Note

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PII: S0008-6215(14)00376-0
DOI: http://dx.doi.org/10.1016/j.carres.2014.10.014
Reference: CAR 6862

To appear in: *Carbohydrate Research*

Received Date: 28 July 2014
Revised Date: 16 October 2014
Accepted Date: 17 October 2014

Please cite this article as: Liu, Y., Nishimoto, M., Kitaoka, M., Facile enzymatic synthesis of sugar 1-phosphates as substrates for phosphorylases using anomeric kinases, *Carbohydrate Research* (2014), doi: http://dx.doi.org/10.1016/j.carres.2014.10.014

Facile enzymatic synthesis of sugar 1-phosphates as substrates for phosphorylases using anomeric kinases

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Highlights

- We describe one-pot enzymatic synthesis of three sugar 1-phosphates.
- Anomeric kinases were used in the presence of an ATP-regeneration system.
- These sugar 1-phosphates were available as crystals of particular salts.
- These sugar 1-phosphates are donor substrates for phosphorylases.

Abbreviations: αGal1*P*, α-D-galactose 1-phosphate; αGlc1*P*, α-D-glucose 1-phosphate, αGlcNAc1*P*, 2-acetamido-2-deoxy-α-D-glucose 1-phosphate; αMan1*P*, α-D-mannose 1-phosphate; βGlc1*P*, β-D-glucose 1-phosphate; GH, glycoside hydrolase; GlcNAc, 2-acetamido-2-deoxy-α-D-glucose; GT, glycosyltransferase; PEP, phosphoenolpyruvic acid; TLC, thin layer chromatography

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Abstract

Three sugar 1-phosphates that are donor substrates for phosphorylases were produced at the gram scale from phosphoenol pyruvic acid and the corresponding sugars by the combined action of pyruvate kinase and the corresponding anomeric kinases in good yields. These sugar 1-phosphates were purified through two electrodialysis steps. α-D-Galactose 1-phosphate was finally isolated as crystals of dipotassium salts. α-D-Mannose 1-phosphate and 2-acetamido-2-deoxy-α-D-glucose 1-phosphate were isolated as crystals of bis(cyclohexylammonium) salts.

Key words

sugar 1-phosphate, anomeric kinase, pyruvate kinase, electrodialysis, substrate for phosphorylase

Phosphorylases are a class of enzymes that phosphorolyze glycosyl linkages to generate sugar 1-phosphates[1-4]. Because their reactions are reversible and highly regiospecific, they are expected to be practical catalysts for producing particular oligosaccharides. Because sugar 1-phosphates are donor substrates for reverse phosphorolysis, their availability is important for the utilization of phosphorylases.

According to the CAZy classification based on their amino acid sequences, phosphorylases belong to one of the following families: glycosyltransferase (GT) 4 and 35 and glycoside hydrolase (GH) 13, 65, 94, 112, and 130[5]. The majority of phosphorylases phosphorolyze α- or β-D-glucosides to generate α- or β-D-glucose 1-phosphate (αGlc1*P* or βGlc1*P*) with anomeric retention or inversion[1-4]. Preparative methods of αGlc1*P* or βGlc1*P* as pure crystalline forms such as those in salts have been well documented. These compounds have been generated by phosphorylases from commercially produced sugar resources. αGlc1*P* has been reported to be produced from starch by glycogen phosphorylase as a dipotassium salt[6]. It has also been produced from sucrose by sucrose phosphorylase[7]. βGlc1*P* has been produced as a bis(cyclohexylammonium) salt from trehalose by inverting trehalose phosphorylase[8].

Several phosphorylases act on glycosides other than glucosides. The GH112 family comprises phosphorlyases that act on β-D-galactosides to generate α-D-galactose 1-phosphate (αGal1*P*) (**1**), including 1,3-β-galactosyl-*N*-acetylhexosamine phosphorylase (EC 2.4.1.211)[9, 10] and 1,4-β-D-galactosyl-L-rhamnose phosphorylase (EC 2.4.1.247)[11]. The GH130 family comprises phosphorylases that act on β-D-mannosides to generate α-D-mannose 1-phosphate (αMan1*P*) (**2**), including 1,4-β-mannosyl-glucose phosphorylase (EC 2.4.1.281)[12], β-1,4-mannooligosaccharide phosphorylase (EC 2.4.1.319)[13], and 1,4-β-mannosyl-*N*-acetylglucosamine phosphorylase (EC 2.4.1.320)[14, 15]. The GH94 family mainly comprises phosphorylases that act on β-D-glucosides, although it also includes *N*,*N*′-diacetylchitobiose phosphorylase (EC 2.4.1.280)[16] to generate 2-acetamido-2-deoxy-α-D-glucose 1-phosphate (αGlcNAc1*P*) (**3**).

Although sugar 1-phosphates can be chemically synthesized[17, 18], enzymatic methods are preferred for their practical synthesis on a large scale. However, it is difficult to produce sugar 1-phosphates using phosphorylases except for αGlc1*P* and βGlc1*P* because there are no abundantly available resources to be phosphorolyzed by known enzymes. Although an attempt to change cellobiose phosphorylase into lactose phosphorylase to obtain αGal1*P* from lactose has been reported[19], the catalytic efficiency of the mutant enzyme was too low to be used as a practical catalyst.

Anomeric kinases are enzymes that phosphorylate the anomeric position of sugars with the consumption of ATP. The enzyme galactokinase (EC 2.7.1.6) produces αGal1*P* from D-galactose (**4**)[20]. αMan1*P* and αGlcNAc*P* can be produced by *N*-acetylhexosamine 1-kinase (EC 2.7.1.162) from D-mannose (**5**) and 2-acetamido-2-deoxy-D-glucose (GlcNAc) (**6**), respectively[21]. However, it is often noted that kinases are inhibited by high concentrations of ATP, their phosphate donor substrate[22, 23]. The substrate inhibition effect restricts the concentration of the substrate to low values, thus resulting in low production efficiency. To avoid this problem, we propose to perform the kinase reaction in the presence of an ATP-regenerating system[24]. Pyruvate kinase (EC 2.7.1.40) with phosphoenolpyruvic acid (PEP) (**7**) is one of the most commonly used ATP-regenerating systems[24]. This enzyme transfers the phosphate group of PEP to ADP to generate pyruvic acid (**8**) and ATP. We confirmed that the reactions of both galactokinase and *N*-acetylhexosamine 1-kinase with 1 mM ATP in the presence of the ATP-regeneration system were much faster than those with 50 mM ATP (Fig. 1). Here we document the facile synthesis of αGal1*P*, αGlcNAc1*P*, and αMan1*P* using kinases and the ATP-regenerating system, followed by the isolation of these compounds by electrodialysis[25] and crystallization.

The enzymatic reactions are described in Scheme 1. Each sugar 1-phosphate was generated with good yields (>90%) from PEP by the combination of pyruvate kinase and each corresponding anomeric kinase. The resultant reaction mixtures contained sugar 1-phosphates, pyruvic acid, ATP/ADP, and unreacted monosaccharide. The first electrodialysis using a 100 Da molecular mass cutoff membrane effectively removed pyruvic acid from the mixture. After the second electrodialysis with a 300 Da molecular mass cutoff membrane, sugar 1-phosphates were recovered in the dialysate solution. The electrodialysis is scale-up ready by increasing the surface area of the membranes and replaces the chromatographic step often reported for the purification of sugar 1-phosphates. After the purification, the countercations were replaced using a cation exchange resin, and the sugar 1-phosphates were crystallized as dipotassium salts or bis(cyclohexylammonium) salts in water/ethanol or water/acetone as pure sugar 1-phosphates.

With the protocol mentioned above, αGal1*P*, αGlcNAc1*P*, and αMan1*P*, which are donor substrates for phosphorylases, are easily prepared at the gram scale. The facile production of these compounds will advance the research on phosphorylases by supplying their substrates.

1. Experimental procedure

1.1. Enzymes and chemicals

Pyruvate kinase from rabbit muscle was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Galactokinase[26] and *N*-acetylhexosamine 1-kinase[21] from *Bifidobacterium longum* subsp. *longum* JCM1217 were prepared as described previously. PEP monosodium salt and ATP disodium salt were purchased from Wako Pure Chemicals Industry (Osaka, Japan). Other chemicals used were of reagent grade.

1.2. The reactions of anomeric kinases with and without the ATP-regeneration system

The enzymatic reactions were carried out in reaction mixtures (100 μL) at 30 °C. The composition of each reaction mixture without the ATP-regeneration system was 50 mM ATP (pH adjusted to 7.0 with NaOH), 5 mM $MgCl_2$, 50 mM monosaccharide (D-galactose and GlcNAc), and anomeric kinase (9.4 μg/mL galactokinase and 100 μg/mL *N*-acetylhexosamine 1-kinase, respectively). That with the ATP-regeneration system was 1 mM ATP, 50 mM PEP (pH adjusted to 7.0 with NaOH), 5 mM $MgCl_2$, 50 mM monosaccharide (D-galactose and GlcNAc), anomeric kinases (9.4 μg/mL galactokinase and 100 μg/mL *N*-acetylhexosamine 1-kinase, respectively), and 0.42 U/mL pyruvate kinase. After the reactions for 20 h, aliquots (1 μL) of the reaction mixtures were spotted on a thin layer chromatography (TLC) plate (Kieselgel 60 F254; Merck, Darmstadt, Germany). The plate was developed with acetonitrile–water (3:1 v/v), soaked in 5% sulfuric acid–methanol solution, and heated in an oven until the spots of the carbohydrates became sufficiently visible.

1.3. Synthesis of sugar 1-phosphate

Each reaction mixture (100 mL) contained 50 mM PEP, each sugar (51 mM D-galactose, 51 mM GlcNAc, 60 mM D-mannose), 1.0 mM ATP, 5.0 mM $MgCl_2$, 0.45 U/mL pyruvate kinase, and each anomeric kinase (15 μg/mL galactokinase for D-galactose, 210 and 105 μg/mL *N*-acetylhexosamine 1-kinase for D-mannose and GlcNAc), with the pH adjusted to 7.0 with 1 M NaOH. The reaction mixtures were incubated at 30 °C for 24 h (D-galactose and GlcNAc) or 72 h (D-mannose). The reaction was monitored by TLC. After the reaction completed, the reaction mixtures were heated until boiling in a microwave oven to inactivate the enzymes. After cooling, the reaction mixtures were centrifuged to remove the precipitated proteins.

1.4. Electrodialysis

Electrodialysis was performed with Microacylizer S1 (Astom Co., Tokyo, Japan).

First, the reaction mixture was electrodialyzed through the electrodialysis cartridge AC-110 (molecular mass cutoff 100 Da, Sunactis, Osaka, Japan) against 100 mL of 1 % $NaNO_3$ solution until the current was lower than 0.01 A at 3.9 V. At this stage, pyruvic acid was removed but sugar 1-phosphate remained in the mixture. Then, the mixture was electrodialyzed through the electrodialysis cartridge AC-220 (molecular mass cutoff 300 Da, Sunactis) against 30 mL of distilled water until the electrical conductivity was 0.25 mS/cm. Sugar 1-phophate was recovered in the dialysate.

1.5. αGal1*P* (**1**) dipotassium salt

The phosphorylation of D-galactose with galactokinase resulted in the formation of 48.4 mM αGal1*P* in 100 mL (total: 4.84 mmol, 97% yield from PEP). After electrodialysis, 4.37 mmol of αGal1*P* was recovered. Then, cations were removed from the dialysate using 10 g of Dowex 50 (strongly acidic cation exchange resin, H^+ type, Wako Pure Chemicals Industry), and the pH was adjusted to 8.5 using 2 M KOH. The solution was concentrated to 5 mL using a rotary evaporator, followed by dropwise addition of 35 mL of ethanol to crystalize αGal1*P* as a dipotassium salt. Finally, 1.25 g of αGal1*P* 2K (3.72 mmol) was obtained. $[\alpha]_D^{25}$ = +89° (c = 0.50, H_2O). ^{1}H NMR (D_2O): δ 5.47 (dd, 1H, $J_{1,2}$ 3.7, $J_{1,P}$ 7.4 Hz, H-1), 4.17 (dddd, 1H, $J_{5,P}$ 0.5, $J_{4,5}$ 1.2, $J_{5,6b}$ 4.6, $J_{5,6a}$ 7.7 Hz, H-5), 3.97 (dd, 1H, $J_{4,5}$ 1.2, $J_{3,4}$ 3.4 Hz, H-4), 3.89 (dd, 1H, $J_{3,4}$ 1.1, $J_{2,3}$ 10.4 Hz, H-3), 3.76–3.71 (m, 2H, H-2, H-6a), 3.69 (dd, 1H $J_{5,6b}$ 4.6, $J_{6a,6b}$ 11.7 Hz, H-6b). ^{13}C NMR (D_2O): δ 95.5 (d, $J_{1,P}$ 5.3 Hz, C-1), 72.3 (s, C-5), 71.4 (s, C-3), 71.2 (s, C-4), 70.8 (d, $J_{2,P}$ 6.5 Hz, C-2), 63.1 (s, C-6).

1.6. αMan1*P* (**2**) bis(cyclohexylammonium) salt

The phosphorylation of D-mannose with *N*-acetylhexosamine 1-kinase resulted in the formation of 46.7 mM αMan1*P* in 100 mL (total 4.67 mmol, 93% yield from PEP). After electrodialysis, 4.07 mmol of αMan1*P* was recovered. Then, cations were removed from the dialysate using 10 g of Dowex 50 (H^+ type), and the pH was adjusted to 8.5 using cyclohexylamine. The solution was concentrated to 5 mL using a rotary evaporator, followed by dropwise addition of 40 mL of acetone to crystalize αMan1*P* as a bis(cyclohexylammonium) salt. Attempts to crystalize αMan1*P* as a disodium salt and dipotassium salt were not successful. Finally, 1.13 g of αMan1*P* bis(cyclohexylammonium) salt (2.46 mmol) was obtained. $[\alpha]_D^{25}$ = +31° (c = 0.50, H_2O). ^{1}H NMR (D_2O): δ 5.31 (dd, 1H, $J_{1,2}$ 1.8, $J_{1,P}$ 8.8 Hz, H-1), 3.95–3.92 (m, 2H, H-2, H-3), 3.90–3.85 (m, 2H, H-5, H-6a), 3.73–3.69 (m, 1H, H-6b), 3.61–3.56 (m, 1H, H-4), 3.17–3.10 (m, 2H, cyclohexylamine H-1), 2.01–1.93 (m, 4H, cyclohexylamine H-2a/6a),

1.82–1.75 (m, 4H, cyclohexylamine H-3a/5a), 1.67–1.61 (m, 2H, cyclohexylamine H-4a), 1.38–1.26 (m, 8H, cyclohexylamine, H-2b/6b, H-3b/5b), 1.20–1.11 (m, 2H, cyclohexylamine H-4b). ^{13}C NMR (D_2O): δ 96.6 (d, $J_{1,P}$ 4.4 Hz, C-1), 74.5 (s, C-5), 72.8 (d, $J_{2,P}$ 6.9 Hz, C-2), 71.8 (s, C-3), 68.8 (s, C-4), 62.9 (s, C-6), 52.1 (s, cyclohexylamine C-1), 32.0 (s, cyclohexylamine C-2/6), 26.0 (s, cyclohexylamine C-3/5), 25.5 (s, cyclohexylamine C-4).

1.7. αGlcNAc1*P* (**3**) bis(cyclohexylammonium) salt

The phosphorylation of GlcNAc with *N*-acetylhexosamine 1-kinase resulted in the formation of 47.3 mM αGlcNAc1*P* in 100 mL (total: 4.73 mmol, 95% yield from PEP). After electrodialysis, 4.35 mmol of αGlcNAc1*P* was recovered. Then, cations were removed from the dialysate using 10 g of Dowex 50 (H^+ type), and the pH was adjusted to 8.5 using cyclohexylamine. The solution was concentrated to 5 mL using a rotary evaporator, followed by dropwise addition of 35 mL of acetone to crystalize αGlcNAc1*P* as a bis(cyclohexylammonium) salt. Attempts to crystalize αGlcNAc1*P* as a disodium salt and dipotassium salt were not successful. Finally, 1.48 g of αGlcNAc1*P* bis(cyclohexylammonium) salt (2.96 mmol) was obtained. $[\alpha]_D^{25}$ = +59° (c = 0.53, H_2O). ^{1}H NMR (D_2O): δ 5.33 (dd, 1H, $J_{1,2}$ 3.2, $J_{1,P}$ 7.6 Hz, H-1), 3.94 (ddd, 1H, $J_{5,6a}$ 2.2, $J_{5,6b}$ 5.1, $J_{4,5}$, 10.1 Hz, H-5), 3.90 (ddd, 1H, $J_{2,P}$ 1.8, $J_{1,2}$ 3.2, $J_{2,3}$ 10.5 Hz, H-2), 3.87 (dd, 1H, $J_{5,6a}$ 2.3, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.78 (dd, 1H, $J_{3,4}$ 9.1, $J_{2,3}$ 10.4 Hz, H-3), 3.76 (dd, 1H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.46 (dd, 1H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.1 Hz, H-4), 3.17–3.10 (m, 2H, cyclohexylamine H-1), 2.04 (s, 3H, Ac), 2.01–1.93 (m, 4H, cyclohexylamine H-2a/6a), 1.82–1.75 (m, 4H, cyclohexylamine H-3a/5a), 1.67–1.61 (m, 2H, cyclohexylamine H-4a), 1.38–1.26 (m, 8H, cyclohexylamine, H-2b/6b, H-3b/5b), 1.20–1.11 (m, 2H, cyclohexylamine H-4b). ^{13}C NMR (D_2O): δ 176.4 (s, $\underline{C}OCH_3$), 94.4 (d, $J_{1,P}$ 5.1 Hz, C-1), 73.8 (s, C-5), 73.2 (s, C-3), 71.8 (s, C-4), 62.5 (s, C-6), 55.9 (d, $J_{2,P}$ 7.4 Hz, C-2), 52.1 (s, cyclohexylamine C-1), 32.0 (s, cyclohexylamine C-2/6), 26.0 (s, cyclohexylamine C-3/5), 25.5 (s, cyclohexylamine C-4), 23.8 (s, $CO\underline{C}H_3$).

1.8. Quantification of anions

Sugar 1-phosphates, phosphate, and pyruvic acid were quantified using an ion chromatography system (ICA-2000, DKK-Toa Corporation, Tokyo, Japan) equipped with a conductivity detector with a chemical suppressor (ICA-200C, DKK-Toa Corporation). Anions were separated through a PCI-205 column (DKK-Toa Corporation) using an aqueous solution containing 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ as the mobile phase at 37 °C with a flow rate of 1.0 mL/min. Sodium cations

were removed by the suppressor using 10 mM H_2SO_4 at a flow rate of 0.65 mL/min.

1.9. Structural analyses

NMR spectra were recorded with Bruker Avance 500 spectrometer (Bruker Biospin, Rheinstetten, Germany) at 298 K in 99.9 % D_2O. t-BuOH (1.23 and 31.3 ppm) was added to each sample as the internal reference standard for the ^{1}H and ^{13}C NMR chemical shifts, respectively. Each signal was assigned by analyzing two-dimensional NMR spectra [double-quantum-filtered correlation spectroscopy (DQF-COSY) and heteronuclear single-quantum-coherence (HSQC)].

Acknowledgements

This work was supported by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry. The authors thank the staff of the Instrumental Analysis Center for Food Chemistry of the National Food Research Institute for recording the NMR spectra. The authors would like to thank Enago (www.enago.jp) for the English language review.

References

1. Nakai, H.; Kitaoka, M.; Svensson, B.; Ohtsubo, K. *Curr. Opin. Chem. Biol.*, **2013**, *17*, 301-309.
2. Kitaoka, M.; Hayashi, K. *Trends Glycosci. Glycotechnol.*, **2002**, *14*, 35-50.
3. Luley-Goedl, C.; Nidetzky, B. *Biotechnol. J.*, **2010**, *5*, 1324-38.
4. O'Neill, E. C.; Field, R. A. *Carbohydr. Res.*, **2014**, *in press*.
5. Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henrissat, B. *Nucleic Acids Res.*, **2014**, *42*, D490-D495.
6. McCready, R. M.; Hassid, W. Z. *J. Am. Chem. Soc.*, **1944**, *66*, 560-563.
7. Goedl, C.; Schwarz, A.; Minani, A.; Nidetzky, B. *J. Biotechnol.*, **2007**, *129*, 77-86.
8. Van der Borght, J.; Desmet, T.; Soetaert, W. *Biotechnol. J.*, **2010**, *5*, 986-993.
9. Derensy-Dron, D.; Krzewinski, F.; Brassart, C.; Bouquelet, S. *Biotechnol. Appl. Biochem.*, **1999**, *29*, 3-10.
10. Kitaoka, M.; Tian, J.; Nishimoto, M. *Appl. Environ. Microbiol.*, **2005**, *71*, 3158-3162.
11. Nakajima, M.; Nishimoto, M.; Kitaoka, M. *J. Biol. Chem.*, **2009**, *284*, 19220-19227.
12. Senoura, T.; Ito, S.; Taguchi, H.; Higa, M.; Hamada, S.; Matsui, H.; Ozawa, T.;

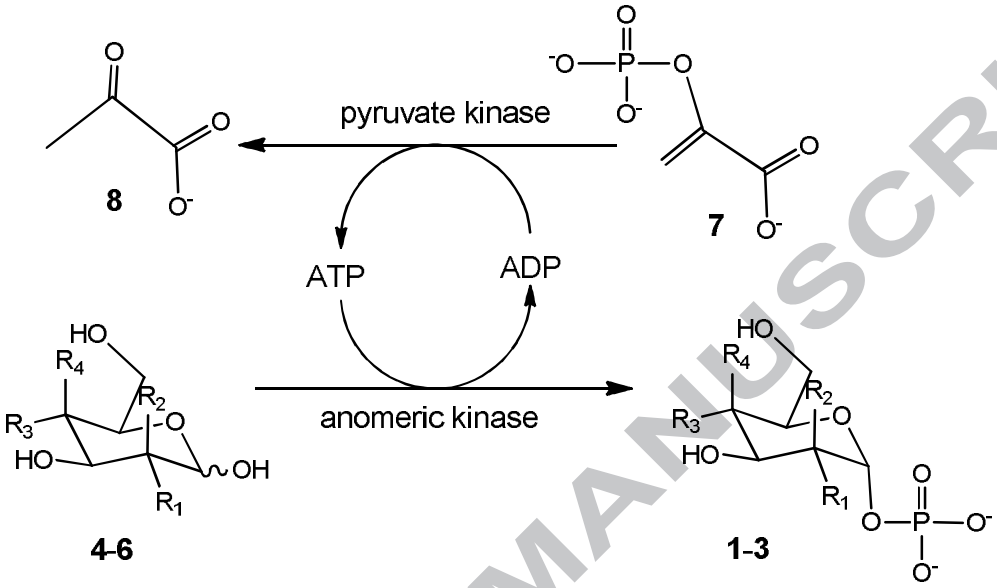
Jin, S.; Watanabe, J.; Wasaki, J. *Biochem. Biophys. Res. Commun.*, **2011**, *408*, 701-706.

13. Kawahara, R.; Saburi, W.; Odaka, R.; Taguchi, H.; Ito, S.; Mori, H.; Matsui, H. *J. Biol. Chem.*, **2012**, *287*, 42389-42399.
14. Nihira, T.; Suzuki, E.; Kitaoka, M.; Nishimoto, M.; Ohtsubo, K.; Nakai, H. *J. Biol. Chem.*, **2013**, *288*, 27366-27374.
15. Ladeveze, S.; Tarquis, L.; Cecchini, D. A.; Bercovici, J.; Andre, I.; Topham, C. M.; Morel, S.; Laville, E.; Monsan, P.; Lombard, V.; Henrissat, B.; Potocki-Veronese, G. *J. Biol. Chem.*, **2013**, *288*, 32370-32383.
16. Park, J. K.; Keyhani, N. O.; Roseman, S. *J. Biol. Chem.*, **2000**, *275*, 33077-33083.
17. Posternak, T. *J. Am. Chem. Soc.*, **1950**, *72*, 4824-4825.
18. Timmons, S. C.; Jakeman, D. L. *Carbohydr. Res.*, **2008**, *343*, 865-874.
19. De Groeve, M. R.; De Baere, M.; Hoflack, L.; Desmet, T.; Vandamme, E. J.; Soetaert, W. *Protein Eng. Des. Sel.*, **2009**, *22*, 393-399.
20. Holden, H. M.; Rayment, I.; Thoden, J. B. *J. Biol. Chem.*, **2003**, *278*, 43885-43888.
21. Nishimoto, M.; Kitaoka, M. *Appl. Environ. Microbiol.*, **2007**, *73*, 6444-6449.
22. Cabrera, R.; Baez, M.; Pereira, H. M.; Caniuguir, A.; Garratt, R. C.; Babul, J. *J. Biol. Chem.*, **2011**, *286*, 5774-5783.
23. Giles, I. G.; Poat, P. C.; Munday, K. A. *Biochem. J.*, **1976**, *157*, 577-589.
24. Zhao, H.; van der Donk, W. A. *Curr. Opin. Biotechnol.*, **2003**, *14*, 583-589.
25. van de Merbel, N. C. *J. Chromatogr. A*, **1999**, *856*, 55-82.
26. Nihira, T.; Nakajima, M.; Inoue, K.; Nishimoto, M.; Kitaoka, M. *Anal. Biochem.*, **2007**, *371*, 259-261.

Figure legend

Fig. 1. TLC analysis of the reactions of anomeric kinases with and without the ATP-regeneration system.

A, the reaction of galactokinase with D-galactose; B, the reaction of *N*-acetylhexosamine 1-kinase with GlcNAc. Lane 1, standard ATP; lanes 2 and 4, without the ATP-regenerating system (50 mM ATP), t = 0 and 20 h, respectively; lanes 3 and 5, with ATP-regeneration system (1 mM ATP), t = 0 and 20 h, respectively.



	R_1	R_2	R_3	R_4
1,4	OH	H	H	OH
2,5	H	OH	OH	H
3,6	NHAc	H	OH	H

Scheme 1

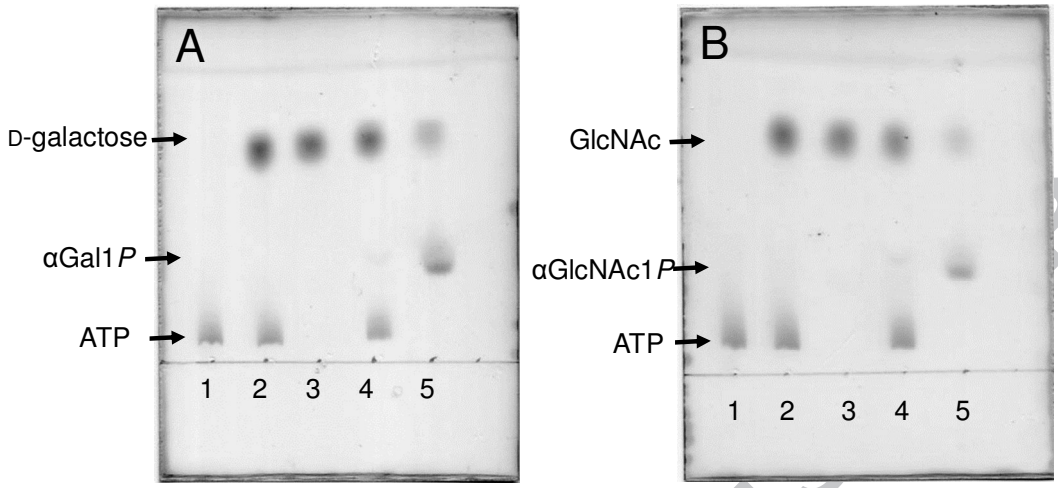
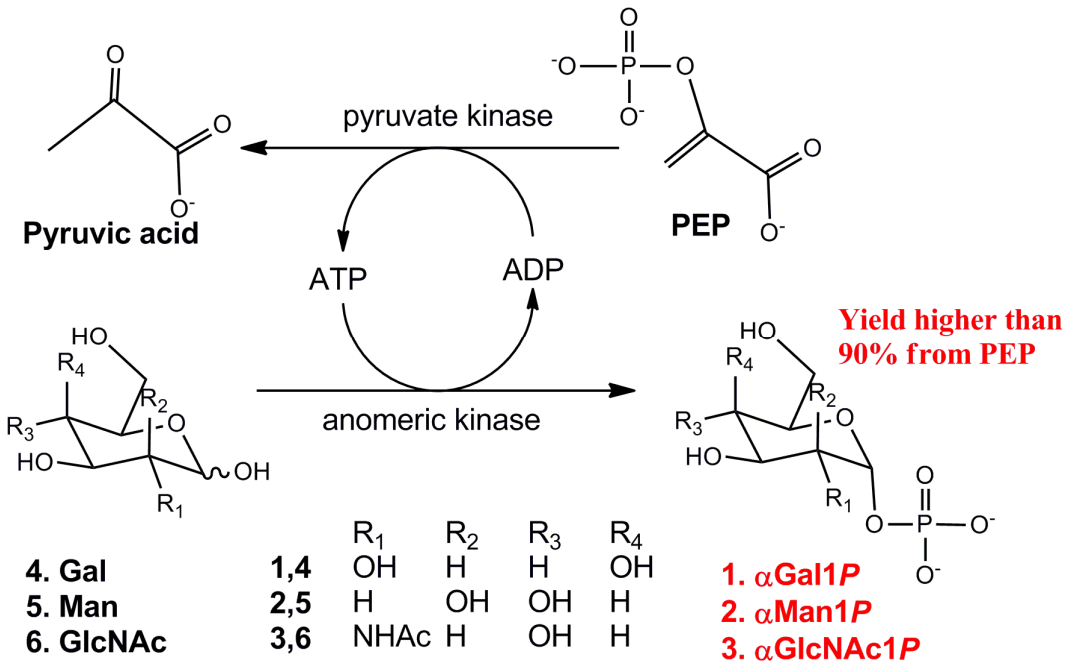


Fig. 1

Graphical abstract



Highlights

- We describe one-pot enzymatic synthesis of three sugar 1-phosphates.
- Anomeric kinases were used in the presence of an ATP-regeneration system.
- These sugar 1-phosphates were available as crystals of particular salts.
- These sugar 1-phosphates are donor substrates for phosphorylases.