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Dioxalanones as Synthetic Intermediates. Part 6. Synthesis of 3-Deoxy-D-*manno*-2-octulosonic acid (KDO), 3-Deoxy-D-*arabino*-2-heptulosonic acid (DAH) and 2-Keto-3-deoxy-D-gluconic acid (KDG)

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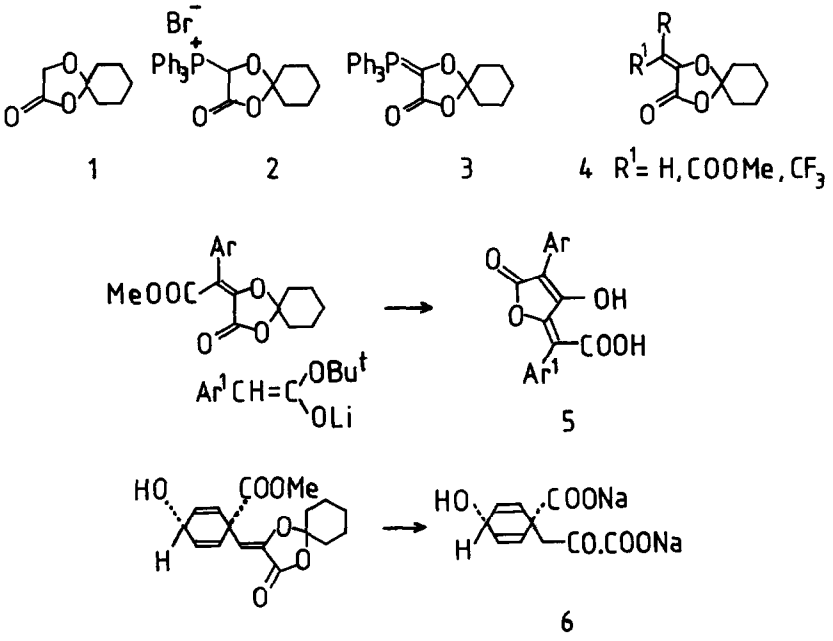
Abstract

Three biosynthetically significant α-keto acids KDO (7), DAH (8) and KDG (9) have been synthesised via 5-ylidene-1,3-dioxalan-4-one intermediates formed by Wittig reactions of protected monosaccharide-derived aldehydes with the Wittig reagent (3).

The chemistry of the 5-ylidene-1,3-dioxalan-4-one system illustrated in Scheme 1 has been exploited in a series[1-5] of natural product syntheses which are dependent on two aspects of the heterocyclic system which is at the same time a doubly protected α-keto acid and an activated α-keto acid function (ν_{max} 1795 cm^{-1}). Previous syntheses in this area have exploited the nucleophilic attack of substituted acetic ester anions to set up a general synthesis[2,3,4] of tetronic acids (5). Furthermore it had been established that 5-ylidene-1,3-dioxalan-4-ones could be quantitatively converted to salts of the corresponding α-keto acid by treatment with an equivalent of dilute aqueous base.[5] This allowed the design of an efficient synthesis[1] of the disodium salt of prephenic acid (6) which has a key role in the biosynthesis of the aromatic amino acids phenylalanine and tyrosine.

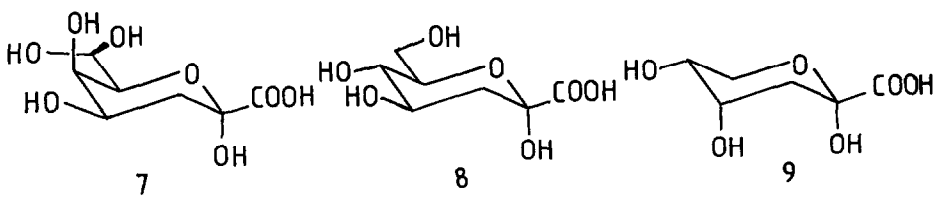
Monosaccharide-derived 2-keto-3-deoxycarboxylic acids

In the preliminary studies on the 5-ylidene-1,3-dioxalan-4-one system, which had been claimed but not established prior to our work, it was found that this system was *relatively* stable to acidic cleavage compared with acetonide protection of 1,2-diols for example. Thus this observation, coupled with the rapid transformation of (4) to the *salts* of 2-keto-3-deoxycarboxylic acids, suggested that synthons derived from protected monosaccharide aldehydes and the Wittig reagent (3)[1,6] could be useful for



Scheme 1

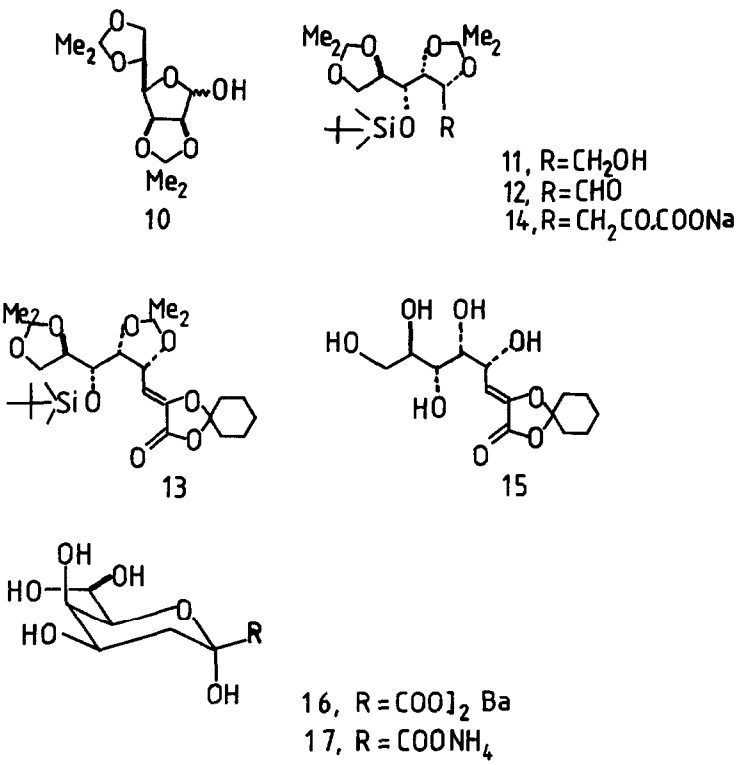
establishing general, enantioselective syntheses of biologically significant targets such as 3-deoxy-D-*manno*-2-octulosonic acid (KDO, 7), 3-deoxy-D-*arabino*-2-heptulosonic acid (DAH, 8) and 2-keto-3-deoxy-D-gluconic acid (KDG, 9).



*3-Deoxy-D-*manno*-2-octulosonic acid* (KDO, 7)

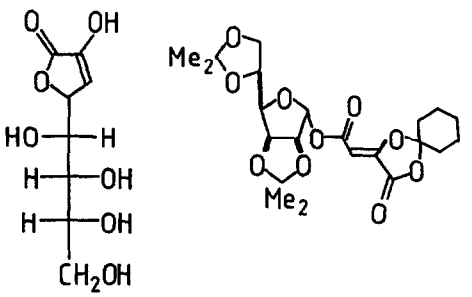
KDO (7) was discovered as the 8-phosphate in 1959 by Levin and Racker.[7] It was found that D-ribose-5-phosphate was transformed into D-arabinose-5-phosphate before condensation with phosphoenolpyruvate to give KDO (7) by a process mediated by an aldolase enzyme.[8] Later it was established[9] that KDO (7) is an important constituent of the cell wall lipopolysaccharide (LPS) in a number of Gram negative bacterial strains. The crucial role that KDO plays in the biosynthesis of Gram negative cell wall LPS has obvious consequences for an approach to antibacterial chemotherapy based on the inhibition of KDO (7) uptake by means of KDO analogues. Thus KDO (7) has been a target for synthesis in recent years.[10]

There are two key stages in our general strategy for the synthesis of the target α-keto acids. The first, variable stage, is the preparation of a protected aldehyde from a monosaccharide derivative incorporating the



Scheme 2

chirality present in the required product. In the second, crucial stage, the protected aldehyde reacts with the Wittig reagent (3) to incorporate a masked α-keto acid function. The specific route selected for the synthesis of KDO (7) is illustrated in Scheme 2 where the Wittig reagent (3) brings about conversion of the D-*manno*-protected aldehyde (12)[11] to the desired 5-ylidene-1,3-dioxalan-4-one (13) as a mixture of E and Z forms (2:1 respectively). Choice of the acetonide and t-butyldimethylsilyl protecting groups was made with the knowledge that KDO is unstable to prolonged acid treatment which affords the 2-keto γ-lactone (18).[12] The 4-hydroxyl function of D-mannose had to be protected in an acyclic derivative since direct use of (10) in a Wittig reaction with (3) afforded an undesired product to which the structure (19) has been ascribed. The required aldehyde (12) has already been used[11] in synthesis and was made from the 2,3:5,6-di-O-isopropylidene-D-*manno*-furanose (10). It was found that oxidation of the primary alcohol (11) using the Pfitzner-Moffatt method[13] was best achieved using the water-soluble reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride instead of dicyclohexylcarbodiimide which caused problems in purification of (12). The Wittig reaction to give (13) was performed using the aldehyde (12) obtained directly from the above oxidation to obviate epimerisation α to the aldehyde function.



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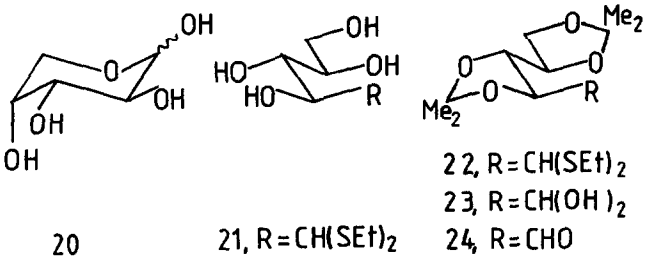
Although hydrolysis of (13) using aqueous NaOH gave the Na salt (14), the loss of the UV chromophore resulted in difficult monitoring of the chromatography of (14) and the subsequent KDO (7) formed by acid cleavage of the protecting groups. Thus the acid-labile protecting groups in (13)were removed by treatment with 90% aqueous acetic acid at 90°C for 1 hour. Removal of the solvent *in vacuo*, followed by trituration of the residue, afforded the desired penultimate intermediate (15) isolated as the E isomer. This compound was found to be hygroscopic and, therefore, was purified under anhydrous conditions. Reaction of (15), in aqueous methanol solution, with 0.5 equivalent of $Ba(OH)_2$ gave the Ba^{2+} salt (16) which could easily be transformed into the known, well characterised NH_4^+ salt (17)[14] by treatment with $(NH_4)_2SO_4$ followed by filtration of $BaSO_4$. Lyophilisation of the filtrate afforded the NH_4^+ salt which was crystallised as plates from 85% aqueous ethanol. The ^{13}C n.m.r. spectrum of (17) exhibited eight major signals attributable to the α-pyranoside form[15] of KDO (7).

3-Deoxy-D-arabino-2-heptulosonic acid (DAH, 8)

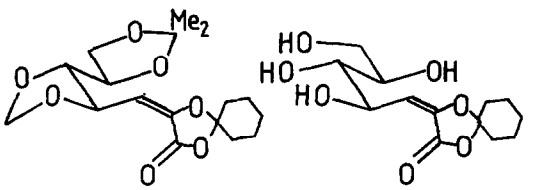
Previously we had synthesised disodium prephenate (6) which is the last seven carbon non-aromatic precursor of phenylalanine and tyrosine in the shikimate pathway of biosynthesis. As part of a larger programme we also wished to synthesise the first seven carbon shikimate intermediate, namely DAH (8), formed by condensation of D-erythrose 4-phosphate and phosphoenolpyruvate.[8] The shikimate pathway is employed by plants and microorganisms to furnish aromatic compounds essential for growth of these hosts but which cannot by synthesised *de novo* by animals. Thus inhibition of the shikimate pathway offers a non toxic approach to agricultural and pharmaceutical products. In recent years research[16] has been directed towards the development of enzyme targeted inhibitors of the shikimate pathway.

The initial phase in the synthesis of DAH (8),[17] illustrated in Scheme 3, involved the construction of the protected aldehyde (24) starting from D-arabinose (20). Treatment of the diethyldithioacetal[18] of D-arabinose (21) with acetone, containing 3% conc H_2SO_4, afforded the required 2,3:4,5-di-O-isopropylidene derivative (22)[18] which was then reacted with $HgO/HgCl_2$ in acetone with vigorous stirring. The HgO is present in order to neutralise any HCl formed which would cause undesired rearrangement of

the acetonide systems. The integrity of (24) was supported by the work by Buchanan *et al*[20] on the correlation between the size of acetonide rings and the chemical shifts in the C-13 n.m.r. spectrum of the $(CH_3)_2C$ carbon resonances. The product of this reaction was the hydrate (23) of the



20 21, R=CH(SEt)$_2$ 22, R=CH(SEt)$_2$
23, R=CH(OH)$_2$
24, R=CHO



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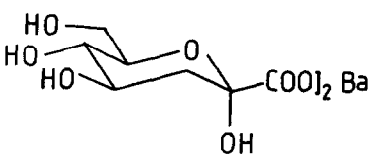

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Scheme 3

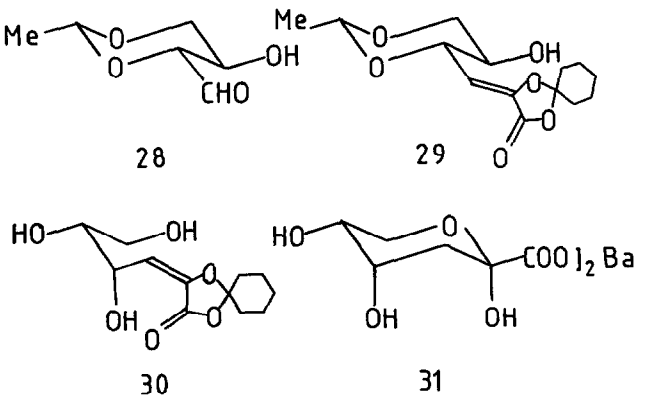
desired aldehyde (24) required for the Wittig reaction. Distillation of (23) to give the aldehyde (24)[19] was effected prior to reaction with (3) which yielded the 5-ylidene-1,3-dioxalan- 4-one (25) as a mixture of geometrical isomers from which the Z form could be isolated as the major product by equilibration of the Z/E mixture (1:2 → 14:1) using iodine in petrol at reflux. Although both E and Z isomers were suitable for further elaboration to (8), it was deemed advisable to work initially with a single isomer to facilitate characterisation of the next product. Deprotection of the acetonide functions in (25) was achieved by treatment with trifluoroacetic acid in aqueous ethanol at reflux to give the penultimate intermediate (26). Finally the ylidenedioxalanone system was unmasked by 0.5 equivalent of $Ba(OH)_2$ in aqueous methanol to reveal the target DAH (8) as the Ba^{2+} salt dihydrate (27).

In order to demonstrate the retention of stereochemical integrity of the arabinose chiral centres during this synthesis, the Ba^{2+} salt of 3-deoxy-L-*arabino*-2-heptulosonic acid was synthesised in the above manner from L-arabinose.

3-Deoxy-2-ketogluconic acid (KDG, 9)

In 1952, Entner and Doudoroff[21] identified a new biochemical pathway in bacteria for the oxidative metabolism of glucose, possibly involving the intermediacy of the 6-phosphate of KDG (9). Both KDG (9) and the 6-phosphate have since been discovered to be intermediates in a diverse set of bacterial metabolic pathways which include the metabolism of uronic acids.[22] KDG (9) has also been encountered in studies of the bacterium responsible for soft-rot disease in many plant species.[23] The original syntheses of KDPG were enzymatic[22] and later work on KDG (9) very often lacked stereochemical control. We sought to develop a synthetic route to KDG (9) from a D-erythrose derivative and the Wittig reagent (3), according to the general synthetic strategy discussed previously in the cases of KDO (7) and DAH (8). This route to (9) is illustrated in Scheme 4 in which the key aldehyde intermediate was selected as 2,4-O-ethylidene-D-erythrose (28).[24]

It emerged later that the choice of the readily accessible aldehyde (28), prepared from $NaIO_4$ treatment of 4,6-ethylidene-D-glucose,[25] was unfortunate since the desired cleavage of the 5-ylidene-1,3-dioxalan-4-one (29) proved to be difficult to control. The Wittig reaction between (28) and (3) proceeded smoothly to afford (29) as a 9:1 mixture of Z and E geometrical isomers. Deprotection of (29) using acetic or trifluoroacetic acid resulted in degradation. However treatment of (29) with a 1% solution of iodine in methanol[26] at reflux gave the desired triol (30) in low yield. Hydrolysis of (30) using 0.5 equivalent of $Ba(OH)_2$ in aqueous methanol afforded the Ba^{2+} salt monohydrate of KDG (9) having analytical data consistent with the required structure (31).



Scheme 4

EXPERIMENTAL

Melting points were recorded on a Reichert 7905 melting point apparatus and are uncorrected. Optical rotations were measured on a AA1000 polarimeter using a 20 cm cell. Thin layer chromatography (tlc) was carried out on plastic sheets precoated with silica gel 60 Gf-254 (Merck). Solvent systems are quoted in the text. Visualisation of the compounds was

achieved by a suitable combination of the following methods: iodine vapour, UV absorption at 254 nm, potassium permanganate and bromophenol blue sprays. Infra-red spectra were recorded on a Perkin Elmer 781 spectrophotometer in the solvent indicated or by the KBr disc technique using polystyrene as the standard (1603 cm^{-1}). Ultraviolet spectra were recorded, in the distilled solvent indicated, on a Pye-Unicam SP8-400 spectrophotometer. High resolution and low resolution fast atom bombardment (FAB) spectra were measured on a Kratos MS50TC machine. Proton n.m.r. spectra were recorded on either Bruker WP80 (80 MHz), WP200 (200 MHz) or WH360 (360 MHz) machines in the solvent indicated using tetramethylsilane (TMS) as the external standard (δ = 0.000). Carbon-13 n.m.r. spectra were recorded on a Bruker WP200 machine operating at 50.3 MHz. Samples were dissolved in the solvent indicated and chemical shifts were measured relative to TMS assigned at zero. Elemental analyses were carried out on a Carlo Erba elemental analyser model 1106. All solvents were distilled before use and the following were dried using the reagents given in parentheses when required: chloroform (phosphorus pentoxide), dichloromethane (calcium hydride), diethyl ether, benzene and toluene (sodium wire), N,N-dimethylformamide (calcium hydride), ethanol (magnesium-iodine). Acetone was distilled with addition of potassium permanganate, dried over potassium carbonate and stored in the presence of molecular sieves.

Cyclohexanespiro-2'-(1',3'-dioxolan)-4'-one (1)

A solution of dried, distilled cyclohexanone (16.98 g, 0.173 mol) in dry dichloromethane (60 ml) was added dropwise to a solution of trimethylsilyl (trimethylsilyloxy) acetate (49.5 g, 0.225 mol) and trimethylsilyl trifluoromethanesulphonate (2.44 g, 0.011 mol) in dichloromethane (350 ml) at -78°C. The mixture was stirred at this temperature for four hours before addition of pyridine (17.8 g, 0.225 mol). After allowing it to warm to room temperature the solution was poured into saturated aqueous sodium bicarbonate (200 ml), extracted with ether (2 x 300 ml) then the organic phase dried over anhydrous sodium sulphate. After filtration and concentration of the solution *in vacuo*, the colourless residue was distilled to afford the dioxolanone (1) as a colourless oil (25.6 g, 95%); b.p. 79°C at 0.5 mm Hg; Rp 0.3 (10% EtOAc/hexane); $\nu(CH_2Cl_2)$ 2950 (m) (CH), 2870 (w), 1795 (s) (C=O), 1105 (s) cm^{-1}; δ_H ($CDCl_3$, 80 MHz) 4.33 (2H, s, H-5,5'), 1.90-1.30 (10H, broad cyclohexyl); m/z (FAB) 157. HRMS 157.08645, $C_8H_{13}O_3$ (MH^+) requires 157.08646. Lit.[5] b.p. 70°C at 0.5 mmHg.

2,3:5,6-Di-O-isopropylidene-4-tert-butyldimethylsilyloxyaldehydo-D-mannose (12)

The alcohol (11)[11] (14.1 g, 0.038 mol) in dry DMSO (70 ml) and toluene (70 ml) was stirred under nitrogen at room temperature and treated with 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride (21.6 g, 0.113 mol), pyridine (10.4 g, 0.132 mol) in toluene (30 ml) and TFA (2.06 g, 0.018 mol) in toluene (35 ml). The solution was stirred overnight then partitioned between ethyl acetate and water. The organic phase was washed with 2N hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine and then dried over anhydrous sodium sulphate to yield the aldehyde (12) as a pale yellow oil (12.2 g, 87%); R_F 0.28 (17% EtOAc/hexane); ν(neat) 2930 (s) (CH), 1739 (s) (C=O), 1475 (m), 1465 (m), 1383 (s), 1373 (s) cm^{-1}; δ_H (CD_2Cl_2, 200 MHz) 9.74 (d J 2.3 Hz, H-1), 4.49 (dd J 6.0, 2.3 Hz, H-2), 4.25-3.76 (4H, complex), 3.68 (1H, m), 1.53 (3H, s, CMe_2), 1.39 (3H, s, CMe_2), 1.35 (3H, s, CMe_2), 1.32 (3H, s, CMe_2), 0.87 (9H, s, $SiBu^t$), 0.10 (3H, s, SiMe), 0.09 (3H, s, SiMe); δ_C (CD_2Cl_2, 50.3 MHz), 199.0 (q, C-1), 110.1, 109.9 (q, CMe_2), 81.4, 80.9, 76.9, 72.2, 68.2 (C-2,3,4,5,6), 27.3, 26.1, 25.5, 25.3 (CCH_3, CMe_2), 25.9 (CH_3, Si^tBu), 18.5 (q, Si^tBu), -3.9, -4.6 (CH_3, $SiMe_2$); m/z (FAB) 375, 317, 277, 259, 229, 187, 171. HRMS 375.22026, $C_{18}H_{34}O_6Si$ (MH^+) requires 375.22027.

(4R,5R,6R,7R)-4,5:7,8-Di-O-isopropylidene-6-tert-butyl-dimethylsilyloxyhexylidenecyclohexanespiro-2'-(1',3'-dioxolan)-4'one (13)

DABCO (7 g, 0.063 mol) and the phosphonium bromide (2) (27.7 g, 0.056 mol) in dry toluene (140 ml) was stirred at 40°C under an atmosphere of nitrogen for 5 minutes. The aldehyde (12) (16.75 g, 0.045 mol) in toluene (100 ml) was added in 10 ml portions over 5 minutes as the temperature was raised to 80°C. After 30 minutes the suspension was cooled to 4°C and

filtered. The filtrate was concentrated *in vacuo* and the residue applied to a dry flash column (SiO_2: hexane/EtOAc gradient) to afford the desired product (13) as a mixture of E and Z geometrical isomers (16.9 g, 74%); R_F 0.31, 0.25 (17% EtOAc/hexane). The isomers were separated using a wet flash column (SiO_2: petrol/ether gradient); E isomer: (Found C, 60.8; H, 8.6 calc. for $C_{26}H_{44}O_8Si$ C, 60.9; H, 8.6%); $[\alpha]_D^{20}$ + 44.6° (c 1.0 in MeOH); R_F 0.31 (17% EtOAc/hexane); ν (CH_2Cl_2) 2990 (s) (CH), 2945 (s), 1792 (s) (C=O), 1453 (m), 1385 (s), 1375 (s) cm^{-1}; λ_{max} (MeOH) 252 nm, ϵ 11440; δ_H ($CDCl_3$, 250 MHz) 5.75 (1H, dd J 10.7, 4.4 Hz, H-4), 5.52 (1H, d J 10.7 Hz, H-3), 3.97-3.88 (5H, complex, H-5,6,7,8,8'), 1.83- 1.64 (8H, broad, cyclohexyl), 1.49 (3H, s, CMe_2), 1.49-1.42 (2H, broad, cyclohexyl), 1.34 (6H, s, CMe_2), 1.27 (3H, s, CMe_2), 0.90 (9H, s, $SiBu^t$), 0.13 (3H, s, SiMe), 0.12 (3H, s, SiMe); δ_C (CD_2Cl_2, 50.3 MHz), 162.3 (q, C-1), 140.1 (q, C-2), 112.8 (q, cyclohexyl), 109.0, 108.8 (q, 2 x CMe_2), 105.2 (CH, C-3), 80.1, 76.5, 71.7, 71.5 (CH, C-4,5,6,7), 65.2 (CH_2, C-8), 36.5, 36.3 (CH_2, cyclohexyl C-2',6'), 28.4, 26.3, 26.0, 25.4 (CH_3, 4 x CMe_2), 25.7 (CH_3, $SiBu^t$), 24.3 (CH_2, C-4'), 23.1, 23.1 (CH_2, C-3',5'), 18.6 (q, $SiBu^t$), -4.1, -4.3 (CH_3, SiMe), m/z (FAB) 512, 462, 440, 410, 398, 326, 213, 147.

(4R,5R,6R,7R)-4,5,6,7,8-Pentahydroxyhexylidenecyclohexanespiro-2'-(1'3'-dioxolan)-4'-one (15)

The olefin (13) (680 mg, 1.33 mmol) was dissolved in 90% aqueous acetic acid (2 ml) and added to 90% aqueous acetic acid (25 ml) at 90°C. The solution was stirred for two hours at 90°C then overnight at room temperature. Solvent was removed *in vacuo* and after repeated addition and removal of a 1:1 mixture of toluene and heptane the residue was triturated with cold 1:1 petrol/ether to afford (15) as an off-white solid. This was washed with 1:1 ether/ethyl acetate and crystallised from hot isopropanol (160 mg, 38%); m.p. 151-152°C; (Found: C, 52.8; H, 7.2. $C_{14}H_{22}O_8$ requires C, 52.8; H, 7.2%), $[a]_D^{20}$ -6,0° (c 0.3 in MeOH); R_F 0.08 (100% EtOAc); ν(KBr disc) 3360 (s) (OH), 2962 (m) (CH), 1793 (s), (C=O) cm^{-1}; λ_{max} (MeOH) 248 nm, ϵ 10270; δ_H (CD_3OD, 200 MHz) 5.75 (1H, d J 9.0 Hz, H-3), 4.70 (1H, dd J 8.9, 7.6 Hz, H-4), 3.92-3.68 (5H, complex, H-5,6,7,8,8'), 1.96-1.91 (4H, broad, cyclohexyl), 1.87-1.78 (4H, broad, cyclohexyl), 1.65-1.57 (2H, broad, cyclohexyl); δ_C (CD_3OD, 50 MHz), 162.4 (q, C-1), 139.0 (q, C-2), 111.7 (q, C-1'), 109.0 (CH, C-3), 71.3, 70.9, 69.5, 66.1 (CH, C-4,5,6,7), 63.0 (CH_2, C-8), 34.9 (CH_2, C-2'6'), 23.2 (CH_2, C-4'), 22.0 (CH_2, C-3'5'); m/z (FAB) 318, 301, 283, 262, 242, 211, 203, 198.

3-Deoxy-D-manno-2-octulosonic acid, barium salt (16)

To the pentaol (15) (346 mg, 1.09 mmol) in methanol (5 ml) was added barium hyroxide (176 mg, 0.54 mmol) in water (25 ml). The solution was stirred overnight when tlc showed reaction to be complete. Solvent was removed *in vacuo*, the residue redissolved in water (20 ml) and lyophilised to afford the salt (16) as a white, fluffy powder (quantitative); m.p. 168° dec.; (Found: C, 29.6; H, 4.6. $C_{16}H_{20}O_{16}Ba.2H_2O$ requires C, 29.7; H, 4.6%); $[\alpha]_D^{20}$ + 49.9° (c 2 in H_2O); ν(KBr disc) 3360 (s) (OH), 2940 (CH), 1605 (C=O) 1417 cm^{-1}; δ_H (D_2O, 200 MHz), 4.50-4.38 (m), 4.18 (m), 4.08-3.98 (m), 3.95-3.59 (m), 2.57 (dd J 14.3, 7.3 Hz), 2.33 (m), 2.10-1.83 (m); δ_C (D_2O, 100 MHz, major conformer) 176.7 (1, C-1), 96.5 (q, C-2), 71.2, 69.6, 66.7, 66.2 (CH, C-4,5,6,7), 62.9 (CH_2, C-8), 33.7 (CH_2, C-3); HRMS (FAB) 613.03492, $C_{16}H_{27}O_{16}Ba$ (MH^+) requires 613.03489.

3-Deoxy-D-manno-2-octulosonic acid, ammonium salt (17)

The barium salt (16) (207 mg, 0.32 mmol) was dissolved in water (10 ml) and to the solution was added solid ammonium sulphate (44.8 mg, 0.32 mmol). The solution was stirred at room temperature for one hour then the resultant suspension filtered through a plug of celite. The filtrate was lyophilised to afford the ammonium salt (17) as a white, fluffy solid which crystallised from hot 85% aqueous ethanol (166 mg, 95%); m.p. 121-124°C; (Found: C, 35.3; H, 7.1; N, 5.3. $C_8H_{17}O_8N.1H_2O$ requires C, 35.2; H, 7.0; N, 5.1%), $[\alpha]_D^{20}$ + 38.6° (c 1.1 in H_2O); ν(KBr disc) 3490 (s) (NH) 3300 (s) (OH) 2950 (m) (CH) 1597 (s) (C=O) cm^{-1}; δ_H (D_2O, 200 MHz), 4.56-4.42 (m), 4.16 (dd J 6.0, 2.1 Hz), 4.09-3.99 (m), 3.93-3.75 (m), 3.70-3.56 (m), 2.57 (dd J 14.2, 6.7 Hz), 2.33 (m), 2.09-1.81 (m); δ_C (D_2O, 50 MHz), 176.8 (q, C-1), 96.4 (q, C-2), 71.2, 69.3, 66.6, 66.2 (CH, C-4,5,6,7), 63.0 (CH_2,

C-8), 33.7 (CH_2, C-3); m/z (FAB) 256, 239, 232, HRMS 256.10320, $C_8H_{18}O_8N$ (MH^+) requires 256.10323. Lit.,[14] m.p. 119-122°C, $[\alpha]_D^{20}$ + 39.2° (c 1.7, H_2O).

3-Deoxy-4,5:7,8-di-O-isopropylidene-6-tert-butyldimethylsilyloxy-D-manno-2-octulosonic acid, sodium salt (14)

The olefin (13) (420 mg, 0.82 mmol) was dissolved in methanol (2 ml) and to the solution was added sodium hydroxide (32.8 mg, 0.82 mmol) in water (1 ml). The solution was stirred for two hours at room temperature after which time solvent was removed *in vacuo*. The residue was washed with a mixture of ether and petrol (1:1) and water and the aqueous phase removed and lyophilised to yield the required compound (14) (quantitative) m.p. 113°C; ν(KBr disc), 2955 (s) (CH), 2945 (CH), 1650 (m) (C=C) 1630 (s) (C=O) 1385 (s) cm^{-1}; λ_{max} (MeOH) 252 nm, ϵ 6246; δ_H (Z-isomer) (D_2O, 100 MHz), 7.05 (1H, m, H-3), 4.48 (1H, m, H-4), 4.32-4.05 (3H, m), 4.00-3.80 (2H, m), 1.42, 1.34, 1.32, 1.27 (3H, s, 4 x CMe_2), 0.96 (9H, s, $SiBu^t$), 0.13, 0.10 (3H, s, 2. SiMe); δ_C 171.3 (q, C-1), 153.6 (CH, C-2), 126.9 (CH, C-3), 11.5 (q, CMe_2), 109.5 (q, CMe_2), 74.6, 74.1, 72.2, 66.8 (CH, C-4,5,6,7), 64.5 (CH_2, c-8), 25.9 (CH_3, $SiBu^t$), 27.8, 26.2, 25.9, 24.9 (CH_3, 4 x CMe_2), 18.1 (q, $SiBu^t$), -4.5, -5.2 (CH_3, 2 x SiMe); m/z (FAB) 455, 415, 387, 369, 347, 325. HRMS 455.20770, $C_{20}H_{36}O_8NaSi$ (MH^+) requires 455.20773.

2,3:4,5-Di-O-isopropylidene-aldehydo-D-arabinose (24)

The bis acetonide (22)[19] (17.3 g, 0.051 mol) was dissolved in acetone (160 ml) and stirred mechanically with HgO (25.8 g), $HgCl_2$ (25.8 g) and water (13 ml) for two hours at 30°C, 1 hour at 50°C and 2 hours at reflux. After cooling, the slurry was filtered through celite and the filtrate concentrated *in vacuo*. The residue was dissolved in chloroform (400 ml) and the solution shaken with saturated aqueous potassium iodide (2 x 400 ml). The organic phase was washed with water (300 ml) and then dried over sodium sulphate. Filtration and concentration of the filtrate *in vacuo* afforded a yellow oil. The required compound (24) was isolated pure by distillation (8.35 g, 71%); b.p. 72°C at 0.1 mm Hg; $[\alpha]_D^{20}$ - 16.2° (c 1.5, $CHCl_3$); R_F 0.28 (50% EtOAc/- hexane); ν_{neat} 3040 (s), 2865 (m), 2775 (w), 1740 (s) (C=O), 1384 (s) cm^{-1}; δ_H ($CDCl_3$, 200 MHz), 9.74 (d, J 1.0 Hz, H-1), 4.39 (dd, J 6.0, 1.1 Hz, H-2), 4.17-3.95 (4H, complex, H-3,4,5,5'), 1.46, 1.41, 1.36, 1.33 (12H, CMe_2); δ_C ($CDCl_3$, 100 MHz), 199.8 (CH, C-1), 111.8, 110.0 (q, CMe_2), 83.2, 77.7, 76.4 (CH, C-2,3,4), 66.9 (CH_2m C-5), 27.0, 26.7, 26.2, 25.1 (CH_3, CMe_2); m/z (FAB) 231, 173, 143. HRMS 231.12322 $C_{11}H_{19}O_5$ (MH^+) requires 231.12324. Lit.,[19] $[\alpha]_D^{20}$ - 16.1° (c 4.5 in $CHCl_3$).

4R,5S,6R)-4,5:6,7-(Di-O-isopropylidene)-pentylidenecyclohexanespiro-2'-(1',3'-dioxolan)-4'-one (25)

To the phosphorane (3) generated from the phosphonium salt (2) (8.44 g, 0.017 mol) and DABCO (2.1 g, 0.019 mol) in dry benzene (35 ml) under nitrogen at 60°C was added aldehyde (24) (4.04 g, 0.017 mol) in benzene (35 ml) in 5 ml portions. the mixture was stirred at 60°C for 10 minutes and room temperature for 30 minutes after which time the pale yellow suspension was cooled and filtered. The filtrate was concentrated *in vacuo* and the residue applied to a dry flash column (SiO_2 : 12% ether/petrol). The desired product was obtained as a 2:1 mixture of E and Z geometrical isomers (5.0 g, 80%); R_F 0.46, 0.41 (50% ether/petrol).

To the above olefin (25) (1.0 g, 2.7 mmol) in petrol (50 ml) was added iodine (56 mg, 0.22 mmol) and the mixture stirred at reflux under a 400 A tungsten filament light for two hours. The solution was cooled and ether (50 ml) added. The solution was washed with aqueous sodium thiosulphate solution, brine then the organic phase was dried over anhydrous magnesium sulphate, filtered and the filtrate concentrated *in vacuo*. The residue was purified by dry flash chromatography (SiO_2 : 8% EtOAc/hexane) to yield mostly Z-olefin (25) (14:1 Z:E by proton NMR) (0.92 g, 92%); $[\alpha]_D^{20}$ - 12.8° (c 0.1 in $CHCl_3$); R_F 0.40 (50% ether/petrol); ν(CH_2Cl_2) 2940 (s) (CH), 1795 (s) (C=O), 1690 (m) (C=C) cm^{-1}; λ_{max} (MeOH) 251 nm, ϵ 10660; δ_H ($CDCl_3$, 250 MHz), 5.61 (1H, d ^3J 9.0 Hz, H-3), 4.78 (1H, dd J 9.0, 7.5 Hz, H-2), 4.18-4.06 (2H, m), 3.91 (1H, dd ^3J 8.2, 5.2 Hz), 3.82 (1H, dd ^3J 7.3, 6.7 Hz), 1.88-1.67 (8H, broad, cyclohexyl), 1.53-1.46 (2H, broad, cyclohexyl), 1.44 (6H, s, CMe_2), 1.38 (3H, s, CMe_2), 1.33 (3H, s, CMe_2); δ_C

162.3 (q, C-1), 140.6 (q, C-2); 112.8 (q, C-1'), 110.1, 109.6 (q, 2 x CMe_2), 106.2 (CH, C-3), 81.2, 76.4, 73.9 (CH, C-4,5,6), 66.8 (C, C-7), 36.4, 36.1 (CH_2, C-2',6'), 26.9, 26.6, 25.3 (CH_3, 4 x CMe_2), 24.3 (CH_2, C-4'), 23.0 (C, C-3',5'); m/z (FAB) 367, 354, 319, 312, 296, 253, 238, 183, 170, 144. HRMS 367.17566, $C_{19}H_{27}O_7$ requires 367.17566.

(4R,5S,6R)-4,5,6,7-Tetrahydroxy-pentylidenecyclohexane-spiro-2'-(1',3'-dioxolan)-4'one (26)

The olefin (25) (1.0 g, 2.7 mmol) was dissolved in ethanol (110 ml) containing trifluoroacetic acid (16 ml) and water (10 ml). The solution was stirred at 80°C for 14 hours then solvent was removed *in vacuo* with a cold finger trap. After repeated addition and removal *in vacuo* of a 1:1 mixture of toluene and heptane, the residue was triturated with ethyl acetate to obtain a white solid which was crystallised from hot isopropanol (390 mg, 50%); m.p. 131°C; (Found: C, 54.2; H, 7.9. Calc. for $C_{13}H_{20}O_7$: C, 54.2; H, 7.9%); $[\alpha]_D^{20}$ + 31.0° (c 1.0 in MeOH); R_F 0.1 (100% EtOAc); ν(KBr disc) 3340 (s) (OH), 2955 (m), (CH), 1794 (s) (C=O) CM^{-1}; λ_{max} (MeOH) 248 nm, ϵ 10840; δ_H (D_2O, 200 MHz), 5.74 (1H, d J 8.5 Hz, H-3), 4.81 (1H, dd ^3J 8.8, 2.6 Hz. H-4), 3.82-3.60 (4H, complex), 1.88-1.85 (4H, broad, cyclohexyl), 1.74-1.63 (4H, broad, cyclohexyl), 1.50-1.45 (2H, broad, cyclohexyl); δ_C (CD_3OD, 50 MHz), 162.5 (q, C-1), 137.6 (q, C-2), 112.0 (q, C-1'), 108.7 (CH, C-3), 73.2, 70.7, 65.2, (CH, C-4,5,6), 62.7 (CH_2, C-7), 34.9 (CH_2, C-2',6'), 23.1 (CH_2, C-4'), 21.9 (CH_2, C-3',5'); m/z (FAB) 290, 289, 271, 219, 211, 198, 181, 145. HRMS 289.12874, $C_{13}H_{21}O_7$ (MH^+) requires 289.12872.

3-Deoxy-D-arabino-2-heptulosonic acid, barium salt (27)

To the tetraol (26) (40 mg, 0.17 mmol) in methanol (1 ml) was added barium hydroxide (27.5 mg, 0.085 mmol) in water (4 ml). The solution was stirred for two hours at room temperature before removal of solvent *in vacuo*. The residue was triturated with ether to afford a solid which was dissolved in water then lyophilised to afford the salt (27) as a white, fluffy powder (quantitative); m.p. 185°C (dec.); (Found: C, 28.3; H, 4.2. Calc. for $C_{14}H_{22}O_{14}$ Ba. $2H_2O$: C, 28.6; H, 4.4%); $[\alpha]_D^{20}$ + 33.0° (C 1.0 in H_2O); ν(KBr disc) 3380 (s) (OH), 2925 (m) (CH), 1604 (s) (C=O) 1070 (s) cm^{-1}; δ_H (D_2O, 200 MHz), 4.01-3.78 (4H, m), 3.48 (1H, m), 2.23 (1H, dd J 13.1, 5.0 Hz, H-3e), 1.82 (1H, dd J 12.8, 12.2 Hz, H-3a); δ_C (D_2O, 100 MHz), 177.4 (q, C-1), 97.2 (q, C-2), 74.5, 71.5, 69.7 (CH, C-4,5,6), 61.4 (C, C-7), 40.0 (CH_2, C-3); m/z (FAB) 553, 497, 467, 453, 429, 245. HRMS 553.01375, $C_{14}H_{23}O_{14}Ba$ (MH^+) requires 553.01376.

(4S,5R)-4,6-O-Ethylidene-5-hydroxybutylidenecyclohexanespiro-2'-(1'-3'-dioxolan)-4'-one (29)

A stirred solution of sodium metaperiodate (11.5 g, 0.504 mol) in water (88 ml), cooled by an ice bath to 15-25°C, was treated with a solution of 4,6-O-ethylidene-D-glucose[25] (5.15 g, 0.025 mol) in water (50 ml) in 5 ml portions, over a period of 1 hour. After each addition, the pH of the solution was returned to pH 4.2 (methyl orange end point) by addition of 2N sodium hydroxide. After 3 additional hours, the solution was brought to pH 7.5 and then lyophilised. Ethyl acetate (3 x50 ml) was used to extract the desired product (28)[24] from the dried residue and concentration of the combined extracts afforded (28) as an oil; ν(CH_2Cl_2) 3460 (s) (OH), 2998 (s) (CH), 2872 (s), 1738 (s) (C=O) cm^{-1}. Phosphonium bromide (2) (16.990 g, 0.034 mol) and DABCO (14.144 g, 0.037 mol) in dry benzene (150 ml) were heated to 40°C under an atmosphere of nitrogen. The mixture was left for 5 minutes then the temperature was raised to 70°C before the aldehyde (28) (5.0 g, 0.034 mol) in benzene (50 ml) was added in 5 ml portions over 10 minutes. After a further 20 minutes, the pale yellow suspension was cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* and the residue applied to a dry flash column (SiO_2: 20% EtOAc/hexane). The desired product (29) was obtained as a mixture of geometrical isomers (9:1 Z/E) (7.3 g, 75%); $[\alpha]_D^{20}$ - 36.6° (C 2.0 in MeOH); R_F 0.7 (20% EtOAc/hexane); ν(CH_2Cl_2) 3600 (w) (OH), 2950 (s) (CH), 2870 (s), 1785 (s) (C=O), 1690 (m) cm^{-1}; λ_{max} (MeOH) 249 nm, ϵ 11290; δ_H ($CDCl_3$, 200 MHz), 5.46 (1H, d J 9.2 Hz, H-3), 4.99 (1H, dd 9.1, 9.1 Hz, H-4), 4.77 (1H, q J 5.1 Hz, CHMe), 4.17 (1H, m, H-4), 3.45 (2H, m, H-6,6'), 1.87-1.69 (8H, broad, cyclohexyl), 1.51-1.42 (2H, broad, cyclohexyl), 1.32 (3H, d J 5.1 Hz,

$CHCH_3$); δ_C ($CDCl_3$, 50.3 MHz), 162.4 (q, C-1), 140.9 (q, C-2), 112.3 (q, CMe_2), 109.1 (CH, C-3), 98.7 (CH, CHMe), 74.7 (CH, C-5), 70.8 (CH_2, C-6), 66.1 (CH, C-4), 36.0, 35.7, 23.9, 22.5 (CH_2, 5 x cyclohexyl CH_2), 20.3 (CH_3, CHMe); m/z 285, 279, 267, 254, 241, 223, 197, 141. HRMS 285.13382, $C_{14}H_{21}O_6$ (MH^+) requires 285.13380.

(4S,5R)-4,5,6-Trihydroxybutylidenecyclohexanespiro-2'-(1',3'-dioxolan)-4'-one (30)

The olefin (29) (3.047 g, 10.7 mmol) in a 1% (w/v) solution of iodine in methanol (150 ml) was heated to 60°C and stirred for five hours, after which time no starting material remained. A solution of sodium thiosulphate (50 ml) was added to the cooled solution then solvent was removed *in vacuo*. The residue was partitioned between ethyl acetate and brine and the organic phase dried over anhydrous sodium sulphate. After concentration of the solution *in vacuo*, the residue was applied to a dry flash column (SiO_2: 100% EtOAc) and the required product (30) obtained as an oil (0.15 g, 9.2%); (Found: C, 55.7; H, 7.4 Calc. for $C_{12}H_{18}O_6$: C, 55.8; H, 7.0%); R_F 0.11 (100% EtOAc); ν (CH_2Cl_2) 3330 (s) (OH), 2958 (m) (CH), 1795 (s) (C=O) cm^{-1}; λ_{max} (MeOH) 248 nm, ϵ 10065; δ_H (MeOD, 200 MHz), 5.73 (1H, d J 8.9 Hz, H-3), 4.60 (1H, dd J 9.0, 5.1 Hz, H-4), 3.79-3.54 (3H, complex, H-5,6,6'), 1.96-1.73 (8H, broad, cyclohexyl), 1.66-1.55 (2H, broad, cyclohexyl); δ_C (MeOD, 50.3 MHz), 162.2 (q, C-1), 138.8 (q, C-2), 111.8 (q, C-1'), 107.7 (CH, C-3), 74.2, 66.5 (CH, C-4,5), 62.3 (CH_2, C-6), 35.0 (CH_2, C-2',3'), 23.2 (CH_2, C-4'), 22.0 (CH_2, C-3',6'); m/z (FAB) 259, 258, 241, 167, 133. HRMS 259.11814, $C_{12}H_{19}O_6$ (MH^+) requires 259.11815.

2-Keto-3-deoxygluconic acid, barium salt (31)

To the triol (30) (55 mg, 0.21 mmol) in methanol (1 ml) was added barium hydroxide (33.6 mg, 0.11 mmol) in water (4 ml). The solution was stirred for two hours at room temperature before removal of solvent *in vacuo*. The residue was redissolved in water then lyophilised to afford the salt (31) as a white, fluffy powder (quantitative); m.p. 180°C (dec.); (Found: C, 28.6; H, 4.3. Calc. for $C_{12}H_{18}O_{12}Ba.H_2O$: C, 28.3; H, 3.9%); $[\alpha]_D^{20}$ + 18.9° (C 1.0 in H_2O); ν (KBr disc) 3400 (s) (OH), 2951 (m) (CH), 1605 (s) (C=O), 1428 (m) cm^{-1}; δ_H 4.25-3.45 (complex), 2.63-2.13 (m), 2.10-1.83 (m); δ_C (D_2O, 50.3 MHz) 176.7 (q, C-1), 99.5 (q, C-2), 67.3, 65.0 (CH, C-4,5), 64.3 (CH_2, C-6), 39.2 (CH_2, C-3); m/z (FAB) 493, 451, 429, 423, 413, 245. HRMS 492.99264, $C_{12}H_{19}O_{12}Ba$ (MH^+) requires 492.99267.

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