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Isomerization of D-Glucose with Glucose-isomerase. A Mechanistic Study

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The isomerization of D-glucose to D-fructose by the enzyme glucose-isomerase (E.C. 5.3.1.5) is shown to proceed without participation of solvent molecules for a soluble as well as an immobilized form of the enzyme. The stereospecificity of the enzymatic reaction has been determined using D-glucose labeled with deuterium in the 1- or 2-position, respectively. Both are isomerized to D-fructose labeled with deuterium at C-1, the former with the 1*S* configuration and the latter with the 1*R* configuration. The reaction has been followed by ^{2}H and ^{13}C NMR spectroscopy; from the results it is concluded that both α-D-glucopyranose and β-D-fructofuranose are substrates for the enzyme. In addition, the substrate specificity for the immobilized enzyme has been investigated and it has been shown that 3-, 5- and 6-deoxy-D-glucose together with 3-*O*- and 6-*O*-methyl-D-glucose are substrates for the enzyme, whereas 4-deoxy- and 4-*O*-methyl-D-glucose cannot be isomerized.

The isomerization of D-glucose to a mixture of D-glucose and D-fructose can be accomplished enzymatically. For technical reasons the water soluble glucose-isomerase (SGI) is often transformed (*e.g.* by crosslinking with glutaric aldehyde) into an immobilized form (IGI). In this form, work-up procedures are simplified and the enzyme can be used in a continuous process. In the present paper, we have investigated the mechanism of the isomerization using both SGI and IGI (performing the isomerization in H_2O and D_2O) and using ^{2}H-labeled derivatives of D-glucose as substrates. The results obtained are compared with studies previously reported by Rose *et al.*[1,2] for similar enzymes. A detailed discussion of the reaction mechanism of glucose-isomerase has been given by Dyson and Noltmann[3] and by Alworth in two reviews.[4,5] The substrate specificity has furthermore been studied with IGI in order to gain further insight into the reaction mechanism and in order to investigate the possibility of using immobilized enzymes in synthetic carbohydrate chemistry. Finally, it has been determined by ^{13}C NMR spectroscopy, which anomeric form of D-glucose and D-fructose functions as the substrate for the soluble form of the enzyme.

RESULTS AND DISCUSSION

Equilibrium and solvent exchange of the isomerization. Treatment of D-glucose in water (60 %) at pH 8.5 and 65 °C for 3 h with IGI led to a mixture of 55 % D-glucose and 45 % of D-fructose as seen from a ^{13}C NMR spectrum of the reaction mixture. Similar results were obtained when D-fructose was used as a substrate in the reaction. If the experiment was conducted in heavy water (D_2O) it was not possible by ^{13}C NMR spectroscopy to detect incorporation of deuterium atoms in the products during the first 2–3 h of isomerization. ^{13}C NMR spectroscopy, however, is a fairly insensitive method for the measurement of deuterium incorporation below ~10 %, and hence ^{2}H NMR spectra of the reaction mixture were measured. This method is a very sensitive probe for the detection of even very small amounts of deuterium.[6] The results are shown in Table 1. Treatment of D-glucose with IGI at pD 8.5 in D_2O for 1 and 24 h results in 7 and 40 % incorporation of deuterium, respectively, whereas treatment of D-glucose or D-fructose under the same reaction conditions, but without added IGI, results in deuterium incorporation of 7 and 15 %, respectively. These results indicate that some solvent exchange occurs due to the basic conditions used for optimum

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Table 1. Solvent exchange of D-glucose and D-fructose under different reaction conditions at 65 °C.

Substrate	Enzyme	Time/h	pH	Incorporation of deuterium in products/%[a]
D-Glucose	IGI	1	8.5	7
D-Glucose	IGI	24	8.5	40
D-Glucose	IGI	24	7.0	21
D-Glucose	SGI	24	7.0	2
D-Glucose		24	8.5	7
D-Fructose		24	8.5	15

[a] Measured by ^2H NMR spectroscopy relative to internal acetone-d_6.

enzyme activity. D-Glucose was therefore isomerized in D_2O at neutral pD with SGI and IGI. ^2H NMR spectra showed a deuterium incorporation of approximately 21 % in the experiment using IGI and practically no incorporation using SGI. This indicates that SGI reacts through a mechanism without exchange with solvent molecules even under equilibrating reaction conditions. The incorporation in the experiment with IGI is most likely caused by the base added in the immobilization process.* These results are in accord with results published by Rose *et al.*[7] for D-xylose isomerase from *Lactobacillus brevis*, but differ from results using glucose-6-phosphate-isomerase which under equilibrating conditions exchange with the solvent.[8,9]

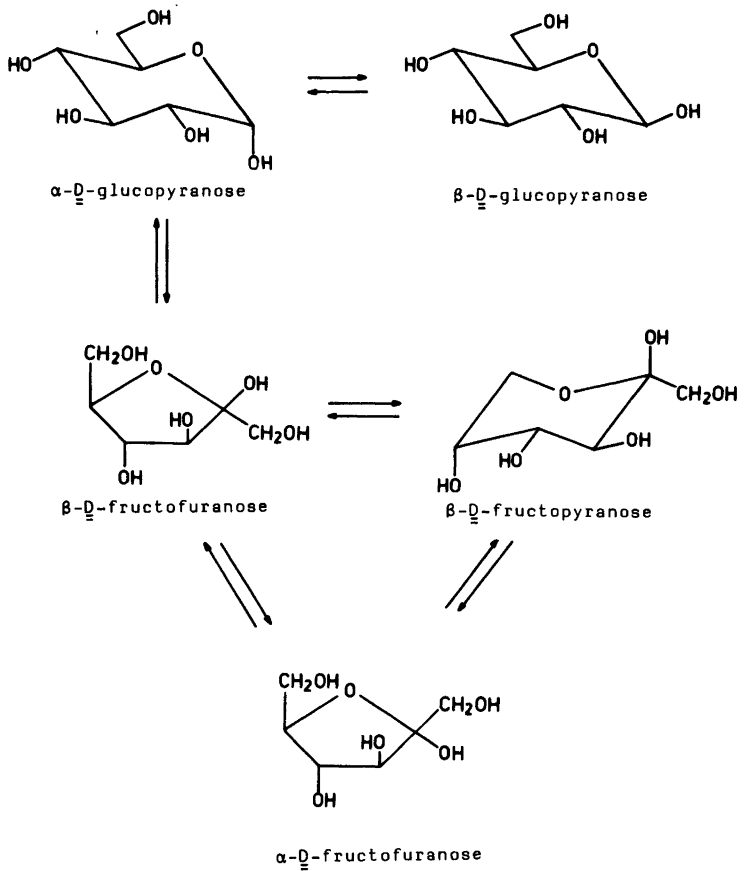
Stereochemistry of the isomerization reaction. (1-$^2H_1$)-D-Glucose (*1*) was synthesized by sodium amalgam reduction of δ-D-gluconolactone in D_2O as described.[10] Isomerization of *1* with SGI at pH 7.0 and at 65 °C in H_2O led to a mixture of (1-$^2H_1$)-D-glucose (*1*) and (1-$^2H_1$)-D-fructose (*2*) without loss of deuterium in agreement with the results described above. The reaction was followed by ^2H NMR spectroscopy, but it was not possible to detect any other intermediates than those shown in Scheme 1.

A complete separation of D-glucose from D-fructose by ion exchange column chromatography is not possible for preparative uses, but the products can be separated as their diisopropylidene derivatives *3a* and *4a*. Treatment of the crude product from the isomerization of *1*, adsorbed on silica gel, with acetone and concentrated sulfuric acid, gives a mixture of the two diisopropylidene derivatives *3a* and *4a* in 60 % yield from which *4a* can be crystallized in 7 % yield. Alternatively, *3a* and *4a* can be converted into the acetates *3b* and *4b* which can be separated by chromatography on silica gel raising the yield of *4b* to ~20 %. The ^1H NMR spectrum of *4b* showed no resonances centered at δ 3.82, typical for H-1 in the unlabeled compound.

(2-$^2H_1$)-D-Glucose (*5*) was synthesized from D-glucose- or D-fructose-6-phosphate.[11] Incubation of either of these compounds in D_2O with glucose-6-phosphate-isomerase[8] under equilibrating conditions gives a 9:1 mixture of (2-$^2H_1$)-D-glucose-6-phosphate and (1-$^2H_1$-*R*)-D-fructose-6-phosphate. (2-$^2H_1$)-D-glucose can be isolated after dephosphorylation with alkaline phosphatase by crystallization in 64 % overall yield. When (2-$^2H_1$)-D-glucose (*5*) is isomerized with SGI and IGI, similar results are obtained as described above. In this case ^2H NMR spectra of the reaction mixture using SGI do not allow a separate identification of the individual signals from the components in the mixture due to overlap of the signals which all resonate between δ 4.0 and 4.5. (The natural line width of the deuterium signal is ~45 Hz measured at 41.43 MHz).

Separation of the products is performed through the diisopropylidene derivatives *7a* and *8a* as described above. A ^1H NMR spectrum of *7b* shows no H-1 proton resonating at δ 3.95. Fig. 1A presents the 270 MHz ^1H NMR spectrum of 3-*O*-acetyl-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose, whereas Fig. 1B portrays a $^1H-\{^1H\}$-difference n.O.e. experiment[12] with irradiation of H-3; the result shows that the H-1 proton resonating at higher field is close to H-3. Inspection of a Dreiding model indicates that the *pro-S* proton at C-1 of the fructose derivative is closer to H-3. This suggests that the proton resonating at 3.82 ppm is the *pro-S* proton and that the proton resonating at 3.95 ppm

*Information provided by Novo A/S, Copenhagen, Denmark.

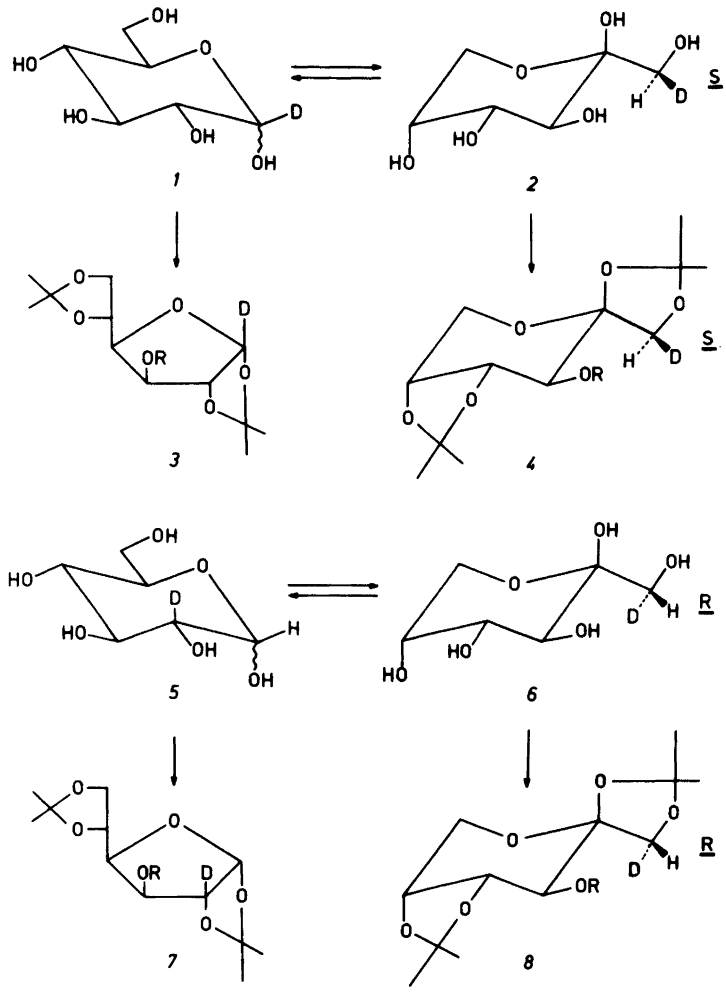


Scheme 1.

is the *pro-R* proton. Similar experiments were conducted with IGI and (1-$^2H_1$) and (2-$^2H_1$)-D-glucose as substrates and with the same results. Thus the enzyme [both in the soluble (SGI) and immobilized (IGI) form] transfers the 1-*pro-R* proton of D-fructose to the 2-position of D-glucose during the isomerization in a complete stereospecific fashion without incorporation of solvent molecules even under equilibrating conditions and with the same stereochemical course as that described for glucose-6-phosphate-isomerase.[13]

Substrate specificity of glucose-isomerase. Subsequent monosaccharides were synthesized in order to study the substrate specificity towards IGI; the results are presented in Table 2. It is seen that minor modifications at C-3 of D-glucose to 3-deoxy- or 3-*O*-methyl-D-glucose do not influence the enzymatic reaction very much. Both of these compounds as well as 3-*O*-methyl-D-fructose are substrates for the enzyme, whereas inversion of the hydroxy group at C-3 to D-allose arrests the reaction. Similarly, the 6-position can be modified to 6-deoxy-, 6-*O*-methyl-, 6-thio-D-glucose [14] or removed completely (D-xylose) without interfering with the substrate character. However, the 4-position appears to be very important for the enzymatic reaction because 4-deoxy-, 4-*O*-methyl- and 4,6-di-*O*-methyl-D-glucose or D-galactose cannot be isomerized by the enzyme. Finally, 5-deoxy-D-glucose appears to be a substrate for the enzyme, but the results must be interpreted with care because this compound is very base labile and rearranges easily to 5-deoxy-D-fructose under basic conditions.

Anomer specificity of glucose-isomerase. The anomerization of D-glucose and D-fructose is fast at the optimum temperature for isomerization with glucose-isomerase.[15] This fact makes it



a; R=H

b; R=Ac

difficult to obtained the information about the anomeric specificity of the enzyme. A computer simulation of the reactions outlined in Scheme 1, assuming pseudo first order kinetics with rate constants fitted to experimental data, has therefore been carried out.[16,17] This shows that deviations from equilibrium concentrations of anomers on the substrate side of the reaction is too small to be detected experimentally. On the other hand, the simulation reveals that it is possible within the first 10 min of the reaction to detect a build-up of the anomer released in the enzymatic reaction. The product concentration during this period is, however, very small and measurements, which are carried out by ^{13}C NMR spectroscopy shall therefore be interpretated with care. The optimum time for experimental proof of the anomeric specifity is expected to be 5–7 min after the start of the reaction. Fig. 2B shows a ^{13}C NMR spectrum of a reaction mixture 5 min after addition of SGI to a 3M solution of mutarotated D-fructose in D_2O. It is seen that the concentration of the α-anomer of D-glucose is significantly higher than that prevailing at equilibrium, Fig. 2A. Furthermore the results from a series of ^{13}C NMR measurements, which are presented in Table 3, show a concentration of

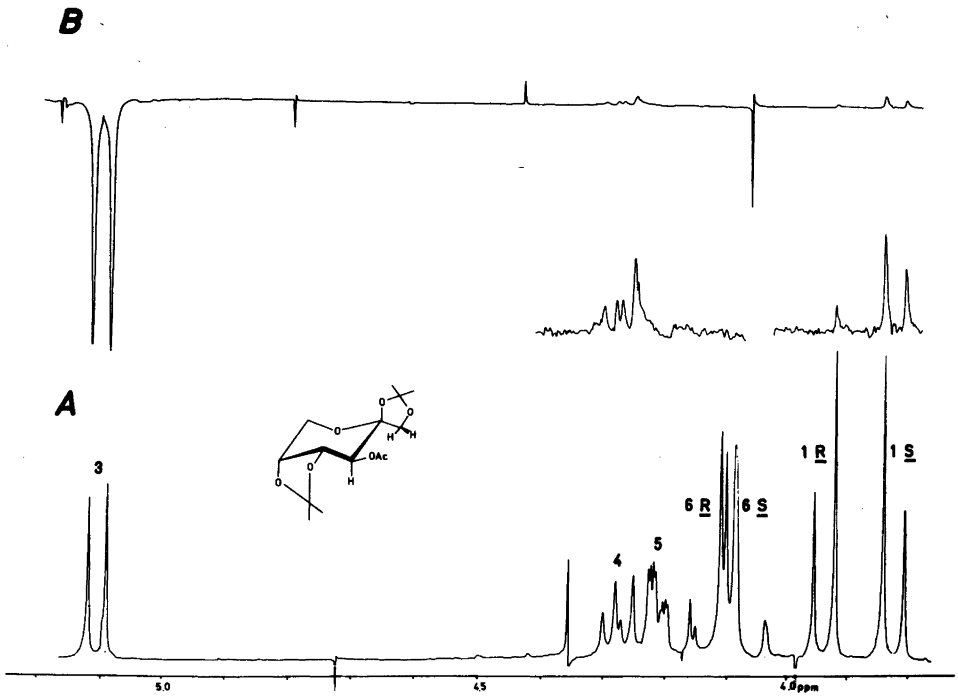


Fig. 1. Partial 270 MHz ^{1}H NMR spectrum of 3-*O*-acetyl-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose in deuteriochloroform. A. Showing the normal spectrum with the assignments indicated above the resonances. B. Difference n.O.e. experiment with saturation of H-3, showing the enhancements of H-4 and H-1 (*pro-S*).

Table 2. Isomerization of different substrates with immobilized glucose-isomerase.

Substrate	pH	Conversion/ %[a]
D-Glucose	8.5	43.9
L-Glucose	8.5	0
D-Mannose	8.5	0
D-Allose	8.5	0
D-Galactose	7.3	0
D-Xylose	8.5	20
3-Deoxy-D-*ribo*-hexose	8.5	38
4-Deoxy-D-*xylo*-hexose	8.5	0
5-Deoxy-D-*xylo*-hexose	7.2	64[b]
6-Deoxy-D-glucose	8.5	15
3-*O*-Methyl-D-glucose	7.1	11[c]
3-*O*-Methyl-D-fructose	8.5	6[d]
4-*O*-Methyl-D-glucose	7.3	0
6-*O*-Methyl-D-glucose	7.5	21
4,6-Di-*O*-methyl-D-glucose	8.5	0
5-Thio-D-glucose	8.5	0
6-Thio-D-glucose	8.5	100[e]

[a] Determined by ^{13}C NMR spectroscopy on the reaction mixtures after incubation for 24 h at 65 °C. [b] Incubation for 40 h gave 100% conversion. Incubation at pH 8.5 for 72 h without enzyme also gave 100% conversion, but under these conditions unknown decomposition products were formed. [c] Incubation for 96 h gave 22% conversion. [d] Incubation for 192 h gave 25% conversion, which indicates that equilibrium is not obtained. [e] Ref. 14.

α-D-glucopyranose larger than that present at equilibrium in the spectra obtained within the first 15 min of the reaction. Based on these results α-D-glucopyranose is thus the substrate for glucose-isomerase. Similar experiments show that β-D-fructofuranose is released, when a solution of mutarotated D-glucose is used as substrate, Table 3. These findings are in accord with results obtained by Schray and Rose[2] and Feather *et al.*[18] for D-xylose-isomerase isolated from *Streptomyces sp.*

EXPERIMENTAL

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter.

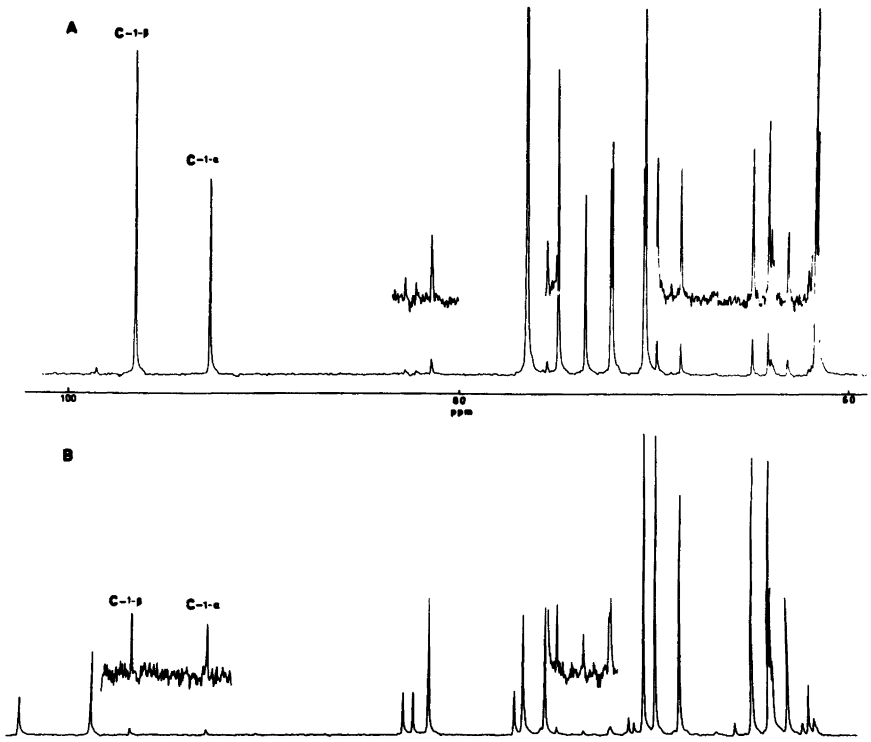


Fig. 2. A. 67.89 MHz ^{13}C NMR spectrum of mutarotated D-glucose in D_2O at 65 °C after incubation with SGI for 10 min. B. 67.89 MHz ^{13}C NMR spectrum of mutarotated D-fructose in D_2O at 65 °C after incubation with SGI for 5 min. The anomeric signals for the α- and β-D-glucopyranoses are indicated C-1-α and C-1-β, respectively.

Table 3. Isomerization of D-glucose and D-fructose with soluble glucose-isomerase at 65 °C.

	D-Fructose		D-Glucose	
Time (min)	α-Glucose/ Total glucose	β-Fructofuranose/ Total fructose	α-Glucose/ Total glucose	β-Fructofuranose/ Total fructose
0	–	–	–	–
5	0.50	0.29	0.40	0.38
10	0.48	0.31	0.40	0.36
14	0.43	0.30	0.43	0.33
18	0.40	0.30	0.40	0.33
23	0.41	0.29	0.42	0.32
28	0.40	0.29	0.42	0.30
38	0.40	0.29	0.41	0.30
48	0.39	0.29	0.42	0.30
58	0.40	0.30	0.41	0.29
73	0.40	0.31	–	–
108	0.41	0.30	0.41	0.30

^1H, ^{13}C and ^2H NMR spectra were obtained on Bruker WH-90, HX-90 and HX-270 NMR instruments. Preparative thin-layer chromatography was performed on silica gel PF_{254} (Merck) on 1 mm layers and compounds were detected by charring with a hot wire. The immobilized glucose-isomerase (IGI), Sweetzyme Q, and purified soluble form (SGI) of the same enzyme (E.C. 5.3.1.5) were obtained from Novo A/S, Copenhagen, Denmark. Glucose-6-phosphate-isomerase (E.C. 5.3.1.8) and alkaline phosphatase (E.C. 3.1.3.1) were from Boehringer Mannheim AG.

^{13}C NMR kinetic measurements. Kinetic experiments were carried out on 3M solutions (3 ml) of mutarotated substrates in D_2O (pD ~ 7) at 65 °C in a 10 mm tube measuring the ^{13}C NMR signals on a Bruker HX-270 NMR instrument operating at 67.89 MHz. The enzyme (200 mg in 0.5 ml D_2O) was added and spectra obtained as soon as possible after temperature equilibrium was established. The accuracy of the integrated ^{13}C NMR signals is considered 10 %.

(1-$^2H_1$)-D-Glucose (1).[10] To a solution of δ-D-gluconolactone (4.07 g) in D_2O (30 ml) was added a small amount of 2M D_2SO_4 until pD 3–4 was obtained. The solution was cooled to 0 °C and sodium amalgam (4 %, 100 g), divided into two portions, was added, while the pD was kept between 3 and 4 with 2M D_2SO_4. When the consumption of acid stopped, the solution was decanted from the deposited mercury, diluted and treated with a mixed bed ion exchange resin (Amberlite MB-3) to remove inorganic salts and unreacted lactone. Evaporation of the water gave 2.01 g (49 %) of syrupy *1*. Crystallization from ethanol containing a small amount of methanol gave 1.60 g (39 %) of crystalline (1-$^2H_1$)-α-D-glucopyranose (*1*), m.p. 144–146 °C. The isotopic purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and proved to be better than 97 %.

(2-^2H)-D-Glucose (5).[11] D-Fructose-6-phosphate monosodium salt (2.0 g) was evaporated twice with D_2O (10 ml) and then dissolved in D_2O (10 ml) and pD adjusted to 7 with solid sodium carbonate (~375 mg). Glucose-6-phosphate-isomerase (400 μl, activity 2 mg/ml) was added and the reaction mixture left overnight at 37 °C. The reaction mixture was then heated to 100 °C in order to denature the enzyme, filtered through carbon and evaporated to dryness. The residue was dissolved in water (46 ml), tris buffer (12 ml, 1M tri-(hydroxymethyl)aminomethane) and alkaline phosphatase (60 μl) were added and the reaction mixture left for two days at 37 °C. The reaction mixture was then treated with a mixed bed ion exchange resin and the eluate concentrated. The residue was crystallized from ethanol containing a small amount of methanol and gave (2-$^2H_1$)-α-D-glucopyranose (*5*), 728 mg (57 %), m.p. 145 °C. The isotopic purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and proved to be better than 98 %.

Isomerization of (1-$^2H_1$)-D-glucose (1) with IGI and SGI. Compound *1* (0.3 g) was dissolved in water (1.5 ml), $MgSO_4$, H_2O (0.5 mg) and IGI (90 mg) were added and the reaction stirred for 4 h at 65 °C. The reaction mixture was filtered through carbon and evaporated leaving the crude product (286 mg). The residue was dissolved into methanol (10 ml) and silica gel (3 g) added, followed by evaporation to dryness. The silica gel was suspended in dry acetone (30 ml) and H_2SO_4 (25 μl) was added. The suspension was stirred at room temperature for 1.5 h, the silica gel filtered off, and the filtrate neutralized with sodium hydroxide in water (1 % solution). The neutral solution was evaporated to dryness and the residue partitioned between water and chloroform. The organic phase was washed twice with water, dried ($MgSO_4$), filtered and evaporated leaving 236 mg of product. Crystallization from ether–pentane gave 30 mg (7 %) of (1-$^2H_1$-*R*)-1,2-4,5-di-*O*-isopropylidene-β-D-fructopyranose (*4a*), m.p. 117–118 °C. The mother liquor consisted of *4a* and (1-$^2H_1$)-1,2-5,6-di-*O*-isopropylidene-α-D-glucofuranose (*3a*), as seen from a ^1H NMR spectrum. The products were characterized through their ^1H, ^2H and ^{13}C NMR parameters and the isotopic purity of the compounds proved to be better than 90 %. Alternatively, *3a* and *4a* could be separated by preparative thin-layer chromatography using ethylacetate–pentane (3:7) as eluant after acetylation with acetic anhydride in pyridine. Similar results were obtained when *1* (200 mg) was isomerized with SGI (20 mg) under the same reaction conditions as mentioned above. *4a* could be isolated (29 mg, 10 % yield), m.p. 117 °C. The purity of the compound was checked by ^1H and ^{13}C NMR spectroscopy and the isotopic purity proved to be better than 95 %.

Isomerization of (2-$^2H_1$)-D-glucose (5) with IGI and SGI. Compound *5* (302 mg) was treated with IGI (90 mg) as described above and *8a* could be crystallized out after work-up in 8 % (36 mg) yield, m.p. 113–115 °C. Penta-*O*-acetyl-(2-$^2H_1$)-α-D-glucopyranose could be isolated in 14 % (90 mg) yield from the mother liquor after acetolysis. The products were characterized through their ^1H, ^2H and ^{13}C NMR parameters and the isotopic purity proved to be better than 90 %. Similar results were obtained when SGI was used as enzyme and *8a* was isolated in 11 % yield. The isotopic purity was better than 95 % as seen from a ^1H NMR spectrum.

Isomerization with IGI or SGI. Substrate (150–500 mg) was dissolved in H_2O (or D_2O) (2 ml), IGI (or SGI) (100 mg) added, followed by pH or pD adjustment (if applicable) with sodium carbonate. The mixture was stirred at 65 °C for the time in-

dicated in Table 1 or 2. The reaction mixtures were analyzed by ^{13}C NMR spectroscopy after filtration through carbon. The integrals were considered to be accurate with 5 %.[19]

*Synthesis of substrates. 3-Deoxy-*D-ribo-*hexose.* Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside [20] (1.0 g) was hydrolyzed with HCl (0.1 M, 20 ml) at 100 °C for 48 h. The reaction mixture was neutralized with a mixed bed ion exchange resin and evaporated leaving 3-deoxy-D-*ribo*-hexose as a syrup, which was characterized through its ^{13}C NMR data.[21]

*4-Deoxy-*D-xylo-*hexose.* Methyl 4-deoxy-α-D-*xylo*-hexopyranoside (0.554 g)[22] was hydrolyzed and worked up as described above. The syrupy 4-deoxy-D-*xylo*-hexose was characterized through its ^{13}C NMR data (D_2O). β-anomer: 97.1 ppm (C-1), 73.3 (C-2), 71.3 (C-3), 35.1 (C-4), 76.9 (C-5), 64.5 (C-6). α-anomer: 93.6 ppm (C-1), 69.3 (C-2), 67.8 (C-3), 35.1 (C-4), 74.1 (C-5), 64.6 (C-6).

*5-Deoxy-*D-xylo-*hexose.* 5-Deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose[23] (180 mg) was hydrolyzed with acetic acid (50 %, 20 ml) at 100 °C for 1 h. The syrupy 5-deoxy-D-*xylo*-hexose was characterized through its ^{13}C NMR parameters (D_2O). α-anomer: 96.5 ppm (C-1), 77.0 (C-2,C-3), 81.6 (C-4), 31.9 (C-5), 59.5 (C-6). β-anomer: 102.5 ppm (C-1), 77.0 (C-2), 76.1 (C-3), 79.8 (C-4), 32.6 (C-5), 59.5 (C-6).

*4-O-Methyl-*D-*glucose.* 1.6-Anhydro-4-*O*-methyl-β-D-glucopyranose[24] (917 mg) was hydrolyzed with 1M H_2SO_4 (15 ml) at 100 °C for 4 h, filtered through carbon and neutralized with a mixed bed ion exchange resin. Evaporation gave 4-*O*-methyl-D-glucose as a syrup which was characterized through its ^{13}C NMR parameters.[25]

*6-O-Methyl-*D-*glucose.* Methylation of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose (1.45 g) with methyl iodide (6 ml) in dimethylformamide (20 ml) using barium oxide as base at 80 °C for 3 h gave after work-up 0.91 g (60 %) of crystalline 3.5-*O*-benzylidene-1,2-*O*-isopropylidene-6-*O*-methyl-α-D-glucofuranose, m.p. 88–90 °C. Hydrolysis with 1M H_2SO_4 gave after neutralization with a mixed bed ion exchange resin and evaporation, 6-*O*-methyl-D-glucose (289 mg, 62 %), m.p. 139–143 °C. The product was further characterized through its ^{13}C NMR data.[25]

*3-O-Methyl-*D-*fructose.* 1,2-4,5-Di-*O*-isopropylidene-β-D-fructopyranose was methylated as described above in 70 % yield. Acidic hydrolysis of the syrup obtained gave 3-*O*-methyl-D-fructose also as a syrup, which was characterized through its ^{13}C NMR parameters (D_2O). β-anomer, pyranose: 64.7 ppm (C-1), 99.0 (C-2), 78.2 (C-3), 70.7 (C-4), 70.2 (C-5), 64.1 (C-6), 61.7 (OMe). α-anomer, furanose: 63.6 ppm (C-1), 104.8 (C-2), 92.0 (C-3), 74.7 (C-4), 81.9 (C-5), 61.7 (C-6), 58.9 (OMe). β-anomer, furanose: 64.5 ppm (C-1), 102.2 (C-2), 85.2 (C-3), 74.9 (C-4), 81.5 (C-5), 62.9 (C-6), 59.3 (OMe).

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REFERENCES

1. Rose, I. A. *Enzyme*, 3rd Ed. *2* (1970) 281.
2. Schray, K. J. and Rose, I. A. *Biochemistry 10* (1971) 1058.
3. Dyson, J. E. D. and Noltmann, E. A. *J. Biol. Chem. 243* (1968) 1401.
4. Alworth, W. L. *Stereochemistry and its Application in Biochemistry*, Wiley, New York 1975, pp. 244–260.
5. Alworth, W. L. In Harmon, R. E., Ed., *Asymmetry in Carbohydrates*, Dekker, New York 1980, p. 31.
6. Hutchinson, C. R., Heckendorf, A. H., Straughn, J. L., Daddona, P. E. and Cane, D. E. *J. Am. Chem. Soc. 101* (1979) 3358.
7. Rose, I. A., O'Connell, E. L. and Mortlock, R. P. *Biochim. Biophys. Acta 178* (1969) 376.
8. Rose, I. A. and O'Connell, E. L. *J. Biol. Chem. 236* (1961) 3086.
9. Simon, H. and Medina, R. *Z. Naturforsch. Teil B 21* (1966) 496.
10. Topper, Y. J. and Stetten, D. *J. Biol. Chem. 189* (1951) 191.
11. a. Topper, Y. J. *J. Biol. Chem. 225* (1957) 419; b. Lowenstein, J. M. *Methods Enzymol. 6* (1963) 828.
12. Kotovych, G., Aarts, G. H. M. and Bock, K. *Can. J. Chem. 58* (1980) 1206.
13. Rose, I. A. and O'Connell, E. L. *Biochem. Biophys. Acta 42* (1960) 159.
14. Chmielewski, M., Chen, M.-S. and Whistler, R. L. *Carbohydr. Res. 49* (1976) 479.
15. Shallenberger, R. S. *Pure Appl. Chem. 50* (1978) 1409.
16. Anderson, L. and Garver, J. C. *Am. Chem. Soc. Symp. Ser. 117* (1973) 20.
17. *DYNAMO*, Northern European Universities Computing Center, Utility Program.
18. Feather, M. S., Desphande, V. and Lybyer, M. J. *Biochim. Biophys. Res. Commun. 38* (1970) 859.
19. Bock, K., Pedersen, C. and Thøgersen, H. *Acta Chem. Scand. B 35* (1981) 441.
20. Vis, E. and Karrer, P. *Helv. Chim. Acta 37* (1954) 378.
21. Pfeffer, P. E., Parrish, F. W. and Unruh, J. *Carbohydr. Res. 84* (1980) 13.
22. Siewert, G. and Westphal, O. *Justus Liebigs Ann. Chem. 720* (1968) 161.
23. Gramera, R. E., Ingle, T. R. and Whistler, R. L. *J. Org. Chem. 29* (1963) 2074.
24. Bochkov, A. F. and Voznyi, Y. V. *Carbohydr. Res. 32* (1974) 1.
25. Usui, T., Yamacka, N., Matsuda, K., Tuzimura, K., Sugiyama, H. and Seto, S. *J. Chem. Soc. Perkin Trans. 1* (1973) 2425.

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