A Pseudoisomerization Route to Aldose Sugars Using Aldolase Catalysis

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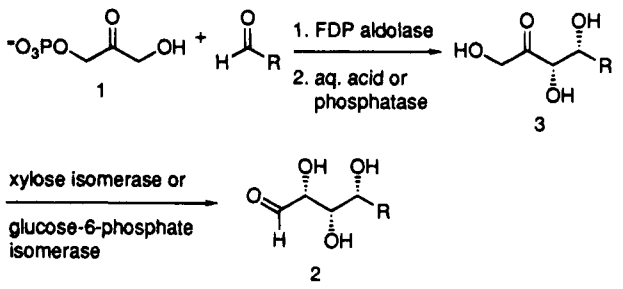
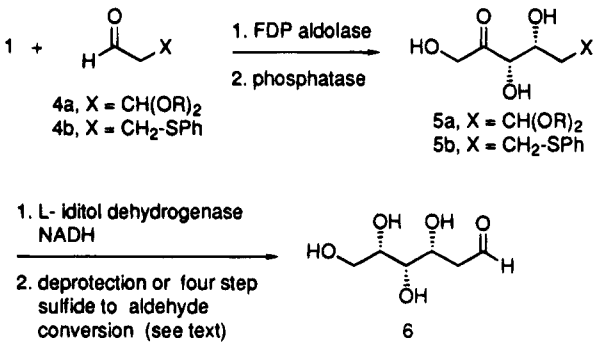
The dihydroxyacetone phosphate (DHAP) (**1**) utilizing enzymes have been widely used in the synthesis of ketose sugars and related sugar derivatives.[1-3] Enzymatic synthesis provides a very versatile synthetic method due both to the availability of different aldolases giving differing stereoisomeric products and to the broad specificity of the aldolases for their aldehyde substrate. Enzymatic synthesis of the even more prevalent aldose sugars has been much more limited. Several 2-deoxy aldoses have been prepared using deoxyribose-5-phosphate aldolase or a pyruvate-utilizing aldolase followed by decarboxylation.[4,5] Enzymatic synthesis of normal aldoses **2** having a C-2 hydroxyl has been achieved by enzymatic isomerization of aldolase-derived ketose sugars **3** (Scheme 1).[6,7] However, the substrate specificity of the isomerases is somewhat limited, so that only a fraction of the ketoses produced using aldolase catalysis can be isomerized enzymatically to the corresponding aldose. Furthermore, the isomerization reaction is reversible and, as a ketone is generally more stable than an isomeric aldehyde, the equilibrium produces substantial aldose isomer only if the aldose sugar can exist in a very stable aldopyranose form.[7] For example, 5-Deoxy-D-glucose, which cannot exist in a pyranose form, is much less stable than the corresponding ketose, 5-deoxy-D-fructose. As a result, no aldose is formed from 5-deoxy-D-fructose in the presence of xylose isomerase, but incubation of 5-deoxy-D-glucose with xylose isomerase results in quantitative conversion to the ketose.[7] While the substrate specificity limitations of ketose to aldose conversion may be partially overcome by the availability of additional isomerase enzymes,[8] no general solution to the equilibrium issue is apparent.

In order to expand the availability of aldose sugars from aldolase products, other means of converting aldolase products to aldose sugars have been sought. An inverted strategy has been developed, employing an aldolase-catalyzed condensation of DHAP (**1**) with a monoprotected dialdehyde **4a** (Scheme 2). Hydrolysis of the phosphate ester gives a ketose **5a** having a protected aldehyde at the terminal carbon.[9] Carbonyl reduction using L-iditol dehydrogenase and deprotection of the aldehyde provides an aldose sugar product **6**. In related work, a terminal phenylthio aldehyde **4b** was used as

Scheme 1



Scheme 2



the substrate, which after phosphatase treatment gave a terminal phenylthio ketose **5b**.[10] Carbonyl reduction, followed by a four-step sequence of acetylation, oxidation of the phenyl sulfide to the sulfoxide, Pummerer rearrangement, and deprotection again gave the aldose sugar **6**.[10] Another approach to aldose sugars from ketose sugars is simple base-catalyzed isomerization. However, such nonenzymatic isomerization methods typically give low yields and mixtures of stereoisomers and they suffer from the same equilibrium limitations encountered with enzymatic isomerization.[11,12]

We recently developed a method for synthesis of 1-deoxy-1-thioketoses using the 1-thio analog of dihydroxyacetone phosphate as a substrate for fructose diphosphate aldolase.[13,14] We envisioned that a 1-deoxy-1-thioketose could be converted to an aldose sugar by a pseudoisomerization procedure, consisting of reduction of the ketone to an alcohol and conversion of the sulfide to an aldehyde. We report here the demonstration of this new method for the conversion of a dihydroxyacetone phosphate-utilizing aldolase product to an aldose sugar.

To illustrate this procedure, 5-deoxy-D-mannose (5-deoxy-D-*lyxo*-hexose) (**7**) was chosen as a synthetic target. This sugar has been previously reported in a protected form, prepared from 2-deoxy-D-glucose, and used in the synthesis of a macrocyclic lactone antibiotic.[15] Like 5-deoxy-D-glucose, 5-deoxy-D-mannose cannot exist in a pyranose form and is expected to be much less stable than the corresponding ketose, 5-deoxy-D-fructose.[7] This precludes formation of 5-deoxy-D-mannose by an aldolase/isomerase sequence.

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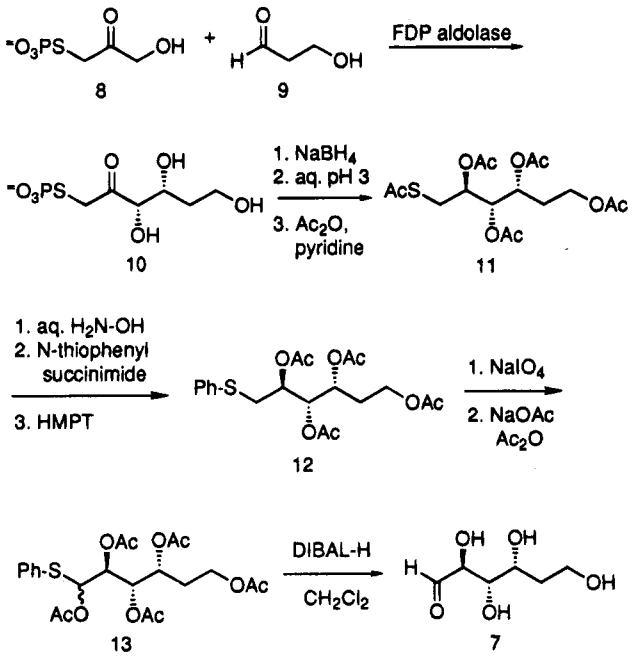
Scheme 3



The synthesis of **7** began with condensation of mercaptohydroxyacetone *S*-phosphate (**8**)[13] with 3-hydroxypropanal (**9**)[7] catalyzed by fructose diphosphate aldolase, to give the 1-deoxy-1-thioketose-1-phosphate **10** (Scheme 3). This product was not isolated but was subjected to sodium borohydride reduction, acid-catalyzed hydrolysis of the thiophosphate ester, and reaction with acetic anhydride in pyridine to give the acetylated thioalditol **11**. ^{1}H-NMR analysis of crude **11** indicated a 2:1 ratio of C-2 epimers, formed in the sodium borohydride reduction, with **11** being the major isomer. Relative stereochemistry was assigned by analysis of vicinal ^{1}H-NMR coupling constants.[16] The single diastereomer **11** was purified by silica gel chromatography and was isolated in 28% overall yield from **8**. Selective deprotection of the thiol group was achieved by reaction with aqueous hydroxylamine to give the free thiol. The thiol was converted to the phenyl disulfide by reaction with *N*-(phenylthio)succinimide,[17,18] which was followed by desulfurization with HMPT[19] to give the phenyl sulfide derivative **12** in 65% overall yield for the three steps from **11**. Efforts to introduce the phenyl group in a single step using a Pd-catalyzed arylation were unsuccessful.[20] The remaining steps were similar to those used in the inverted strategy for aldose synthesis.[10] The sulfide was oxidized to the sulfoxide[21] and then reacted with sodium acetate in acetic anhydride to give the Pummerer rearrangement product **13** as a mixture of C-1 epimers in 95% yield from **12**. Deprotection with DIBAL-H gave the target compound **7** in 22% yield from **13**.

The pseudoisomerization provides a novel route to 5-deoxy-D-mannose, a compound not accessible using an isomerase. While this pseudoisomerization sequence involves several steps, each step is relatively easy to perform and proceeds in acceptable yield. Furthermore the pseudoisomerization is more general than enzymatic isomerization, avoiding limitations of substrate specificity and unfavorable equilibria. Perhaps the most problematic step is the carbonyl reduction, which gives only a 2:1 preference for the anti isomer and requires separation of diastereomers. Furthermore, there is no apparent method for preferentially forming the syn isomer. Enzymatic reduction with L-iditol dehydrogenase has been utilized in the inverted strategy to obtain exclusively the syn isomer.[9] We have found that 1-deoxy-1-thio-D-xylulose, and presumably other 1-deoxy-1-thioketoses, are not accepted as substrates by this enzyme. Other hydride reducing agents and conditions have been investigated for the reduction of ketose phosphate sugars and somewhat higher diastereoselectivities have been obtained.[22] However, significant diastereoselectivity has only been obtained for the anti isomer.

The overall sequence for the synthesis of **7** is similar to the inverted strategy.[9,10] In one form of the inverted strategy the aldehyde is generated at the terminal carbon in the final steps from a phenylthio group originally present in the aldehyde substrate.[10] In the pseudoisomerization strategy the aldehyde is generated at C-1, using the thiol group originating from the thio analog of dihydroxyacetone phosphate **8** as the aldehyde surrogate. These two methods are quite complementary. In the inverted strategy, the functionality of the final four carbons is imposed by the nature of the reaction, as they originate from dihydroxyacetone phosphate and the aldehyde carbonyl carbon. The inverted strategy thus allows variability in structure of carbons between C-1 and the fourth carbon from the terminus. In the presently described pseudoisomerization method carbons 1–4 are imposed by the reaction, with potential for variability of functionality at C-5 and higher. These two strategies should thus be useful for the synthesis of aldose sugar analogs modified at different positions. There are reports in the literature of the syntheses of several 5-deoxyhexoses by nonenzymatic manipulation of preexisting monosaccharide structures.[23] Construction of such sugars using an enzymatic aldol condensation coupled with pseudoisomerization provides an attractive alternative. A variety of structures and stereochemical orientations with a range of functionality at C-5 and higher are potentially obtainable by choice of aldolase and aldehyde substrate, without limitations of substrate specificity and equilibrium often associated with enzymatic isomerization.

Supporting Information Available: Experimental procedures and characterization data for all compounds (4 pages).

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