arabino*-oct-2-ulos-3-enonate 19

A solution of the aldehyde **17**[17] (4.0 g) and (ethoxyoxalyl)methylenetriphenylphosporane **18** (5.2 g) in dry benzene (180 ml) was heated under reflux for 5 h. The pale yellow solution was taken to dryness and the residue was triturated with diethyl ether (100 ml) and the mixture cooled to 0 °C. The precipipated triphenylphosphine oxide was filtered off and washed with cold diethyl ether (2 × 20 ml). The combined filtrate and washings were concentrated to a syrupy mixture which was chromatographed in diethyl ether-hexane (40 → 70%), giving a pale yellow solid. Recrystallisation from diethyl ether-hexane furnished the enone **19** (3.8 g, 73%) as small white crystals, m.p. 71.5—72.5 °C; $[\alpha]_D^{23}$ + 24.4° (*c* 1.1, chloroform); R_F 0.50 [petroleum ether-diethyl ether (1:2 v/v)]; ν_{max} 1745 (ester C=O) and 1685 cm^{-1} (conjugate ketone C=O), 1640 cm^{-1} (C=C); λ_{max} 232 nm (ε 6.5 × 10^3); δ 1.36 (3H, t, *J* 7 Hz); 2.01 (9H, bs), 2.14 (3H, s), 4.05 (1H, dd, *J* 12.5 and 4 Hz), 4.19 (1H, dd, *J* 12.5 and 3 Hz), 4.26 (3H, q, *J* 7 Hz), 5.14 (1H, ddd, *J* 9, 4 and 3 Hz), 5.38 (1H, dd, *J* 9 and 2.5 Hz), 5.72 (1H, ddd, *J* 3.8, 2.5 and 1 Hz), 6.61 (1H, dd, *J* 16 and 1 Hz), 6.91 (1H, dd, *J* 16 and 3.8 Hz). (Found: C, 52.1; H, 5.8. $C_{18}H_{24}O_{11}$ requires C, 51.9; H, 5.8%).

Ethyl 5,6,7,8-tetra-*O*-acetyl-3,4-dideoxy-D-*arabino*-oct-2-ulosonate 20

A solution of the enone **19** (2.5 g) in ethyl acetate (70 ml) was hydrogenated over palladium-on-charcoal (10%, 200 mg) at room temperature and atmospheric pressure until one mole equivalent of hydrogen was absorbed (ca. 135 ml at 25 °C and 760 torr). The mixture was filtered and the filtrate concentrated to a syrup which crystallised when triturated with a few drops of diethyl ether at 0 °C. Recrystallisation from diethyl ether-hexane gave white needles (2.1 g, 84%) with m.p. 52—53 °C; $[\alpha]_D^{20}$ + 25.5° (*c* 2.0, chloroform); R_F 0.40 [petroleum ether-diethyl ether (1:2 v/v)]; $\nu_{max.}$ 1740-1760 cm^{-1} (C=O); δ 1.37 (3H, t, *J* 7 Hz); 1.67-1.98 (2H, m), 2.05, 2.06, 2.07, 2.15(12H, 4s), 2.81(1H, ddd, *J* 19, 8 and 5.7 Hz), 2.96 (1H, td, *J* 19, 8 and 8 Hz), 4.34 (1H, dd, *J* 12.5 and 4.8 Hz), 4.43 (1H, dd, *J* 12.5 and 2.5 Hz), 4.52 (3H, q, *J* 7 Hz), 5.13 (1H, ddd, *J* 7, 4.5 and 2.8 Hz), 5.17 (1H, ddd, *J* 8.6, 4.8 and 2.5 Hz), 5.32 (1H, dd, *J* 8.6 and 2.8 Hz). (Found: C, 52.0; H, 6.3. $C_{18}H_{24}O_{11}$ requires C, 51.7; H, 6.3%).

Ethyl *E*-3,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-D-*arabino*-oct-2-ulos-3-enonate 22

A solution of the aldehyde **21**[19] (6.0 g) and (ethoxyoxalyl)methylenetriphenylphosporane (10 g) in dry toluene (200 ml) was heated under reflux for 2.5 h. The pale yellow solution was taken to dryness and the residue was triturated with diethyl ether (150 ml) and the mixture cooled to 0 °C. The precipipated triphenylphosphine oxide was filtered off and washed with cold diethyl ether (2 × 30 ml). The combined filtrate and washings were concentrated to a syrupy mixture which was chromatographed [petroleum ether-diethyl ether (2:1 v/v)] to give the enone **22** (5.8 g, 68%) as a pale yellow syrup; $[\alpha]_D^{24}$ - 6.7° (*c* 1.0, chloroform); R_F 0.50 [petroleum ether-diethyl ether (2:1 v/v)]; ν_{max} 1730 (ester C-O), 1705 and 1682 (conjugate

ketone C=O), 1634 cm^{-1} (C=C); λ_{max} 243 nm (ε 6.5 × 10^3); δ 1.32 (3H, s), 1.35 (3H, t, *J* 7 Hz, obscured by the Me signals), 1.36, 1.39 (×2) (9H, 2s), 3.62 (1H, t, *J* 7.8 Hz), 3.88-4.20 (3H, m), 4.32 (3H, q, *J* 7 Hz), 4.61 (1H, ddd, *J* 7.8, 4 and 0.8 Hz), 6.88 (1H, dd, *J* 16 and 0.8 Hz), 7.22 (1H, dd, *J* 16 and 4 Hz). (Found: C, 58.9; H, 7.5. $C_{18}H_{24}O_{11}$ requires C, 58.5; H, 7.4%).

Ethyl 3,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-D-*arabino*-oct-2-ulosonate 23

A solution of the enone **22** (4.1 g) in ethyl acetate (100 ml) was hydrogenated over palladium-on-charcoal (10%, 300 mg) at room temperature and atmospheric pressure until one mole equivalent of hydrogen was absorbed (ca. 280 ml at 25 °C and 760 torr). The mixture was filtered and the filtrate concentrated to a colourless syrup (3.9 g, 95%); $[\alpha]_D^{18}$ + 14.3° (*c* 7.2, chloroform); R_F 0.40 [petroleum ether-diethyl ether (2:1 v/v)]; ν_{max} 1730 cm^{-1} (C=O); λ_{max} 243 nm (ε 6.5 × 10^3); δ (d_5-pyridine) 1.18 (3H, t, *J* 7 Hz), 1.29 (6H, s), 1.34, 1.37 (6H, 2s), 1.85-2.26 (2H, m), 3.02-3.20 (2H, m, D_2O exchangeable), 3.52-4.25 (7H, m, with q at 4.14, *J* 7 Hz). (Found: C, 59.1; H, 8.2. $C_{18}H_{24}O_{11}$ requires C, 59.2; H, 7.9%).

Calcium 3,4-dideoxy-D-*arabino*-oct-2-ulosonate 2

The oct-2-ulosonate **23** (2.0 g) was stirred with aqueous TFA (30% v/v, 20 ml) at room temperature overnight. After evaporation of the solvent, the residual TFA was removed by azeotropic distillation under reduced pressure with water (3 ×) and the resulting syrup was dried over KOH pellets. The syrup (1.4 g) was dissolved in water (15 ml) and the pH of the solution adjusted to 10 with gradual addition of saturated aqueous $Ca(OH)_2$. After 1 h at room temperature, the solution was neutralised by stirring with Dowex 50W-8X resin (H^+, prewashed with water), decolourised with activated charcoal (ca. 100mg), filtered through a thin pad of kieselguhr, and the filtrate was concentrated to give the calcium salt **2** (1.3 g, 83%) as a white amorphous solid, $[\alpha]_D^{23}$ + 7.8° (4 min)(*c* 0.6, water, no mutarotation observed); R_F 0.25, 0.35 [EtOAc-acetic acid-water (2:1:1 v/v)]; ν_{max} 3200-3400 (OH), 1610 cm^{-1} (carboxylate C=O). (Found: C, 36.7; H, 5.9. $C_{16}H_{26}O_{14}Ca{\cdot}2H_2O$ requires C, 36.1; H, 5.8%).

3-Hydroxy-3-(3',4',5',6'-tetraacetoxy-D-*arabino*-hexyl)-quinoxaline 24

The calcium salt **2** (0.51 g) in water (10 ml) was decationised by stirring with Dowex 50W-8X resin (H^+, 3 g, prewashed with water) at room temperature for 10 min. Filtration and concentration of the filtrate gave a syrup which was heated under reflux for 1 h with a solution of *o*-phenylenediamine (0.25 g) in methanol (30 ml) containing 3 drops of glacial acetic acid. The product crystallised out during the course of the reaction and the mixture was subsequently cooled to 0 for 2 h. The crystals were collected, washed with cold methanol (10 ml), dried and dissolved in a solution of DMF (4 ml), pyridine (4 ml) and acetic anhydride (8 ml) at room temperature overnight. The product was isolated in the conventional way and recrystallised from aqueous methanol to give the tetraacetate **24** (0.66 g, 73%) as colourless needles with m.p. 167.5—168 °C; $[\alpha]_D^{22}$ + 4.9° (*c* 0.5, chloroform); R_F 0.35 [toluene-EtOAc (1:1 v/v)]; δ 2.06, 2.07, 2.08, 2.11(12H, 4s), 1.91-2.20 (2H, m), 2.94 (1H, ddd, *J* 15, 8.7 and 6 Hz), 3.08 (1H, ddd, *J* 15, 8.7 and 6.4 Hz), 4.20 (1H, dd, *J* 12.4 and 5.1 Hz), 4.29 (1H, dd, *J* 12.4 and 2.8 Hz), 5.20 (1H, ddd, *J* 8.3, 5.1 and 2.8 Hz), 5.37-5.42 (1H, m), 5.45 (1H, dd, *J* 8.3 and 2.8 Hz), 7.31-7.37 (2H, m), 7.47-7.51 (1H, m), 7.80-7.85 (1H, m), 11.85 (1H, bs). (Found: C, 57.0; H, 5.7; N, 6.1. $C_{22}H_{26}N_2O_9$ requires C, 57.1; H, 5.7; N, 6.1%).

Ethyl *E*-3,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-D-*xylo*-oct-2-ulos-3-enonate 27

A solution of the aldehyde **26**[19] (3.7 g) and (ethoxyoxalyl)methylenetriphenylphosporane (10 g) in dry toluene (100 ml) was heated under reflux for 3 h. The reaction was worked up as described for compound **23** to give a syrupy mixture which was chromatographed [petroleum ether-diethyl ether (2:1 v/v)] to furnish the enone **27** (3.4 g, 64%) as a pale yellow syrup; $[\alpha]_D^{25}$ - 26.6° (*c* 0.6, chloroform); R_F 0.50 [petroleum ether-diethyl ether (2:1 v/v)]; ν_{max} 1735 (ester C=O), 1710 and 1685 (conjugate ketone C=O), 1635 cm^{-1} (C=C); λ_{max} 244 nm (ε 6.1 × 10^3); δ (C_6D_6) 1.94 (3H, t, *J* 7.5 Hz), 1.24 (6H, s), 1.32, 1.35 (6H, 2s), 3.49 (1H, dd, *J* 8 and 2.2 Hz) 3.70-3.86 (3H, m), 3.92 (3H, q, *J* 7.5 Hz), 4.58 (1H, dd, *J* 8 and 2.8 Hz), 6.94 (1H, d, *J* 15Hz), 7.10 (1H, dd, *J* 15 and 2.8 Hz). (Found: C, 58.4; H, 7.3. $C_{16}H_{24}O_7$ requires C, 58.5; H, 7.4%).

Ethyl 3,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-D-*xylo*-oct-2-ulosonate 28

A solution of the enone **27** (2.4 g) in ethyl acetate (50 ml) was hydrogenated over palladium-on-charcoal (10%, 120 mg) at room temperature and atmospheric pressure until one mole

equivalent of hydrogen was absorbed (ca. 164 ml at 25 °C and 760 torr). The mixture was filtered and the filtrate concentrated to give a quantitative yield of the analytically pure oct-2-ulosonate **28** as a colourless syrup; $[\alpha]_D^{23}$ - 34.2° (*c* 1.1, chloroform); R_F 0.40 [petroleum ether-diethyl ether (2:1 v/v)]; ν_{max} 1710-1730 cm^{-1} (C=O); δ 1.37 (3H, t, *J* 7 Hz); 1.39 (9H, bs), 1.44 (3H, s), 1.82 (1H, dddd, *J* 14.3, 9, 7.7 and 6.3 Hz), 2.01 (1H, dddd, *J* 14.3, 8.1, 6.6 and 2.9 Hz), 2.99 (3H, q, *J* 18.8, 7.7 and 6.6 Hz), 3.11 (1H, ddd, *J* 18.8, 8.1 and 6.3 Hz), 3.71 (1H, dd, *J* 8.3 and 4.6 Hz), 3.84 (1H, dd, *J* 8.1 and 7 Hz), 3.95 (1H, ddd, *J* 8.9, 8.3 and 2.9 Hz), 4.06 (1H, dd, *J* 8.1 and 6.8 Hz), 4.15 (1H, dt, *J* 7, 6.8 and 4.6 Hz), 4.33 (2H, q, *J* 7 Hz). (Found: C, 58.5; H, 7.9. $C_{18}H_{24}O_{11}$ requires C, 59.2; H, 7.9%).

Calcium 3,4-dideoxy-D-*xylo*-oct-2-ulosonate 3

The oct-2-ulosonate **28** (1.7 g) was stirred with aqueous TFA (30% v/v, 20 ml) at room temperature overnight. After evaporation of the solvent, the residual TFA was removed by azeotropic distillation under reduced pressure with water (3 ×) and the resulting syrup was dried over KOH pellets. The syrup (1.2 g) was dissolved in water (10 ml) and the pH of the solution adjusted to 10 with gradual addition of saturated aqueous $Ca(OH)_2$. After 1 h at room temperature, the solution was neutralised by stirring with Dowex 50W-8X resin (H^+, prewashed with water), decolourised with activated charcoal (ca. 100mg), filtered through a thin pad of kieselguhr, and the filtrate was concentrated to give the calcium salt **3** (1.3 g, 83%) as a white amorphous solid, $[\alpha]_D^{26}$ - 5.4° (6 min) → - 4.1° (40 min)(*c* 0.6, water); R_F 0.25, 0.35 [EtOAc-acetic acid-water (2:1:1 v/v)]; $\nu_{max.}$ 3100-3400 (OH), 1615 cm^{-1} (carboxylate C=O). (Found: C, 38.0; H, 5.6. $C_{16}H_{26}O_{14}\,Ca{\cdot}1.25H_2O$ requires C, 38.1; H, 5.7%).

3-Hydroxy-3-(3',4',5',6'-tetraacetoxy-D-*xylo*-hexyl)-quinoxaline 29

A solution of the calcium salt **3** (0.25 g) in water (5 ml) was decationised by stirring with Dowex 50W-8X resin (H^+, prewashed with water) at room temperature for 10 min. Filtration and evaporation of the water from the filtrate gave a syrup which was heated under reflux for 1 h with a solution of *o*-phenylenediamine (0.12 g) in methanol (15 ml) containing 2 drops of glacial acetic acid. The cooled solution was concentrated to a brown syrup which was acetylated with acetic anhydride (4 ml) and pyridine (4 ml) at room temperature overnight. The product was isolated in the conventional way and recrystallised from aqueous methanol to furnish the tetraacetate **29** (0.27 g, 58%) as colourless needles with m.p. 154—155 °C; $[\alpha]_D^{22}$ + 13.2° (*c* 0.6, chloroform); R_F 0.35 [toluene-EtOAc (1:1 v/v)]; δ 2.05, 2.09, 2.10, 2.13(12H, 4s), 2.14-2.35 (2H, m), 2.96 (1H, ddd, *J* 15, 8.7 and 6 Hz), 3.07 (1H, ddd, *J* 15, 8.7 and 7.3 Hz), 4.39 (1H, dd, *J* 12.4 and 5.5 Hz), 4.40 (1H, dd, *J* 12.4 and 4.1 Hz), 5.29-5.38 (2H, m), 5.43 (1H, t, *J* 5.2 Hz), 7.30-7.36 (2H, m), 7.47-7.52 (1H, m), 7.80-7.84 (1H, m), 11.81 (1H, bs). (Found: C, 57.0; H, 5.7; N, 6.3. $C_{22}H_{26}N_2O_9$ requires C, 57.1; H, 5.7; N, 6.1%).

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