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ENZYMATIC SYNTHESES OF RARE KETOSE 1-PHOSPHATES [1]

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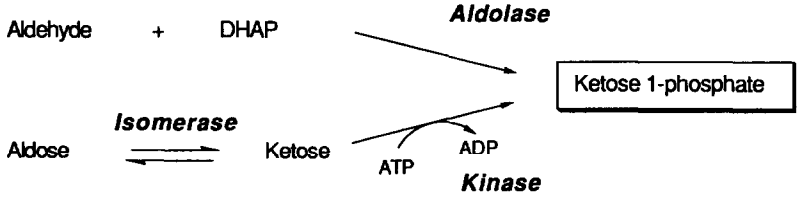
Key Words: L-rhamnulose 1-phosphate aldolase; L-fuculose 1-phosphate aldolase; L-rhamnose isomerase; L-fucose isomerase; L-rhamnulose kinase

Abstract: Ketose 1-phosphates are obtained from aldehydes and dihydroxyacetone phosphate by enzymatic aldol addition, and from aldoses (or ketoses) by isomerization/phosphorylation. The microbial enzymes employed are readily available by overexpression.

Microbial metabolisms comprise a remarkable repertoire for the catabolic utilization of a large variety of carbohydrates as the sole source of carbon and energy.[3] A common feature of most of these degradative sequences is the conversion of sugars and polyols into ketose 1-phosphates to facilitate the breakdown of the carbon backbone by aldol cleavage into smaller units that can enter the central metabolism. Since the phosphorylated ketoses frequently function as the inducers of respective metabolic enzymes, they are thus primary targets for the investigation of mechanistic and regulatory aspects of sugar metabolism.[4] Here we report on two enzymic routes (Scheme 1) that make a number of rare ketose 1-phosphates readily available. While the first route — a *de novo*, asymmetric synthesis — employs a suitable aldolase for the highly diastereoselective addition of dihydroxyacetone phosphate (DHAP) to certain aldehydes, the second route — an ex-chiral-pool synthesis — starts from commercially available ketoses or aldoses and makes use of a ketose kinase in combination with an appropriate ketol isomerase. All enzymes are derived from L-rhamnose/L-fucose metabolism and are accessible in quantity by overexpression in recombinant *E. coli* strains.

Scheme 1. General synthesis of ketose 1-phosphates by aldol addition or by isomerization/phosphorylation



Thus, for any desired product the complementarity of both routes allows a choice for the most suitable from different possible starting materials as determined by its costs or respective reaction kinetics. While stereoisomers related to D-fructose or L-sorbose configuration are easily obtainable by using D-fructose 1,6-bisphosphate aldolase or hexokinase,[5] protocols for the preparation of other stereoisomers related to rare sugars were scarce so far. This restriction is now largely removed with the availability of enzymes capable of generating or transforming a range of diastereomers. In contrast to typically multi-step chemical synthesis, the enzymic approach presented avoids the need for protecting group introduction and manipulation and provides high overall product yields by convenient one-pot procedures.

Route 1: Aldol addition. We have recently accomplished the cloning for overexpression and characterization of the L-rhamnulose 1-phosphate aldolase (RhuA; EC 4.1.2.19) and L-fuculose 1-phosphate aldolase (FucA; EC 4.1.2.17) from *E. coli* and have demonstrated their value for asymmetric synthesis in a building block fashion.[6] Both enzymes, which belong to aldolases of class II (Zn^{2+} dependent), seem to be specific for DHAP as one substrate but tolerate a significant variety of aldehydes as substrate analogues. RhuA generates vicinal diol units having the (3*R*,4*S*)-*trans* configuration (L-*threo*) while FucA forms products with (3*R*,4*R*)-*cis* configuration (D-*erythro*). Besides a mechanism-based stereospecificity for the absolute (3*R*)-configuration,[7] the enzymes have a virtually complete diastereoselectivity for hydroxylated aldehydes. Crucial for practical large-scale preparations and supplementary to the current aims is the discovery that both microbial aldolases display an overwhelming kinetic preference (>95:5) for L-enantiomeric 2-hydroxyaldehydes. Thus, when starting from racemic substrates supplied in excess this allows the single-step preparation of diastereomerically pure L-ketose 1-phosphates **2** / **3** with definition of three contiguous chiral centers (Scheme 2). Alternatively, individual diastereomers belonging to the D- or L-series may be obtained in a pure state from enantiomerically pure aldehydes or as a 1:1 mixture from a stoichiometrically employed racemate. The results for additions of DHAP to a set of hydroxyaldehydes and derivatives are compiled in Table 1.[8] We point out that this technique should prove particularly useful in the preparation of specifically labeled compounds for more detailed studies of carbohydrate structure and metabolism.

Scheme 2. Highly diastereoselective enzymic aldol additions with kinetic resolution of racemic aldehydes

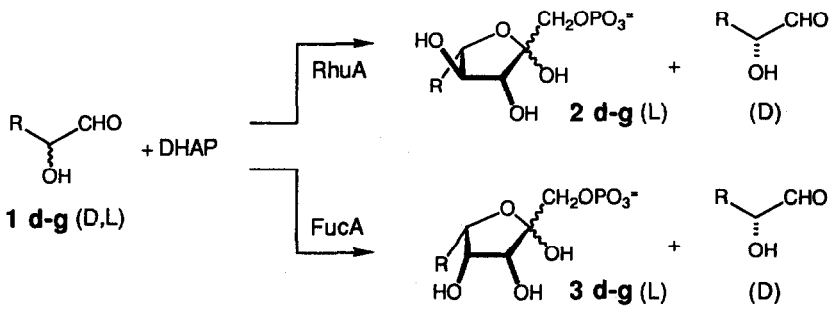


Table 1. Synthesis of ketose 1-phosphates by aldolase-catalyzed addition of DHAP to selected aldehydes[8]

Config.	Aldehyde R=	Enzyme	Aldol adduct	Yield (isol.) [%]
-	H (**1a**)	RhuA	L-xylulose 1-phosphate (**2a**)	82 [6]
-	"	FucA	D-ribulose 1-phosphate (**3a**)	85 [6]
L	CH_2OH (**1b**)	RhuA	L-fructose 1-phosphate (**2b**)	91 [6]
L	"	FucA	L-tagatose 1-phosphate (**3b**)	86 [6]
D	CH_2OH (**1c**)	RhuA	D-sorbose 1-phosphate (**2c**)	84 [6]
D	"	FucA	D-psicose 1-phosphate (**3c**)	82 [6]
D,L	CH_3 (**1d**)	RhuA	L-rhamnulose 1-phosphate (**2d**)	91
D,L	"	FucA	L-fuculose 1-phosphate (**3d**)	85
D,L	CH_2OCH_3 (**1e**)	RhuA	6-*O*-methyl-L-fructose 1-phosphate (**2e**)	77
D,L	"	FucA	6-*O*-methyl-L-tagatose 1-phosphate (**3e**)	83
D,L	CH_2N_3 (**1f**)	RhuA	6-deoxy-6-azido-L-fructose 1-phosphate (**2f**)	97
D,L	"	FucA	6-deoxy-6-azido-L-tagatose 1-phosphate (**3f**)	80
D,L	CH_2F (**1g**)	RhuA	6-deoxy-6-fluoro-L-fructose 1-phosphate (**2g**)	95
D,L	"	FucA	6-deoxy-6-fluoro-L-tagatose 1-phosphate (**3g**)	86

Route 2: Isomerization/Phosphorylation. For this alternative technique we have overproduced and purified the L-rhamnulose kinase (RhuK; EC 2.7.1.5) from *E. coli*,[9] an ATP-dependent enzyme involved in L-rhamnose degradation which has recently been cloned,[10] and have investigated its substrate acceptance. Unlike the functionally related hexokinases the enzyme was found to provide a relatively broad tolerance for structural modifications of its natural substrate L-rhamnulose, requiring only absolute (3*R*)-stereochemistry for activity but allowing for inversion, (de)oxygenation or substitution at the remaining positions (Table 2).

The scheme is completed by two ketol isomerases, the L-rhamnose isomerase (RhaI; EC 5.3.1.14) and the L-fucose isomerase (FucI; EC 5.3.1.3) from *E. coli*.[9] The enzymes which are easily isolated from appropriately constructed overexpression clones[4,11] catalyze *in vivo* the isomerization of L-rhamnose and L-fucose, respectively, but were found to accept in addition a variety of stereochemically related aldoses for conversion into the corresponding ketoses (Scheme 3, Table 2).[8] In common with the RhuK, both isomerases require a fixed stereochemistry only up to position C-3, *i.e.* (2*R*,3*R*)-configuration for RhaI and (2*S*,3*R*)-configuration for FucI, but are flexible towards configurational inversion or derivatization at further stereocenters.

Scheme 3. Enzymic synthesis of ketose 1-phosphates from aldoses by isomerization/phosphorylation

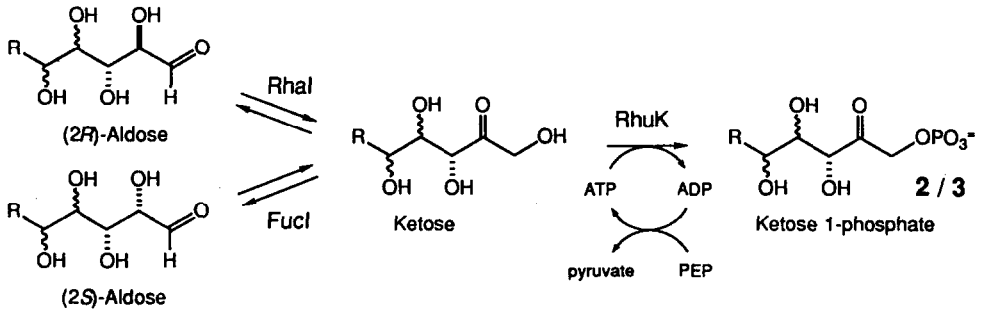


Table 2. Synthesis of ketose 1-phosphates by enzymic isomerization/phosphorylation of aldoses[8]

Aldose	Isomerase			Ketose	RhuK		Ketose 1-phosphate	
	Type	K_M [a] [mM]	V_{max} [%]		K_M [b] [mM]	V_{max} [%]	Compd	Total Yield [%]
L-lyxose	RhaI	16	87	L-xylulose	1	87	**2a**	78
D-ribose	RhaI	8	5	D-ribulose	11	24	**3a**	82
L-mannose	RhaI	55	46	L-fructose	3	77	**2b**	76
L-talose	RhaI	– [d]	<1	L-tagatose	21	19	**3b**	75
D-gulose	RhaI	– [d]	>3	D-sorbose	26	16	**2c**	72
D-allose	RhaI	26	4	D-psicose	20	23	**3c**	90
L-rhamnose	RhaI	1.5	100 [c]	L-rhamnulose	0.2	100 [c]	**2d**	90
L-fucose	FucI	130	100 [c]	L-fuculose	3	56	**3d**	78

a) for aldose b) for ketose c) set to 100% for reference d) not determined

Although multiple variations may considerably decrease substrate affinity (Table 2; high K_M) and conversion rate (low V_{max}), these adverse effects can easily be compensated by higher dosage of substrates and the abundant enzymic catalysts. Accordingly, this method for synthesis of rare ketose 1-phosphates is applicable to a range of diastereomeric hexoses or pentoses that, depending on individual availability, may serve as an alternative to the corresponding ketoses as starting materials. While the isomerization equilibria may be unfa-

vorable for specific cases, the combination with the thermodynamically favorable phosphoryl transfer from ATP drives the reactions to completion. Particularly for large-scale synthesis, an efficient *in situ* cofactor regeneration is required, for which we have preferably used the system based on PEP/pyruvate kinase.

In conclusion, we have demonstrated that enzymic aldol addition or combined isomerization and phosphorylation are both practical routes for the straightforward synthesis of sensitive, rare intermediates of carbohydrate metabolism or, as with azides **2f** and **3f**, that of precursors for potent glycosidase inhibitors.[13] It is appropriate to note that with the ready availability of additional aldolases[6] and ketol isomerases also the scope is further broadened for an enzymic aldolization/isomerization[12] strategy as a general tool for the *de novo* synthesis of aldoses from DHAP and simple aldehydic precursors. Further work along this line is in progress.

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