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SYNTHESIS OF L-IDURONIC ACID DERIVATIVES BY EPIMERISATION OF ANANCOMERIC D-GLUCURONIC ACID ANALOGUES

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ABSTRACT

Derivatives of L-iduronic acid were prepared by epimerisation of D-glucuronic acid derivatives that were constrained to adopt a conformation having C-6 in an axial position, so that the L-iduronic acid derivatives would be thermodynamically more stable. Methyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-β-L-idofuranuronate was conveniently obtained in 23% yield by crystallisation from the epimerising mixture and was characterised either by reduction to the corresponding alcohol or by catalytic hydrogenation followed by spontaneous cyclisation to give 1,2-*O*-isopropylidene-β-L-idofuranurono-6,3-lactone. A synthesis of methyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronate in two steps from D-glucofuranurono-6,3-lactone was devised. Other candidates for epimerisation, including other esters, amides, and nitriles, were also examined. In all cases, the yields were reduced by competing decomposition, probably involving β-elimination.

INTRODUCTION

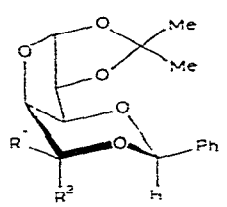
A source of L-iduronic acid derivatives was required for use in the synthesis of artificial substrates for the enzyme α-L-idosiduronase[1]. A synthetic route based on isomerisation at C-5 of D-glucuronic acid to give L-iduronic acid is attractive, because of the availability of the former. A claim[2] to have achieved this isomerisation in aqueous alkali was later shown[3] to be invalid; the five products obtained arose by isomerisation involving the aldehyde group at C-1. When the aldehyde group was masked in a D-glucopyranosiduronic acid derivative, the corresponding L-iduronic acid derivative was obtained[4] in low yield by base-promoted isomerisation, but the major product arose by β-elimination. In another example[5], a D-glucuronic acid derivative was isomerised to a mixture containing the corresponding L-iduronic acid derivative, but the components were not separated. In contrast, di-*O*-methylene derivatives of D-glucaric and D-mannaric acids are readily converted[6] into the corresponding L-idaric acid derivates. It has been suggested[7] that this facile isomerisation is due to the presence of axial carboxyl groups in the D-glucaric and D-mannaric acid

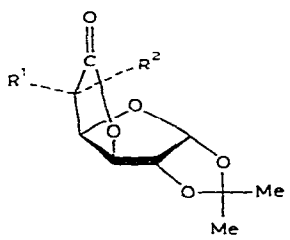
derivatives, which results in conversion into the more stable L-idaric acid derivatives having equatorial carboxyl groups.

It thus appears that the essential features in any derivative of D-glucuronic acid to be used for efficient isomerisation to L-iduronic acid are that the aldehyde group should be masked and that the carboxyl group should be constrained to an axial position. Both of these features are embodied in the 1,2:3,5-diacetals of D-glucuronic acid, provided that they have similar conformations to their reduced analogues[8], and consequently a number of these derivatives have been synthesised and their epimerisation studied.

RESULTS AND DISCUSSION

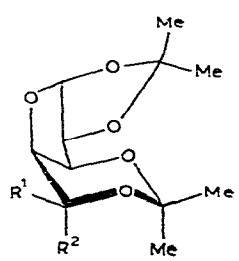
Methyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronate[9] (**1**) was prepared by an improved synthesis involving reaction of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone[10] (**2**) with α,α-dimethoxytoluene, thus providing a substrate for epimerisation in two steps from the commercially available D-glucofuranurono-6,3-lactone. When a solution of the ester **1** in dry methanol containing sodium methoxide was stored in a refrigerator, the *ido*-epimer **3** crystallised out (23% yield after recrystallisation). Chromatography of the combined mother liquor gave the unchanged ester **1** in 33% yield in addition to more of **3** (10%). Confirmation of the *ido* configuration was obtained by conversion of **3** into the known[11] alcohol **4** by





1 $R^1 = H, R^2 = CO_2Me$
3 $R^1 = CO_2Me, R^2 = H$
4 $R^1 = CH_2OH, R^2 = H$
6 $R^1 = H, R^2 = CO_2CH_2Ph$
7 $R^1 = H, R^2 = CO_2H$
8 $R^1 = CO_2CH_2Ph, R^2 = H$
9 $R^1 = H, R^2 = CO_2Et$
10 $R^1 = CO_2Et, R^2 = H$
11 $R^1 = H, R^2 = CONH_2$
12 $R^1 = CONH_2, R^2 = H$
13 $R^1 = H, R^2 = CN$
14 $R^1 = CN, R^2 = H$
17 $R^1 = CO_2H, R^2 = H$

2 $R^1 = H, R^2 = OH$
5 $R^1 = OH, R^2 = H$



15 $R^1 = H, R^2 = CO_2Me$
16 $R^1 = CO_2Me, R^2 = H$

reduction with lithium aluminium hydride, and into 1,2-*O*-isopropylidene-α-L-idofuranurono-6,3-lactone[12] (**5**) by catalytic hydrogenolysis.

In base-promoted equilibration of methyl cyclohexanecarboxylates, the axial ester represents $<10\%$ of the mixture[13], and it was expected that equilibration of such esters as **1** and **3** would give a mixture containing an even smaller proportion of the axial epimer, because the C-O bond is shorter than the C-C bond and this should make the axial epimer relatively more strained in substituted 1,3-dioxanes. The recovery of 33% of **1** was therefore surprising.

In an experiment designed to clarify this apparent anomaly, **1** was dissolved in MeOD containing sodium methoxide, and the resulting epimers were isolated by column chromatography. N.m.r. spectroscopy showed that the *ido*-isomer **3** was deuterated at position **5**, as expected, whereas the *gluco*-ester **1** was not deuterated. Thus, **1** in the mixture is unreacted material and equilibrium has not been reached. When the ester **3** was dissolved in methanolic sodium methoxide, t.l.c. showed that no **1** was formed, but slow-moving decomposition products were produced. A pure sample of the slow-moving material could not be obtained and consequently this was not identified; decomposition of **3** by β-elimination would be expected to be facile because of the antiperiplanar disposition of groups[14]. Unlike other examples of β-elimination[4], in which the products were reasonably stable and were isolated, β-elimination in this case should involve cleavage of the furanoid ring and the 1,2-*O*-isopropylidene ring, thus liberating the free aldehyde at C-1, which could undergo further alkaline degradation involving cleavage of the 3,5-acetal ring as well. Such alkaline degradation would consume base and thus account for the fact that conversion was not complete.

In the successful epimerisation of hexaric acid derivatives[6], the product crystallised quickly from the reaction mixture and this could be an important factor in obtaining good yields. Consequently, in an effort to increase the yield in the epimerisation, other compounds were explored in the hope that a pair of epimers could be found with the *ido*-isomer much less soluble than the *gluco*-isomer in the epimerisation solvent so that it would precipitate. Such precipitation should avoid side reactions. Benzyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronate (**6**) was prepared by esterification of the acid **7**. Reaction with sodium benzylate in benzyl alcohol gave the *ido*-epimer **8** in low yield.

Ethyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronate (**9**) was prepared from 1,2-*O*-isopropylidene-α-D-glucofuranuronic acid[15], benzaldehyde, and triethyl orthoformate. Epimerisation in ethanolic sodium ethoxide gave the *ido*-isomer **10**, but it was found that **9** is less soluble in ethanol than **10**.

The facile epimerisation of amides has been reported[6]. Therefore, 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronamide (**11**) was prepared by reaction of the methyl ester **1** with aqueous ammonia. Because epimerisation may accompany amide formation under these conditions[6], the *gluco* configuration of **11** was confirmed by independent synthesis from 1,2-*O*-isopropylidene-α-D-glucofuranuronamide[16] and benzaldehyde under acid conditions that should not cause epime-

risation. The *ido*-amide **12** was also prepared by the action of aqueous ammonia on **3**. Epimerisation of **11** with methanolic sodium methoxide gave **12**, but again low conversion was observed.

In a further attempt to find a substrate for efficient epimerisation, 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranurononitrile (**13**) was prepared, in 67% yield, from the amide **11** by reaction with benzenesulphonyl chloride in pyridine. The nitrile **14** was similarly prepared from the amide **12**. The *ido*-nitrile **14** was obtained by base-promoted epimerisation of the *gluco*-nitrile **13**, but the yield was low and extensive decomposition of both **13** and **14** was observed.

In extending the search for a pair of compounds of which the *gluco*-epimer was more soluble, attention was turned to methyl 1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranuronate (**15**), which has been reported[17] as a syrup and consequently was expected to be readily soluble. When this compound was prepared from lactone **2** and purified, it crystallised. The corresponding *ido*-epimer **16** was also made from the lactone **5**, Neither **15** nor **16** could be epimerised on treatment with sodium methoxide; only decomposition was observed.

Coxon has studied[8] the conformation of derivatives of 1,2:3,5-di-*O*-benzylidene-α-D-glucofuranose and has concluded that the 3,5-acetal ring adopts an "O-inside", chair conformation in which C-6 of the sugar is axial. Thus, a low value (1.35–1.5 Hz) of $J_{4,5}$ was observed and was considered to be diagnostic of this conformation with H-4 and H-5 in equatorial positions. In the alternative, "H-inside" chair conformation, these atoms would be axial and thus expected to give a higher value of $J_{4,5}$. The p.m.r. signal for H-5 in compounds **1**, **6**, **9**, **11**, and **13** was easily identified and in each case appeared as a singlet. This low value of $J_{4,5}$ therefore indicates an "O-inside" chair conformation for the compounds studied.

Thus, the original assumption that these compounds having the *gluco* configuration should be thermodynamically less stable than their *ido*-epimers is probably valid, but the failure to obtain high yields in the isomerisations is due to lability under the basic conditions studied.

For the di-*O*-isopropylidene acetals **15** and **16**, the presence of two methyl groups on the acetal carbon introduces steric strain since, in a chair conformation, one methyl group must be axial. In the *gluco*-isomer, this involves a 1,3-diaxial interaction in either chair conformation[8]. This strain causes considerable deformation of the molecule away from the chair conformation, as indicated by the value (5.5 Hz) of $J_{4,5}$. This strain could be relieved by a β-elimination reaction and may account for the tendency of **15** and **16** to decompose rather than epimerise.

The reason for the differences in lability between the compounds studied here and those studied previously[6] is probably not an electronic effect associated with the leaving group in the β-elimination because, in both groups of compound, O-4 is engaged in an acetal linkage. The most obvious difference between the two cases is that O-4 is engaged in a five-membered ring in the more labile compounds. Thus, β-elimination will involve greater relief of steric strain in the former class and may be responsible for the increased tendency to decompose.

TABLE I

^{13}C-N.M.R. DATA[a] FOR SOME EPIMERS CONTAINING A 3,5-*O*-BENZYLIDENE RING

Compound	*Ph*-C-*H*	C(CH_3)$_2$	*C-1′*	*C-4′*	*C-2′/ C-3′*	*C*(CH_3)$_2$	*OCH*$_3$	*C-1*	*C-2/C-3/C-4*	*C-5*	*C-6*
1	96.0	112.1	137.0, 129.2, 128.3, 126.2			26.7, 26.1	52.6	105.2	83.8, 77.6, 72.3	73.6	169.7
3	99.2	112.2	136.6, 129.3, 128.3, 126.3			26.8, 26.3	52.6	105.8	83.4, 79.3, 72.4	75.1	167.7
11	96.4	112.1	136.8, 129.6, 128.5, 126.2			26.7, 26.2		104.9	83.8, 77.8, 71.4	74.7	171.4
12	98.9	112.4	136.8, 129.5, 128.4, 126.2			26.8, 26.4		105.7	83.2, 79.5, 72.0	76.1	170.0
13	96.2	112.8	135.8, 129.7, 128.4, 126.2			26.8, 26.1		105.8	83.3, 77.4, 72.3	64.6	115.1
14	99.5	112.7	135.8, 129.7, 128.4, 126.2			26.8, 26.2		105.9	83.4, 78.7, 71.2	65.6	114.5

[a]Chemical shifts in p.p.m. downfield from Me_4Si.

The ^{13}C-n.m.r. data in Table I show that the benzylidene carbon atoms resonate at relatively higher field for the *gluco*-isomers (**1**, **11**, **13**) than for the corresponding *ido*-isomers (**3**, **12**, **14**), as expected[18], but the observed values fall outside the ranges given as typical for monocyclic acetals; the *gluco*-isomers give signals to lower field than expected and the *ido*-isomers give signals to higher field, so that the differences between the epimers are smaller than expected. In addition, in the *gluco*-isomers (**1**, **11**, **13**), C-3 and C-5 resonated to relatively higher field, whereas C-6 resonated to lower field than the corresponding atoms in the *ido*-isomers (**3**, **12**, **14**).

EXPERIMENTAL

General methods. — Melting points are uncorrected. 1,4-Dioxane was dried over calcium hydride. Dry ethanol refers to ethanol distilled from calcium oxide. Dry ether refers to diethyl ether stored over sodium wire. Dry methanol refers to methanol that had been distilled from magnesium methoxide in methanol. Light petroleum refers to the fraction having b.p. 60–80°. Dry pyridine refers to pyridine distilled from phosphorus pentoxide and stored over potassium hydroxide. Organic solutions were dried with anhydrous magnesium sulphate. Zinc chloride was dried at 110°.

Thin-layer chromatography (t.l.c.) was carried out by upward irrigation on silica gel (Merck, 7731), and detection was effected by iodine vapour or vanillin–sulphuric acid. Column chromatography was carried out by downward irrigation on silica gel (Merck, 7734), using toluene–ether mixtures.

^{1}H-N.m.r. spectra were recorded with Perkin–Elmer R-14 or Varian X-L 100 spectrometers under normal working conditions for solutions in chloroform-*d*, unless otherwise specified, with tetramethylsilane as internal reference. All coupling constants refer to measured splittings. ^{13}C-N.m.r. spectra ($CDCl_3$) were recorded with a Jeol JNM FX60 FT spectrometer under normal working conditions with tetramethylsilane as internal reference. Optical rotations were determined with a Perkin–Elmer 141 polarimeter (1-dm tube).

Benzyl 3,5-O-benzylidene-1,2-O-isopropylidene-α-D-glucofuranuronate (**6**). — 3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranuronic acid[19] (**7**, 5 g) was dissolved in pyridine (200 mL), and toluene-*p*-sulphonyl chloride (6 g) was added, with cooling in ice. Benzyl alcohol (1.6 mL) was added, and the solution was kept cold for 1 h and then poured into an ice and water mixture (800 mL). The precipitate was recrystallised from ethanol, to yield **6** (5.45 g, 85%), m.p. 114°, $[\alpha]_D^{23}$ +18.7° (*c* 0.7, chloroform); ^{1}H-n.m.r. data: δ 7.37 (m, 10 H, Ph), 6.04 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.79 (s, 1 H, PhC*H*), 5.41, 5.32 (q, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.96 (s, 1 H, H-5), 4.65 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.46, 4.41 (2 s, 2 H, H-3,4), 1.50, and 1.32 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{23}H_{24}O_7$: C, 67.0; H, 5.9. Found: C, 66.9; H, 5.6.

Benzyl 3,5-O-benzylidene-1,2-O-isopropylidene-β-L-idofuranuronate (**8**). — A solution of **6** (400 mg) in benzyl alcohol (2 mL) was treated with sufficient of a satur-

ated solution of sodium benzylate in benzyl alcohol and toluene (3:1) to afford pH 9. More sodium benzylate solution was added after 1 day to maintain the pH. After 2 days, the solid was filtered off, the solution was evaporated, and the solid residue (171.5 mg) was subjected to column chromatography, to yield **6** (122 mg, 30.5%), and **8** (23 mg, 6%), m.p. 174–175°, $[\alpha]_D^{21}$ −4.0° (*c* 0.5, chloroform); ^{1}H-n.m.r. data: δ 7.34 (m, 10 H, Ph), 6.03 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 5.36, 5.24 (q, 2 H, J_{gem} 12 Hz, OCH_2Ph), 4.75 (s, 1 H, H-5), 4.64 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.49 (s, 2 H, H-3,4), 1.49, and 1.34 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{23}H_{24}O_7$: C, 67.0; H, 5.9. Found: C, 66.7; H, 6.2.

1,2-O-Isopropylidene-α-D-glucofuranuronic acid. — 1,2-*O*-Isopropylidene-α-D-glucofuranurono-6,3-lactone[10] (**2**, 9 g) was stirred overnight with anhydrous sodium hydrogencarbonate (9 g) in water (90 mL). The solution was concentrated as much as possible without precipitation, acidified with conc. hydrochloric acid, and extracted with ethyl acetate (10 × 90 mL). The organic layers were evaporated and the residue was recrystallised from ethyl acetate, to yield the title compound (4.39 g, 45.0%); m.p. 140–142°; lit.[15] m.p. 145–146°.

Methyl 3,5-O-benzylidene-1,2-O-isopropylidene-α-D-glucofuranuronate (**1**). — (*a*) A mixture of 1,2-*O*-isopropylidene-α-D-glucofuranuronic acid (250 g), toluene-*p*-sulphonic acid (25 g), and α,α-dimethoxytoluene (1.2 L) was stirred overnight. The solution was then diluted with chloroform (1.5 L), extracted with saturated, aqueous sodium hydrogencarbonate (2.5 L), washed with water, and dried. The solvents were evaporated and crystallisation of the syrupy residue from methanol yielded **1** (193.1 g, 53.8%), m.p. 106°, $[\alpha]_D^{23}$ +18.0° (*c* 0.5, chloroform); lit.[9] m.p. 106°, $[\alpha]_D^{25}$ +16.1° (chloroform); ^{1}H-n.m.r. data: δ 7.4 (m, 5 H, Ph), 6.05 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.84 (s, 1 H, PhC*H*), 4.92 (s, 1 H, H-5), 4.64 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.44 (s, 2 H, H-3,4), 3.86 (s, 3 H, OMe), 1.54, and 1.35 (2 s, each 3 H, CMe_2).

(*b*) The lactone **2** (5 g) and toluene-*p*-sulphonic acid (0.5 g) were taken in α,α-dimethoxytoluene (25 mL) and shaken into solution, and then kept at room temperature for 2 days. Work-up as in (*a*) gave **1** (1.635 g, 20.1%), m.p. 106°.

Methyl 3,5-O-benzylidene-1,2-O-isopropylidene-β-L-idofuranuronate (**3**). — Sodium (20.5 mg, 0.89 mmol) was added to the minimum volume of dry methanol, and the mixture was added to a solution of **1** (3 g, 8.9 mmol) in dry methanol (70 mL) which was then seeded with **3**. The mixture was stored in a refrigerator and a similar amount of sodium methoxide solution was added after 1 day. After a further day, the crystals were filtered off and the solid (1.6 g) was recrystallised from methanol, to yield **3** (689 mg, 23.0%). Column chromatography of the material in the combined mother liquor yielded **1** (981 mg, 32.7%), and **3** (311 mg, 10.3%), m.p. 188–189°, $[\alpha]_D^{23}$ +13.7° (*c* 0.75, chloroform); ^{1}H-n.m.r. data: δ 7.4 (m, 5 H, Ph), 6.05 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 4.72 (s, 1 H, H-5), 4.62 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.49 (s, 2 H, H-3,4), 3.86 (s, 3 H, OMe), 1.54, and 1.33 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{17}H_{20}O_7$: C, 60.7; H, 6.0. Found: C, 61.0; H, 6.0.

Methyl 3,5-O-benzylidene-1,2-O-isopropylidene-β-L-[5-^{2}H]idofuranuronate. — Sodium (9 mg) was added to a solution of **1** (900 mg) in MeOD (21 mL). After 2

days, the solvent was evaporated and toluene was distilled from the residue, which was then subjected to column chromatography, to yield **1** (370 mg, 41.1%), m.p. 104–105° (^{1}H-n.m.r. data identical to those of authentic **1**); and the title compound, m.p. 188°, the ^{1}H-n.m.r. data of which were similar to those of **3**, except for the absence of the signal at δ 4.72 (s, 1 H, H-5).

Attempted epimerisation of **3**. — Sodium (2.1 mg, 0.09 mmol) was added to the minimum volume of dry methanol and the mixture was added to a solution of **3** (300 mg, 0.9 mmol) in dry methanol (35 mL). The reaction was monitored by t.l.c. during 5 days. No material with a mobility similar to that of **1** was observed, but material with lower mobility was formed.

*3,5-O-Benzylidene-1,2-O-isopropylidene-β-*L*-idofuranuronic acid* (**17**). — A mixture of **3** (500 mg, 1.5 mmol) and water (20 mL) containing sodium hydroxide (59.5 mg, 1.5 mmol) was stirred overnight. The mixture was then filtered, the filtrate was acidified with dilute hydrochloric acid, and the resulting precipitate was filtered off, washed with water, and dried, to yield **17** (202 mg, 42.4%), m.p. 205°, $[\alpha]_D^{19}$ +21.8° (*c* 0.8, acetone); ^{1}H-n.m.r. data: δ 7.57 (m, 5 H, Ph), 6.00 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 4.84 (s, 1 H, OH), 4.70 (s, 1 H, H-5), 4.60 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.49 (s, 2 H, H-3,4), 1.51, and 1.31 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{18}O_7$: C, 59.6; H, 5.6. Found: C, 59.9; H, 5.8.

*3,5-O-Benzylidene-1,2-O-isopropylidene-β-*L*-idofuranose* (**4**). — A mixture of lithium aluminium hydride (75 mg) in dry ether (30 mL) was boiled under reflux for 1 h. The supernatant liquid was decanted into a second flask, and a solution of **3** (250 mg) in dry 1,4-dioxane (15 mL) and dry ether (15 mL) was added dropwise under anhydrous conditions. The solution was boiled under reflux for 2 h and then cooled in ice, and water (0.15 mL) was added, followed by 0.15% aqueous sodium hydroxide (0.15 mL) and water (0.45 mL). The mixture was filtered, the residual solids were washed with ether, and the washings were added to the filtrate, which was then evaporated. The resulting syrup was partitioned between chloroform and water, the organic layer was dried and evaporated, and crystallisation of the residue from ether–light petroleum yielded **4** (166.5 mg, 72.7%), m.p. 131–132°; lit.[11] m.p. 120–121°; ^{1}H-n.m.r. data: δ 7.60 (m, 5 H, Ph), 6.02 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.51 (s, 1 H, PhC*H*), 4.61 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.41 (d, 1 H, $J_{3,4}$ 2 Hz, H-3), 4.26–3.70 (m, 4 H, H-4,5,6,6′), 2.22 (broad s, OH), 1.50, and 1.30 (2 s, each 3 H, CMe_2).

*1,2-O-Isopropylidene-β-*L*-idofuranurono-6,3-lactone* (**5**). — A mixture of **3** (2.5 g) and 10% palladium-on-charcoal (1 g) in methanol (200 mL) and acetic acid (5 mL) was shaken under hydrogen overnight. After filtration and evaporation of the solvents, toluene was distilled from the residue to remove acetic acid, and the remaining syrup was crystallised from ethyl acetate–light petroleum, to yield **5** (1.1 g, 66.7%), m.p. 137–138°; lit.[12] m.p. 137–138°; ^{1}H-n.m.r. data (acetone-d_6): δ 5.95 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.63 (s, 1 H, OH), 4.98, 4.96 (2 d, 2 H, $J_{3,4}$ 3 Hz, H-3,4), 4.83 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.16 (s, 1 H, H-5), 1.32, and 1.47 (2 s, each 3 H, CMe_2).

*Ethyl 3,5-O-benzylidene-1,2-O-isopropylidene-α-*D*-glucofuranuronate* (**9**). — Toluene-*p*-sulphonic acid (1.125 g) was added to a suspension of **2** (2 g) in benzal-

dehyde (10 mL) and triethyl orthoformate (35 mL), and the mixture was shaken until dissolution occurred. After 2 weeks, dichloromethane (55 mL) was added, and the solution was extracted with saturated, aqueous sodium hydrogencarbonate (100 mL), washed with water (100 mL), dried, and evaporated. The residue was crystallised from ethanol, to yield **9** (0.79 g, 42.4%), m.p. 114°, $[\alpha]_D^{23}$ +19.3° (*c* 0.59, chloroform); ^{1}H-n.m.r. data: δ 7.40 (m, 5 H, Ph), 6.10 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.90 (s, 1 H, PhC*H*), 4.89 (s, 1 H, H-5), 4.65 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.44 (s, 2 H, H-3,4), 4.30 (q, 2 H, $J_{\alpha,\beta}$ 7 Hz, CH_2CH_3), 1.54, 1.34 (2 s, each 3 H, CMe_2), and 1.34 (t, 3 H, $J_{\alpha,\beta}$ 7 Hz, CH_2CH_3).

Anal. Calc. for $C_{18}H_{22}O_7$: C, 61.7; H, 6.3. Found: C, 62.0; H, 6.6.

*Ethyl 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-β-*L*-idofuranuronate* (**10**). — Sodium (5.2 mg, 0.23 mmol) was added to the minimum of dry ethanol, and the mixture was added to a solution of **9** (400 mg, 1.14 mmol) in dry ethanol (20 mL). After 3 days, the mixture was worked-up by column chromatography, to yield **9** (157.5 mg, 39.4%), and **10** (35.6 mg, 8.9%), m.p. 147–148° (from ether–light petroleum), $[\alpha]_D^{23}$ +11.4° (*c* 0.5, chloroform); ^{1}H-n.m.r. data: δ 7.38 (m, 5 H, Ph), 6.03 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.53 (s, 1 H, PhC*H*), 4.70 (s, 1 H, H-5), 4.63 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.50 (s, 2 H, H-3,4), 4.33 (q, 2 H, $J_{\alpha,\beta}$ 7 Hz, CH_2CH_3), 1.53, 1.34 (2 s, each 3 H, CMe_2), and 1.34 (t, 3 H, $J_{\alpha,\beta}$ 7 Hz, CH_2CH_3).

Anal. Calc. for $C_{18}H_{22}O_7$: C, 61.7; H, 6.3. Found: C, 61.9; H, 6.1.

*3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-*D*-glucofuranuronamide* (**11**). — A suspension of **1** (2.5 g) in conc. ammonia (25 mL) was stirred for 3 h at room temperature. More ammonia (25 mL) was then added, and the mixture stirred for a further 3 h and then cooled in ice. The product was filtered off, washed with ice-cold water until the filtrate was neutral, and dried, to yield **11** (2.1 g, 88%), m.p. 140–141° (from chloroform–light petroleum), $[\alpha]_D^{23}$ +13.2° (*c* 0.6, chloroform); ^{1}H-n.m.r. data: δ 7.44 (m, 5 H, Ph), 6.59–6.42 (broad s, 1 H, NH), 6.42–6.26 (broad s, 1 H, NH), 6.05 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.57 (s, 1 H, PhC*H*), 4.81, 4.45 (2 s, 2 H and 1 H, H-3,4,5), 4.63 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 1.54, and 1.33 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{19}NO_6$: C, 59.8; H, 6.0; N, 4.4. Found: C, 59.7; H, 5.9; N, 4.3.

(*b*) 1,2-*O*-Isopropylidene-α-D-glucofuranuronamide[16] (25 g) and dried zinc chloride (25 g) were shaken with benzaldehyde (100 mL) for 5 h. The solution was then poured into water (1 L) and light petroleum (1 L), the mixture was shaken vigorously, and the solid that formed at the interface was filtered-off, washed with light petroleum, and dried in air. Crystallisation of the resulting solid from chloroform–light petroleum yielded **11** (11.0 g, 29.0%), m.p. 136–137°.

*3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-β-*L*-idofuranuronamide* (**12**). — (*a*) A suspension of **3** (500 mg) in conc. ammonia (5 mL) was stirred for 3 h at room temperature. More ammonia (5 mL) was then added, and the mixture was stirred for a further 3 h and then cooled in ice. The product was filtered off, washed with ice-cold water until the filtrate was neutral, and dried, to yield **12** (342.3 mg, 71.6%), m.p. 164°, $[\alpha]_D^{23}$ +41.4° (*c* 0.55, chloroform); ^{1}H-n.m.r. data: δ 7.45 (m, 5 H, Ph),

6.60–6.40 (broad s, 1 H, NH), 6.03 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 6.00–5.80 (broad s, 1 H, NH), 5.57 (s, 1 H, PhC*H*), 4.75–4.50 (m, 4 H, H-2,3,4,5), 1.52, and 1.33 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{19}NO_6$: C, 59.8; H, 6.0; N, 4.4. Found: C, 59.6; H, 5.8; N, 4.4.

(*b*) Sodium (6.9 mg, 0.3 mmol) was added to the minimum of dry methanol, and the mixture was added to a solution of **11** (960 mg, 3.0 mmol) in dry methanol. After 1 day, t.l.c. indicated that the epimerisation had occurred, and a seed of the *ido*-epimer was added. After a further 2 days, crystallisation had not occurred, and the solution was then neutralised with Amberlite IR-120 (H^+) resin and evaporated, and the resulting solid was subjected to column chromatography, to yield **11** (546.8 mg, 60.7%), and **12** (114.6 mg, 12.7%).

3,5-O-Benzylidene-1,2-O-isopropylidene-α-D-glucofuranurononitrile (**13**). — This compound was prepared by the method of Weidmann[16]. A solution of benzenesulphonyl chloride (16 mL) in dry pyridine (12.5 mL) was slowly added under anhydrous conditions to an ice-cold solution of **11** (10 g) in dry pyridine (30 mL). The solution was stored in a refrigerator for 1 week and then transferred to an ice-bath, and a few drops of water were cautiously added. The mixture was then poured into ice–water and extracted with chloroform. The extract was dried, the chloroform was evaporated, toluene was distilled from the residue, and the residual pyridine was removed under high vacuum. Crystallisation of the syrup from methanol yielded **13** (6.0 g, 66.6%), m.p. 104°, $[\alpha]_D^{22}$ +10.2° (*c* 0.8, chloroform); ^{1}H-n.m.r. data: 7.45 (m, 5 H, Ph), 6.05 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.86 (s, 1 H, PhC*H*), 5.23 (s, 1 H, H-5), 4.65, 4.27 (2 m, 2 H + 1 H, H-2,3,4), 1.48, and 1.29 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{17}NO_5$: C, 63.4; H, 5.7; N, 4.6. Found: C, 63.3; H, 5.9; N, 4.5.

3,5-O-Benzylidene-1,2-O-isopropylidene-β-L-idofuranurononitrile (**14**). — (*a*) The amide **12** (0.8 g) was treated with benzenesulphonyl chloride (1.3 mL) as described for **11**. Purification by column chromatography and crystallisation from methanol yielded **14** (510.5 mg, 67.6%), m.p. 127°, $[\alpha]_D^{22}$ +25.0° (*c* 0.7, chloroform); ^{1}H-n.m.r. data: δ 7.45 (m, 5 H, Ph), 6.07 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.46 (s, 1 H, PhC*H*), 4.97 (d, 1 H, $J_{5,4}$ 3 Hz, H-5), 4.63 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.46 (d, 1 H, $J_{3,4}$ 3 Hz, H-3), 4.22 (t, 1 H, $J_{4,3}$ 3, $J_{4,5}$ 3 Hz, H-4), 1.51, and 1.32 (2 s, each 3 H, CMe_2).

Anal. Calc. for $C_{16}H_{17}NO_5$: C, 63.4; H, 5.7; N, 4.6. Found: C, 63.7; H, 5.6; N, 4.4.

(*b*) Sodium (6.9 mg, 0.3 mmol) was added to the minimum of dry methanol, and the mixture was added to a solution of **13** (910 mg, 3 mmol) in dry methanol. After 30 min, the solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and evaporated, and the resulting syrup was fractionated by column chromatography, to yield **13** (529 mg, 58.2%) and **14** (88 mg, 9.7%).

Methyl 1,2:3,5-di-O-isopropylidene-α-D-glucofuranuronate (**15**). — Toluene-*p*-sulphonic acid (0.1 g) and **2** (1 g) were shaken in 2,2-dimethoxypropane (5 mL) until dissolution occurred. After 2 days, the solution was diluted with chloroform

(20 mL), extracted with saturated, aqueous sodium hydrogencarbonate (25 mL), washed with water (25 mL), and dried. Evaporation of the solvents and column chromatography of the resulting syrup yielded a chromatographically homogeneous syrup (761.5 mg), crystallisation of which from ether–light petroleum yielded **15** (568.8 mg, 42.7%), m.p. 59–60°, $[\alpha]_D^{22}$ +31.9° (*c* 0.85, chloroform); lit.[17] syrup, no $[\alpha]_D$ reported; ^{1}H-n.m.r. data: δ 6.04 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.64 (q, 1 H, $J_{4,3}$ 3, $J_{4,5}$ 5.5 Hz, H-4), 4.58 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.29 (d, 1 H, $J_{3,4}$ 3 Hz, H-3), 4.25 (d, 1 H, $J_{5,4}$ 5.5 Hz, H-5), 3.85 (s, 3 H, OMe), 1.51, 1.41, and 1.34 (3 s, 3 H, 6 H, and 3 H, 2 CMe_2).

Anal. Calc. for $C_{13}H_{20}O_7$: C, 54.2; H, 7.0. Found: C, 54.25; H, 7.2.

*Methyl 1,2:3,5-di-O-isopropylidene-β-*L*-idofuranuronate* (**16**). — The lactone **5** (700 mg) was treated with toluene-*p*-sulphonic acid (70 mg) and 2,2-dimethoxypropane (3.5 mL) as described for **15**. Purification of the resulting syrup by column chromatography followed by recrystallisation from ether–light petroleum yielded **16** (597.7 mg, 69.5%), m.p. 119°, $[\alpha]_D^{22}$ +7.6° (*c* 0.5, chloroform): ^{1}H-n.m.r. data: δ 5.95 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.65 (d, *J* 3 Hz, 1 H), 4.49 (d, 1 H, $J_{2,1}$ 4 Hz, H-2), 4.40–4.29 (m, 2 H), 3.80 (s, 3 H, OMe), 1.46, 1.43, and 1.28 (3 s, 6 H, 3 H, and 3 H, 2 CMe_2).

Anal. Calc. for $C_{13}H_{20}O_7$: C, 54.2; H, 7.0. Found: C, 54.0; H, 7.0.

Attempted epimerisation of **15** *and* **16**. — Separate solutions of **15** (25 mg) and **16** (25 mg) in dilute, methanolic sodium methoxide (0.5 mL) were monitored by t.l.c. (toluene–ether, 1:1). The original spots (R_F 0.7 and 0.5, respectively) decreased in intensity and slow-moving material was formed, but no epimerisation had occurred after 7 days.

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