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Facile Enzymatic Synthesis of Phosphorylated Ketopentoses

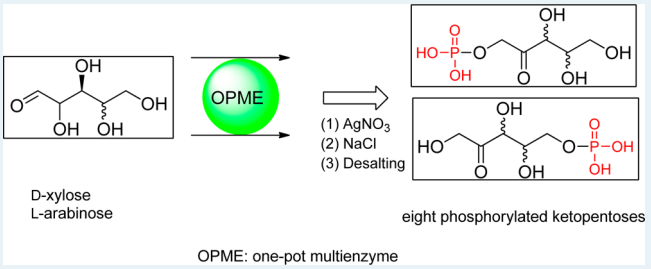
Liuqing Wen,[†] Kenneth Huang,[†] Yunpeng Liu,*[,†,‡] and Peng George Wang*[,†,‡]

[†]Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, United States

[‡]National Glycoengineering Research Center, Shandong University, Jinan 250100, China

Supporting Information

ABSTRACT: An efficient and convenient platform for the facile synthesis of phosphorylated ketoses is described. All eight phosphorylated ketopentoses were produced using this platform starting from two common and inexpensive aldoses (D-xylose and L-arabinose) in more than 84% isolated yield (gram scale). In this method, reversible conversions (isomerization or epimerization) were accurately controlled toward the formation of desired ketose phosphates by targeted phosphorylation reactions catalyzed by substrate-specific kinases. The byproducts were selectively removed by silver nitrate precipitation avoiding the tedious and time-consuming separation of sugar phosphate from adenosine phosphates (ATP and ADP). Moreover, the described strategy can be expanded for the synthesis of other sugar phosphates.



KEYWORDS: *biocatalysis, enzymatic synthesis, phosphorylated ketopentoses, one-pot reactions, silver nitrate precipitation*

Of all possible structures, only 12 pentoses naturally occur, comprising eight aldopentoses (D-xylose, D-lyxose, D-ribose, D-arabinose, L-xylose, L-lyxose, L-ribose, and L-arabinose) and four corresponding ketopentoses (D-xylulose, D-ribulose, L-xylulose, and L-ribulose).[1] The ketopentoses can be phosphorylated at the C-1 or C-5 position, resulting in eight phosphorylated ketopentoses (Figure 1). It is now well-established that abnormal levels of phosphorylated ketopentoses in mammals are directly correlated to a wide range of diseases such as diabetes, cancer, atherosclerosis, and cystic fibrosis.[2−7] Uyeda and co-workers found that xylulose 5-phosphate acts as a glucose signaling compound, recruiting and activating a specific protein serine/threonine phosphatase (PPase), which is responsible for the activation of transcription of the L-type pyruvate kinase gene and lipogenic enzyme genes.[8−10] Enzymes involved in phosphorylated ketopentoses metabolism are exciting potential targets for therapeutic treatment.[11−14] Additionally, phosphorylated ketopentoses are important intermediates in sugar metabolism pathway. All pentoses including aldopentose and ketopentose that could be utilized by bacteria or other organisms must first be converted to their phosphorylated ketopentose forms to enable metabolic function.[15−18] In these metabolism pathways, ketopentose 1-phosphates are split into glycolaldehyde and dihydroxyacetone phosphate, which is an intermediate in the glycolytic pathway, by aldolases.[19−21] Ketose 5-phosphates will be epimerized to D-xylulose 5-phospahte or D-ribulose 5-phosphate, which are key participants in the pentose phosphate pathway (PPP), by epimerases.[22−24] PPP is a universal metabolic process present in bacteria, plants, and animals,[25] and its main function is to produce reducing power and building blocks for cell growth.[26] Because of their critical position in the sugar metabolism pathway, phosphorylated ketopentoses are also the starting materials in synthetic chemistry.[27] For example, D-xylulose 5-phosphate can act as the starting material for the synthesis of heptose in bacteria.[28] Likewise, D-ribulose 5-phosphate can be used for the synthesis of 3-deoxy-D-manno-octulosonic acid (KDO).[29] Therefore, phosphorylated ketopentoses have great potential for applications in investigating the mechanistic and

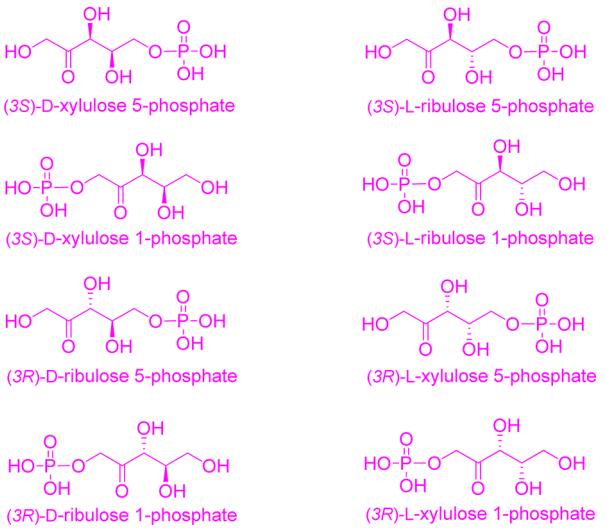


Figure 1. Eight phosphorylated ketopentoses.

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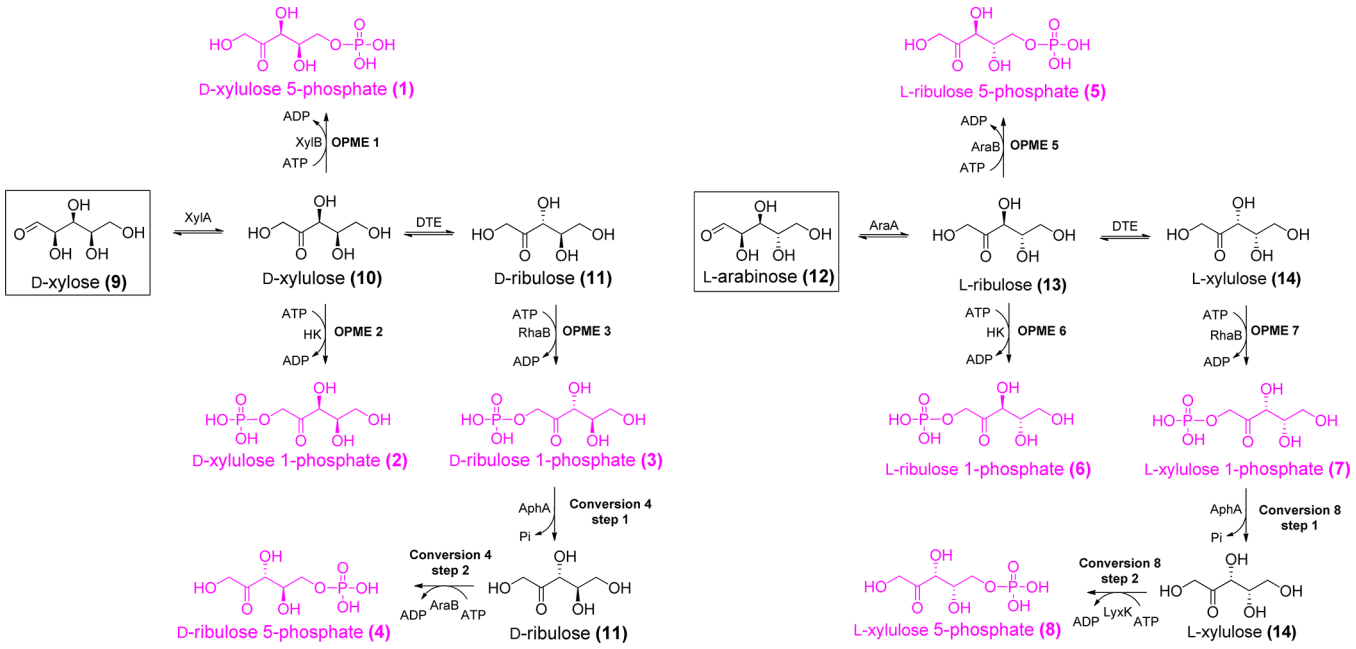
Scheme 1. Total Synthesis of Phosphorylated Ketopentoses from D-Xylose and L-Arabinose



regulatory aspects of sugar metabolism, identification and characterization of new enzymes in nature, and being raw materials in synthetic chemistry.[30−32] A platform for the highly efficient and convenient synthesis of phosphorylated ketopentoses is therefore of considerable interest.

Regarding the biological and synthetic applications of phosphorylated ketopentoses, multiple methods have been developed. The chemical phosphorylation of ketoses involves protection/deprotection steps.[33−35] Alternatively, enzymatic catalysis (kinase) with mild reaction conditions and high reaction efficiency is able to proceed regio- and stereoselectively without requiring protection.[36−41] However, ketopentoses are cost prohibitive for preparative-scale synthesis. The strategy includes enzymatic isomerization in a one-pot fashion to enable the use of cheaper aldose as starting materials to prepare phosphorylated ketoses,[34,42−44] but the availability of substrate-specific kinases is a prerequisite. Moreover, enzymatic phosphorylation requires the separation of sugar phosphate from adenosine phosphates (ATP and ADP) to obtain a sugar phosphate in pure form. The two most common methods used for sugar phosphate purification are ion-exchange chromatography and barium precipitation,[34,45,46] but both methods are labor-intensive and time-consuming. Additionally, enzymatic synthesis of phosphorylated ketopentoses employing transketolase,[47−49] aldolase,[42,45,50] or oxidase[51] has also been suggested. However, these methods suffer from both expensive starting materials and extensive purification steps. Therefore, while some phosphorylated ketopentoses are commercially available, they are extremely expensive (D-ribulose 5-phosphate, $1245/25 mg, Sigma-Aldrich; D-xylulose 5-phosphate, $1150/10 mg, Carbosynth). Studies of this fundamental class of carbohydrates have been hindered by a lack of efficient preparation methods to readily provide substantial amounts of the desired products.

In this work, an efficient and convenient platform for phosphorylated ketose syntheses is described. All eight phosphorylated ketopentoses were produced from D-xylose and L-arabinose utilizing this platform (Scheme 1). Our strategies are mainly based on "isomerization → phosphorylation" and "isomerization → epimerization → phosphorylation" cascade reactions. We combined the thermodynamically unfavorable bioconversions of D-xylose to D-series of ketopentose (D-xylulose and D-ribulose), or L-arabinose to L-series of ketopentose (L-ribulose and L-xylulose) with phosphorylation reactions by substrate-specific kinases to prepare the desired phosphorylated ketopentoses (**1** to **3**, and **5** to **7**). D-ribulose 5-phosphate (**4**) and L-xylulose 5-phosphate (**8**) were synthesized by a two-step strategy.

D-Xylose and L-arabinose are the two common pentoses found in substantial quantities in nature.[52] Although the reactions are reversible, aldose-ketose isomerization and ketose−ketose epimerization has been the main method for ketose preparation.[52] Nevertheless, separation of the desired ketose from its isomeric mixture is difficult. To avoid such separation, we combined these reversible reactions with targeted phosphorylation reactions catalyzed by kinases in a one-pot fashion to prepare phosphorylated ketopentoses instead of directly phosphorylating the ketoses. These reactions are known as one-pot multienzyme reaction (OPME).[53] The phosphorylation reaction coupled with the reversible conversions (isomerization or epimerization) drives the reactions (isomerization or epimerization) toward the formation of ketoses in their phosphorylated forms until a high conversion ratio was reached.

In order to establish the basis of OPME reactions for the preparation of D-xylulose 5-phosphate from D-xylose, and L-ribulose 5-phosphate from L-arabinose, the substrate specificity of D-xylulose kinase (XylB) and L-ribulose kinase (AraB) from *E. coli*,[39,41] were tested (see Supporting Information). No detectable activity of XylB toward D-xylose, or AraB toward L-arabinose was found (Table 1), indicating their potential for OPME reactions. To test the practicability of the designed OPME reactions, analytical reactions were first carried in 50 ul system containing conversion-related enzymes (Table 2), starting materials and ATP. Reactions without isomerase were performed as negative controls. The reactions were tested by

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Table 1. Substrate Specificity of Kinases[a]

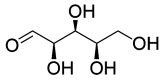
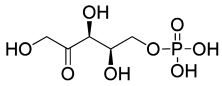
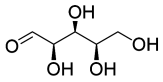
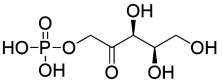
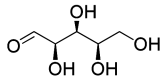
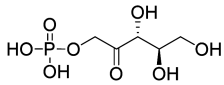
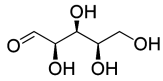
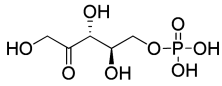
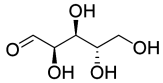
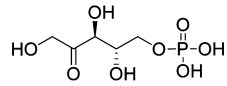
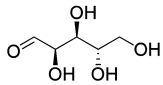
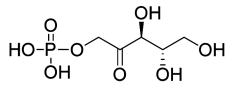
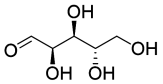
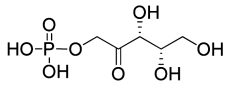
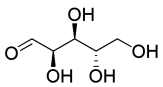
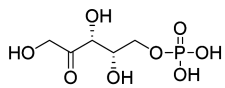
substrate	XylB activity (%)	AraB activity (%)	LyxK activity (%)	RhaB[b] activity (%)	HK[b] activity (%)
L-arabinose	ND	ND	ND	ND	ND
D-xylose	ND	ND	ND	0.1	ND
L-xylulose	7.3	1.3	100	100	NA
D-ribulose	4.9	139	2.5	111	NA
L-ribulose	16.9	100	2.5	ND	100
D-xylulose	100	3.4	1.6	0.3	349

[a]Substrate specificity was studied by the reactions that were performed at 37 °C for 10 min in 50 ul reaction mixture containing a Tris-HCl buffer (100 mM, pH 7.5), 20 mM of sugar standards, 20 mM of ATP, 5 mM of Mg^{2+}, and 10 μg of enzymes. [b]Data from ref 56. NA: not assayed. ND: no detectable activity was observed.

TLC (EtOAc/MeOH/H2O/HOAc = 5:2:1.4:0.4). After observing sugar phosphates formation by TLC and no reactions in control samples, preparative scale syntheses were performed.

Preparative-scale syntheses were routinely performed in gram scale (Table 2). D-Xylose was incubated with D-xylose isomerase (XylA) from *E. coli*[54] and XylB in the presence of ATP (1.25 molar equiv) as phosphate donor to prepare D-xylulose 5-phosphate (OPME 1, Scheme 1). L-Arabinose was incubated with ATP (1.25 molar equiv), L-arabinose isomerase (AraA) from *Bacillus subtilis*,[55] and AraB to prepare L-ribulose 5-phosphate (OPME 5, Scheme 1). No buffer was used in consideration to purification. The reaction pH was held near 7.5, where all enzymes are quite active, using sodium hydroxide as the reaction occurred. Both reactions were allowed to proceed until a conversion ratio exceeding 99% was reached (as confirmed by HPLC). Afterward, the silver nitrate precipitation method[56] was used to purify D-xylulose 5-phosphate and L-ribulose 5-phosphate. In detail, silver nitrate was added to the solution to precipitate ATP and ADP selectively until no new precipitation was observed. Precipitates were removed by centrifugation. Sodium chloride was added to precipitate residual silver ions, and silver chloride precipitate was removed by centrifugation. The entire separation process can be finished in less than 15 min. After desalting by a P-2 column, D-xylulose 5-phosphate and L-ribulose 5-phosphate were isolated in more

Table 2. Total Synthesis of Phosphorylated Ketopentoses from D-Xylose and L-Arabinose Using the Strategy Shown in Scheme 1

Entry	Starting material	Enzymes	Scheme	Product	Isolated yield (%)	Scale (mg)	Purity[a] (%)
1	 D-xylose	XylA XylB	OPME 1	 D-xylulose 5-phosphate **(1)**	91	1143	98.4
2	 D-xylose	XylA HK	OPME 2	 D-xylulose 1-phosphate **(2)**	92	1159	>99
3	 D-xylose	XylA DTE RhaB	OPME 3	 D-ribulose 1-phosphate **(3)**	94	1183	98.2
4	 D-xylose	XylA DTE RhaB AphA AraB	Conversion 4	 D-ribulose 5-phosphate **(4)**	85	1291	99
5	 L-arabinose	AraA AraB	OPME 5	 L-ribulose 5-phosphate **(5)**	92	1164	99.5
6	 L-arabinose	AraA HK	OPME 6	 L-ribulose 1-phosphate **(6)**	93	1170	>99
7	 L-arabinose	AraA DTE RhaB	OPME 7	 L-xylulose 1-phosphate **(7)**	96	1204	>99
8	 L-arabinose	AraA DTE RhaB AphA LyxK	Conversion 8	 L-xylulose 5-phosphate **(8)**	84	1265	99

[a]Defined as the percentage of desired phosphorylated ketose out of the sum of all possible isomers (as confirmed by HPLC).

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than 90% yield (Table 2) comparable to the yield for ion-exchange purification methods. Compared to the barium precipitation method, by which sugar phosphate was isolated as a barium salt in the presence of barium and ethanol,[34,46] no additional steps to remove toxic ions and no accurate pH control are necessary. Moreover, silver is safer than barium. The isolated products were confirmed by NMR and MS analysis (see Supporting Information). Compared to the previous synthesis,[34,44,49] we not only obtained a higher isolated yield but also avoided the tedious purification step.

To assess product purity, the phosphate groups on both phosphorylated sugars were hydrolyzed by acid phosphatase. The resultant monosaccharides were analyzed by HPLC (see Supporting Information). A product distribution of D-xylulose to D-xylose (98.4:1.6) was observed indicating the isolated product of D-xylulose 5-phosphate contains 1.6% of D-xylose 5-phosphate (as also observed by NMR). Similarly, a product distribution of L-ribulose to L-arabinose (99.5:0.5) was observed indicating the isolated product of L-ribulose 5-phosphate contains 0.5% of L-arabinose 5-phosphate (as also observed by NMR). The existence of aldoses may because XylA and AraA can incorrectly isomerize D-xylulose 5-phosphate and L-ribulose 5-phosphate to a certain extent.

To prepare D-xylulose 1-phosphate and L-ribulose 1-phosphate from D-xylose and L-arabinose using OPME reactions, an enzyme that could phosphorylate D-xylulose and L-ribulose at the C-1 position but not D-xylose and L-arabinose is required. Human fructosekinase (HK) catalyzes the phosphorylation of ketoses to ketose 1-phosphates.[57] Recently, we found HK could specifically recognize D-xylulose and L-ribulose but not D-xylose and L-arabinose.[56] Analytical reactions containing conversion-related enzymes (Table 2) were performed and tested as described above. Once the formation of sugar phosphates were observed by TLC and no further reactions in control samples seen, preparative scale syntheses were performed. D-Xylose was incubated with ATP (1.25 molar equiv), XylA, and HK to prepare D-xylulose 1-phosphate (OPME 2, Scheme 1). L-Arabinose was incubated with ATP (1.25 molar equiv), AraA, and HK to prepare L-ribulose 1-phosphate (OPME 6, Scheme 1). Both reactions were monitored by TLC and HPLC. Once no detectable amounts of the starting aldoses were observed (conversion ratio exceeding 99%), silver nitrate precipitation was used to purify the sugar phosphates. After desalting by a P-2 column, D-xylulose 1-phosphate and L-ribulose 1-phosphate were isolated in more than 90% yield (Table 2). The products were confirmed by NMR and MS analysis (see Supporting Information). The purity was analyzed in the same manner mentioned above (see Supporting Information). Because the C-1 position was blocked by the phosphate group, the side reactions were avoided to result in a product purity exceeding 99% (Table 2).

D-Ribulose 1-phosphate and L-xylulose 1-phosphate were prepared from D-xylose and L-arabinose using OPME 3 and OPME 7 (Scheme 1). Although D-ribulose 1-phosphate and L-xylulose 1-phosphate have great potential applications, they have been difficult to prepare in substantial quantities. For example, it has been reported that D-ribulose 1-phosphate can be isomerized from D-ribose 1-phopshate, which results from the phosphorylation of D-ribose, by MTR 1-P isomerase.[58] However, this process is impractical, requiring not only a sugar phosphate purification step but also a protracted isomer separation. D-ribulose and L-xylulose have a (3*R*)-configuration which is different from D-xylose and L-arabinose. To prepare D-ribulose 1-phosphate from D-xylose, and L-xylulose 1-phosphate from L-arabinose, isomerization catalyzed by isomerase (XylA or AraA) and a C-3 epimerization catalyzed by D-tagatose 3-epimerase (DTE) from *Pseudomonas* Sp, ST-24[59] were combined in one-pot. These two reversible conversions were coupled with L-rhamnulose kinase (RhaB) from *Thermotoga maritima* MSB8, a novel enzyme that requires ketoses with (3*R*)-configuration,[56] resulting in the formation of (3*R*)-ketose 1-phosphates. Once the starting aldoses were no longer detected, D-ribulose 1-phosphate and L-xylulose 1-phosphate were purified by silver nitrate precipitation in more than 90% yield (Table 2) and confirmed by NMR and MS analysis (see Supporting Information). A product distribution of D-ribulose, D-xylulose and D-xylose (98.2:1.2:0.6) was observed indicating the purity of D-ribulose 1-phosphate is 98.2%. For L-xylulose 1-phosphate analysis, no detectable L-arabinose or L-ribulose was found indicating L-xylulose 1-phosphate has a purity exceeding 99%. Such conversions are important because most of sugars that naturally occur in large amounts are (3*S*)-aldoses.[52] Therefore, the reactions described herein (OPME 3 and OPME 7) represent a novel strategy for the preparation of (3*R*)-ketoses from (3*S*)-aldoses directly.

D-Ribulose 5-phosphate and L-xylulose 5-phosphate also have been difficult to prepare in quantity. The methods known in the literature for the synthesis of D-ribulose 5-phosphate are the phosphorylation of D-ribulose,[34] the isomerization of D-ribose 5-phosphate, and the oxidization of D-gluconate 6-phosphate.[51] L-Xylulose 5-phosphate could be prepared by phosphorylating L-xylulose.[38] Nevertheless, these methods also suffer from expensive starting materials or a tedious purification step. In this work, D-ribulose 5-phosphate and L-xylulose 5-phosphate were prepared from D-xylose and L-arabinose by a two-step strategy (conversion 4 and conversion 8, Scheme 1) due to an inability to identify kinases that could specifically phosphorylate D-ribulose and L-xylulose at the C-5 position. In the first conversion step, D-ribulose and L-xylulose were obtained by hydrolyzing the phosphate groups of D-ribulose 1-phosphate and L-xylulose 1-phosphate, both of which were prepared from D-xylose and L-arabinose using OPME 3 and OPME 7, by acid phosphatase (AphA) from *E. coli*,.[60] AraB is welL-known as L-ribulose kinase,[41] but it also displays high activity toward D-ribulose (Table 1). Thus, in the second conversion step, D-ribulose was incubated with AraB to prepare D-ribulose 5-phosphate. L-xylulose was incubated with L-xylulose kinase (LyxK) from *E. coli*[38] to prepare L-xylulose 5-phosphate. The products were purified by using silver nitrate precipitation. After desalting by using a P-2 column, the products were isolated in more than 84% yield with regard to D-xylose and L-arabinose (Table 2), respectively. Purity analysis indicated both D-ribulose 5-phosphate and L-xylulose 5-phosphate have a purity of 99%.

In summary, a novel method for the efficient and convenient synthesis of phosphorylated ketoses was established. This method relies on substrate-specific kinases and a convenient sugar phosphates purification method (silver nitrate precipitation). Starting from two common and inexpensive aldoses (D-xylose and L-arabinose), all phosphorylated ketopentoses were produced utilizing this strategy with high yield and purity without a tedious sugar phosphate separation step. ATP is also commercially affordable due to the increased industrial production over the past decades,[61,62] making the transformation reaction described herein of particular interest for

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large-scale preparation. The precipitate (silver−adenosine phosphates complex) produced during purification process can be redissolved with ammonium hydroxide, and the adenosine phosphates (ATP and ADP) or silver ions can be then recycled, reducing the preparation cost when this method was applied on large-scale purification.

Moreover, on the basis of the availability of D-xylulose 5-phosphate and D-ribulose 5-phosphate in considerable quantities, we have further established a biosynthetic strategy for the efficient synthesis of KDO and ADP-heptose, which are the building block of the lipopolysaccharide and have been difficult to obtain (results will be reported in due course). We anticipate that this strategy will accelerate an understanding of both biological roles and synthetic applications of phosphorylated ketopentoses. Future studies will enable the identification of new substrate-specific kinases to be used in more phosphorylated sugar syntheses, providing a powerful set of tools for carbohydrate research.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b02234.

Experimental procedure and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: yliu65@gsu.edu.

*E-mail: pwang11@gsu.edu.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Granstrom, T. B.; Takata, G.; Tokuda, M.; Izumori, K. *J. Biosci. Bioeng.* **2004**, *97*, 89−94.

(2) Ciou, S. C.; Chou, Y. T.; Liu, Y. L.; Nieh, Y. C.; Lu, J. W.; Huang, S. F.; Chou, Y. T.; Cheng, L. H.; Lo, J. F.; Chen, M. J.; Yang, M. C.; Yuh, C. H.; Wang, H. D. *Int. J. Cancer* **2015**, *137*, 104−115.

(3) Gillies, R. J.; Robey, I.; Gatenby, R. A. *J. Nucl. Med.* **2008**, *49* (Suppl 2), 24S−42S.

(4) Young, M. E.; McNulty, P.; Taegtmeyer, H. *Circulation* **2002**, *105*, 1861−1870.

(5) Huck, J. H.; Verhoeven, N. M.; Struys, E. A.; Salomons, G. S.; Jakobs, C.; van der Knaap, M. S. *Am. J. Hum. Genet.* **2004**, *74*, 745−751.

(6) Solinas, G.; Naugler, W.; Galimi, F.; Lee, M. S.; Karin, M. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 16454−16459.

(7) Wetmore, D. R.; Joseloff, E.; Pilewski, J.; Lee, D. P.; Lawton, K. A.; Mitchell, M. W.; Milburn, M. V.; Ryals, J. A.; Guo, L. *J. Biol. Chem.* **2010**, *285*, 30516−30522.

(8) Kabashima, T.; Kawaguchi, T.; Wadzinski, B. E.; Uyeda, K. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 5107−5112.

(9) Nishimura, M.; Fedorov, S.; Uyeda, K. *J. Biol. Chem.* **1994**, *269*, 26100−26106.

(10) Kawaguchi, T.; Takenoshita, M.; Kabashima, T.; Uyeda, K. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 13710−13715.

(11) Caruthers, J.; Bosch, J.; Buckner, F.; Van Voorhis, W.; Myler, P.; Worthey, E.; Mehlin, C.; Boni, E.; DeTitta, G.; Luft, J.; Lauricella, A.; Kalyuzhniy, O.; Anderson, L.; Zucker, F.; Soltis, M.; Hol, W. G. *Proteins: Struct., Funct., Genet.* **2006**, *62*, 338−342.

(12) Stern, A. L.; Naworyta, A.; Cazzulo, J. J.; Mowbray, S. L. *FEBS J.* **2011**, *278*, 793−808.

(13) Alves-Ferreira, M.; Guimaraes, A. C. R.; Capriles, P. V. D. Z.; Dardenne, L. E.; Degrave, W. M. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 1100−1110.

(14) Fullam, E.; Pojer, F.; Bergfors, T.; Jones, T. A.; Cole, S. T. *Open Biol.* **2012**, *2*, 110026.

(15) Shin, I.; Kim, K. S. *Chem. Soc. Rev.* **2013**, *42*, 4267−4269.

(16) Fraser-Reid, B. *Top. Curr. Chem.* **2011**, *301*, xi−xiii.

(17) Beerens, K.; Desmet, T.; Soetaert, W. *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 823−834.

(18) Ahmed, Z. *Electron. J. Biotechnol.* **2001**, *4*, 13−14.

(19) Badia, J.; Gimenez, R.; Baldoma, L.; Barnes, E.; Fessner, W. D.; Aguilar, J. *J. Bacteriol.* **1991**, *173*, 5144−5150.

(20) Nam Shin, J. E.; Kim, M. J.; Choi, J. A.; Chun, K. H. *J. Biochem. Mol. Biol.* **2007**, *40*, 801−804.

(21) Bais, R.; James, H. M.; Rofe, A. M.; Conyers, R. A. *Biochem. J.* **1985**, *230*, 53−60.

(22) Shi, R.; Pineda, M.; Ajamian, E.; Cui, Q. Z.; Matte, A.; Cygler, M. *J. Bacteriol.* **2008**, *190*, 8137−8144.

(23) Samuel, J.; Luo, Y.; Morgan, P. M.; Strynadka, N. C. J.; Tanner, M. E. *Biochemistry* **2001**, *40*, 14772−14780.

(24) Akana, J.; Fedorov, A. A.; Fedorov, E.; Novak, W. R. P.; Babbitt, P. C.; Almo, S. C.; Gerlt, J. A. *Biochemistry* **2006**, *45*, 2493−2503.

(25) Kruger, N. J.; von Schaewen, A. *Curr. Opin. Plant Biol.* **2003**, *6*, 236−246.

(26) Shibuya, N.; Inoue, K.; Tanaka, G.; Akimoto, K.; Kubota, K. *Oncology* **2015**, *88*, 309−319.

(27) Stincone, A.; Prigione, A.; Cramer, T.; Wamelink, M. M.; Campbell, K.; Cheung, E.; Olin-Sandoval, V.; Gruning, N.; Kruger, A.; Tauqeer Alam, M.; Keller, M. A.; Breitenbach, M.; Brindle, K. M.; Rabinowitz, J. D.; Ralser, M. *Biol. Rev.* **2015**, *90*, 927−963.

(28) Li, T.; Wen, L.; Williams, A.; Wu, B.; Li, L.; Qu, J.; Meisner, J.; Xiao, Z.; Fang, J.; Wang, P. G. *Bioorg. Med. Chem.* **2014**, *22*, 1139−1147.

(29) Camci-Unal, G.; Mizanur, R. M.; Chai, Y. H.; Pohl, N. L. B. *Org. Biomol. Chem.* **2012**, *10*, 5856−5860.

(30) Patra, K. C.; Hay, N. *Trends Biochem. Sci.* **2014**, *39*, 347−354.

(31) Tsouko, E.; Khan, A. S.; White, M. A.; Han, J. J.; Shi, Y.; Merchant, F. A.; Sharpe, M. A.; Xin, L.; Frigo, D. E. *Oncogenesis* **2014**, *3*, e103.

(32) Horecker, B. L. *J. Biol. Chem.* **2002**, *277*, 47965−47971.

(33) Forget, S. M.; Bhattasali, D.; Hart, V. C.; Cameron, T. S.; Syvitski, R. T.; Jakeman, D. L. *Chem. Sci.* **2012**, *3*, 1866−1878.

(34) Huang, H.; Pandya, C.; Liu, C.; Al-Obaidi, N. F.; Wang, M.; Zheng, L.; Keating, S. T.; Aono, M.; Love, J. D.; Evans, B.; Seidel, R. D.; Hillerich, B. S.; Garforth, S. J.; Almo, S. C.; Mariano, P. S.; Dunaway-Mariano, D.; Allen, K. N.; Farelli, J. D. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, E1974−E1983.

(35) Breuer, M.; Hauer, B. *Curr. Opin. Biotechnol.* **2003**, *14*, 570−576.

(36) Simpson, F.; Wood, W. *J. Am. Chem. Soc.* **1956**, *78*, 5452−5453.

(37) Neuberger, M. S.; Hartley, B. S.; Walker, J. E. *Biochem. J.* **1981**, *193*, 513−524.

(38) Sanchez, J. C.; Gimenez, R.; Schneider, A.; Fessner, W. D.; Baldoma, L.; Aguilar, J.; Badia, J. *J. Biol. Chem.* **1994**, *269*, 29665−29669.

(39) Wungsintaweekul, J.; Herz, S.; Hecht, S.; Eisenreich, W.; Feicht, R.; Rohdich, F.; Bacher, A.; Zenk, M. H. *Eur. J. Biochem.* **2001**, *268*, 310−316.

(40) LeBlanc, D. J.; Mortlock, R. P. *J. Bacteriol.* **1971**, *106*, 82−89.

(41) Lee, L. V.; Gerratana, B.; Cleland, W. W. *Arch. Biochem. Biophys.* **2001**, *396*, 219−224.

(42) Fessner, W.-D.; Badia, J.; Eyrisch, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, *33*, 5231−5234.

(43) Fessner, W. D.; Schneider, A.; Eyrisch, O.; Sinerius, G.; Badia, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1183−1192.

(44) Kim, J. E.; Zhang, Y. H. *Biotechnol. Bioeng.* **2016**, *113*, 275−282.

DOI: 10.1021/acscatal.5b02234
ACS Catal. 2016, 6, 1649−1654

(45) Fessner, W. D.; Badia, J.; Eyrisch, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, *33*, 5231−5234.
(46) Wong, C.-H.; McCurry, S. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7938−7939.
(47) Zimmermann, F. T.; Schneider, A.; Schorken, U.; Sprenger, G. A.; Fessner, W. D. *Tetrahedron: Asymmetry* **1999**, *10*, 1643−1646.
(48) Solovjeva, O. N.; Kochetov, G. A. *J. Mol. Catal. B: Enzym.* **2008**, *54*, 90−92.
(49) Shaeri, J.; Wright, I.; Rathbone, E. B.; Wohlgemuth, R.; Woodley, J. M. *Biotechnol. Bioeng.* **2008**, *101*, 761−767.
(50) Guerard-Helaine, C.; Debacker, M.; Clapes, P.; Szekrenyi, A.; Helaine, V.; Lemaire, M. *Green Chem.* **2014**, *16*, 1109−1113.
(51) Pontremoli, S.; Mangiarotti, G. *J. Biol. Chem.* **1962**, *237*, 643−645.
(52) Izumori, K. *J. Biotechnol.* **2005**, *118*, S89−S90.
(53) Yu, H.; Lau, K.; Thon, V.; Autran, C. A.; Jantscher-Krenn, E.; Xue, M.; Li, Y.; Sugiarto, G.; Qu, J.; Mu, S.; Ding, L.; Bode, L.; Chen, X. *Angew. Chem., Int. Ed.* **2014**, *53*, 6687−6691.
(54) Rozanov, A. S.; Zagrebelny, S. N.; Beklemishev, A. B. *Appl. Biochem. Microbiol.* **2009**, *45*, 31−37.
(55) Kim, J. H.; Prabhu, P.; Jeya, M.; Tiwari, M. K.; Moon, H. J.; Singh, R. K.; Lee, J. K. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1839−1847.
(56) Wen, L.; Huang, K.; Wei, M.; Meisner, J.; Liu, Y.; Garner, K.; Zang, L.; Wang, X.; Li, X.; Fang, J.; Zhang, H.; Wang, P. G. *Angew. Chem., Int. Ed.* **2015**, *54*, 12654−12658.
(57) Asipu, A.; Hayward, B. E.; O'Reilly, J.; Bonthron, D. T. *Diabetes* **2003**, *52*, 2426−2432.
(58) Imker, H. J.; Fedorov, A. A.; Fedorov, E. V.; Almo, S. C.; Gerlt, J. A. *Biochemistry* **2007**, *46*, 4077−4089.
(59) Itoh, H.; Okaya, H.; Khan, A. R.; Tajima, S.; Hayakawa, S.; Izumori, K. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 2168−2171.
(60) Rossolini, G. M.; Thaller, M. C.; Pezzi, R.; Satta, G. *FEMS Microbiol. Lett.* **1994**, *118*, 167−173.
(61) Liu, Z.; Zhang, J.; Chen, X.; Wang, P. G. *ChemBioChem* **2002**, *3*, 348−355.
(62) Zhao, H.; van der Donk, W. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 583−589.

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ACS Catal. 2016, 6, 1649−1654