Published in final edited form as:

Tetrahedron Lett. 2016 August 24; 57(34): 3819–3822. doi:10.1016/j.tetlet.2016.07.015.

Two-step enzymatic synthesis of 6-deoxy-L-psicose

Liuqing Wen^{a,*}, Kenneth Huang^a, Yuan Zheng^a, Junqiang Fang^{b,*}, Shukkoor Muhammed Kondengaden^a, and Peng George Wang^{a,*}

^aDepartment of Chemistry, Georgia State University, Atlanta, GA 30303. USA

^bNational Glycoengineering Research Center, Shandong University, Jinan, Shandong 250100. People's Republic of China

Abstract

Rare sugars offer a plethora of applications in the pharmaceutical, medicinal, and industries, as well as in synthetic chemistry. However, studies of rare sugars have been hampered by their relative scarcity. In this work, we describe a two-step strategy to efficiently and conveniently prepare 6-deoxy-L-psicose from L-rhamnose. In the first reaction step, the isomerization of L-rhamnose (6-deoxy-L-mannose) to L-rhamnulose (6-deoxy-L-fructose) catalyzed by L-rhamnose isomerase (RhaI), and the epimerization of L-rhamnulose to 6-deoxy-L-psicose catalyzed by D-tagatose 3-epimerase (DTE) were coupled with selective phosphorylation reaction by fructose kinase from human (HK), which selectively phosphorylate 6-deoxy-L-psicose at C-1 position. 6-deoxy-L-psicose 1-phosphate was purified by a silver nitrate precipitation method. In the second step, the phosphate group of the 6-deoxy-L-sorbose 1-phosphate was hydrolyzed with acid phosphatase (AphA) to produce 6-deoxy-L-psicose in 81% yield with respect to L-rhamnose. This method allows that the 6-deoxy-L-psicose to be obtained from readily available starting materials with high purity and without having to undergo isomer separation.

Graphical abstract

Keywords

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

*Corresponding authors. lwen2@gsu.edu (L. Q. Wen), fangjunqiang@sdu.edu.cn (J. Q. Fang), or pwang11@gsu.edu (P.G. Wang).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary Data

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

The International Society of Rare Sugars (ISRS) has defined the namesake “rare sugars” as monosaccharides that are found in small amounts in nature.¹ Under this classification, every hexose and pentose, excepting D-xylose, D-ribose, L-arabinose, D-galactose, D-glucose, D-mannose, and D-fructose, are termed rare sugars.² Despite their relatively low natural abundance, rare sugars still have an enormous potential for applications in synthetic, pharmaceutical, and medicinal chemistry.³ For example, L-form rare sugars, compared to their D-enantiomer counterparts, have widely been used for the production of antiviral and anticancer agents in medical fields due to their improved biological activity and lower toxicity.⁴ Some rare sugars such as D-allose can function as anti-tumor, anti-inflammatory, and anti-oxidative reagent.⁵ D-psicose has been shown to inhibit hepatic lipogenous enzyme activity, helping reduce abdominal fat accumulation.⁶ D-tagatose was shown to be a potentially important drug for treating type 2 diabetes, and has been approved by the FDA as a food additive.⁷ In the food industry, rare sugars are well known as low-calorie supplements. For example, D-psicose has about 70% of the sweetness of sucrose, but only 0.3% of the calories.⁸

Rare sugars cannot be extracted from natural sources due to their low abundance. Therefore, numerous chemical and enzymatic methods have been explored to synthesize rare sugars from common sugars.^{1c,9} Chemical strategies for rare sugars tend to require multiple protection/deprotection and complicated purification steps, suffering from low yield as a result.¹⁰ As an alternative, enzymatic synthesis with mild conditions and high efficiency has distinct advantages in both regio- and stereo-selectivity.^{2b} Izumori *et al.* have previously shown that the total synthesis of rare sugars by using isomerization, epimerization, oxidization or reduction reactions is achievable.^{2a,11} For example, aldose-ketose isomerization catalyzed by a single isomerase has been widely used to produce ketose from aldose as most common sugars are aldoses.¹² Nevertheless, aldose-ketose isomerization is very unfavorable for ketose formation in the final reaction as ketose has a higher energy state than aldose.¹³ In most instances, the conversion ratios are no more than 40%.¹⁴ Attempts to improve aldose-ketose isomerization include the addition of borate to break the aldose-ketose reaction equilibrium,¹⁵ the discovery of novel enzymes in nature,¹⁶ and directed evolution¹⁷ have also been suggested. Nevertheless, these methods still suffer from low yield, or an isomer separation manipulation, which is a labor-intensive and time-consuming process due to the similar properties of the isomer pair. Ketose-ketose epimerization catalyzed by D-psicose 3-epimerase (DPE) or D-tagatose 3-epimerase (DTE), which was firstly discovered by Izumori and co-workers,¹⁸ is another important reaction for rare sugar synthesis. However, this process also suffers from low yield and isomer separation manipulation. Oxidization or reduction reactions require co-enzyme such as NAD⁺/NADH, which are commercially too expensive for large scale synthesis. Therefore, the study of rare sugars is still hindered due to the lack of an efficient and convenient preparation method.

Recently, we have established a convenient, efficient and cost-effective platform for ketose synthesis, by which 10 non-readily available ketoses (L-ribulose, D-xylulose, D-ribulose, L-xylulose, D-tagatose, D-sorbose, D-psicose, L-tagatose, L-fructose and L-psicose) were prepared from common and inexpensive starting materials.¹⁹ The main advantage of this method is that the products could be obtained in both high yield and purity without having to undergo an isomer separation step. The principle of this strategy is based on a

“phosphorylation→dephosphorylation” cascade reaction. Thermodynamically unfavorable aldose-ketose conversions or ketose-ketose epimerizations were combined with selective phosphorylation of the target ketose by substrate-specific kinases, which results in a clean conversion of aldoses to the desired ketoses. The intermediate of ketose phosphates were purified by a silver nitrate precipitation method, in which sugar phosphates can be cleanly separated from adenosine phosphates (ATP and ADP).²⁰ L-fuculose, L-rhamnulose, and 6-deoxy-L-sorbose were subsequently produced.²¹ In this work, 6-deoxy-L-psicose was prepared from L-rhamnose using the similar two-step strategy (Scheme 1).

6-deoxy-L-psicose is a 6-deoxy rare sugar and also the C-3 epimer of L-rhamnulose. 6-deoxy sugars are building blocks of a variety of natural products, including antifungals,²² antibiotics,²³ and anticancer agents.²⁴ 6-deoxy-L-psicose is the intermediate for the preparation of 6-deoxy-L-allose and 6-deoxy-L-altrose.²⁵ The more potential applications of 6-deoxy-L-psicose have not been reported, possibly due to its limited availability. Chemical 6-deoxy reaction from corresponding ketoses require complicated protection/deprotection steps, resulting in a low yield.²⁶ The only enzymatic strategy reported for 6-deoxy-L-psicose synthesis is by Shompoosang *et al*, to the best of our knowledge.²⁵ They incubated L-rhamnose with L-rhamnose isomerase (RhaI) and D-tagatose 3-epimerase (DTE) to produce 6-deoxy-L-psicose. When the final reaction equilibrium was observed, a mixture containing L-rhamnose, L-rhamnulose and 6-deoxy-L-psicose (55: 35: 15) was obtained. After a tedious purification step, 6-deoxy-L-psicose was obtained in only 8.7% yield with respect to L-rhamnose. Therefore, an efficient and convenient strategy capable of producing 6-deoxy-L-psicose in considerable amount would be of great interest to accelerate the study of 6-deoxy-L-psicose.

To produce 6-deoxy-L-psicose from L-rhamnose by using the proposed two-step strategy, two prerequisites must first be met: 1) the availability of a D-tagatose 3-epimerase (DTE) to catalyze the interconversion of L-rhamnulose and 6-deoxy-L-psicose, but not the interconversion between 6-deoxy-L-psicose 1-phosphate and L-rhamnulose 1-phosphate; and 2) the availability of a kinase that specifically phosphorylates 6-deoxy-L-psicose but not L-rhamnulose and L-rhamnose. The discovery of DTE, which epimerize ketoses at C-3 position, by Izumori *et al* has made it possible to achieve the interconversion of (3*S*)-sugars and (3*R*)-sugars.¹⁸ They demonstrated that DTE from *Pseudomonas* Sp, St-24 failed to recognize D-fructose 6-phosphate and D-ribulose 5-phosphate.¹⁸ We recently found that DTE from *Pseudomonas* Sp, St-24 also fails to use many ketose phosphates as the substrate.^{19–20} Therefore, we proposed that it may not recognize 6-deoxy-L-psicose 1-phosphate as substrate in this work. This assumption is supported by the purity analysis of 6-deoxy-L-psicose (Table 1). To find a kinase that specifically phosphorylates 6-deoxy-L-psicose but not L-rhamnulose and L-rhamnose, substrate specificity of many kinases was studied in this work (data not shown). Finally, we found that fructose kinase (HK) from human accords well with the requirement of the described Scheme. Substrate specificity study indicated that HK could efficiently phosphorylate 6-deoxy-L-psicose, with only trace activity (<0.1 %) towards L-rhamnulose and no detectable activity towards L-rhamnose (Table 1). The high specificity of HK towards 6-deoxy-L-psicose indicated its potential for applications in one-pot multienzyme (OPME) reaction for the preparation of 6-deoxy-L-psicose from L-rhamnose.

To test this potential, analytical scale reaction system (164 ug in 50 ul) containing conversion-related enzymes (RhaI, DTE and HK) was performed. Reactions without RhaI (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-psicose 1-phosphate was observed in the reaction group and no reaction was observed in either control 1 or control 2 (Figure 1), indicating the feasibility of the designed OPME reaction.

Preparative scale synthesis was carried in a 400 ml reaction solution containing 20 mM L-rhamnose. As much as possible to consume L-rhamnose, 1.25 equivalent of ATP was added. The reaction pH was held near 7.5 using 1 M of sodium hydroxide as the reaction was ongoing. The reaction was carefully shaken at 37°C for 48 hours to allow the formation of 6-deoxy-L-psicose 1-phosphate. Enzymes were supplemented every 12 hours. Once reaction finished, 95% of L-rhamnose was consumed (as confirmed by HPLC). Afterwards, ATP and ADP was selectively precipitated by using silver nitrate precipitation method,²⁰ while 6-deoxy-L-psicose 1-phosphate is still in solution. 6-deoxy-L-psicose 1-phosphate was separated from the remaining L-rhamnose by using Bio-Gel P-2 column to afford final product in 90% yield with regard to L-rhamnose. In the second reaction step, 6-deoxy-L-psicose 1-phosphate obtained in first reaction step was dissolved in water and the solution pH was adjusted to 5.5 using 1 M of HCl. Then, acid phosphatase was added to hydrolyze the phosphate group of 6-deoxy-L-psicose 1-phosphate to afford 6-deoxy-L-psicose. After desalting by using Bio-Gel P-2 column, 6-deoxy-L-psicose was obtained in 80% yield with regard to L-rhamnose (Table 2).

The obtained product was confirmed by NMR, HRMS and HPLC analysis (see Supporting Information). The predicted peak ($[M+Na]^+$ 187.0582) was well observed on high resolution mass spectrum. The product purity was analyzed by HPLC using HPX-87H column or Sugar-Pak 1 column equipped with evaporative light scattering detector (Figure 2). HPLC analysis using HPX-87H column indicated that no detectable L-rhamnose was found (as also confirmed by ¹H-NMR). HPLC analysis using Sugar-Pak 1 column showed that 1.5 % of L-rhamnulose was found in final product. This may result from the long reaction time and the trace activity of HK towards L-rhamnulose. Nevertheless, this purity is comparable to that obtained from traditional method.

In summary, on the basis of the successful identification of a kinase that could specifically phosphorylate 6-deoxy-L-psicose, we were able to utilize an “isomerization→epimerization→phosphorylation→dephosphorylation” cascade reaction to prepare 6-deoxy-L-psicose from L-rhamnose. Using a desalting column (Bio-Gel P-2 column), 6-deoxy-L-psicose with high purity (98.5%) was obtained in high yield (81%). This method represents the most efficient and convenient strategy for 6-deoxy-L-psicose synthesis. Moreover, L-rhamnose and ATP are commercially inexpensive. The recycle of silver ions and ADP is also possible in this reaction.²⁰ These advantages make the preparation process described herein of particular interest for large-scale production. We anticipate that this work will facilitate the studies of potential applications of 6-deoxy-L-psicose. Future studies will enable the identification of new kinases to be used in more sugar syntheses, providing a powerful set of tools for carbohydrate research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was financially supported by National Institute of Health of USA (R01 GM085267 and R01 AI083754), basic Research Program of China (973 Program, No.2012CB822102) and the Key Grant Project of Chinese Ministry of Education (No.313033).

References and notes

1. (a) Tang SY. *Journal of Proteomics & Enzymology*. 2013; 2012(b) Beerens K, Desmet T, Soetaert W. *J Ind Microbiol Biotechnol*. 2012; 39:823. [PubMed: 22350065] (c) Ahmed Z. *Electronic Journal of Biotechnology*. 2001; 4:13.
2. (a) Izumori K. *J Biotechnol*. 2005; 118:S89.(b) Beerens K, Desmet T, Soetaert W. *J Ind Microbiol Biotechnol*. 2012; 39:823. [PubMed: 22350065]
3. (a) Li B, Varanasi S, Relue P. *Green Chem*. 2013; 15:2149.(b) Ishida Y, Kakibuchi K, Kudo R, Izumori K, Tajima S, Akimitsu K, Tanaka K. *Acta Hort*. 2012; 927:929.(c) Maki-Arvela P, Salmi T, Holmbom B, Willfor S, Murzin DY. *Chem Rev*. 2011; 111:5638. [PubMed: 21682343] (d) Granstrom TB, Izumori K, Leisola M. *Appl Microbiol Biotechnol*. 2007; 74:273. [PubMed: 17216458] (e) Bolouri Moghaddam MR, Van den Ende W. *J Exp Bot*. 2012; 63:3989. [PubMed: 22553288] (f) Kano A, Gomi K, Yamasaki-Kokudo Y, Satoh M, Fukumoto T, Ohtani K, Tajima S, Izumori K, Tanaka K, Ishida Y, Tada Y, Nishizawa Y, Akimitsu K. *Phytopathology*. 2010; 100:85. [PubMed: 19968553] (g) Calvo MB, Figueroa A, Pulido EG, Campelo RG, Aparicio LA. *International journal of endocrinology*. 2010(h) Wen L, Zheng Y, Li T, Wang PG. *Bioorg Med Chem Lett*. 2016; 26:2825. [PubMed: 27173798]
4. (a) Mathe C, Gosselin G. *Antiviral Res*. 2006; 71:276. [PubMed: 16797735] (b) Koeller KM, Wong CH. *Nat Biotechnol*. 2000; 18:835. [PubMed: 10932151] (c) Gumina G, Song GY, Chu CK. *FEMS Microbiol Lett*. 2001; 202:9. [PubMed: 11506901] (d) Okano K. *Tetrahedron*. 2009; 65:1937.(e) Perali RS, Mandava S, Bandi R. *Tetrahedron*. 2011; 67:4031.
5. (a) Lim YR, Oh DK. *Appl Microbiol Biotechnol*. 2011; 91:229. [PubMed: 21655980] (b) Yamada K, Noguchi C, Kamitori K, Dong Y, Hirata Y, Hossain MA, Tsukamoto I, Tokuda M, Yamaguchi F. *Nutr Res*. 2012; 32:116. [PubMed: 22348460] (c) Ueki M, Asaga T, Chujo K, Ono J, Iwanaga Y, Taie S. *J Biosci Bioeng*. 2008; 105:481. [PubMed: 18558338] (d) Kano A, Fukumoto T, Ohtani K, Yoshihara A, Ohara T, Tajima S, Izumori K, Tanaka K, Ohkouchi T, Ishida Y. *Journal of experimental botany*. 2013:ert282.
6. Chung YM, Hyun Lee J, Youl Kim D, Hwang SH, Hong YH, Kim SB, Jin Lee S, Hye Park C. *Journal of food science*. 2012; 77:H53. [PubMed: 22339545]
7. (a) Lu Y, Levin GV, Donner TW. *Diabetes Obes Metab*. 2008; 10:109. [PubMed: 17941870] (b) Espinosa I, Fogelfeld L. *Expert Opin Investig Drugs*. 2010; 19:285.
8. (a) Oshima H, Kimura I, Zumori K. *Food Sci Technol Res*. 2006; 12:137.(b) Matsuo T, Suzuki H, Hashiguchi M, Izumori K. *J Nutr Sci Vitaminol (Tokyo)*. 2002; 48:77. [PubMed: 12026195]
9. Wei MH, Li ZJ, Li TH, Wu BL, Liu YP, Qu JY, Li X, Li L, Cai L, Wang PG. *Acs Catalysis*. 2015; 5:4060.
10. Emmadi M, Kulkarni SS. *Natural Product Reports*. 2014; 31:870. [PubMed: 24700208]
11. Granstrom TB, Takata G, Tokuda M, Izumori K. *J Biosci Bioeng*. 2004; 97:89. [PubMed: 16233597]
12. Zheng YJ, Merz KM, Farber GK. *Protein Eng*. 1993; 6:479. [PubMed: 8415575]
13. Ekeberg D, Morgenlie S, Stenstrom Y. *Carbohydr Res*. 2007; 342:1992. [PubMed: 17606255]
14. (a) Yeom SJ, Ji JH, Kim NH, Park CS, Oh DK. *Appl Environ Microbiol*. 2009; 75:4705. [PubMed: 19447949] (b) Patel DH, Wi SG, Lee SG, Lee DS, Song YH, Bae HJ. *Appl Environ Microbiol*. 2011; 77:3343. [PubMed: 21421786] (c) Yoon RY, Yeom SJ, Kim HJ, Oh DK. *J Biotechnol*. 2009; 139:26. [PubMed: 18984017] (d) Menavuvu BT, Poonperm W, Takeda K, Morimoto K, Granstrom

- TB, Takada G, Izumori K. Journal of bioscience and bioengineering. 2006; 102:436. [PubMed: 17189171] (e) Leang K, Takada G, Fukai Y, Morimoto K, Granstrom TB, Izumori K. Biochim Biophys Acta. 2004; 1674:68. [PubMed: 15342115]
15. Mendicino JF. J Am Chem Soc. 1960; 82:4975.
16. Vester JK, Glaring MA, Stougaard P. Microbial cell factories. 2014; 13:72. [PubMed: 24886068]
17. Leemhuis H, Kelly RM, Dijkhuizen L. IUBMB life. 2009; 61:222. [PubMed: 19180668]
18. Itoh H, Okaya H, Khan AR, Tajima S, Hayakawa S, Izumori K. Bioscience Biotechnology and Biochemistry. 1994; 58:2168.
19. Wen L, Huang K, Wei M, Meisner J, Liu Y, Garner K, Zang L, Wang X, Li X, Fang J, Zhang H, Wang PG. Angew Chem Int Ed. 2015; 54:12654.
20. Wen LQ, Huang K, Liu YP, Wang PG. Acs Catalysis. 2016; 6:1649.
21. (a) Wen L, Zang L, Huang K, Li S, Wang R, Wang PG. Bioorg Med Chem Lett. 2016; 26:969. [PubMed: 26778148] (b) Wen L, Huang K, Zheng Y, Liu Y, Zhu H, Wang PG. Bioorg Med Chem Lett. 2016
22. Kim WG, Yoon TM, Kwon HJ, Suh JW. J Antibiot (Tokyo). 2006; 59:640. [PubMed: 17191679]
23. Rodriguez L, Aguirrezabalaga I, Allende N, Brana AF, Mendez C, Salas JA. Chem Biol. 2002; 9:721. [PubMed: 12079784]
24. (a) Teruya T, Konishi T, Uechi S, Tamaki H, Tako M. Int J Biol Macromol. 2007; 41:221. [PubMed: 17433432] (b) Aisa Y, Miyakawa Y, Nakazato T, Shibata H, Saito K, Ikeda Y, Kizaki M. Am J Hematol. 2005; 78:7. [PubMed: 15609279]
25. Shompoosang S, Yoshihara A, Uechi K, Asada Y, Morimoto K. Biosci Biotechnol Biochem. 2014; 78:317. [PubMed: 25036688]
26. (a) Yoshihara A, Haraguchi S, Gullapalli P, Rao D, Morimoto K, Takata G, Jones N, Jenkinson SF, Wormald MR, Dwek RA, Fleet GWJ, Izumori K. Tetrahedron-Asymmetry. 2008; 19:739.(b) Trayner BJ, Grant TN, West FG, Cheeseman CI. Bioorganic & Medicinal Chemistry. 2009; 17:5488. [PubMed: 19586773]

Highlights

- An enzyme that specifically recognizes 6-deoxy-L-psicose was identified.
- One-pot multienzyme system was used to convert L-rhamnose to 6-deoxy-L-psicose.
- Product was obtained with high yield and purity without isomer separation step.

Figure 1.

Analytical scale reaction for the formation of 6-deoxy-L-psicose 1-phosphate from L-rhamnose (TLC: EtOAc/MeOH/H₂O/HOAc=5:2:1.4:0.4). The reactions were performed at 37°C for 1 hour in a 50 μ l reaction mixture containing Tris-HCl buffer (100 mM, pH 7.5), 20 mM of sugar standards, 20 mM of ATP, 5 mM of Mg²⁺, and conversion-related enzymes (RhaI, DTE, and HK; 10 μ g each). **1**, reaction without RhaI (Control 1). **2**, reaction without DTE (control 2). **3**, reaction containing RhaI, DTE, and HK.

Figure 2.

A, HPLC profile of 6-deoxy-L-psicose compared with L-rhamnose (HPX-87H column). B, HPLC profile of 6-deoxy-L-psicose compared with L-rhamnulose (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 using ELSD detector.

A. Previous work: enzymatic synthesis of 6-deoxy-L-psicose from L-rhamnose **B. This work: two-step enzymatic synthesis of 6-deoxy-L-psicose from L-rhamnose** **Scheme 1.**

Enzymatic synthesis of 6-deoxy-L-psicose. RhaI: L-rhamnose isomerase. DTE: D-tagatose 3-epimerase. HK: fructose kinase. AphA: acid phosphatase. OPME: one-pot multienzyme.

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

Substrate	HK activity (%)	C-3 configuration
L-rhamnose	ND	<i>R</i>
L-rhamnulose	<0.1	<i>R</i>
6-deoxy-L-psicose	100	<i>S</i>

^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 enzyme. ND: no detectable activity was observed.

Table 2

Enzymatic synthesis of 6-deoxy-L-psicose from L-rhamnose using the two-step strategy shown in Scheme 1^a

Starting material	Enzymes	Intermediate	Product	Total yield (%)	Scale (mg)	Purity ^a (%)
L-rhamnose	Rhal DTE HK AphA	6-deoxy-L-psicose 1-phosphate	6-deoxy-L-psicose	81	1059	98.5

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