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Syntheses of 4-deoxy-D-fructose and enzymatic affinity study

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Abstract

Enantiomerically pure 4-deoxy-D-fructose has been prepared and characterised in a protected form, acidic hydrolysis of which led to an aqueous solution of 4-deoxy-D-fructose. Activities of this compound with enzymes of the glycolysis pathway involved in glucose metabolism make possible access to 4-deoxy-D-fructose-6-phosphate, 4-deoxy-D-glucose-6-phosphate and 4-deoxy-D-gluconate-6-phosphate. © 1998 Elsevier Science Ltd. All rights reserved.

Fructose plays a crucial role in all living organisms, in the free or phosphorylated form. It acts on the primary metabolism and cannot be considered as a therapeutic target. However, close fructose analogues such as deoxy-D-fructoses help one to understand the mechanism of action of the glycolysis pathway enzymes (hexokinase, phosphofructokinase, phosphoglucose isomerase, fructose-1,6-bisphosphate aldolase). They are also important to elucidate specific systems such as: the fructose carrier in trypanosoma[1] or the glucosamine-6 phosphate synthase involved in the biosynthesis of hexosamines required in the construction of microbial cell walls.[2]

In the course of our studies on the chemo-enzymatic syntheses of sugar analogues, we were interested in obtaining 4-deoxy-D-fructose, 4-deoxy-D-fructose-6-phosphate and 4-deoxy-D-fructose-1,6-bisphosphate, the latter being a potential competitive inhibitor of aldolase. Very few of these compounds have been mentioned in the literature. 4-Deoxy-D-fructose was prepared in 1973 by Haylock and Slessor[3] by microbial oxidation of a mixture of 3-deoxy-D-arabinohexitol and 3-deoxy-D-lyxohexitol. However, 4-deoxy-D-fructose was not fully characterised either by derivative preparation or by spectroscopic data. Later, in 1982, Kucar et al.[4] studied the isomerisation of 4-deoxy-D-glucose into 4-deoxy-D-fructose, but this compound was characterised only by its specific rotation ($[\alpha]_D$=3.3 against -3.9^3). Recently, Enders et al.[5] published a synthesis of various protected deoxy ketoses by alkylation of protected dihydroxyacetone by the 'SAMP' method.

This summary of the literature data, suggests that new methods for the synthesis of 4-deoxy-D-fructose (and its phosphorylated derivatives) are necessary. We attempted to obtain this compound by enzymatic

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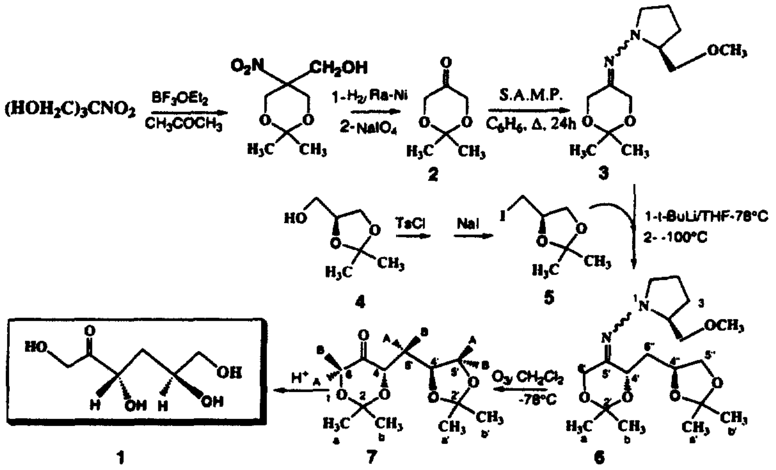
catalysis of transketolase. However, the activities are rather low and we were unable to carry out the synthesis on a preparative scale.[6]

This paper deals with the chemical synthesis of 4-deoxy-D-fructose **1** and the activities of hexokinase, glucose phosphate isomerase and glucose phosphate dehydrogenase, towards this compound.

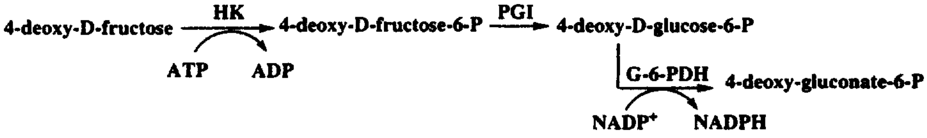
The synthesis (Scheme 1) is based on the stereoselective alkylation of SAMP hydrazone of protected dihydroxyacetone **2**, (S)-(+)-1-(2,2-dimethyl-1,3-dioxan-5-ylidenamino)-2-methoxy methyl pyrrolidine **3** as described by Enders.[7] We synthesized this compound in four steps with an overall yield of 37%, according to described procedures.[8] Alkylation of this compound with (R)-2,2-dimethyl-4-(iodomethyl)-1,3-dioxolane **5** should lead to the desired protected 4-deoxy-D-fructose. To prepare this iodide, commercial (S)-2,2-dimethyl-1,3-dioxolane-4-methanol **4** was converted into the (S)-tosylate[9] by action of tosyl chloride in triethylamine in 85% yield. The iodide was obtained from tosylate by treatment with sodium iodide in acetone, in 75% yield.[10] The metallation of SAMP-hydrazone **3** was achieved by addition of 1.1 equivalent of *t*-butyllithium (1.7 M solution in hexane) at −78°C, in THF. After 2 h at this temperature, the mixture was cooled to −100°C and a slight excess of alkyliodide **5** (1.2 equiv.) was added. After 1.5 h, the mixture was slowly warmed at 20°C, ether was added, and the organic phase was washed and concentrated *in vacuo*, leading to monoalkylated hydrazone **6** as a pale yellow oil in 84% yield.



Scheme 1.

Spectroscopic data[11] are consistent with the expected structure. No trace of the other diastereoisomer was detected by ^1H or ^{13}C NMR spectroscopy. The chiral auxiliary was removed by ozonolysis with a 46% yield, leading to the protected 4-deoxy-D-fructose **7**. Spectroscopic data for this compound were recorded.[12] ^1H NMR signals were attributed by ^1H–^1H NMR correlation. The hydrolysis of the diacetonide was achieved in the presence of acid resin (Dowex) in aqueous solution. The reaction was followed by TLC and was complete after 1 h. 4-Deoxy-D-fructose was not isolated and the solution was used directly for enzymatic assays.

As the first enzyme of the glycolytic pathway, hexokinase (HK) phosphorylates glucose and fructose into glucose-6-phosphate and fructose-6-phosphate, respectively, in the presence of adenosine triphosphate (ATP). We measured the activity of HK towards 4-deoxy-D-fructose according to the literature method.[13] For 1 mM concentration in substrate, the activity of HK was 3% relative to its V_{max} for D-fructose. Due to the probably high value of K_m for this analogue of D-fructose, the activity should increase with the concentration of 4-deoxy-D-fructose, and allow the enzymatic synthesis of 4-deoxy-D-fructose-6-phosphate. We carried out the synthesis on a 50 μmol scale (50 mM in substrate) in the presence of an equimolecular amount of ATP, and 50 U of HK. After 3 h, all starting materials were consumed (Scheme 2). The formation of 4-deoxy-D-fructose-6-phosphate was controlled by coupled reactions of phosphoglucose isomerase (PGI) and glucose-6-phosphate dehydrogenase[13] (G6 PDH) (Scheme 2). The activities of these enzymes at 1 mM concentration in substrate in this assay were 6% and 1%, respectively, relative to their V_{max} for fructose-6-phosphate.



Scheme 2.

In conclusion, enantiomerically pure 4-deoxy-D-fructose has been prepared and characterised in a protected form. Acidic hydrolysis of the protected form led to an aqueous solution of 4-deoxy-D-fructose. This close analogue of D-fructose is a substrate for hexokinase, allowing the synthesis of 4-deoxy-fructose-6-phosphate. Activities of other enzymes of glucose metabolism could make possible the access to other interesting analogues, such as 4-deoxy-D-glucose-6-phosphate.

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11. Selected physical data of **6**: ^{1}H NMR (400 MHz, CDCl$_3$) 1.28 (s, 3H), 1.31 (s, 3H), 1.43 (s, 3H), 1.55 (m, 1H, 5_{5B}), 1.63 (m, 1H, $H_{6''B}$), 1.67–2.03 (m, 4H, 2H_3 and 2H_4), 2.35 (m, 1H, $H_{6''A}$), 2.90–3.27 (m, 4H, H_2, 2H_6 and H_{5A}), 3.35 (s, 3H), 3.58 (dd, 1H, $H_{5''B}$, J_{gem}=7.9, J_{vic}=7.1), 4.02 (dd, 1H, $H_{5''A}$, J_{gem}=8.1, J_{vic}=5.8), 4.09–4.35 (m, 3H, $H_{4'}$, 2$H_{6'}$, $H_{4''}$); ^{13}C NMR (100 MHz, CDCl$_3$) 23.51, 23.63, 24.09, 24.36 (Ca, Cb, Ca′, Cb′), 25.02 and 25.91 (C_3 and C_4), 32.53 ($C_{6''}$), 56.23 (C_7), 58.67 (C_5), 60.32 (C_2), 63.56 ($C_{6'}$), 66.71 ($C_{4''}$), 69.21 ($C_{5''}$), 70.70 ($C_{4'}$), 75.68 (C_6), 100.01 ($C_{2'}$), 107.98 ($C_{2''}$), 100.31 ($C_{5'}$); MS, 341 (M−CH$_3$).
12. Selected physical data of **7**: $[\alpha]_D^{25}$ −149.6 (c CHCl$_3$); lit.[6] $[\alpha]_D^{23}$ −154.7 (neat). MS, 229 (M−CH$_3$). ^{1}H NMR (400 MHz, CDCl$_3$) 1.35 (s, 3H), 1.41 (s, 3H), 1.45 (s, 3H), 1.48 (s, 3H), 1.62 (ddd, 1H, $H_{6'B}$, J_{gem}=14.4, $J_{vic\ 6'B,4}$=10.7, $J_{vic\ 6'B,4'}$=4.9), 2.24 (ddd, 1H, $H_{6'A}$, J_{gem}=14.4, $J_{vic\ 6'A,4'}$=8.3, $J_{vic\ 6'A,4}$=2.6), 3.57 (dd, 1H, $H_{5'B}$, J_{gem}=8.1, $J_{vic\ 5'B,4'}$=7.2 cis), 4.02 (d, 1H, 6H_{6B}, J_{gem}=17.1), 4.04 (dd, 1H, $H_{5'A}$, J_{gem}=8.3, $J_{vic\ 5'A,4'}$=5.8 trans) 4.22 (m, 1H, $H_{4'}$, $J_{vic\ 4',6'A}$=8.2, $J_{vic\ 4',5'B}$=7.0 cis, $J_{vic\ 4',5'A}$=5.9 trans, $J_{vic\ 4',6'B}$=4.9), 4.28 (d, 1H, H_{6A}, J_{gem}=17, $J_{6A,4}$=1.4 Jw), 4.17 (dd, 1H, H_4, $J_{vic\ 4,6'B}$=10.7, $J_{vic\ 4,6'A}$=2.6, $J_{4,6A}$=1.4 Jw); ^{13}C NMR (100 MHz, CDCl$_3$) 23.7, 23.8 (Ca, Cb), 25.9, 27.1 (Ca′, Cb′), 32.4 ($C_{6'}$), 66.6 ($C_{4'}$), 69.7 ($C_{5'}$), 72.0 (C_6), 72.5 (C_4), 101.1 (C_2), 108.8 ($C_{2'}$), 209.1 (C_5).
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