Preparation of Deoxy Sugars via Aldolase-Catalyzed Synthesis of 1-Deoxy-1-thioketoses

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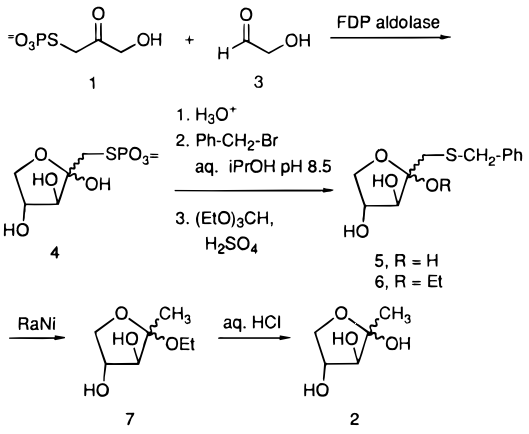
Deoxy sugars are widespread in nature as both biosynthetic intermediates and components of biologically active natural products.[1−3] Deoxy sugars have been prepared by reductive deoxygenation of suitably activated sugar derivatives[4,5] and by total synthesis.[6,7] Dihydroxyacetone phosphate (DHAP)-utilizing aldolases have been used for the synthesis of ketose sugars with a deoxy carbon at C-5 or higher using appropriate aldehyde substrates in the aldolase reaction.[8,9] Similarly, ketose sugars with a deoxy carbon at C-4 or higher have been prepared using transketolase.[10] An inverted strategy has been used to prepare 2-deoxyaldohexoses using aldolase catalysis.[11,12] Some additional 2-deoxy sugars have been prepared using deoxyribose 5-phosphate aldolase or a pyruvate-utilizing aldolase followed by decarboxylation, though neither of these aldolases give the high activities with a wide range of aldehyde substrates characteristic of the DHAP-utilizing aldolases.[13,14] We describe here the use of aldolase-catalyzed synthesis of 1-deoxy-1-thioketose sugars as a novel entry into classes of deoxy sugars not previously accessible by enzymatic methods.

We recently reported the synthesis of the 1-thio analog **1** of dihydroxyacetone phosphate.[15] Subsequently, we and others have demonstrated the use of **1** as a substrate for fructose diphosphate aldolase in enzymatic carbohydrate synthesis.[16,17] We further envisioned that use of **1** in aldolase-catalyzed reactions followed by desulfurization with Raney nickel could provide access to deoxy sugars. As an initial deoxy sugar target we chose 1-deoxy-D-xylulose (**2**). This deoxy sugar is an intermediate in the biosynthesis of thiamine and pyridoxine and has been used in studies of the enzymology of the biosynthesis of these biological cofactors.[18−22] Condensation of **1** with glycoaldehyde **3** catalyzed by fructose diphosphate aldolase formed 1-deoxy-1-thio-D-xylulose 1-phosphate (**4**) (Scheme 1). Progress of the reaction was monitored by enzymatic assay of **1**[15] and by ^{31}P-NMR analysis of the reaction mixture.[23] The product **4** was not isolated but subjected to acid-catalyzed hydrolysis of the thiophosphate and reaction with benzyl bromide to give 1-deoxy-1-thio-D-xylulose as the benzyl sulfide derivative **5**, in 23% overall yield from **1**.[24] The benzyl group provided a handle for purification and characterization of the product. The ethyl glycoside **6** was formed as a mixture of anomers in 52% yield. Subsequent Raney nickel reduction formed the ethyl glycoside of 1-deoxy-D-xylulose **7** in 76% yield. Hydrolysis with aqueous acid gave compound **2** in 46% yield, which gave spectral data in full agreement with that previously reported.[25,26]

Scheme 1



To further demonstrate the versatility of this route to deoxy sugars, we undertook the synthesis of a 2,6-dideoxy sugar. Several 2,6-dideoxyaldohexoses occur as components of natural bioactive compounds, including antibiotics and cardiac glycosides.[27,28] These sugars have not previously been available by enzymatic methods. To address this problem, the half-protected malonic dialdehyde **8** was used in the FDP aldolase-catalyzed condensation with the thiophosphate substrate **1** (Scheme 2). The aldehyde **8** has previously been used in aldolase

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(23) 161.9 MHz ^{31}P-NMR (DOH): **1** δ15.69 (keto form), 17.47 (hydrate); **4** 15.80 (acyclic form), 17.06, 17.58 (cyclic forms).
(24) The modest yield is apparently due in part to decomposition of the product **5**. Yields of peracetylated thioalditol of greater than 50% from **1** have been obtained upon sodium borohydride reduction, thiophosphate hydrolysis, and acetylation of the product **4**.
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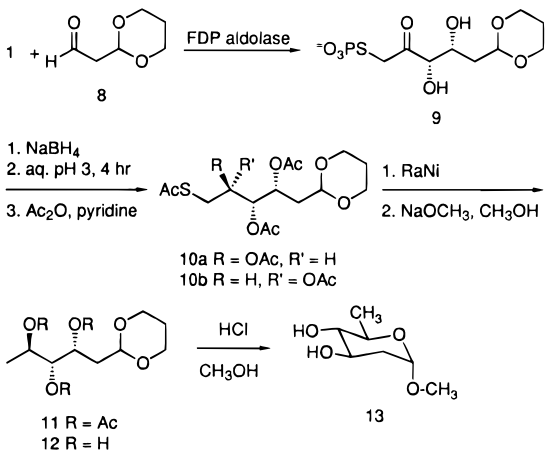
Scheme 2



reactions with DHAP in the inverted strategy for aldose synthesis.[11] The product **9** was not isolated but was subjected to sodium borohydride reduction followed by acid-catalyzed hydrolysis of the thiophosphate ester under mild conditions to avoid acetal hydrolysis. Subsequent reaction with acetic anhydride in pyridine gave a mixture of the acetylated products **10a** and **10b**. The two isomers, formed in a 2:1 ratio as determined by ^{1}H-NMR integration of crude product, were separated by chromatography on silica gel. The configuration at C-2 was assigned by analysis of the vicinal proton coupling constants.[29] The major isomer **10a** was reduced with Raney nickel to give **11** in 70% yield. Deacetylation with sodium methoxide in methanol gave **12** in 95% yield. Acetal cleavage was performed with HCl in methanol to give the dideoxy sugar as the α-methyl glycoside **13** in 56% yield. Methyl glycoside formation facilitated product isolation and characterization. The α-methyl glycoside of 2,6-dideoxy-D-glucose **13** (also known as D-olivose or systematically as 2,6-dideoxy-D-*arabino*-hexose) gave spectral data identical with that previously reported.[30,31]

As C-1 through C-3 of the products of DHAP-utilizing aldolases always arise from DHAP and C-4 from an aldehyde carbonyl carbon, aldol reactions with DHAP offer variability in functionality only at C-5 and higher.[9] The inverted strategy reverses the situation so that the four carbons farthest from C-1 are defined, with variable substitution possible at the lower number carbons (e.g., C-1 and C-2 of a hexose) depending on the aldehyde substrate.[11,12] Reactions with the thio analog of DHAP now permit added variability of functionality at a position that always bears a hydroxyl group when DHAP is used as substrate. This provides additional flexibility in the enzymatic synthesis of deoxy sugars, as demonstrated here in the synthesis of a 1-deoxyketopentose and a 2,6-dideoxyaldohexose. Extension of this methodology to all four DHAP-utilizing aldolases[9] and a range of aldehyde substrates should provide entry into a wide variety of deoxy sugars not previously accessible by enzymatic methods. Work is underway to develop further applications of enzymatically prepared deoxythioketoses as synthons for a variety of sugar structures.

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Supporting Information Available: Experimental procedures and spectral data for all compounds (6 pages).

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