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A Synthesis of 3-Deoxy-D-*gluco*-oct-2-ulosonic Acid

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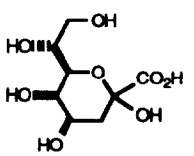
Abstract: Acyclic 2:3,5:6- and 3:4,5:6-di-*O*-isopropylidene-D-glucoses have been converted by four reactions involving a Wittig chain homologation, a catalytic hydrogenation, an acid hydrolysis and an acetonation into 2,3-dideoxy-5:6,7:8-di-*O*-isopropylidene-D-*gluco*-octono-1,4-lactone which underwent a Wasserman reaction and then a hydrolysis to yield 3-deoxy-D-*gluco*-oct-2-ulosonic acid, isolated as its ammonium salt **2**.

3-Deoxy-D-*manno*-oct-2-ulosonic acid (KDO) **1**, a name used synonymously with 3-deoxy-octulosonic acid by some, is a member of a biochemically important class of carbohydrates, namely 3-deoxy-gly-2-ulosonic acids which are sometimes erroneously referred to as 2-keto-3-deoxy-octonic acids.[1] These acids have a significant role in some biosynthetic processes and in the formation of specific biopolymers.[1,2] KDO was reported to be indispensable to the biosynthesis of the Gram-negative bacterial lipopolysaccharide (LPS).[3] Accordingly, compounds that disrupt the KDO metabolism would lead to a selective inhibition of the bacterial LPS biosynthesis and may therefore become a new class of antibacterial agents.[4] 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.[5,6] Although most workers have synthesised analogues of KDO by a chemical or an enzyme-catalysed aldol reaction between oxalacetic acid (and its equivalents) or pyruvate and an appropriate pentose derivative,[7] 3-deoxy-D-*gluco*-oct-2-ulosonic acid (D-*gluco*-KDO) was first synthesised from D-glucose *via* sequential Nef, Kiliani, and oxidation reactions.[8] We would like to explore the enzyme inhibitory potential of KDO analogues and recently we described[9] facile and simple syntheses of calcium salts of 3,4-dideoxy-D-*arabino*-oct-2-ulosonic acid and 3,4-dideoxy-D-*xylo*-oct-2-ulosonic acid. In this paper, we disclose an enantiospecific synthesis of the ammonium salt of D-*gluco*-KDO **2**, a configurational analogue of KDO.



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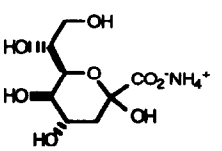
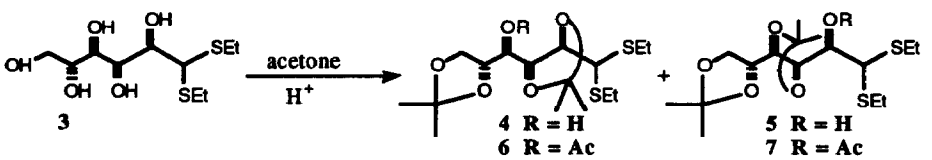


2

Several years ago, we reported[10] a practical synthesis of KDO involving essentially a Wittig chain homologation and a Wasserman reaction as the key steps. In order to demonstrate the applicability and versatility of the synthetic route, D-*gluco*-KDO could be obtained also *via* basically the same reaction sequence. In the present case, the substrate for the Wittig condensation reaction was made from D-glucose by the conventional method used to prepare *aldehydo*-sugars, i.e., sequential thioacetalisation, acetonation, and demercaptalisation. Thus isopropylidentation of D-glucose diethyl dithioacetal **3**[11] with acidic acetone under thermodynamic control afforded, after fractionation on a column, a 96% yield of two syrupy components in a ratio of 3:1. Their ^{13}C-NMR spectra displayed two quaternary ketal carbons in the range δ_C 109.5-110.0, typifying two dioxolane rings since the ketal carbons of 1,3-dioxolanes generally resonate from δ_C 108.5 to 115.7 whereas those of 1,3-dioxanes appear from δ_C 97.1 to 99.5.[12] This piece of evidence led to three possible structures for the diacetonides, namely a 2,3:4,5-, a 2,3:5,6-, and a 3,4:5,6 diacetonide. Under the thermodynamic conditions used, the formation of a 2,3:4,5-diacetonide is unlikely because the OH-4,5 of *erythro*-configuration would give the relatively less stable 1,3-dioxolane derivative having α,β-*cis*-substituents which would destablise the ring on account of non-bonded interactions.[13] Furthermore, the ^{13}C-NMR spectra of the two components revealed that C-6 in each component was deshielded compared with that in the unprotected dithioacetal **3**, signifying that the hydroxyl group at this position was ketalised:[14] hence the possiblity of a 2,3:4,5-diacetonide was ruled out.



3 **4** R = H, **6** R = Ac; **5** R = H, **7** R = Ac

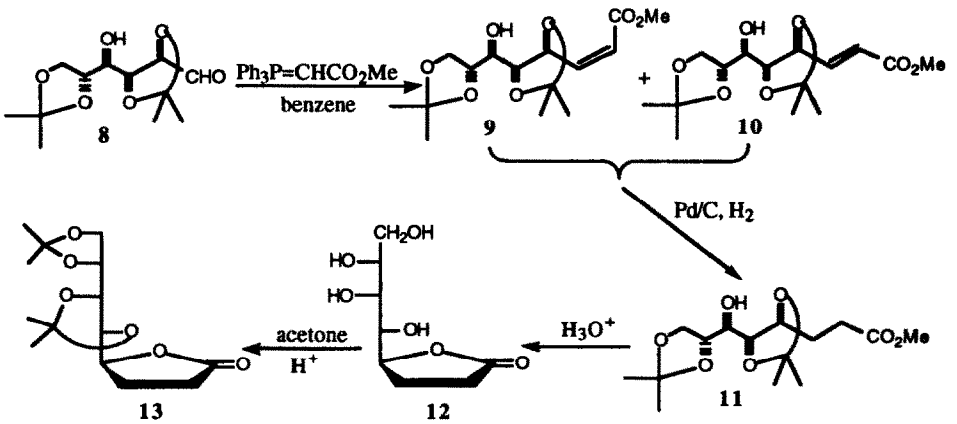
The differentiation between the two diacetonides **4** and **5** was accomplished by examining the ^{1}H-NMR spectra of their corresponding monoacetate derivatives **6** and **7**. The monoacetate, derived from the minor component, whose spectrum exhibited a downfield 1H dd coupled to H-1 and H-3, was ascribed to the 2-*O*-acetyl derivative **7**. The monoacetate from the major component had a spectrum which showed a downfield 1H dd, coupled to H-3 and H-5, was attributed to the 4-*O*-acetyl derivative **6**. The assignment of the protons was confirmed by the spin-decoupling techniques. Hence, acetonation of D-glucose diethyl dithioacetal **3** yielded the 2,3:5,6-di-*O*-isopropylidene derivative **4** as the major product and the 3,4:5,6-di-*O*-isopropylidene derivative **5** as the minor one. Two diacetonides from isopropylidenation of the same dithioacetal **3** have been isolated by Gorin[15] who proposed, but did not prove, that they were 2,3:5,6- and 3,4:5,6- substituted. Only specific optical rotations and elemental analytical result were reported, so it was not surprising that the structures were not rigorously established. In the present work, not only the specific optical rotation values were obtained but also the chromatographic mobility, the IR and NMR spectral parameters of the two diacetonides and their corresponding monoacetates have been recorded and structures ratified.

The two di-*O*-isopropylidene derivatives **4** and **5** were separately dethioacetalised and then subjected to a Wittig chain extension reaction. Demercaptalisation of the dithioacetal **4** with mercuric chloride and red mercuric oxide gave the syrupy aldehyde **8** (61%) which was treated directly with the ylid $Ph_3P{=}CHCO_2Me$ in boiling benzene to furnish, after chromatographic separation, the *Z*- and *E*- enoates, **9** and **10**, in a ratio of 2:1 (70%

combined yield). The assignment of the geometry of the double bond was evident from their ^{1}H-NMR spectra in which the $J_{2,3}$ of **9** was 12 Hz whereas that of **10** was 16 Hz. The ^{13}C-NMR spectra of compounds **9** and **10** were compatible with their assigned structures, showing the required carbonyl and olefinic carbon resonances. The preponderant formation of the *Z*-isomer in this case is not understood.

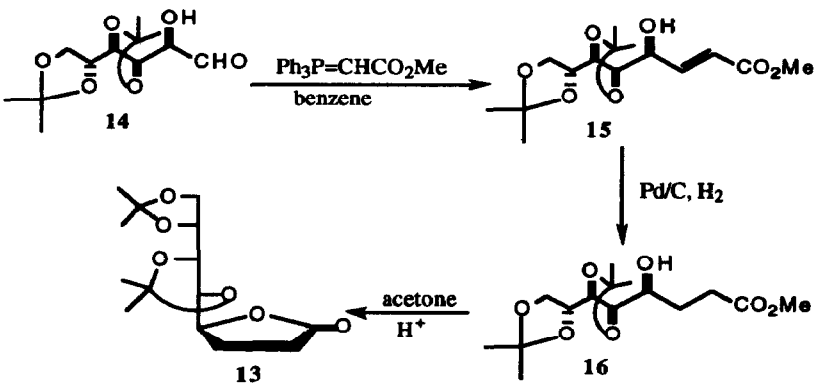
For practical reasons, a mixture of the *Z*- and *E*-isomers, **9** and **10**, was hydrogenated over palladium-on-charcoal to give a quantitative yield of the octonate **11**. The structure of the octonate **11** followed the mode of preparation, and was verified by the ^{13}C-NMR spectrum which exhibited the anticipated highfield carbon signals for C-2 and C-3 and a relatively deshielded carbonyl resonance resulting from the saturation of the double bond.

Removal of the isopropylidene groups from diacetonide **11** with aqueous trifluoroacetic acid (TFA) furnished the crystalline 1,4-lactone **12** in 85% yield. The $\nu_{max.}$ 1760 cm^{-1} was indicative of a five-membered lactone carbonyl which was substantiated by the resonance at δ 182.9 in its ^{13}C-NMR spectrum.



Acetonation of the tetraol **12** in the customary way gave the crystalline di-*O*-isopropylidene derivative **13** in 74% yield. The four highfield methyl singlets in the ^{1}H-NMR spectrum implied the presence of two acetonide protecting groups and this was supported by the correct elemental analyses. The $\nu_{max.}$ at 1777 cm^{-1} and the δ_C at 177.9 justified that the γ-lactone ring had not been ruptured. The two quaternary ketal carbons of the isopropylidene groups resonated at δ_C 110.1 and 110.2, thus confirming that two dioxolane rings were present. The corollary of the above evidence was that there was only one possibility of introducing two five-membered acetonide rings onto the tetraol **12** and so compound **13** must be the 5,6:7,8-di-*O*-isopropylidene derivative. This compound could also be, more efficiently, obtained from the said dithioacetal **5**.

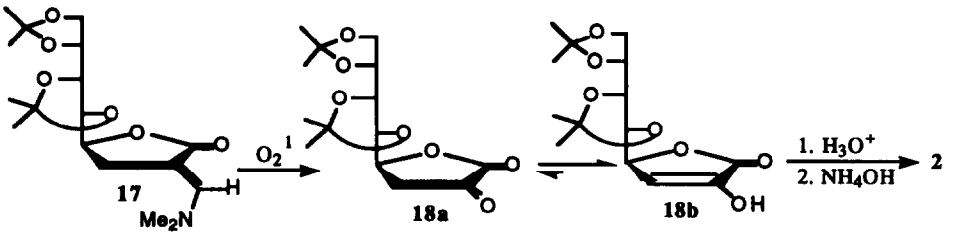
Demercaptalisation of the dithioacetal **5** with mercuric chloride and red mercuric oxide yielded the syrupy aldehyde **14** which immediately was treated with $Ph_3P{=}CHCO_2Me$ in benzene under reflux to furnish, after column chromatographic separation, the crystalline *E*-enonate **15** as the sole product in an overall yield of 73%.



The $J_{2,3}$ (15.5 Hz) of **15** established the *trans*-orientation of the methoxycarbonyl group and the sugar moiety. Catalytic hydrogenation of the double bond in enonate **15** gave a quantitative yield of the pure octonate **16**. The C-1 signal demonstrated the usual downfield shift upon removal of conjugation and this was indicative of a saturated carbonyl group.

Lactonisation of methyl octonate **16** was induced in acidic acetone solution under reflux and afforded (87% yield) the 1,4-lactone **13** identical with that described previously. Cyclisation of the same compound **16** with sodium methoxide, however, gave an equilibrium mixture containing the lactone **13** and starting material in a ratio of *ca.* 3:1 (t.l.c.); whereas the octonate **11**, when subjected to identical base treatment, underwent no observable chemical reaction and starting material was quantitatively recovered. This chemical behaviour is in full concordance with the substitution pattern of the di-*O*-isopropylidene groups in methyl octonates **11** and **16**, and in turn definitely established the structures of the two diacetonides **4** and **5** assigned on the basis of physical evidence.

The protected lactone **13** having been harvested, the next step was to introduce an oxo-function at the α-position. Two methods appear to be suitable for the conversion of lactone **13** into the α-ketolactone **18a**. The first one derived from the recognition that introduction of two sulfur substituents in the position alpha to the lactone carbonyl group (α-bisulfenylation[16]) constitutes a net oxidation of the methylene group to a carbonyl function.[17] Demercaptalisation of the created α-dithioketal would unmask the ketolactone derivative.[18] However, α-bisulfenylation of lactone **13** proved disappointing in practice and none of the desired sulfenylated lactone was detected. The second method was that of Wasserman and Ives[19] which involves conversion of the lactone into the *exo*-enaminolactone followed by oxidative cleavage of the enamine double bond with singlet oxygen to form the α-ketolactone and/or its enol tautomer. This two-step process proved highly successful when first applied to the synthesis of KDO.[10] The versatility of this sequence is now demonstrated by transforming lactone **13** into corresponding α-ketolactone **18a** in an excellent yield. Thus treatment of the protected lactone **13** with commercially available tris(dimethylamino)methane[20] for 20 days afforded the crystalline *exo*-enaminolactone **17** in 84% yield. The ^{1}H-NMR spectrum exhibited the salient features of the *exo*-enamine system: the olefinic proton resonated at δ 7.06 as a triplet, showing long rang allylic couplings to H-3 and H-3' and the 6H singlet of the dimethylamino group was centred at δ 2.07. The alkenic proton was so deshielded that it was reasonable to place it *syn* to the carbonyl function.



Sensitised photooxidation of the *exo*-enaminolactone **17** with singlet oxygen generated *in situ* gave a 90% yield of the α-ketolactone **18a** which existed preponderantly as its enol tautomer **18b**. The $\nu_{max.}$ at 3230 and 1650 cm^{-1} clearly indicated the presence of the enol tautomer **18b**. The ^{13}C-NMR spectrum in $CDCl_3$ displayed the relevant signals contributed by both tautomers. The diagnostic signals for the tautomer **18a** which resonated at δ 191.5, 160.2 and 37.2 were assigned C-1, C-2 and C-3 respectively because their chemical shifts approximated to those of acyclic α-ketoesters whereas the other three lowfield signals of higher intensity at δ 170.4, 143.2 and 116.5 were ascribed respecrtively to C-1, C-2 and C-3 of the enol tautomer **18b** (Table 1). When the ^{13}C-NMR spectrum of the same material was measured in d_6-acetone, only the relevant resonances due to the enol tautomer **18b** were observed, thereby confirming that the additional signals in the ^{13}C-NMR spectrum in $CDCl_3$ were attributable to a tautomer (i.e. **18a**) and not to impurities.

Table 1 ^{13}C-NMR Spectral Parameters of α-Ketolactone Tautomers 18a 18b

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	CMe_2	Me
18a	160.2	191.5	37.2	81.9	77.3	76.2	73.1	68.0	110.3,111.2	25.3, 25.9,
⇅ $CDCl_3$										26.2, 26.7
18b	170.4	143.2	116.5	79.6	77.3	77.5	77.5	68.0	110.3,110.7	27.3
18b	169.8	144.6	117.3	80.6	77.7	78.1	78.3	68.3	110.5,110.8	25.5, 26.7
d_6-acetone										27.1,27

Deacetonation of the lactone tautomers, **18a** and **18b**, with aqueous TFA yield D-*gluco*-KDO which was isolated as its ammonium salt **2**. Its specific rotation value was in reasonable agreement with that reported in literature.[8] The ^1H-NMR spectrum of compound **2** was uninformative but the absence of the isopropylidene methyl signals was compatible with the structure **2**. In its ^{13}C-NMR spectrum, the signals in the anomeric carbon absorption region at δ_C 94.9, 95.7; 102.3, 106.1 (*ca.* 5:3:2:2) suggested the presence of four isomers. The former two were tentatively assigned to the α-D- and β-D-anomers of the pyranose form of ammonium D-*gluco*-KDO **2**, and the latter two were ascribed to the α-D- and β-D-anomers of the furanoid form respectively. This assignment was corroborated by the observation of the corresponding four deoxy-carbon (C-3) resonances at δ_C 33.4, 36.2; 45.9, 47.3 respectively. However, the carbinol resonance region was complex and no rigorous assignment of peaks was possible owing to extensive overlap of signals. The infrared band from 1600 to 1650 cm^{-1} indicated the carboxylate carbonyl group which was substantiated by δ_C 170.9, 174.3 and 175.8.

The overall yield of ammonium D-*gluco*-KDO from D-glucose by this route was *ca.* 21%. This approach, which involves essentially the Wittig reaction for chain lengthening and the Wasserman method for α-oxygenation of a γ-lactone, appears to be a general synthetic sequence for the preparation of KDO analogues from hexoses other than D-mannose.

Experimental

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

3,4:5,6- and 2,3:5,6-*Di*-O-*isopropylidene*-D-*glucose diethyl dithioacetal* **5** and **4**. — D-Glucose diethyl dithioacetal **3**[11] (21 g) was stirred at room temperature with dry acetone (210 ml) containing toluene-*p*-sulphonic acid monohydrate (0.5 g). After 24 h, 2,2-dimethoxypropane (20 ml) was added dropwise over a period of 1 h and the resulting solution was stirred for a further 52 h. The solution was cooled and adjusted to pH 8 with ammonia (S.G. 0.88). Customary work-up gave a syrupy mixture containing two major components with R_f values of 0.3 and 0.5 (diethyl ether:hexanes, 1:2). Fractionation by column chromatography gave the less polar component, 3,4:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal **5** (6.6 g, 24.5%); $[\alpha]_D^{22}$ + 15 (*c*, 1.5 in chloroform); R_f 0.5 (diethyl ether:hexanes, 1:2); $\nu_{max.}$ 3480 cm^{-1} (OH); lit.,[15] $[\alpha]_D$ - 9 (*c*, 1.6 in methanol); δ_H 1.31 (*t*, 6H, 2 SCH_2CH_3, *J* 7 Hz), 1.39 (*s*, Me), 1.46 (*s*, 9H, 3 Me), 2.76 (*q*, 4H, 2 SCH_2CH_3, *J* 7 Hz), 2.98 (*d*, 1H, OH, temp. depend., $J_{OH,2}$ 7 Hz), 3.83 (*dt*, 1H, H-2, $J_{2,3}$ 1.5, $J_{2,1}$ 7, $J_{2,OH}$ 7 Hz), 3.98-4.32 (*m*, 5H, H-1,4,5,6,6'), 4.56 (*dd*, 1H, H-3, $J_{3,2}$ 1.5, $J_{3,4}$ 6 Hz); δ_c 14.5 (2 SCH_2CH_3), 24.4, 25.0, 25.4, 27.3 (4 Me), 26.8 (2 SCH_2CH_3), 56.0 (C-1), 68.0 (C-6), 71.0 (C-2), 77.5, 79.3 (C-3,4,5), 109.9 (2 CMe_2). Found C, 52.67; H, 8.35; S, 17.1. Calcd. for $C_{16}H_{30}O_5S_2$ C, 52.43; H, 8.25; S, 17.5% and the more polar 2,3:5,6-di-*O*-isopropylidene-D-glucose diethyl dithioacetal **4** (19.2 g, 71.4%), $[\alpha]_D^{22}$ - 47.8 (*c*, 2.0 in chloroform); R_f 0.3 (diethyl ether:hexanes, 1:2); $\nu_{max.}$ 3480 cm^{-1} (OH); lit.,[15] $[\alpha]_D$ -44 (*c*, 1.9 in methanol); δ_H 1.31 (*t*, 6H, $2SCH_2CH_3$, *J* 7 Hz), 1.38, 1.47, 1.51 (3*s*, 12H, 4 Me), 2.53 (*d*, 1H, OH, temp.depend., $J_{OH,4}$ 8 Hz), 2.81 (*q*, 4H, 2 SCH_2CH_3, *J* 7 Hz), 3.80 (*m*, 1H, H-4), 3.98-4.50 (*m*, 6H, H-1,2,3,5,6,6'); δ_c 14.5 (2 SCH_2CH_3), 25.0, 25.3, 27.2 (2 SCH_2CH_3 and 4 Me), 53.4 (C-1), 67.0 (C-6), 71.4 (C-4), 76.8. 79.6 (C-2,3,5), 109.5, 110.0 (2 CMe_2). Found C, 52.94; H, 8.37; S, 17.0. Calcd. for $C_{16}H_{30}O_5S_2$ C, 52.43; H, 8.25; S, 17.5%.

4-O-*Acetyl*-2,3:5,6-*di*-O-*isopropylidene*-D-*glucose diethyl dithioacetal* **6**. — Acylation of the alcohol **4** (120 mg) with acetic anhydride in pyridine (1:2, 3 ml) at room temperature overnight afforded a quantitative yield of the syrupy *monoacetate* **6**; $[\alpha]_D^{22}$ - 31.4 (*c*, 1.4 in chloroform); R_f 0.6 (diethyl ether:hexanes, 1:2); $\nu_{max.}$ 1742 cm^{-1} (ester carbonyl); δ_H 1.26, 1.27 (2*t*, 6H, $2SCH_2CH_3$, *J* 7.5 Hz), 1.36, 1.41 (×2), 1.46 (3*s*, 12H, 4 Me), 1.85 (*s*, 3H, OAc), 2.64-2.80 (*m*, 4H, $2SCH_2CH_3$), 3.87 (*d*, 1H, H-1, $J_{1,2}$ 6.2, Hz), 3.92 (*dd*, 1H, H-6', $J_{6',5}$ 6, $J_{6',6}$ 8.8 Hz), 4.04 (*dd*, 1H, H-6, $J_{6,5}$ 6.0 Hz), 4.03 (*dd*, 1H, H-2, $J_{2,3}$ 7.0 Hz), 4.31 (q, 1H, H-5, $J_{5,4}$ 6.0 Hz), 4.42 (*dd*, 1H, H-3, $J_{3,4}$ 2.0 Hz), 5.28 (*dd*, 1H, H-4); δ_c 14.3 (×2) (2 SCH_2CH_3), 20.9 (CH_3CO), 24.5, 25.3, 25.4, 26.6, 26.9, 27.5 (4Me, $2SCH_2CH_3$), 53.2 (C-1), 66.1 (C-6), 71.4 (C-4), 75.5 (C-5), 79.1 (C-2,3), 109.4, 110.7 (2 CMe_2), 170.5 (CH_3CO). Found C, 52.87; H, 7.93; S, 15.52. $C_{18}H_{32}O_6S_2$ requires C, 52.91; H, 7.90; S, 15.70%.

2-O-*Acetyl*-3,4:5,6-*di*-O-*isopropylidene*-D-*glucose diethyl dithioacetal* **7**. — 2-Hydroxy mercaptal **5** (150 mg) was acylated with acetic anhydride (1 ml) in pyridine (2 ml) at room temperature overnight and the product was isolated in the conventional way to give the syrupy *monoacetate* **7** (154 mg, 92%); $[\alpha]_D^{20}$ - 10.1 (*c*, 1.2 in chloroform); R_f 0.6 (diethyl ether:hexanes, 1:2); $\nu_{max.}$ 1745 cm^{-1} (ester C=O). δ_H (C_6D_6)

1.07, 1.11 (2*t*, 6H, $2SCH_2CH_3$, *J* 7.4 Hz), 1.22, 1.36, 1.46, 1.52 (4*s*, 4Me), 1.85 (*s*, 3H, OAc), 2.64 (*quin*, 4H, $2SCH_2CH_3$), 3.87 (*t*, 1H, H-4, $J_{4,3}$, $J_{4,5}$ 7.0 Hz) 3.94-4.03 (*m*, 3H, H-5, 6, 6'), 4.41 (*d*, 1H, H-1, $J_{1,2}$ 9.6 Hz), 5.12 (*dd*, 1H, H-3, $J_{3,2}$ (2.2 Hz), 5.59 (*dd*, 1H, H-2); δ_c 14.1, 14.5 (2 SCH_2CH_3), 21.0 (CH_3CO), 24.6(×2), 24.9, 26.5, 26.8, 27.5 (4 Me, 2 SCH_2CH_3), 52.9 (C-1), 67.6 (C-6), 72.6 (C-2), 77.3 (C-3), 78.4 (C-4), 78.7 (C-5), 110.1(×2) ($2CMe_2$), 170.3 (CH_3CO). Found C, 52.93; H, 7.70; S, 15.46. $C_{18}H_{32}O_6S_2$ requires C, 52.91; H, 7.90; S, 15.70%.

2,3:5,6-*Di*-O-*isopropylidene*-aldehydo-D-*glucose* **8**. — To a vigorously stirred solution of the dithioacetal **4** (6 g) in aqueous acetone (90% *v/v*, 60 ml), were added red mercuric oxide (8.8 g) and mercuric chloride (9.5 g). Vigorous stirring was continued for 2 h at room temperature. The mixture was then filtered through kieselguhr into a flask containing red mercuric oxide (1 g), and the residue was washed with acetone (2 × 30 ml). The combined filtrate and washings were concentrated at a bath temperature of 20-25 °C to a syrup which was triturated with cold chloroform (2 × 40 ml). The suspension was filtered through kieselguhr to remove the insoluble material and the filtrate was washed with water (2 × 30 ml). Each water wash was back extracted with chloroform (40 ml). The combined chloroformic solution was dried and concentrated to yield the syrupy aldehyde **8** (2.6 g, 61%), R_f 0.1 (diethyl ether:hexanes, 3:2). The ^{1}H-NMR spectrum showed, *inter alia*, an aldehydic proton resonance at δ 9.6. This crude material was used in the following synthesis without further purification.

Z- *and* E-*Methyl* 2,3-*dideoxy*-4,5:7,8-*di*-O-*isopropylidene*-D-gluco-*oct*-2-*enonate* **9** and **10**. — The crude aldehyde **8** (2.5 g) and (methoxycarbonylmethylene)-triphenylphosphorane(4g) in dry benzene (80 ml) were heated under reflux for 1 h. After evaporation of the solvent, the residue was triturated with diethyl ether (50 ml) and the mixture cooled to 0 °C for 15 h. The insoluble triphenylphosphine oxide was filtered off and washed with cold ether (2 × 20 ml). The combined filtrate and washings were concentrated to a syrupy mixture which was shown by t.l.c.examination to contain two components with R_f values of 0.3 and 0.4 (diethyl ether:hexanes, 3:2). Separation by gradient elution column chromatography—diethyl ether in hexanes, 50-70%—furnished the less polar Z-*enonate* **9** (1.45 g, 48%) as a colourless syrup, $[\alpha]_D^{22}$ + 67.5 (*c*, 1.0 in chloroform); R_f 0.4 (diethyl ether:hexanes, 3:2); $\nu_{max.}$ 3520 (OH), 1720 (conjugated ester C=O), 1660 cm^{-1} (C=C); δ_H 1.42, 1.44, 1.54 (×2) (3*s*, 4Me), 3.34(*d*, 1H, $J_{OH,6}$ 7.2 Hz, OH), 3.70 (*dt*, 1H, H-6, $J_{6,5}$ 1.5, $J_{6,7}$ 7.0 Hz), 3.89 (*s*, 3H, OMe), 4.06 (*dd*, 1H, H-5, $J_{5,4}$ 8.5 Hz), 4.20-4.40 (*m*, 3H, H-7,8,8'), 5.69 (*dt*, 1H, H-4, $J_{4,3}$ 8.5, $J_{4,2}$ 1.0 Hz), 6.18 (*dd*, 1H, H-2, $J_{2,3}$ 12.0 Hz), 6.52 (*dd*, 1H, H-3); δ_c 25.5, 26.8, 27.0, 27.2 (4 Me), 52.1 (OMe), 67.5 (C-8), 68.9, 73.0, 76.2 79.9 (C-4,5,6,7), 109.5, 110.3 ($2CMe_2$), 112.6 (C-2), 147.0 (C-3) 167.1 (C-1). Found C, 57.0; H, 7.7. $C_{15}H_{24}O_7$ requires C, 57.4; H, 7.7%. This was followed by the isolation of the more polar E-*isomer* **10** (0.67 g, 22%) with m.p. 73-76 °C. Recrystallisation from light petroleum (b.p. 60-80 °C) afforded transparent, regularsized needles with m.p. 79 °C, $[\alpha]_D^{21}$ - 22.2 (*c*, 1.2 in methanol); R_f 0.3 (diethyl ether:hexanes, 3:2); $\nu_{max.}$ 3490 (OH), 1710 (C=O), 1670 cm^{-1} (C=C); $\lambda_{max.}$ 219 (ϵ 8.5 × 10^3); δ_H 1.41, 1.46, 1.52 (×2) (3*s*, 4 Me), 2.55 (*d*, 1H, OH, $J_{OH,6}$ 7.0 Hz), 3.50-4.30 (*m*, 5H, H-5,6,7,8,8'), 3.89 (*s*, OMe), 4.79 (*ddd*, 1H, H-4, $J_{4,5}$ 8.0, $J_{4,3}$ 5.5, $J_{4,2}$ 1.5 Hz) 6.37 (*dd*, 1H, H-2, $J_{2,3}$ 16.0 Hz), 7.16 (*dd*, 1H, H-3); δ_c 25.3, 26.9 (×3) (4 Me), 51.8 (OMe), 67.3 (C-8), 70.3, 76.4 (×2), 80.1 (C-4,5,6,7), 109.8, 110.6 ($2CMe_2$), 122.8(C-2), 144.5

(C-3), 166.7 (C-1). Found C, 57.0; H, 7.7. $C_{15}H_{24}O_7$ requires C, 57.0; H, 7.7%.

Methyl 2,3-*dideoxy*-4,5:7,8-*di*-O-*isopropylidene*-D-gluco-*octonate* **11**. — Hydrogenation of a mixture of *E*- and *Z*-enonates **9** and **10** (1.1 g) in ethyl acetate (50 ml) in the presence of palladium-on-charcoal (10%, 0.2 g) at room temperature and atmospheric pressure for 10 min gave a quantitative yield of the analytically pure *methyl octonate* **11** as a colourless syrup, $[\alpha]_D^{20}$ - 29.3 (*c*, 1.2 in chloroform); R_f 0.25 (diethyl ether:hexanes, 3:2); $\nu_{max.}$ 3480-3500 (OH), 1735 cm^{-1} (non-conjugated ester C=O); δ_H 1.36 (*s*,Me), 1.39(*s*,9H, 3 Me), 1.60-2.05(*m*,2H, H-3,3'),2.40-2.59(*m*,3H, H-2,2' and OH), 3.28-3.45(*m*, 1H, H-6),3.65 (*s*, OMe), 3.81 (*dd*, 1H, H-5, $J_{5,4}$ 7, $J_{5,6}$ 1.8 Hz), 3.93-4.12(*m*, 4H, H-4,7,8,8'); δ_c 25.3,27.5,26.6 (×2) (4 Me), 28.0, 30.6 (C-2,3), 51.8 (OMe), 67.3 (C-8), 70.4, 76.2, 76.6, 80.2 (C-4,5,6,7), 109.4, 109.6 (2*C*Me_2), 173.9 (C-1). Found C, 56.65; H, 8.56. $C_{15}H_{26}O_7$ requires C, 56.59; H, 8.23%.

2,3-*Dideoxy*-D-gluco-*octono*-1,4-*lactone* **12**. — The diacetonide **11** (0.8 g) in aqueous TFA (50% *v*/*v*, 20 ml) was stirred at room temperature overnight. Evaporation of the solvent gave a crystalline residue with m.p. 130-133 °C. Recrystallisation of the solid from 96% aqueous ethanol gave the 1,4-*lactone* **12** (0.44 g, 86%) as small white needles, m.p. 135-137 °C; $[\alpha]_D^{18}$ + 45.3 (*c*, 0.6 in water); R_f 0.3 (EtOAc:AcOH:H_2O, 3:1:1); $\nu_{max.}$ 3300 (OH), 1760 cm^{-1} (γ-lactone carbonyl); $\delta_H(D_2O)$ 1.83-2.44(*m*, 2H, H-3,3'), 2.50-2.77(*m*, 2H, H-2,2'), 3.54-3.95(*m*, 5H, H-5,6,7,8,8'), 4.74 (*q*, 1H, H-4, $J_{4,3} = J_{4,3'} = J_{4,5}$ 7 Hz); δ_c (D_2O) 24.6, 29.5 (C-2,3), 63.7 (C-8), 70.8, 71.5, 72.8, 84.6 (C-4,5,6,7), 182.9 (C-1). Found C, 46.35; H, 6.84. $C_8H_{14}O_6$ requries C, 46.60; H, 6.84%.

2,3-*Dideoxy*-5,6:7,8-*di*-O-*isopropylidene*-D-gluco-*octono*-1,4-*lactone* **13**

(a) From γ-lactone **12** — The tetraol **12** (0.71 g) was acetonated with acidic acetone in the conventional way to give a white solid after work-up. Recrystallisation of the product from light petroleum (b.p. 60-80°) furnished the isopropylidenated 1,4-*lactone* **13** (0.74 g, 75%) as long chunky needles with m.p. 93 °C; $[\alpha]_D^{22}$ + 40.4 (*c*, 0.8 in chloroform); R_f 0.5 (diethyl ether:CH_2Cl_2, 1:5); $\nu_{max.}$ 1775 cm^{-1} (γ-lactone carbonyl); δ_H (C_6D_6) 1.31 (*s*, 6H, 2 Me), 1.39, 1.43 (2*s*) (2 Me), 1.61-2.63(*m*, 4H, H-2,2',3,3'), 3.83 (*dd*, 1H, H-5, $J_{5,4}$ 1.6, $J_{5,6}$ 7.5 Hz), 3.94-4.29 (*m*, 4H, H-6,7,8,8'), 4.57 (*ddd*, 1H, H-4, $J_{4,3}$ 4, $J_{4,5}$ 1.5 Hz); δ_c 25.3, 26.3, 26.7, 27.3 (4 Me), 24.8, 27.9 (C-3,2), 68.2 (C-8), 77.0, 77.5 (×2), 83.1 (C-4,5,6,7), 110.1, 110.2 (2 *C*Me_2), 177.9 (C-1). Found C, 58.92; H, 7.70. $C_{14}H_{22}O_6$ requires C, 58.72; H, 7.75%.

(b) From methyl octonate **16** — Compound **16** (3.1 g) in absolute acetone (60 ml) containing toluene-*p*-sulphonic acid monohydrate (0.1 g) was heated under reflux for 7 h. The cooled solution was worked up in the conventional way to give a pale yellow syrup which was dissolved in hot light petroleum (b.p. 60-80 °C) (*ca.* 30 ml). The solution was cooled slowly to room temperature and rapid crystallisation was induced on seeding to yield the identical *lactone* **13** (2.4 g, 87%) as colourless long needless.

3,4:5,6-*Di*-O-*isopropylidene*-D-*glucose* **14**. — The mercaptal **5** (6.5 g) in aqueous acetone (90% *v*/*v*, 80 ml) was treated with red mercuric oxide (8 g) and mercuric chloride (9 g) in the same way as described for compound **4**. Customary work-up afforded the crude *aldehyde* **14** (4.5 g, 97%) as a syrup, R_f 0.1 (diethyl ether:hexanes, 3:2). This material was used directly in the following synthesis.

E-*Methyl* 2,3-*dideoxy*-5,6:7,8-*di*-O-*isopropylidene*-D-gluco-*oct*-2-*enonate* **15**. — A solution of the crude aldehyde **14** (4.5 g) and (carbomethoxymethylene)-triphenylphosphorane(7g) in dry benzene (120 ml)

was heated under reflux for 3 h. The product was isolated in the conventional way as a syrupy mixture which was fractionated by column chromatography (diethyl ether:hexanes, 2:1) to give the crystalline *E*-enonate **15** (4.1 g, 75%). Recrystallisation from light petroleum (b.p. 60-80 °C) furnished the *title compound* as fluffy centimeter-long needles with m.p. 77-77.5 °C, $[\alpha]_D^{18}$ - 24.2 (*c*, 1.0 in chloroform); R_f 0.5 (diethyl ether:hexanes, 2:1); $\nu_{max.}$ 3350, 1690, 1650 cm^{-1} (OH, C=O, C=C); $\lambda_{max.}$ 215 nm (ϵ 7.5 × 10^3); δ_H 1.41, 1.44, 1.47 (×2) (3*s*, 12H, 4Me), 3.35 (*d*, 1H, OH, $J_{OH,4}$ 8.5 Hz), 3.81 (*s*, OMe), 3.90-4.35 (*m*, 5H, H-5,6,6,8,8'), 4.61 (*dddd*, 1H, H-4, $J_{4,5}$ 3.0, $J_{4,3}$ 4.1, $J_{4,2}$ 2.0 Hz), 6.25 (*dd*, 1H, H-2, $J_{2,3}$ 15.5 Hz), 7.20 (*dd*, 1H, H-3); δ_c 25.2, 26.6, 27.0, 27.1 (4 Me), 51.7 (OMe), 68.0 (C-8), 69.7, 77.0, 77.7, 82.1 (C-4,6,6,7), 110.1, 110.4 (2CMe_2), 121.7 (C-2), 147.7 (C-3), 167.0 (C-1). Found C, 56,91; H, 7.68. $C_{15}H_{24}O_7$ requires C, 56.95; H, 7.65%.

Methyl 2,3-*dideoxy*-5,6:7,8-*di*-O-*isopropylidene*-D-gluco-*octonate* **16**. — The *E*-enonate **15** (3.5 g) in ethyl acetate (70 ml) was hydrogenated over palladium-on-charcoal (10%, 0.37 g) at room temperature and atmospheric pressure for 5 min to give a quantitative yield of the syrupy *methyl octonate* **16**; $[\alpha]_D^{19}$ + 0.3 (*c*, 1.1 in chloroform); R_f 0.4 (diethyl ether:hexanes, 2:1); $\nu_{max.}$ 3450 (OH), 1720 (ester C=0); δ_c 25.3, 26.7, 27.2 (×2) (4 Me), 29.8, 30.7 (C-3,2), 51.6 (OMe), 68.0 (C-8), 69.8, 77.3, 77.7, 83.0 (C-4,5,6,7), 109.7, 110.0 (2 CMe_2), 174.5 (C-1). Found C, 55.96; H, 8.33. $C_{15}H_{26}O_7$ requires C, 56.59; H, 8.23%.

2,3-*Dideoxy*-2-(*dimethylaminomethylene*)-5,6:7,8-*di*-O-*isopropylidene*-D-gluco-*octono*-1,4-*lactone* **17**. — A mixture of the finely powdered lactone **13** (4.2 g) and tris(dimethylamino)methane (Aldrich, 10 g) was stirred at *ca.* 70 °C under a slow stream of dry nitrogen for 20 days. Aqueous work-up procedure yielded a colourless syrup which crystallised instantly when triturated with diethyl ether (5 ml). The crude *exo*-enaminolactone (4.2 g, 84%) had m.p. 123-125 °C. Recrystallisation of a portion of the product furnished an analytical sample as colourless plates with m.p. 126-127 °C, $[\alpha]_D^{23}$ + 128.3 (*c*, 1.1 in chloroform); R_f 0.4 (diethyl ether:ethyl acetate, 4:1); $\nu_{max.}$ 1725 (C=O), 1630 cm^{-1} (C=C); $\lambda_{max.}$ 296 nm (ϵ 6.93 × 10^4); δ_H 1.27, 1.35, 1.41, 1.52 (4*s*, 4 Me), 2.07 (×2) (*s*, NMe_2), 2.66 (*ddd*, 1H, H-3', $J_{3',=CH}$ 1.8, $J_{3',3}$ 14, $J_{3',4}$ 9.7 Hz), 2.98 (*ddd*, 1H, H-3, $J_{3,=CH}$ 1.8, $J_{3,4}$ 5.0 Hz), 3.96 (*dd*, 1H, H-5, $J_{5,4}$ 1.8, $J_{5,6}$ 7.8 Hz), 3.98-4.29 (*m*, 3H, H-6,8,8'), 4.39 (*ddd*, 1H, H-7, $J_{7,6}$ 2.8, $J_{7,8}$ 5.5, $J_{7,8'}$ 8.0 Hz), 4.69 (*ddd*, 1H, H-4), 7.06 (*t*, 1H, =CH); δ_c 25.4, 26.5, 26.8, 27.4 (4 Me), 28.6 (C-3), 41.7 (×2) (NMe_2), 68.1 (C-8), 74.0, 77.1, 77.7, 82.6 (C-4,5,6,7), 87.7 (C-2), 110.0, 110.1 (CMe_2), 175.5 (C-1). Found C, 59.7; H, 8.1; N, 4.1. $C_{17}H_{26}NO_6$ requires C, 59.8; H, 8.0; N, 4.1%.

3-*Deoxy*-5,6:7,8-*di*-O-isopropylidene-D-gluco-*oct*-2-*ulosono*-1,4-*lactone* (**18a** ⇌ **18b**). — Oxygen was bubbled through a solution of the crude *exo*-enaminolactone **17** (2.5 g) in methylene dichloride (90 ml) containing methylene blue (*ca.* 3 mg) and the mixture was irradiated for 80 min at - 72 °C (temperature maintained by a bath of solid carbon dioxide/absolute ethanol) with a ARGAPHOTO-B 240V 500W lamp (Philips PF308 E121) through Pyrex. The solution was then allowed to warm slowly to room temperature and washed with water (3 × 70 ml), decolourised with charcoal (*ca.* 100 mg), dried, filtered, and the filtrate concentrated to a yellow syrup which crystallised from ether. The crude *title compound* (1.98 g, 90%) had m.p. 119-121 °C, $[\alpha]_D^{21}$ + 29.3 (*c*, 0.8 in chloroform); $\nu_{max.}$ 1750, 1735 (C=O), 1650 (C=C), 3230 cm^{-1} (OH); R_f 0.75 (diethyl ether); δ_H (d_5-pyridine) 1.38, 1.39, 1.42, 1.48 (4*s*, 4 Me), 4.09-4.39 (*m*, 5H, H-

5,6,7,8,8'),5.31 (*t*, 1H, H-4, $J_{4,3} = J_{4,5}$ 2 Hz), 6.40 (*d*, 1H, H-3, $J_{3,4}$ 2 Hz); ^{13}C-NMR spectrum, see Table 1. Found C, 55.6 ; H, 7.0. $C_{14}H_{20}O_7$ requires C, 56.0 ; H, 6.7%.

Ammonium 3-*deoxy*-D-gluco-*oct*-2-*ulosonate* **2**. — The crude lactone tautomers **18a** and **18b** (0.75 g) were stirred with a solution of aqueous TFA (10% *v/v*, 10 ml) and ethanol (10 ml) at room temperature overnight. The solvent was evaporated and the residue was dissolved in water (15 ml). The resulting solution was washed with CH_2Cl_2 (3 × 10 ml) and the aqueous phase was neutralised with 0.1N ammonium hydroxide. Concentration of the neutral solution afforded the *ammonium salt* **2** (0.56 g, 88%) as a yellow glass, $[\alpha]_D^{23}$ + 16.5 (*c*, 0.7 in water, no mutarotation observed), lit.[8] $[\alpha]_D$ +12.5→ + 11.9°(12 h constant value)(*c*, 1.0 in water); R_f 0.6, 0.5 (EtOAc:AcOH:H_2O, 3:2:2); $\nu_{max.}$ 1600-1650 cm^{-1} (carboxylate C=O) For NMR spectral data, see discussion. Found C, 37.9 ; H, 6.4 ; N, 4.8. Calc. for $C_8H_{17}NO_8$ C, 37.7 ; H, 6.7 ; N, 5.5%.

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