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A two-step strategy for the preparation of 6-deoxy-L-sorbose

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Abstract

A two-step enzymatic strategy for the efficient and convenient synthesis of 6-deoxy-L-sorbose was reported herein. In the first reaction step, the isomerization of L-fucose (6-deoxy-L-galactose) to L-fuculose (6-deoxy-L-tagatose) catalyzed by L-fucose isomerase (FucI), and the epimerization of L-fuculose to 6-deoxy-L-sorbose catalyzed by D-tagatose 3-epimerase (DTE) were coupled with the targeted phosphorylation of 6-deoxy-L-sorbose by fructose kinase from human (HK) in a one-pot reaction. The resultant 6-deoxy-L-sorbose 1-phosphate was purified by silver nitrate precipitation method. In the second reaction step, the phosphate group of the 6-deoxy-L-sorbose 1-phosphate was hydrolyzed with acid phosphatase (AphA) to produce 6-deoxy-L-sorbose in 81% yield with regard to L-fucose.

Graphical abstract

Keywords

6-deoxy-L-sorbose; Enzymatic synthesis; Phosphorylation; Dephosphorylation; One-pot multienzyme

Rare sugars are defined as monosaccharides that only naturally occur in small amounts.¹ According to the classification by the International Society of Rare Sugars (ISRS), only seven monosaccharides (D-xylose, D-ribose, L-arabinose, D-galactose, D-glucose, D-mannose, and D-fructose) are present in substantial enough quantities to be considered common sugars, whereas all others belong to the “rare sugars” classification.² Rare sugars have garnered much attention over the past decades due to their potential applications in pharmaceutical, medicinal, and synthetic chemistry.^{3–6} For example, L-ribose can be used as a building block for L-enantiomers of nucleoside analogues against cancers and viral

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at

infections.⁷ D-psicose is used as a precursor for the synthesis of xylosylpsicoses, which are promising candidates for prebiotics, cosmetics and therapeutic uses.⁸ In addition, “rare sugars” are also well known for their uses as low calorie sweeteners and functional food bulking agents due to the slow and incomplete absorption of these sugars by the human body.^{3,9-12} As evidenced, D-psicose has approximately 70% of the sweetness but only 0.3% of the energy calories of sucrose.^{10,13} Nevertheless, the study of rare sugars have been hindered largely by their limited availability.

Izumori and co-workers have established a beautiful strategy termed “Izumoring” for rare sugar synthesis, a method by which most of rare sugars have been produced through isomerization, epimerization, oxidization or reduction reactions.¹⁴ However, this strategy tends to suffer from low yield, complicated conversion processes or a tedious purification steps. To address these problems, we recently developed a convenient, efficient and cost-effective platform for ketose synthesis, by which 10 non-readily available ketopentoses (L-ribulose, D-xylulose, D-ribulose, and L-xylulose) and ketohexoses (D-tagatose, D-sorbose, D-psicose, L-tagatose, L-fructose and L-psicose) were prepared from common and inexpensive starting materials with both high yield and purity without having to undergo a tedious isomer separation step.¹⁵ The basic concept of this strategy is based on a “phosphorylation→dephosphorylation” cascade reaction. Thermodynamically unfavorable conversions were combined with targeted phosphorylation reactions by substrate-specific kinases to drive the conversions towards the formation of the desired ketoses. The remaining phosphate donor (ATP) and phosphorylation reaction byproduct (ADP) were selectively removed by silver nitrate precipitation method to afford the intermediate of sugar phosphate. Afterwards, the phosphate group was hydrolyzed with phosphatase to afford the desired product. Subsequently, L-rhamnulose and L-fuculose were also produced by using this strategy, in which both deoxy ketoses were obtained in an yield exceeding 80%.¹⁶ In this work, 6- deoxy L-sorbose was synthesized from L-fucose using the similar strategy.

6-deoxy-L-sorbose can serve as a precursor of furaneol (4-Hydroxy-2,5-dimethyl-3(2H)-furanone), an important compound in the food industry for its caramel-like flavor used in food industry.¹⁷ Moreover, 6-deoxy-L-sorbose is also the starting material for the preparation of 6-deoxy-L-gulose and 6-deoxy-L-idose.¹⁸ The majority of the potential applications of 6-deoxy-L-sorbose have not been fully investigated due to its relative scarcity. Enzymatic synthesis by the condensation of dihydroxyacetone phosphate (DHAP) and L-lactaldehyde employing aldolase has been the primary method for 6-deoxy-L-sorbose preparation (Scheme 1, A), in which 6-deoxy-L-sorbose was obtained in 56% yield.¹⁹ Hecquet and co-workers found that 6-deoxy-L-sorbose could also be prepared from 2,3-dihydroxybutyraldehyde and hydroxypyruvate by using transketolase (TK) (Scheme 1, B).²⁰ Although a 24% yield was achieved, up to 26% of an isomer (6-deoxy-D-fructose) was also present in the resulting solution. Subsequently, they found that TK could exclusively produces 6-deoxy-L-sorbose when hydroxypyruvate and 4-deoxy-L-threose were used as starting materials (Scheme 1, C).²¹ The process was improved by coupling the isomerization reaction of 4-deoxy-L-threose from 4-deoxy-L-erythrulose giving a yield up to 35%.²¹ However, these methods suffer from expensive starting materials, low yields and complicated purification processes. Recently, Shompoosang and co-workers used L-fucose (6-deoxy-L-galactose), a readily available sugar in nature, as starting materials in preparing 6-deoxy-L-

sorbose (Scheme, D).¹⁸ By this process, L-fucose was isomerized to L-fuculose (6-deoxy-L-tagatose) by L-fucose isomerase, and then 6-deoxy-L-sorbose was epimerized from 6-deoxy-L-tagatose by D-tagatose 3-epimerase (DTE). However, since all aldose-ketose isomerization is very unfavorable for ketose formation, the first step only produce 9% isolated yield. The production was improved by reusing the purified L-fucose several times, but only 27% isolated yield was obtained. The second step produces 50% yield resulting in a final 14% isolated yield of 6-deoxy-L-sorbose with regard to L-fucose. Moreover, this method requires a tedious isomer separation process to obtain 6-deoxy-L-sorbose in pure form.

To establish a facile method for 6-deoxy-L-sorbose preparation, we attempt to apply an “isomerization→epimerization→phosphorylation→dephosphorylation” cascade reaction to produce 6-deoxy-L-sorbose from L-fucose directly (Scheme 1, E). FucI catalyzes the isomerization of L-fucose to L-fuculose.²² DTE is a novel epimerase that catalyze the epimerization of ketoses at C-3 position resulting (3*S*)- and (3*R*)-interconversion.²³⁻²⁵ These two enzymes have been used to produced 6-deoxy-L-sorbose as mentioned above. Although these two reactions can be carried in one-pot to simplify the synthetic process, the purification of 6-deoxy-L-sorbose from the reaction mixture containing L-fucose, L-fuculose and 6-deoxy-L-sorbose is very difficult and makes this strategy impractical. In this work, the isomerization of L-fucose to L-fuculose, and epimerization of L-fuculose to 6-deoxy-L-sorbose were accurately controlled by coupling with a targeted phosphorylation reaction of 6-deoxy-L-sorbose in one-pot fashion. The selective phosphorylation of 6-deoxy-L-sorbose could drive both reversible reactions towards the formation of the 6-deoxy-L-sorbose 1-phosphate in the first reaction step. However, to achieve the designed scheme, the prerequisite is the availability of a kinase that specifically recognizes 6-deoxy-L-sorbose but not L-fucose and L-fuculose. Otherwise, the products obtained finally will be a mixture containing many isomers (L-fucose and L-fuculose), which are difficult to be separate. By screening the substrate specificity of many kinases, we recently found that fructose kinase (HK) from human, which phosphorylate ketose to ketose 1-phosphate,²⁶ preferred ketose with (3*S*)-configuration as its substrate.¹⁵ In this work, substrate specificity of HK towards 6-deoxy-L-sorbose ((3*S*)-configuration), L-fucose ((3*R*)-configuration), and L-fuculose ((3*R*)-configuration) was studied (see Supplementary Information). 6-deoxy-L-sorbose could serve as the substrate of HK while no detectable activity of HK towards either L-fucose or L-fuculose was observed (Table 1), indicating its potential for one-pot multienzyme (OPME) reaction to produce 6-deoxy-L-sorbose from L-fucose. Indeed, treatment of 6-deoxy-L-sorbose with HK in the presence of ATP as phosphate donor led to complete conversion to 6-deoxy-L-sorbose 1-phosphate after 2 hour at 37 °C.

Having met the prerequisite, other conversion related enzymes including L-fucose isomerase (FucI) from *Escherichia coli*,²⁷ DTE from *Pseudomonas* Sp, St-24²⁵, and AphA from *Escherichia coli*²⁸ were prepared as described in Supplementary data. To test the potential of HK in OPME reaction, a small scale reaction system (50 ul) containing conversion-related enzymes (FucI, DTE and HK) was performed (reaction group). Reactions without FucI (control 1 group) or DTE (control 2 group) were done as negative controls. The reaction was incubated at 37 °C for 1 hour and monitored by TLC (EtOAc/MeOH/H₂O/HOAc=5:2:1.4:0.4). The formation of 6-deoxy-L-sorbose 1-phosphate was observed in the

reaction group and no reaction was observed in either control 1 or control 2 (Figure S1), indicating the feasibility of the designed OPME reaction. Preparative reactions were performed in the gram scale. To efficiently convert L-fucose, excess ATP (1.25 equiv) was used. For the convenience of the final purification, no buffer was used. The reaction pH was held near 7.5 using sodium hydroxide as the reaction was ongoing. The reaction was allowed to proceed until no detectable L-fucose was observed by HPLC (conversion ratio exceeding 99%) and thereby making isomer separation unnecessary. 6-deoxy-L-sorbose 1-phosphate was purified by using silver nitrate precipitation method.²⁹ The principle of this method is that silver ions could precipitate ATP and ADP while monophosphate sugars could not be precipitated when the sugars are composed of four or more carbons. Therefore, by applying this selective precipitating ability of silver ions, sugar phosphate can be cleanly and easily separated from adenosine phosphates. Afterwards, the solution was desalted by Bio-Gel P-2 column to afford 6-deoxy-L-sorbose 1-phosphate in 92% yield with regard to L-fucose. In the second reaction step, the phosphate group of 6-deoxy-L-sorbose 1-phosphate was hydrolyzed by AphA in pH 5.5. Once no detectable sugar phosphate was observed, the reaction was stopped by adding cooled ethanol. The solution was purified by Bio-Rad P-2 column to afford 6-deoxy-L-sorbose in 81% yield with regard to L-fucose.

The product was confirmed by MS, NMR and HPLC analysis. The predicted peak ($[M + Na]^+$ 187.0582) was well observed on high resolution mass spectrum (see Supplementary Information). NMR spectra are well in accord with the previous reported data.^{19,30} The reported NMR data (¹H-NMR and ¹³C-NMR) of 6-deoxy-L-sorbose is a mixture contain both α and β furanose isomers. However, we observe that 6.7% of deoxy-L-sorbose exists in aqueous solution as linear chain form (as confirmed by ¹H-NMR and ¹³C-NMR). To analyze the product purity, the product was analyzed by HPLC. No detectable L-fucose (as also confirmed by NMR) and L-fucose were observed indicated the product purity exceeding 99% (Figure 1).

In summary, using the “isomerization→epimerization→phosphorylation→dephosphorylation” cascade reaction as a basis, 6-deoxy-L-sorbose was directly prepared from a readily available sugar L-fucose, in 81% isolated yield on the gram scale. By using the silver nitrate precipitation, a desalting column (Bio-Gel P-2 column) allows the product to be isolated with high purity. ATP is also commercially inexpensive owing to increased industrial production over the past decade,³¹ making the preparation process described herein of particular interest for large-scale production. Moreover, the precipitate of silver-adenosine phosphates complex could be redissolved with ammonium hydroxide, making the recycle of adenosine phosphates and silver ions achievable, which will further reduce the preparation cost when this method was applied on large scale purification. We anticipate that this work will accelerate the progress in understanding both biological roles and synthetic applications of 6-deoxy-L-sorbose.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Previous strategies for the synthesis of 6-deoxy-L-sorbose

This work: two step strategy for the efficient and convenient preparation of 6-deoxy-L-sorbose

Scheme 1.

Enzymatic synthesis of 6-deoxy-L-sorbose. DHAP: dihydroxyacetone phosphate. TK: transketolase. FucI: L-fucose isomerase. DTE: D-tagatose 3-epimerase. HK: fructose kinase. AphA: acid phosphatase. OPME: one-pot multienzyme.

Figure 1. HPLC profiles of 6-deoxy-L-sorbose compared with L-fucose (HPX-87H column) and L-fuculose (Sugar-Pak 1 column). The columns were eluted at 60°C with pure water as mobile phase at a flow rate of 0.6 ml/min (ELSD detector).

Table 1Substrate specificity of HK towards several deoxy sugars^a

Substrate	HK activity (%)	C-3 configuration
L-fucose	ND	<i>R</i>
L-fuculose	ND	<i>R</i>
6-deoxy-L-sorbose	100	<i>S</i>

ND: no detectable activity was observed.

^aSubstrate specificity was studied by the reactions that were performed at 37°C for 10 minutes in 50 ul reaction mixture containing a Tris-HCl buffer (100 mM, pH 7.5), 20 mM of sugar standards, 20mM of ATP, 5 mM of Mg²⁺, and 10 ug of enzymes.

Table 2Enzymatic synthesis of 6-deoxy-L-sorbose from L-fucose using the two-step strategy shown in Scheme 1 ^a

Starting material	Enzymes	Intermediate	Step 1 yield (%)	Product	Total yield (%)	Scale (mg)	Purity ^a (%)
L-fucose	FucI DTE HK AphA		92	6-deoxy-L-sorbose	81	1059	>99

^a Defined as the percentage of 6-deoxy-L-sorbose out of the sum of all possible isomers (as confirmed by HPLC).