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Synthetic applications of glucose isomerase: isomerisation of C-5-modified (2*R*,3*R*,4*R*)-configured hexoses into the corresponding 2-ketoses

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Abstract

Immobilised glucose isomerase (EC 5.3.1.5) accepted various (2*R*,3*R*,4*R*)-configured hexoses such as 5-deoxy-D-*ribo*-hexose, 5-azido-5-deoxy-D-allose, as well as the corresponding epimer at C-5, 5-azido-5-deoxy-L-talose, as substrates. From the resulting azidodeoxyketoses, 5-azido-5-deoxy-D-psico- and -L-tagatopyranose, the powerful D-galactosidase inhibitors 2,5-dideoxy-2,5-imino-D-galactitol and -D-altritol were obtained in one additional step.

Keywords: D-Allofuranose; 2,5-Dideoxy-2,5-iminoalditol; Galactosidase inhibitor; Glucose isomerase; L-Tagatopyranose; D-Psicopyranose

1. Introduction

Microbial glucose isomerases (EC 5.3.1.5) catalyse the conversion of D-glucose (**1**) into D-fructose (**2**, Scheme 1) and are, in immobilised form, widely employed on a large scale in the industrial exploitation of this economically important transformation [1]. Most of these enzymes function in vivo as D-xylose isomerases, their K_M values with D-xylose being one to two orders of magnitude smaller than those with D-glucose as substrate [2]. To gain insight into the substrate tolerance of glucose isomerase, several investigators have probed the enzyme with a wide range of unnatural aldoses as well as 2-ketoses. The first successful transformation of an unnatural substrate reported was the conversion of 6-deoxy-6-thio-D-glucose into the corresponding D-fructopyranose [3]. Subsequently, in a detailed investigation, Bock and co-workers discovered that D-glucose derivatives bearing modifications at C-6 or C-3 were converted by the enzyme of a *Streptomyces* species in yields varying between about 10 and 40%. In contrast, epimers of D-glucose such as D-mannose, D-allose, D-galactose, as well as L-idose, were not accepted as substrates [4]. An important finding was the quantitative conversion of 5-deoxy-D-*xylo*-hexofuranose ("5-deoxy-D-glucose") into the corresponding 2-ketohexopyranose. These results were reproduced and extended by Wong and his group, who employed glucose isomerase for the conversion of enzymatically prepared unnatural D-fructoses into the corresponding D-glucopyranose derivatives [5–7]. By this means, diastereomeric mixtures that contained L-sorboses, which were obtained from an aldolase

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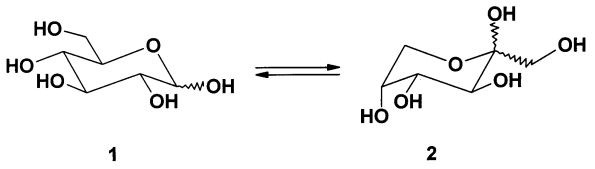


Scheme 1.

or transketolase that catalysed the carbon–carbon bond-forming step, could be conveniently separated, as these compounds were not isomerised to L-idose derivatives [6]. Recently, we were able to report several preparatively useful extensions of the glucose isomerase-catalysed reaction. Following Bock's results, it could be demonstrated that immobilised glucose isomerase (Sweetzyme T) quantitatively converted 5-modified D-glucofuranoses into the corresponding D-fructopyranoses [8], the driving force of the reaction being attributed to the release of ring strain in the starting materials. Furthermore, it was found that L-idofuranoses also undergo this rearrangement to give the corresponding L-*sorbo* isomers in high yields. Based on the observation that the five-membered ring in these aldofuranoses, due to steric crowding of substituents, is obviously highly unstable, we expected that 5-modified D-xylofuranoses, as well as 5,6-dimodified D-glucofuranose derivatives, might give access to rare open-chain 2-ketoses when exposed to Sweetzyme T. Gratifyingly, this could be shown for either series of compounds [9] as well as, very recently, for 5,6-dimodified analogues of L-idose, the isomerisation products of which have proven to be very useful in the synthesis of a range of interesting glycosidase inhibitors [10]. In a search for additional applications of this approach, the behaviour of selected (2*R*,3*R*)-configured aldopentoses towards this enzyme has recently been investigated [11]. From the encouraging results obtained in this study, we concluded that D-allose as well as L-talose derivatives with modifications at C-5, some of which could be highly useful intermediates to prepare glycosidase inhibitors, might also be worthwhile investigating.

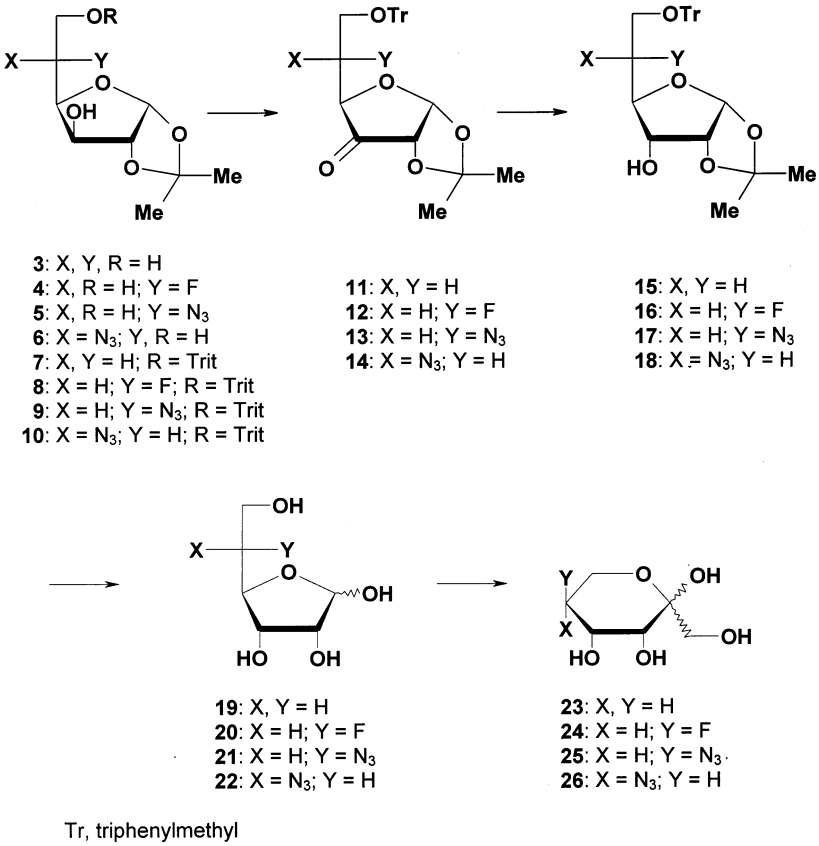
2. Results and discussion

Some D-xylose (D-glucose) isomerases exhibit side activities towards (2*R*,3*R*,4*R*)-configured aldoses such as D-ribose [2,12,13] and, in rare cases as with the enzyme from *Streptomyces albus*, D-allose is also a substrate [14]. Based on previous findings [8,9] that ring enlargement or other means of strain release in the course of the enzyme-catalysed aldose-to-ketose isomerisation enhance the conversion rate, we felt that C-5-modified aldohexoses with the (2*R*,3*R*,4*R*)-configuration might also be accepted as substrates by Sweetzyme T, which also happens to stem from a *Streptomyces* species.

The aldofuranoses employed in this study were prepared from easily available 1,2-*O*-isopropylidene-protected 5-deoxy-D-*xylo*-hexofuranose (**3** [15,16]), 5-deoxyfluoro- (**4** [17]) and 5-azidodeoxy-L-idofuranose (**5** [18]), and 5-azidodeoxy-D-glucofuranose (**6** [18,19]), respectively, according to Scheme 2.

Regioselective O-tritylation of O-6 in these intermediates provided 6-*O*-trityl derivatives **7** [20,21] as well as **8**–**10** which, upon oxidation employing pyridinium chlorochromate in dichloromethane in the presence of molecular sieves and excess sodium acetate as buffer, furnished 3-uloses **11**–**14** in yields between 60 and 70%. Compounds **12**–**14** were found to be unstable due to the β-positioned leaving group at C-5 with respect to the ketone and were immediately employed in the following step. Their reduction with sodium borohydride in methanol proceeded with complete stereoselectivity and furnished the desired (2*R*,3*R*,4*R*)-configured sugar derivatives **15** [20] and **16**–**18**. Conventional deprotection with acidic ion-exchange resin in acetonitrile–water mixtures at 50 °C gave free aldoses **19** [22,23], as well as **20**–**22**, in satisfactory overall yields.

Glucose isomerase-catalysed transformation into the corresponding ketoses **23**–**26** was performed at 65 °C employing a three-fold excess of immobilised enzyme (w/w) in neutral aqueous solutions containing small amounts of magnesium sulfate. Gratifyingly, in all four cases investigated in this study, NMR spectra of the crude reaction mixtures revealed that a

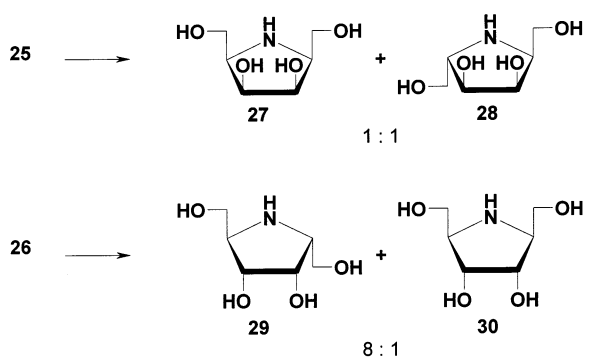


Scheme 2.

3:2 ratio of the respective starting material and the resulting ketopyranose was obtained. From these, the desired products were isolated by chromatography on silica gel employing ethyl acetate as the eluant. Isolated yields ranged from 30 to 37%. The recovered aldofuranoses can be recycled. Consequently, these figures correspond to yields of over 80% by conversion.

Contrasting the results of other workers, a 1:1 mixture of 2,5-dideoxy-2,5-imino-D-galactitol (**27** [24]) and -L-altritol (**28** [25,26]) was formed from 5-azido-5-deoxy-L-tagatopyranose (**25**) by catalytic hydrogenation over palladium-on-charcoal (10%) at ambient pressure and concomitant intramolecular reductive amination. When 5-azido-5-deoxy-D-psicopyranose (**26**) was subjected to the reaction conditions, an 8:1 ratio of 2,5-dideoxy-2,5-imino-D-altritol (**29** [27]) and the corresponding D-*allo*-configured pyrrolidine derivative **30** was obtained (Scheme 3).

In conclusion, immobilised glucose isomerase (Sweetzyme T) was found, for the first time, to be able to convert (2*R*,3*R*,4*R*)-configured aldohexoses modified at C-5 into the corresponding ketopyranoses, the latter being obtained in preparatively useful proportions. As was previously observed with D-*gluco*- and L-*ido*-configured aldofuranoses [8], neither the nature of the substituent at C-5 nor the configuration at this centre appear to have any influence on the efficiency of the reaction. This transformation allows access to a variety of rare ketose derivatives not readily



Scheme 3.

accessible by non-enzymatic methods, some of which could be useful as intermediates in the synthesis of biologically active compounds. This could be shown with the synthesis of compound **27**, which has recently been found to be a powerful inhibitor of β-galactosidase [24], and analogues thereof.

3. Experimental

General methods.—Melting points were recorded on a Tottoli apparatus and are uncorrected. Optical rotations were measured on a Jasco digital polarimeter or with a Perkin–Elmer model 341 spectropolarimeter with a path length of 10 cm. NMR spectra were recorded at 200 as well as 300 MHz (^{1}H), and at 50.29 and 75.47 MHz (^{13}C). $CDCl_3$ was employed as solvent for protected compounds, and D_2O for free sugars. Chemical shifts are listed in δ employing residual, not deuterated, solvent as the internal standard. The signals of the protecting groups were found in the expected regions and are not listed explicitly. TLC was performed on precoated aluminum sheets (E. Merck 5554). TLC plates were stained with concd H_2SO_4 containing 5% vanillin.

Detection of iminoalditols on TLC was performed by employing a mixture of 10% ammonium molybdate (w/v) in 10% aqueous sulfuric acid containing 0.8% cerium sulfate (w/v).

For column chromatography Silica Gel 60 (E. Merck) was used. Mixtures of ethyl acetate and petroleum ether (1:10 to 3:1) were used for TLC and column chromatography of protected compounds, and ethyl acetate, as well as ethyl acetate–MeOH mixtures (10:1 to 4:1), were employed for TLC and chromatography of unprotected sugars. Free inhibitors were chromatographed in 100:100:1 $CHCl_3$–MeOH–concd aq ammonia.

General method for isomerisation reactions with immobilised glucose isomerase.—To a 5% solution of the respective free sugar in distilled water, a few drops of a 1% aq solution of $MgSO_4$ and 4 equiv (w/w) of immobilised glucose isomerase (Sweetzyme T, Novo) were added, and the mixture was spun on a rotary evaporator or shaken at 60 °C for 3–8 h. After the appropriate reaction time, the solids were filtered off, the solution was concentrated under reduced pressure, and the residue was chromatographed on silica gel. Parallel control experiments were conducted with Ca^{2+}-inhibited enzyme, as well as without enzyme, and gave less than 5% conversion after reaction times of 16 and 24 h, respectively, under otherwise identical conditions.

*5-Deoxy-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-α-*D*-xylohexofuranose* (**7**).—Following the reported procedure [28], 5-deoxy-1,2-*O*-isopropylidene-α-D-xylohexofuranose (**3**, 5.00 g, 24.5 mmol) was 6-*O*-tritylated. Compound **7** was obtained as an off-white wax (9.50 g, 87%): $[\alpha]_D^{20}$ +4° (*c* 1.3, chloroform) lit. +3.9° [20]; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.95 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.58 (d, 1 H, H-2), 4.43 (m, 1 H, $J_{4,5}$ 5.1 Hz, $J_{4,5'}$ 6.8 Hz, H-4), 4.12 (s, 1 H, H-3), 3.35 (m, 3 H, H-6, H-6′ OH-3), 2.08 (m, 2 H, H-5, H-5′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 104.3 (C-1), 85.3, 78.7, 75,4 (C-2, C-3, C-4), 60.6 (C-6), 28.4 (C-5). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.31; H, 6.77. Found: C, 75.18; H, 6.81.

*5-Deoxy-5-fluoro-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-β-*L*-idofuranose* (**8**).—Following the procedure for the preparation of compound **7**, 5-deoxy-5-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose (**4**, 2.33 g, 10.5 mmol) was 6-*O*-tritylated. Compound **8** was obtained as a yellow foam (2.94 g, 60%): $[\alpha]_D^{20}$ −87° (*c* 1.8, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.99 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.86 (dddd, 1 H, $J_{4,5}$ 6.9 Hz, $J_{5,6}$ 4.8 Hz, $J_{5,6'}$ 3.9 Hz, $J_{5,F}$ 47.9 Hz, H-5), 4.47 (d, 1 H, H-2), 4.46 (m, 1 H, $J_{3,4}$ 2.6 Hz, $J_{4,F}$ 17.3 Hz, H-4), 4.10 (bs, 1 H, H-3), 3.56 (ddd, 1 H, $J_{6,6'}$ 10.8 Hz, $J_{6,F}$ 20.4 Hz, H-6), 3.44 (ddd, 1 H, $J_{6',F}$ 25.2 Hz, H-6′) 2.49 (bs, 1 H, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 105.0 (C-1), 91.6 ($J_{5,F}$ 174 Hz, C-5), 85.3 (C-2), 80.2, ($J_{4,F}$ 18.6 Hz, C-4), 75.4, ($J_{3,F}$ 6.9 Hz, C-3), 63.2, ($J_{6,F}$ 24.7 Hz, C-6). Anal. Calcd for $C_{28}H_{29}FO_5$: C, 72.40; H, 6.29. Found: C, 72.45; H, 6.30.

*5-Azido-5-deoxy-1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-β-*L*-idofuranose* (**9**).—Following the procedure for the preparation of compound **7**, 5-azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (**5**, 2.78 g, 11.3 mmol)

was 6-*O*-tritylated. Compound **9** was obtained as an off-white foam (5.31 g, 98%): $[\alpha]_D^{20}$ −19° (*c* 0.6, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.94 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.48 (d, 1 H, H-2), 4.15 (dd, 1 H, $J_{2,3}$ 2.6 Hz, $J_{4,5}$ 7.7 Hz, H-4), 4.05 (dd, 1 H, 80.2, $J_{3,OH\text{-}3}$ 4.8 Hz, H-3), 3.70 (m, 1 H, H-5), 3.41 (d, 2 H, $J_{5,6}$ 5.6 Hz, H-6, H-6′) 2.60 (d, 1 H, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 104.8 (C-1), 85.4, 81.1, 75,4 (C-2, C-3, C-4), 64.0, 61.0 (C-5, C-6). Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00. Found: C, 68.89; H, 5.95.

5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-α*-D-*glucofuranose* (**10**).—Following the procedure for the preparation of compound **7**, 5-azido-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose (**6**, 2.35 g, 9.6 mmol) was 6-*O*-tritylated. Compound **10** was obtained as a slightly yellow foam (4.59 g, 98%): $[\alpha]_D^{20}$ +26° (*c* 0.9, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.91 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.51 (d, 1 H, H-2), 4.28 (dd, 1 H, $J_{3,4}$ 2.6 Hz, $J_{3,OH\text{-}3}$ 4.4 Hz, H-3), 4.17 (dd, 1 H, $J_{4,5}$ 8.3 Hz, H-4), 3.88 (ddd, 1 H, H-5), (dd, 1 H, $J_{5,6}$ 3.2 Hz, $J_{6,6'}$ 10.1 Hz, H-6), (dd, 1 H, $J_{5,6'}$ 6.7 Hz, H-6′), 2.45 (d, 1 H, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 105.1 (C-1), 85.2, 78.7, 75,3 (C-2, C-3, C-4), 64.0, 60.2 (C-5, C-6). Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00. Found: C, 68.92; H, 6.13.

5-Deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-α*-D-*erythro-hex-3-ulofuranose* (**11**).—To a 5% solution of compound **7** (7.30 g, 16.3 mmol) in dry CH_2Cl_2, pyridinium chlorochromate (8.5 g, 39.5 mmol), powdered molecular sieves (3 Å) and sodium acetate (7.5 g) were added, and the mixture was stirred under reflux until quantitative conversion of the starting material was indicated by TLC. After removal of solids by filtration, chromium salts were precipitated by addition of diethyl ether. The filtrate was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatographic purification of the residue yielded compound **11** (4.93 g, 68%) as a slightly yellow foam: $[\alpha]_D^{20}$ −5° (*c* 1.8, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.67 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 4.55 (dd, 1 H, $J_{4,5}$ 3.4 Hz, $J_{4,5'}$ 5.8 Hz, H-4), 4.40 (d, 1 H, H-2), 3.37 (ddd, 1 H, $J_{5,6}$ 4.5 Hz, $J_{5',6}$ 9.0 Hz, $J_{6,6'}$ 9.0 Hz, H-6), 3.14 (ddd, 1 H, $J_{5',6}$ 3.6 Hz, $J_{5,6'}$ 9.0 Hz, H-6′), 2.30 (dddd, 1 H, H-5), 2.03 (dddd, 1 H, H-5′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 211,3 (C-3) 103.1 (C-1), 76.5, 76,2 (C-2, C-4), 58.4 (C-6), 31.6 (C-5). Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.66; H, 6.35. Found: C, 75.78; H, 6.43.

5-Deoxy-5-fluoro-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-β*-L-*lyxo-hex-3-ulofuranose* (**12**).—Following the procedure for the preparation of compound **11**, compound **8** (2.84 g, 6.11 mmol) was oxidized to yield unstable product **12** (1.89 g, 67%) as a slightly yellow syrup that was immediately used in the next step.

5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-β*-L-*lyxo-hex-3-ulofuranose* (**13**).—Applying the oxidation procedure to compound **9** (5.20 g, 10.7 mmol) gave product **13** (3.53 g, 68%) as a decomposing yellow syrup that had to be immediately employed for the next step.

5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-α*-D-*ribo-hex-3-ulofuranose* (**14**).—Compound **10** (3.93 g, 8.06 mmol) was oxidized according to the procedure given for the preparation of ketone **11** to furnish unstable ulose **14** (2.57 g, 66%) as an off-white syrup that had to be immediately taken to the next step: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.82 (d, 1 H, $J_{1,2}$ 4.4 Hz, H-1), 4.36 (d, 1 H, H-2), 3.92 (m, 2 H, H-4, H-5), 3.53 (dd, 1 H, $J_{5,6}$ 6.4 Hz, $J_{6,6'}$ 9.4 Hz, H-6), 3.24 (dd, 1 H, $J_{5,6'}$ 8.3 Hz, H-6′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 208.2 (C-3) 103.6 (C-1), 80.0, 79,0 (C-2, C-4), 62.8, 61.1 (C-5, C-6).

5-Deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-α*-D-*ribo-hexofuranose* (**15**).—To a 3% solution of ulose **11** (1.50 g, 3.37 mmol) in dry MeOH, sodium tetrahydridoborate (160 mg, 4.21 mmol) was added portionwise over 3 h. After complete conversion of the starting material, the solution was treated with acidic ion-exchange resin (Amberlite IR-120 [H^+]) and, after removal of the resin by filtration, concentrated several times from MeOH to remove boric acid. Chromatography of the oily residue gave compound **15** (1.37 g, 91%) as a colourless syrup: $[\alpha]_D^{20}$ +18° (*c* 1.6, chloroform), lit +18.5° [20]; ^{1}H NMR (200 MHz, $CDCl_3$): spectral data were identical with reported values [20]; ^{13}C NMR (50.3

MHz, $CDCl_3$): δ 103.8 (C-1), 78.9, 77.8, 76.1 (C-2, C-3, C-4), 60.7 (C-6), 32.7 (C-5). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.31; H, 6.77. Found: C, 75.22; H, 6.83.

5-Deoxy-5-fluoro-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-β*-L-*talofuranose* (**16**).—In analogy to the procedure for **15**, compound **12** (1.89 g, 4.09 mmol) was reduced with sodium tetrahydridoborate (250 mg, 6.59 mmol) to yield product **16** (1.57 g, 83%) as a colourless syrup: $[\alpha]_D^{20}$ −23° (*c* 1.4, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.82 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.83 (dddd, 1 H, $J_{5,F}$ 47.5Hz, H-5), 4.58 (dd, 1 H, H-2), 4.12 (ddd, 1 H, $J_{2,3}$ 5.1 Hz, $J_{3,4}$ 9.0 Hz, $J_{3,OH\text{-}3}$ 10.2 Hz, H-3), 3.92 (ddd, 1 H, $J_{4,5}$ 2.4 Hz, $J_{4,F}$ 24.9 Hz, H-4), 3.61 (ddd, 1 H, $J_{5,6}$ 7.1 Hz, $J_{6,6'}$ 10.6 Hz, $J_{6,F}$ 13.8 Hz, H-6), 3.39 (ddd, 1 H, $J_{5,6'}$ 4.2 Hz, $J_{6',F}$ 25.3 Hz, H-6′) 2.53 (d, 1 H, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 104.4 (C-1), 89.8 ($J_{5,F}$ 179,6 Hz, C-5), 79.7, ($J_{4,F}$ 17.7 Hz, C-4), 78.5 (C-2), 70.9, ($J_{3,F}$ 6.2 Hz, C-3), 63.4, ($J_{6,F}$ 24.6 Hz, C-6). Anal. Calcd for $C_{28}H_{29}FO_5$: C, 72.40; H, 6.29. Found: C, 72.32; H, 6.33.

5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-β*-L-*talofuranose* (**17**).—Sodium tetrahydridoborate reduction (625 mg, 16.7 mmol) of compound **13** (3.53 g, 7.27 mmol) in dry MeOH in the presence of acidic ion-exchange resin (Amberlite IR-120 [H^+]), to avoid azide reduction gave, after chromatography, product **17** (3.07 g, 88%) as a yellow foam: $[\alpha]_D^{20}$ −3° (*c* 1.4, chloroform); ^{1}H NMR (300 MHz, ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.78 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.55 (dd, 1 H, H-2), 4.01 (ddd, 1 H, $J_{2,3}$ 5.3 Hz, $J_{3,4}$ 8.6 Hz, $J_{3,OH\text{-}3}$ 10.4 Hz, H-3), 3.78 (dd, 1 H, $J_{4,5}$ 3.1 Hz, H-4), 3.64 (ddd, 1 H, H-5), 3.52 (dd, 1 H, $J_{5,6}$ 8.0 Hz, $J_{6,6'}$ 9.8 Hz, H-6), 3.45 (dd, 1 H, $J_{5,6'}$ 5.0 Hz, H-6′), 2.41 (d, 1 H, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 104.3 (C-1), 80.0, 78.5, 72.3 (C-2, C-3, C-4), 64.2, 61.3 (C-5, C-6). Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00. Found: C, 68.93; H, 6.12.

5-Azido-5-deoxy-1,2-O-*isopropylidene-6*-O-*triphenylmethyl-α*-D-*allofuranose* (**18**).—Application of the procedure for the preparation of compound **17** to ulose **14** (1.94 g, 4.00 mmol) employing sodium tetrahydridoborate (334 mg, 8.79 mmol) yielded allose derivative **18** (1.57 g, 81%) as a slightly yellow foam: $[\alpha]_D^{20}$ +13° (*c* 0.8, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.81 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.58 (dd, 1 H, $J_{2,3}$ 4.8 Hz, H-2), 4.08 (m, 1 H, H-3), 3.91 (m, 2 H, H-4, H-5), 3.45 (dd, 1 H, $J_{5,6}$ 3.9 Hz, $J_{6,6'}$ 10.1 Hz, H-6), 3.39 (dd, 1 H, $J_{5,6'}$ 6.8 Hz, H-6′), 2.64 (d, 1 H, $J_{3,OH\text{-}3}$ 8.7 Hz, OH-3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 104.0 (C-1), 80.0, 79.3, 71.9 (C-2, C-3, C-4), 63.4, 62.9 (C-5, C-6). Anal. Calcd for $C_{28}H_{29}N_3O_5$: C, 68.98; H, 6.00. Found: C, 69.12; H, 6.08.

5-Deoxy-D-*ribo-hexofuranose* (**19**).—A 1% solution of compound **15** (700 mg, 1.57 mmol) in 50% aq CH_3CN was stirred with acidic ion-exchange resin (Amberlite IR-120 [H^+]) at 40 °C for 24 h. After removal of the resin and concentration of the filtrate under reduced pressure, the residue was chromatographed to give free sugar **19** (225 mg, 88%) as a colourless syrup: ^{1}H NMR (300 MHz, D_2O): δ 5.39 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1α), 5.24 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1β), α/β ratio 1:2; ^{13}C NMR (75.5 MHz, D_2O): δ 101.9 (C-1β), 96.8 (C-1α), 80.3, 76.3, 75,0 (C-2β, C-3β, C-4β), 80.4, 74.5, 71,4 (C-2α, C-3α, C-4α), 59.5 (C-6β), 59.4 (C-6α), 37.4 (C-5β), 36.2 (C-5α). Anal. Calcd for $C_8H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.76; H, 7.43.

5-Deoxy-5-fluoro-L-*talofuranose* (**20**).—Hydrolysis of compound **16** (800 mg, 1.72 mmol) following the procedure for **19** yielded free deoxyfluorotalose **20** (247 mg, 79%) as a colourless oil: ^{1}H NMR (300 MHz, D_2O): δ 5.39 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1β), 5.24 (s, 1 H, H-1α), 4.71 (m, 1 H, $J_{5,F}$ 44.9 Hz, H-5α), 4.37 (dd, 1 H, $J_{2,3}$ 4.7 Hz, $J_{3,4}$ 7.0 Hz, H-3α), α/β ratio 3:1; ^{13}C NMR (75.5 MHz, D_2O): δ 102.1 (C-1α), 97.5 (C-1β), 94.4 ($J_{5,F}$ 175 Hz, C-5α), 93.9 ($J_{5,F}$ 174 Hz, C-5β), 81.4 ($J_{4,F}$ 17.7 Hz, C-4β), 81.0 ($J_{4,F}$ 17.6 Hz, C-4α), 75.8 (C-2α), 71.3 (C-2β), 71.1 ($J_{3,F}$ 6.3 Hz, C-3α), 71.0 ($J_{3,F}$ 5.6 Hz, C-3β), 62.0 ($J_{6,F}$ 21.8 Hz, C-6α, C-6β). Anal. Calcd for $C_6H_{11}FO_5$: C, 39.56; H, 6.09. Found: C, 39.62; H, 6.21.

5-Azido-5-deoxy-L-*talofuranose* (**21**).—Hydrolytic removal of protecting groups from azidodeoxy sugar **17** (540 mg, 1.10 mmol), according to the procedure for **19** furnished compound **21** (187 mg, 83%) as a colourless syrup: ^{1}H NMR (300 MHz, D_2O): δ 5.45 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1β), 5.31 (s, 1 H, H-1α), α/β

ratio 2:1; ^{13}C NMR (75.5 MHz, D_2O): δ 102.2 (C-1α), 97.6 (C-1β), 82.2 (C-4β), 82.0 (C-4α), 76.0, 72.3 (C-2α, C-3α), 72.1, 71.5 (C-2β, C-3β), 65.7 (C-6α), 64.7 (C-6β), 62.6, 62.1 (C-5α, C-5β). Anal. Calcd for $C_6H_{11}N_3O_5$: C, 35.13; H, 5.40. Found: C, 35.01; H, 5.43.

5-Azido-5-deoxy-D-*allofuranose* (**22**).—Compound **18** (790 mg, 1.62 mmol) was subjected to the hydrolysis of protecting groups as reported above to yield free azidodeoxyallose **22** (259 mg, 78%) as a colourless oil: ^{1}H NMR (300 MHz, D_2O): δ 5.42 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1α), 5.27 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1β), α/β ratio 5:2; ^{13}C NMR (75.5 MHz, D_2O): δ 102.0 (C-1β), 97.2 (C-1α), 82.9 (C-4α), 81.7 (C-4β), 76.2, 71.8, 66.3 (C-2β, C-3β, C-6β), 71.8, 70.8, 65.5 (C-2α, C-3α, C-6α), 61.9 (C-5α, C-5β). Anal. Calcd for $C_6H_{11}N_3O_5$: C, 35.13; H, 5.40. Found: C, 35.09; H, 5.49.

5-Deoxy-D-erythro-*hex-2-ulopyranose* (**23**).—Following the general procedure, compound **19** (150 mg, 0.91 mmol) was exposed to immobilised glucose isomerase (Sweetzyme T, 500 mg) for 8 h to yield ketose **23** (55.1 mg, 37%): ^{1}H NMR (300 MHz, D_2O), major anomer: δ 4.14 (ddd, 1 H, $J_{5',6}$ 3.0 Hz, $J_{5,6}$ 5.2 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 3.94 (ddd, 1 H, $J_{5',6'}$ 2.9 Hz, $J_{5,6'} < 1$ Hz, H-6′), 3.82–3.70 (m, 2 H, H-3, H-4), 3.74 (d, 1 H, $J_{1,1'}$ 11.7 Hz, H-1), 3.51 (d, 1 H, H-1′), 1.88 (m, 1 H, H-5), 1.71 (m, 1 H, H-5′), anomeric ratio 11:3; ^{13}C NMR (75.5 MHz, D_2O): δ 101.9, 99.1 (C-2α,β), 69.2, 69.0, 67.4, 66.4, 65.4, 64.3, 60.1,56.2 (C-1, C-3, C-4, C-6α,β), 31.6, 28.1 (C-5α,β). Anal. Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.77; H, 7.41.

5-Deoxy-5-fluoro-L-*tagatopyranose* (**24**).—Aldose **20** (167 mg, 0.92 mmol) was isomerised to ketose **24** (58.8 mg, 35%) following the general procedure: ^{1}H NMR (300 MHz, D_2O), major anomer: δ 4.76 (m, 1 H, $J_{5,F}$ 45.0 Hz, H-5), 4.14 (m, 1 H, H-6), 3.96 (m, 2 H, H-3, H-4), 3.83 (m, 1 H, H-6′), 3.74, 3.73 (2d, 1 H, H-1), 3.55, 3.50 (2d, 1 H, H-1′), anomeric ratio 3:2; ^{13}C NMR (75.5 MHz, D_2O): δ 99.1, 99.0 (C-2α,β), 90.9 ($J_{5,F}$ 174 Hz), 89.2 ($J_{5,F}$ 174 Hz) [C-5α,β)], 71.4 ($J_{3,F}$ 8.9 Hz), 64.4 [C-3α,β], 70.4 ($J_{4,F}$ 17.4 Hz), 69.0 ($J_{4,F}$ 27.3 Hz) [C-4α,β], 64.6, 64.4 (C-1α,β), 60.4 ($J_{6,F}$ 29.9 Hz), 59.3 ($J_{6,F}$ 19.6 Hz) [C-6α,β]. Anal. Calcd for $C_6H_{11}FO_5$: C, 39.56; H, 6.09. Found: C, 39.41; H, 6.02.

5-Azido-5-deoxy-L-*tagatopyranose* (**25**).—Application of the general isomerisation procedure to azidodeoxyaldose **21** (187 mg, 0.91 mmol) furnished ketose **25** (62.7 mg, 34%): ^{1}H NMR (300 MHz, D_2O), major anomer: δ 4.03–3.88 (m, 3 H, H-3, H-6, H-6′), 3.87-3.76 (m, 2 H, H-1, H-4), 3.68 (m, 1 H, H-5), 3.58 (d, 1 H, J 11.8 Hz, H-1′), anomeric ratio 8:1; ^{13}C NMR (75.5 MHz, D_2O), major anomer: δ 99.2, (C-2) 70.9, 70.5 (C-3, C-4), 64.9 (C-6), 61.4 (C-1), 59.2 (C-5). Anal. Calcd for $C_6H_{11}N_3O_5$: C, 35.13; H, 5.40. Found: C, 35.02; H, 5.47.

5-Azido-5-deoxy-D-*psicopyranose* (**26**).—Compound **22** (177 mg, 0.86 mmol) was isomerised according to the general procedure to yield psicose derivative **26** (58.8 mg, 33%) as a colourless syrup: ^{1}H NMR (300 MHz, D_2O), anomeric ratio 1:1: δ 4.35 (t, 1 H, J 2.6 Hz), 4.23 (t, 1 H, J 4.0 Hz), 4.13 (dd, 1 H, J 2.0, 12.9 Hz), 3.97 (m, 2 H), 3.85 (dd, 1 H, J 1.8, 12.9 Hz), 3.84–3.68 (m, 6 H), 3.57 (d, 1 H, J 11.9 Hz), 3.45 (d, 1 H, J 11.7 Hz); ^{13}C NMR (75.5 MHz, D_2O): δ 99.2, 98.7 (C-2α,β), 71.2, 70.0, 66.6, 66.5, 64.9, 64.1, 62.0, 60.6(C-1, C-3, C-4, C6α,β), 58.1, 57.0 (C-5α,β). Anal. Calcd for $C_6H_{11}N_3O_5$: C, 35.13; H, 5.40. Found: C, 35.07; H, 5.49.

2,5-Dideoxy-2,5-imino-D-*galactitol* (**27**) *and* -L-*altritol* (**28**).—To a 1% methanolic solution of azidodeoxyketose **25** (62.7 mg, 0.31 mmol), palladium-on-carbon (10%, 50 mg) was added and the mixture was stirred at ambient temperature under an atmosphere of hydrogen. (Caution: MeOH–Pd/C mixtures are a fire hazzard!) After 1 h, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a 1:1 mixture of known iminoalditol **27** and epimer **28** (45.9 mg, 92%). Compound **27**: ^{1}H NMR (300 MHz, D_2O): δ 4.40 (dd, 2 H, $J_{3,4}$ 1.6 Hz, $J_{2,3}$ 4.4 Hz, H-3, H-4), 3.89 (dd, 2 H, $J_{1,2}$ 5.3 Hz, $J_{1,1'}$ 11.7 Hz, H-1, H-6), 4.13 (dd, 2 H, $J_{1',2}$ 6.8 Hz, H-1′, H-6′), 3.48 (m, 2 H, H-2,H-5); ^{13}C NMR (75.5 MHz, D_2O): δ 72.1 (C-3, C-4), 61.2 (C-2, C-5), 60.5 (C-1, C-6); lit. [24]: ^{1}H NMR (500 MHz, CD_3OD) δ 3.55–3.65 (m, 2 H), 3.88 (dd, 2 H, J 8.0, 12.0 Hz), 3.92 (dd, 2 H, J 5.0, 12.0 Hz), 4.3–4.4 (m, 2 H); ^{13}C NMR (125 MHz, CD_3OD): δ 59.45, 63.08, 71.69. Compound **28**: ^{13}C NMR (75.5 MHz D_2O): δ 73.8, 72.4 (C-3, C-4), 62.5, 61.8, 61.5, 60.6 (C-1, C-2, C-5, C-6);

lit. [25]: ^{13}C NMR, (50 MHz, CD_3OD): δ 61.8 (CH_2), 62.4 (CH), 62.5 (CH_2), 64.0 (CH), 73.4 (CH), 74.6 (CH).

2,5-Dideoxy-2,5-imino-D-*altritol* (**29**) *and* -D-*allitol* (**30**).—Hydrogenolysis of azidodeoxyketose **26** (58.8 mg, 0.29 mmol) yielded an 8:1 mixture of iminodeoxyalditol **29** (37.4 mg, 80%) and its epimer at C-2, compound **30**. Inhibitor **29** was characterised as both the free base and the corresponding hydrochloride. Compound **30** could not be isolated from this mixture in pure form. Complex **29**: $[\alpha]_D^{20}$ + 49° (*c* 0.9, water), lit. [27]: + 51.5° (*c* 1, water); ^1H NMR (300 MHz, D_2O): δ 4.21 (dd, 1 H, $J_{2,3}$ 4.0 Hz, $J_{3,4}$ 4.2 Hz, H-3), 4.03 (dd, 1 H, $J_{4,5}$ 8.5 Hz, H-4), 3.82 (dd, 1 H, $J_{1,2}$ 6.7 Hz, $J_{1,1'}$ 11.0 Hz, H-1), 3.78 (dd, 1 H, $J_{5,6}$ 4.1 Hz, $J_{6,6'}$ 11.7 Hz, H-6), 3.67 (dd, 1 H, $J_{5,6'}$ 5.9 Hz, H-6′), 3.66 (dd, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 3.34 (m, 1 H, H-2), 3.13 (m, 1 H, H-5); ^{13}C NMR (75.5 MHz, D_2O): δ 74.6, 73.0 (C-3, C-4), 63.0, 62.4, 61.5, 60.7 (C-1, C-2, C-5, C-6). Anal. Calcd for $C_6H_{13}NO_4$: C, 44.17; H, 8.03. Found: C, 44.07; H, 8.19. Compound **29**·**HCl**: ^1H NMR (300 MHz, D_2O): δ 4.37 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 3.9 Hz, H-3), 4.30 (dd, 1 H, $J_{4,5}$ 9.1 Hz, H-4), 4.03 (dd, 1 H, $J_{1,2}$ 5.4 Hz, $J_{1,1'}$ 12.1 Hz, H-1), 4.00 (dd, 1 H, $J_{5,6}$ 3.5 Hz, $J_{6,6'}$ 12.5 Hz, H-6), 3.93 (dd, 1 H, $J_{2,1'}$ 8.0 Hz, H-1′), 3.87 (dd, 1 H, $J_{5,6'}$ 5.8 Hz, H-6′), 3.78 (m, 1 H, H-2), 3.66 (m, 1 H, H-5); ^{13}C NMR (75.5 MHz, D_2O): δ 72.3, 71.2 (C-3, C-4), 63.1, 62.7, 59.3, 58.6 (C-1, C-2, C-5, C-6). Anal. Calcd for $C_6H_{13}NO_4$·HCl: C, 36.10; H, 7.07. Found: C, 35.96; H, 7.18.

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