Tetrahedron: Asymmetry Vol. 4, No. 6, pp. 1183-1192, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

6-Deoxy-L-*lyxo*- and 6-Deoxy-L-*arabino*-hexulose# 1-phosphates. Enzymic Syntheses by Antagonistic Metabolic Pathways[1]

Wolf-Dieter Fessner,* Achim Schneider, Oliver Eyrisch, Gudrun Sinerius, and Josefa Badía[2]

Department of Organic Chemistry and Biochemistry, University of Freiburg,
Albertstr. 21, 7800 Freiburg, Germany

(*Received* 10 *February* 1993; *accepted* 16 *March* 1993)

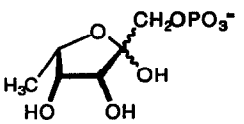
Abstract: Efficient large-scale syntheses of the title compounds (up to 0.1 mole) from readily available precursors have been accomplished along destructive as well as constructive biosynthetic routes by employing overexpressed enzymes from *E. coli*. The first route, which consists of an aldose isomerization–ketose phosphorylation sequence, utilizes natural L-fucose and L-rhamnose as the respective sources of chirality. The key reaction of the second route, which starts from racemic 2-hydroxypropanal and achiral dihydroxyacetone phosphate, is a diastereospecific aldol addition with complete kinetic resolution.

INTRODUCTION

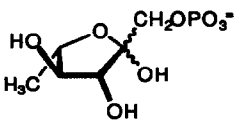
Enzymic techniques in some areas supplement, in others quite successfully compete with, existing methodologies of asymmetric synthesis. The use of aldolases for the concise, highly enantio- and diastereocontrolled *de novo*-synthesis of carbohydrates and related multifunctionalized compounds[3] is a prominent example of a field where enzymes prove to be particularly suitable and valuable biocatalysts for organic synthesis.[4] The biogenetic evolution of a complete stereochemical set of four aldolases that utilize dihydroxyacetone phosphate (DHAP aldolases) allowed us to establish a flexible building block scheme according to which the choice of an aldehyde precursor and the correct enzymic catalyst provides full control over constitution and absolute configuration of the products.[5] Thus, a D-tagatose 1,6-bisphosphate aldolase (TagA; EC 4.1.3.-) has been obtained from certain wild-type *E.-coli* strains,[6] and the three remaining types of DHAP aldolases — D-fructose 1,6-bisphosphate (FruA; EC 4.1.3.13),[7] L-fuculose 1-phosphate (FucA; EC 4.1.3.17)[8,9] and L-rhamnulose 1-phosphate aldolase (RhuA; EC 4.1.3.19)[8] — have recently been made readily available by recombinant DNA methodology. The facile access to abundant catalyst quantities of high purity[10] has removed restrictions for synthetic applications of DHAP aldolases on larger scale. As a result, this development has created a need for the corresponding natural substrates, *i.e.* the 6-deoxy-L-*lyxo*- (**1**) and 6-deoxy-L-*arabino*-hexulose# 1-phosphates (**2**) for the FucA and

Trivial names "fuculose" (6-deoxy-L-tagatose) and "rhamnulose" (6-deoxy-L-fructose) are used throughout the text to maintain consistence with enzyme nomenclature, although their usage is normally discouraged.

RhuA enzymes, as assay standards for determination of aldolase activity and as reference samples for the evaluation of substrate analogs or potential enzyme inhibitors. Although several literature reports on the preparation of compounds **1** and **2** are available based on biocatalytic (mutant strains of bacteria[11] and crude enzyme preparations[12,13,14]) or chemical methods,[15] none satisfies all criteria desirable for the synthesis of a reference material, that is, an indisputable quality of the product, convenience and overall efficiency of the protocol, and a supply of sufficient quantities for multiple tests (»100 mg). Also, since ketose 1-phosphates such as **1** and **2** frequently serve a regulatory function in carbohydrate metabolism as the inducers of appropriate enzymes[16] but no simple procedure of a broader scope exists for their synthesis, we were led to explore in more detail the conceivable options for enzymic routes based on recently cloned[17] and overexpressed microbial enzymes from fucose and rhamnose metabolism.[18] Here we present the outcome of our studies, exemplified in the practical enzymic syntheses of sugar phosphates **1** and **2**.



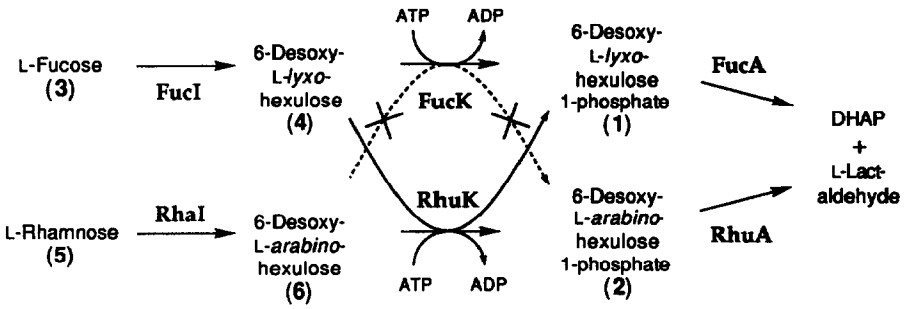
1



2

RESULTS AND DISCUSSION

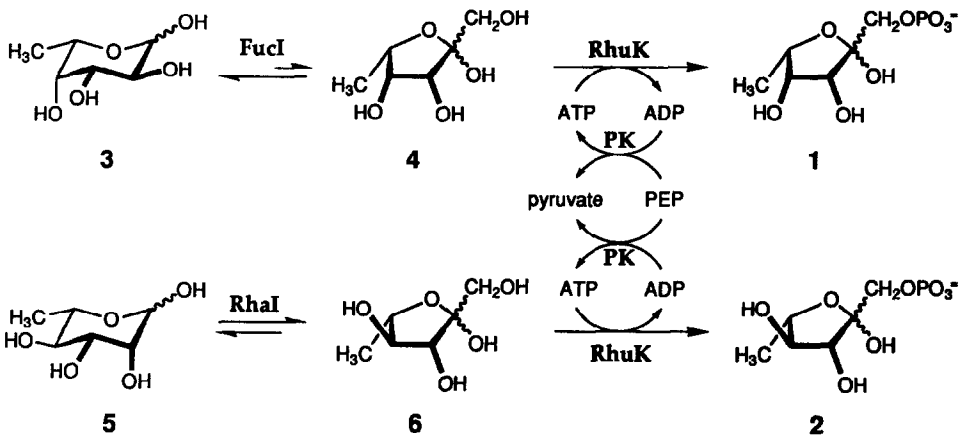
The deoxysugars L-fucose (**3**) and L-rhamnose (**5**) can be used as a source of carbon and energy by a wide range of microorganisms. Catabolism of both aldoses proceeds along parallel pathways that comprise an isomerization to the corresponding ketoses fuculose (**4**) and rhamnulose (**6**), phosphorylation at the expense of ATP with formation of the ketose 1-phosphates **1** and **2**, and their (reversible) cleavage by an aldolase into DHAP and L-lactaldehyde (Scheme 1).[16] Thus, a biosynthetic preparation of **1** and **2** may follow the first steps of the catabolic sequence or may alternatively proceed in reverse of the final aldol cleavage.



Scheme 1. *Microbial catabolism of 6-deoxysugars. FucI = fucose isomerase; FucK = fuculokinase; FucA = fuculose 1-phosphate aldolase; RhaI = rhamnose isomerase; RhuK = rhamnulokinase; RhuA = rhamnulose 1-phosphate aldolase; DHAP = dihydroxyacetone phosphate.*

First Route: Isomerization–Phosphorylation

For the chemical preparation of ketoses the base-catalyzed isomerization of a corresponding aldose mediated by boiling pyridine is usually the preferred method for the mildness of its conditions. In case of the 6-deoxy-sugars fucose and rhamnose, the restriction of the corresponding ketose isomers to the formation of furanoid rings adversely influences the thermodynamic relationship to the aldose precursors and limits the quantity of the desired products at equilibrium (~10% **4**, ~60% **6**). Moreover, it has been shown by HPLC analysis that during the isomerization of **5** to **6** a number of other structural isomers are produced concomitantly.[19] For equilibrations involving **4** the situation must be expected to be further impaired due to its contrathermodynamic all-*cis* substitution pattern. The need for purification of **4** and **6** by chromatography[20] is a practical limitation, as are reported yields of ~35%[21] and 63%[19] based on the consumed aldose. Obviously, an enzymic *in situ* isomerization would be more suitable not only because of its higher selectivity but also because its coupling to a thermodynamically favorable enzymic phosphoryl transfer from ATP would serve to constantly drain the equilibrium of the ketose component and allow a complete conversion to product (Scheme 2).



Scheme 2. Enzymic synthesis of 6-deoxyketose 1-phosphates from aldoses. FucI = fucose isomerase; RhuK = rhamnulokinase; RhaI = rhamnose isomerase; PK = pyruvate kinase; PEP = phosphoenol pyruvate.

As the enzymes involved in bacterial fucose and rhamnose catabolism display a selectivity for the stereochemistry of their respective substrates sufficient to preclude crossreactivity among the two pathways, access to all individual enzymes seemed mandatory. However, the rhamnulokinase (RhuK; EC 2.7.1.5) from *E. coli* is exceptional for its apparent nonspecificity. It had been reported that, unlike the functionally equivalent fuculokinase (FucK; EC 2.7.1.51),[11] this RhuK also accepts the isomeric L-fuculose at roughly one third of its maximum catalytic activity with L-rhamnulose as natural substrate.[22] Using highly purified enzyme we found that fuculose in fact is processed at even better than half-maximal velocity (56% of V_{max}). Thus, this kinase seemed particularly well suited to fulfill a twofold synthetic purpose (Scheme 1) and, in the event, considerably reduced the task of biocatalyst isolation. The purification of rhamnose isomerase (RhaI; EC 5.3.1.14) from a recombinant strain of *E. coli* has been detailed lately.[23] Literature procedures for the purification of fucose isomerase (FucI; EC 5.3.1.3)[24] and RhuK[13,22] from *E. coli* were also available but tedious and not very

effective. Hence, for the present purpose we took advantage of recently constructed recombinant strains of *E. coli* that overexpress the RhuK[17] and FucI.[25] Due to the resultant high percentage in the total soluble cell protein (up to 30%), these enzymes could be readily isolated in pure form by simple 3 and 2 standard chromatographic steps, respectively.

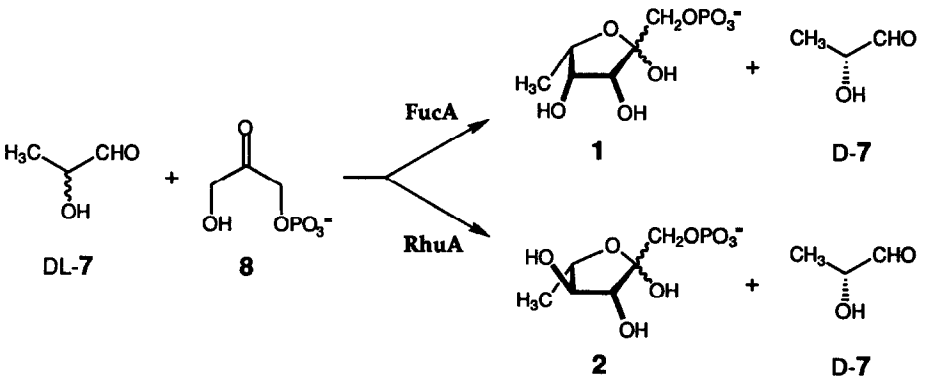
In accordance with literature data,[24] the FucI was found to have a relatively high K_m value of 130 mM for its natural substrate **3**. For preparative applications on smaller scales (up to ~10 mmol) the convenience of using the phosphoryl donor ATP in stoichiometric amounts outweighs the extra expense. An upper limit for its initial concentration was determined at ca. 40 mM because of the strong inhibition of the RhuK by the ADP released. As the concentration of the sugar co-substrates rapidly decreases as the reaction progresses, the resultant low, sub-K_m concentrations of **3** necessitated to use larger amounts of the FucI in order to compensate for reaction rates considerably below V_{max} and to guarantee a steady substrate feed for the ensuing phosphorylation. This complication did not occur in the syntheses that employ the RhaI since this enzyme shows a much higher affinity for its natural substrate, **5** (K_m = 1.5 mM). The affinity of the RhuK for the ketoses **4** and **6** is of a comparable magnitude with kinetic constants determined at K_m = 3.0 and 0.2 mM which ensures a fast consumption of the intermediates even at low stationary concentrations.

Purification of products **1** and **2** from the unreacted sugars and ADP was achieved by simple ion exchange chromatography applying a stepwise gradient of triethylammonium hydrogencarbonate as a volatile buffer. The sugar phosphates were obtained as crystalline, stable bis(cyclohexylammonium) salts of analytical purity. For preparations on a larger scale, the cofactor costs demand to add an *in situ* regeneration scheme for ATP, for which we chose to use the pyruvate kinase–phosphoenol pyruvate system[26] because of the high donor potential and hydrolytic stability of the enol phosphate which minimizes contamination of the products by inorganic phosphate. The work-up procedure had to be modified to achieve a separation of the organophosphates from stoichiometric pyruvate. First attempts to elute pyruvate at lower ionic strength from the ion exchange resin proved to be less effective, particularly at larger scales, due to the similar elution profiles of the components. Barium salts co-precipitated both anions. Direct crystallization of the cyclohexylammonium salt of **2** from aqueous ethanol provided a facile (though at 60% yield not fully satisfying) solution to the problem since pyruvate seemed to form only soluble salts or condensation products. Recovery of the sugar phosphates in high yield finally resulted when pyruvate was first decomposed by incubation with pyruvate decarboxylase (EC 4.1.1.1) into volatile fragments which were readily removed by evaporation prior to product crystallization. This technique is especially important in case of the fuculose derivative **1** which has a much lower tendency to crystallize than **2**.

Second Route: Aldol Addition

Reversal of the catabolic aldol cleavage of sugar phosphates **1** and **2** to L-lactaldehyde (L-**7**) and DHAP (**8**) is thermodynamically preferred due to the formation of a C–C bond and a furanose hemiacetal structure at the expense of two carbonyl double bonds. While the stereochemistry of the aldol products at both termini of the new C–C bond is completely determined by the FucA and RhuA enzymes,[3,5,8] the stereogenetic nature of the addition would cause the formation of diastereomers if the chiral aldehyde was used in racemic form. Enantiomerically pure L-**7** can be prepared from the 'nonproteinogenic' D-enantiomer of threonine by ninhydrin induced oxidative decarboxylation.[27] However, the expense of the precursor and the inconvenience of repeated ion-ex-

change treatments required for purification of the aldehyde make this procedure impractical on a scale larger than a few millimoles. The crucial observation that both microbial aldolases generally display an overwhelming kinetic preference for the L-configurated antipodes of 2-hydroxyaldehydes — in contrast to results obtained for the aldolase from rabbit muscle which offers only very limited if any capability for kinetic resolutions[28] — renders the aldol route (Scheme 3) a practical alternative to the isomerization–phosphorylation method. Thus, reactions could be run directly from *rac*-**7** which is readily prepared from inexpensive 1,1-dimethoxypropanone.[29] When the aldehyde was supplied in excess of two equivalents, L-ketose phosphates **1** and **2** (>90% d.e.; no contamination by D-configurated diastereomers detectable within the analytical limits of ^{1}H NMR at 400 MHz) were obtained by determination of all three independent, contiguous centers of chirality within a single enzyme catalyzed reaction step. Both compounds were isolated in excellent yield and in a diastereomerically pure form by crystallization of the bis(cyclohexylammonium) salts. In addition, the corresponding unphosphorylated ketoses were obtained by acid phosphatase (EC 3.1.3.2) catalyzed hydrolysis of **1** and **2** to provide pure samples of **4** and **6**, respectively. This result again demonstrates the potential of asymmetric aldol additions for the facile *de novo* synthesis of rare or, particularly, that of sensitive sugars.



***Scheme 3.** Enzymic synthesis of 6-deoxyketose 1-phosphates by aldol addition including kinetic resolution. FucA = fuculose 1-phosphate aldolase; RhuA = rhamnulose 1-phosphate aldolase.*

Now that methods are established for the facile preparation of the ketose 1-phosphates **1** and **2** in quantity, along with a straightforward access to large amounts of a number of enzymes useful for their preparation, the value of the DHAP aldolases, in particular that of FucA and RhuA, as synthetic tools can be widely assessed. Thus, it is envisaged that the ready availability and practical utility of these biocatalysts will further stimulate the development of enzymic strategies towards the synthesis of related polyfunctionalized and physiologically active compounds.

ACKNOWLEDGMENTS

We are grateful for financial support of this research by the *Deutsche Forschungsgemeinschaft* (Fe 244/2-2), the *Wissenschaftliche Gesellschaft Freiburg*, and the *Fonds der Chemischen Industrie*, and for scholarships to O.E. and A.S. by the *Ministerium für Wissenschaft und Kunst, Baden-Württemberg*.

EXPERIMENTAL

General. ATP disodium salt and Tris buffer were purchased from Serva. Growth media, gel electrophoresis materials, and all other chemicals came from Fluka. Phosphoenolpyruvate was prepared according to the procedure of Hirschbein et al.[30] Dihydroxyacetone phosphate was prepared chemically from dihydroxyacetone according to the procedure of Pederson et al.[31] or enzymically from glycerol 1-phosphate by a novel protocol based on glycerol phosphate oxidase.[32] Ion exchange resins (100-200 mesh) were from Bio-Rad. Media for protein chromatography were obtained from Pharmacia Biosystems. Assay enzymes, pyruvate kinase (rabbit muscle, type II), and pyruvate decarboxylase (brewers yeast) were obtained from Sigma. Acid phosphatase (from potato) was a gift of Boehringer Mannheim GmbH. Rhamnose isomerase[23] and the two aldolases[8] were purified according to previously described protocols. Analytical thin-layer chromatography was performed on Merck silica gel plates 60 GF_{254} using a 1 : 1 mixture of satd. ammonia–ethanol for development, and anisaldehyde stain for detection. Sugars were analyzed by HPLC on a Nucleosil 5-NH_2 column (250 x 4 mm) with UV detection at 200 nm using 80% aqueous acetonitrile as eluent. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. ^1H, ^{13}C, and ^{31}P NMR spectra (sodium 3-trimethylsilyl-[D_4]-propionate, acetonitrile, and phosphoric acid standards ($\delta_H = 0.00$, $\delta_C = 1.30$, and $\delta_P = 0.00$ ppm)) were recorded by Drs. D. Hunkler and W. Deck.

Growth of bacteria and preparation of cell extracts. Cells were grown aerobically to late logarithmic phase at 37°C in a 10-L fermentor in LB medium supplemented with ampicillin (100 μg mL^{-1}) and were harvested by continuous centrifugation (Sharples centrifuge). Cells were resuspended in 4 volumes of preparation buffer (20 mM Tris, 10 mM mercaptoethanol, 1 mM $MnCl_2$, pH 7.3) and disrupted by three-fold passage through a French press. Cellular debris was removed by centrifugation at 24 000 *g* for 15 min and the clear supernatant was used for enzyme purification.

Purification of fucose isomerase. The cell-free extract of *E. coli* strain JM105-pKKFI-2 (ref. 26; 250 mL from 50 g of cells) was applied to a DEAE-Sepharose column (5 x 32 cm) equilibrated with preparation buffer. After the column was washed with buffer containing 150 mM NaCl (500 mL), protein was eluted with a linear gradient of NaCl (150–600 mM, 2.0 L). Active fractions were pooled and concentrated to a volume of 15 mL by ultrafiltration. The concentrate was applied to a Sephadex G-150 column (5 x 78 cm) equilibrated with preparation buffer containing 100 mM NaCl and active fractions were again pooled and concentrated by ultrafiltration to a volume of 7 mL containing the pure enzyme (total activity 110 000 U).

Purification of rhamnulokinase. The cell-free extract of *E. coli* strain JA121/pJB4.3 (ref. 17; 520 mL from 117 g of cells, 4700 U) were chromatographed on a DEAE-Sepharose column (5 x 32 cm) equilibrated with preparation buffer. After washing the column with buffer containing 150 mM NaCl (850 mL), a linear gradient of NaCl (150–350 mM, 2.3 L) was applied to elute the protein. Active fractions were combined (3350 U) and adjusted to a concentration of 250 mM $(NH_4)_2SO_4$ by slow addition of finely ground salt. This solution was subjected to Phenyl-Sepharose chromatography (3.5 x 23 cm), and the kinase was eluted using a decreasing $(NH_4)_2SO_4$-gradient (250–0 mM, 700 mL) followed by pure buffer (400 mL). Pooled activity (380 mL, 3320 U) was concentrated to a volume of 20 mL by ultrafiltration and subjected to Sephadex-G150 chromatography as above. Concentration of active fractions to a volume of 10 mL yielded 3200 U of total activity.

Enzyme assays. Rhamnose isomerase and fucose isomerase activities were assayed by measuring ketose formation in reaction mixtures containing preparation buffer and L-rhamnose or L-fucose at 10 or 120 mM concentration, respectively, by the cystein–carbazol reaction.[33] Color intensity was measured after 1 h at the absorbance maximum of 545 nm. 1 Unit of enzyme is expressed as the quantity required to produce 1 μmol of L-rhamnulose or L-fuculose per minute at 25°C. Kinetic parameters were derived by measuring the reaction rate at substrate concentrations spanning the range of 0.25 – 4 K_M. Rhamnulokinase activity was measured photometrically by coupling ADP formation to consumption of NADH by using pyruvate kinase–phosphoenolpyruvate and lactate dehydrogenase.[22] 1 Unit is defined as the amount of enzyme required to release 1 μmol of ADP per minute at 25°C. Activity of aldolases was determined photometrically by an assay coupled to glycerophosphate dehydrogenase catalyzed NADH oxidation.[8] 1 Unit catalyzes the cleavage of 1 μmol of L-ketose 1-phosphate per minute at 25°C.

L-Fuculose 1-phosphate (**1**) *from L-fucose.* An aqueous solution (500 mL) containing L-Fucose (**3**, 1.64 g, 10 mmol) and ATP (Na_2 salt trihydrate, 6.05 g, 10 mmol) was adjusted to pH 8.0 with 2 M NaOH. After addition of FucI (300 U) and RhuK (150 U), the solution was allowed to stand at room temperature. Conversion was monitored by t.l.c. (R_f(**3**) = 0.51; R_f(**1**) = 0.30). After complete consumption of **3** (ca. 24 h), the mixture was filtered through charcoal and passed through an anion exchange column (Dowex AG1-X8, HCO_3^- form, 100 mL). The column was washed with water (200 mL), and the product was eluted with 0.2 M triethylammonium hydrogencarbonate buffer. Repeated concentration from water (3 x 50 mL), ion exchange to the free acid (Dowex AG50W-X8, H^+ form, 100 mL), and neutralization with cyclohexylamine followed by crystallization from 90% aqueous ethanol provided the colorless bis(cyclohexylammonium) salt of **1** (3.45 g, 78%); mp 146°C (dec), $[\alpha]_D$ + 0.5 (*c* 2, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 1.23 (d, 6-Hα), 1.29 (d, 6-Hβ), 3.71-3.83 (m, 1-H_aα, 1-H_aβ, 1-H_bβ), 3.95 (dd, 1-H_aα), 4.10-4.19 (m, 4-Hβ, 5-Hβ), 4.22-4.27 (m, 3-Hα, 4-Hα), 4.30 (d, 3-Hβ), 4.38 (dq, 5-Hα), $J_{1a,P}$ = 7.4, $J_{1aa,1ba}$ = 12.0, $J_{3\alpha,4\alpha}$ = 5.3, $J_{3\beta,4\beta}$ = 4.5, $J_{4\alpha,5\alpha}$ = 3.7, $J_{5\alpha,6\alpha}$ = 6.7, $J_{5\beta,6\beta}$ = 6.1 Hz; ^{13}C NMR (100.6 MHz, D_2O): δ 14.4 (C-6α), 14.8 (C-6β), 65.7 (d, C-1α), 66.4 (C-1β), 72.4 (C-3β), 72.8 (C-3α,-4β), 75.9 (C-4α), 76.5 (C-5β), 78.5 (C-5α), 102.1 (d, C-2β), 104.6 (d, C-2α); $J_{C\text{-}1\alpha,P}$ = 3.5, $J_{C\text{-}2\alpha,P}$ = 6.6, $J_{C\text{-}1\beta,P}$ = 4.5, $J_{C\text{-}2\beta,P}$ = 8.2 Hz; ^{31}P-NMR (80 MHz, D_2O): δ 4.03 (β), 4.41 (α). Integration showed the ratio of α and β anomers to be 2 : 5. Anal. Calcd for $C_{18}H_{39}N_2O_8P$ (442.15): C, 48.36; H, 8.81; N, 6.31. Found: C, 48.89; H, 8.82; N, 6.33.

L-Fuculose 1-phosphate (**1**) *from L-lactaldehyde.* An aqueous solution (40 mL) of DHAP and L-lactaldehyde[27] (1 mmol each) was adjusted to pH 6.8 with 1 M NaOH, and FucA (30 U) was added. After complete conversion of DHAP (**8**) determined by t.l.c. (R_f(**8**) = 0.39; R_f(**1**) = 0.30), the mixture was worked up as above to furnish **1** as the bis(cyclohexylammonium) salt (380 mg, 86%).

L-Fuculose 1-phosphate (**1**) *from DL-lactaldehyde.* DL-Lactaldehyde dimethylacetal[29] (6.0 g, 50 mmol) in 50 mL of water was hydrolyzed by treatment with cation exchange resin (Dowex AG50W-X8, H^+ form) at 60°C for 8 h. After filtration, an aqueous solution of DHAP (80 mL, 10 mmol) was added. The mixture was adjusted to pH 6.8 with 1 M NaOH and incubated with FucA (300 U) at room temperature. After complete conversion (t.l.c. and enzymic assay for DHAP), the product was isolated as above to yield 3.76 g of **1** (85%).

L-Rhamnulose 1-phosphate (**2**) *from L-rhamnose (small scale).* A solution (100 mL) of L-Rhamnose (**5**, 1.82 g, 10.0 mmol) in Tris buffer (20 mM; pH 8.0) containing mercaptoethanol (20 mM) and $MgCl_2$ (5 mM) was deoxygenated with a stream of nitrogen and was incubated with rhamnose isomerase (200 U) at 37°C. After 2 h, ca. 30% of ketose had been formed (HPLC). A solution of ATP (3.03 g; 5.0 mmol) in the same solvent (50 mL) and rhamnulokinase (200 U) were added and the mixture was further incubated for 3 h after which t.l.c. showed complete consumption of ATP. The solution was diluted to 400 mL and passed down an anion exchange column (Dowex AG1-X8, HCO_3^-, 30 mL). After washing with water (150 mL), the sugar containing eluates were neutralized by addition of Dowex AG50W-X8 (H^+) and concentrated to recycle unconverted **5**. Product was eluted with 0.2 M triethylammonium hydrogencarbonate buffer (300 mL). After repeated concentration from water (3 x 50 mL), ion exchange to the free acid (Dowex AG50W-X8, H^+ form, 100 mL), and neutralization with cyclohexylamine, the product was crystallized from 80% aqueous ethanol to yield the bis-(cyclohexylammonium) salt of **2** as fine colorless needles (1.75 g; 79%); mp 165°C (dec), $[\alpha]_D$ + 2 (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O): δ 1.33 (d, 6-Hβ), 1.33 (d, 6-Hα), 3.92-3.72 and 4.16-4.02 (2 m, rel. integr. 1:3, 5 H), $J_{5\alpha,6\alpha} = J_{5\beta,6\beta} = 6.1$ Hz; ^{13}C NMR (100.6 MHz, D_2O): δ 18.1 (s, C-6α), 19.2 (s, C-6β), 65.2 (s, C-1α), 66.5 (s, C-1β), 76.4 (s, 2 Cβ), 78.0 (s, 1 Cα), 79.5 (s, 1 Cβ), 81.2 (s, 1 Cα), 82.7 (s, 1 Cα), 101.0 (d, C-2β), 104.5 (d, C-2α); $J_{C\text{-}1\alpha,P} = 3.9$, $J_{C\text{-}1\beta,P} = 3.8$, $J_{C\text{-}2\alpha,P} = 6.2$, $J_{C\text{-}2\beta,P} = 7.4$ Hz; ^{31}P NMR (80MHz, D_2O) δ 4.15 (β), 4.74 (α). Integration showed the ratio of α and β anomers to be 1 : 2.6. Anal. Calcd for $C_{18}H_{39}N_2O_8P$ (442.15): C, 48.36; H, 8.81; N, 6.31. Found: C, 48.42; H, 8.85; N, 6.30.

L-Rhamnulose 1-phosphate (**2**) *from L-rhamnose (large scale).* A solution of **5** (19.0 g, 104 mmol) in Tris buffer (1 L, as above) was incubated with rhamnose isomerase (2000 U) at 21°C overnight after which ca. 25% of ketose had been formed (HPLC). After addition of a solution of ATP (600 mg, 1.0 mmol) and phosphoenol pyruvate (K salt,[30] 20.6 g, 100 mmol) in the same buffer (500 mL, adjusted to pH 8 by addition of 2 M KOH), rhamnulokinase (1400 U) and pyruvate kinase (1000 U) were added. After standing at room temperature for 8 h, t.l.c. showed complete consumption of ATP. At this point, the solution was separated into unequal parts for separate work-up procedures. One tenth of the volume was processed by anion exchange chromatography (Dowex AG1-X8, HCO_3^-, elution with stepwise gradients of 0–250 mM in steps of 50 mM) in a futile attempt to separate pyruvate. The combined eluates were incubated with pyruvate decarboxylase (20 U total, added in portions of 5 U every 12 h) for 48 h, after which enzymic assay indicated >90% cleavage of pyruvate. After ion exchange into the cyclohexylammonium form, crystallization of the remainder from 90% aqueous ethanol provided pure **2** (4.1 g, 93%). — The remaining nine tenth of the product solution were filtered through charcoal and converted into the cyclohexylammonium form by ion exchange. After concentration, the semi-solid residue was dissolved in water (250 mL), ethanol (750 mL) was added, and the mixture was allowed to crystallize at 0°C to give a first crop of product (13.8 g). Further crystallization from 90% ethanol provided a second and third crop (7.6 g and 2.4 g; total yield 23.8 g, 60%).

L-Rhamnulose 1-phosphate (**2**) *from L-lactaldehyde.* Solutions of DHAP (Li salt, 115 mg, 0.5 mmol, 5 mL) and L-lactaldehyde[27] (100 mM, 10 mL) were combined and the pH was adjusted to 6.8. After addition of RhuA (50 U) the reaction mixture was allowed to stand at room temperature until t.l.c. showed complete conversion of DHAP (1 h). Work-up was performed by ion-exchange techniques as above to furnish colorless needles of **2** (215 mg, 96%).

L-Rhamnulose 1-phosphate (2) *from DL-lactaldehyde.* DL-Lactaldehyde dimethylacetal[29] (960 mg, 8 mmol) was hydrolyzed by stirring an aqueous solution (50 mL) with ion exchange resin (Dowex AG50W-X8, H^+ form, 5 mL) at 50°C for 8 h. After filtration, DHAP (Li salt, 460 mg, 2 mmol) was added and the pH adjusted to 6.8 with 1 N NaOH. RhuA (100 U) was added, and the solution was allowed to stand at room temperature until t.l.c. and enzymic assay for DHAP showed complete conversion (1.5 h). Filtration through charcoal and usual work-up gave pure **2** (811 mg, 91%).

Fuculose (4) *and rhamnulose* (6). An aqueous solution of sugar phosphate **1** (300 mg, 0.7 mmol, 40 mL) or **2** (500 mg, 1.1 mmol, 50 mL) was adjusted to pH 6.0 by adding a small quantity of acidic ion exchange resin. After filtration, the solution was incubated overnight with acid phosphatase (200 and 300 U) at room temperature. When hydrolysis was complete (t.l.c., ca. 15 h), the solution was neutralized. Silica gel (2 g) was added, the slurry was concentrated to dryness, and the residue was placed on top of a chromatography column containing silica gel. Elution with $CHCl_3$–methanol (2:1) provided pure ketoses **4** (66 mg, 60%) or **6** (178 mg, 96%) as a colorless semisolid or syrup, respectively, whose physical data were in accordance with literature values.[14]

REFERENCES AND NOTES

1. Enzymes in Organic Synthesis, part 6. For part 5, see ref. 18.
2. Permanent address: Department of Biochemistry, School of Pharmacy, University of Barcelona, Barcelona 08028, Spain.
3. Fessner, W.-D. *Kontakte (Darmstadt)* **1992** (3), 3-9; **1993** (1), in press.
4. Toone, E.J.; Simon, E.S.; Bednarski, M.D.; Whitesides, G.M. *Tetrahedron* **1989**, *45*, 5365-5422. Drueckhammer, D.G.; Hennen, W.J.; Pederson, R.L.; Barbas, C.F.; Gautheron, C.M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499-525. David, S.; Augé, C.; Gautheron, C. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 175-237.
5. Fessner, W.-D. A Building Block Strategy for Asymmetric Synthesis: The DHAP Aldolases. In *Microbial Reagents in Organic Synthesis*, Servi, S. (Ed.), NATO ASI Series C, Vol. 381, Kluwer Academic, Dordrecht, 1992, pp. 43-55.
6. Fessner, W.-D.; Eyrisch, O. *Angew. Chem.* **1992**, *104*, 76-78; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 56-58. Eyrisch, O.; Sinerius, G.; Fessner, W.-D. *Carbohydr. Res.* **1993**, in press.
7. von der Osten, C.H.; Sinskey, A.J.; Barbas, C.F.; Pederson, R.L.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1989**, *111*, 3924-3927. Alefounder, P.R.; Baldwin, S.A.; Perham, R.N.; and Short, N.J. *Biochem. J.* **1989**, *257*, 529-534.
8. Fessner, W.-D.; Sinerius, G.; Schneider, A.; Dreyer, M.; Schulz, G.E.; Badía, J.; Aguilar, J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 555-558.
9. Ozaki, A.; Toone, E.J.; von der Osten, C.H.; Sinskey, A.J.; Whitesides, G.M. *J. Am. Chem. Soc.* **1990**, *112*, 4970-4971.
10. FucA and RhuA enzymes will soon be available commercially from *Boehringer Mannheim* Biochemicals.

11. Chiu, T.C.; Otto, R.; Power, J.; Feingold, D.S. *Biochim. Biophys. Acta* **1966**, *127*, 249-251; Fan, D.-F.; Sartoris, M.; Chiu, T.H.; Feingold, D.S. *Methods Enzymol.* **1975**, *41*, 91-94.
12. Heath, E.C.; Ghalambor, M.A. *J. Biol. Chem.* **1962**, *237*, 2423-2426.
13. Wilson, D.M.; Ajl, S. *J. Bacteriol.* **1957**, *73*, 415-420; Takagi, Y., Sawada, H. *Biochim. Biophys. Acta* **1964**, *92*, 18-25.
14. Liu, K.K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H. *J. Org. Chem.* **1991**, *56*, 6280-6289.
15. Zhou, P.; Lajoie, G.; Honek, J.F.; Nambiar, P.T.C.; Ward, O.P. *Carbohydr. Res.* **1992**, *232*, 177-181.
16. Lin, E.C.C. Dissimilatory Pathways for Sugars, Ployols, and Carboxylates. In Escherichia coli *and* Salmonella typhimurium*: Cellular and Molecular Biology*; Neidhardt, F.C.; Ingraham, J.L.; Low, K.B.; Magasanik, B.; Schaechter, M.; Umbarger, H.E. Eds.; American Society for Microbiology: Washington, D.C., 1987; pp. 244-284.
17. Badía, J.; Baldomà, L.; Aguilar, J.; Boronat, A. *FEMS Microbiol. Lett.* **1989**, *65*, 253-258.
18. Fessner, W.-D.; Badía, J.; Eyrisch, O.; Schneider, A.; Sinerius, G. *Tetrahedron Lett.* **1992**, *33*, 5231-5234.
19. Ennifar, S.; El Khadem, H.S. *Carbohydr. Res.* **1989**, *193*, 303-306.
20. Attempted phosphorylation of a sample of crude fuculose, generated by the pyridine isomerization procedure and reduced in fucose content by fractional crystallization from ethanol, failed to produce any sugar phosphate but only led to precipitation of protein.
21. Barnett, J.; Reichstein, T. *Helv. Chim. Acta* **1937**, *20*, 1529-1536.
22. Chiu, T.H.; Feingold, D.S. *Biochim. Biophys. Acta* **1964**, *92*, 489-497.
23. Badía, J.; Gimenez, R.; Baldomà, L.; Barnes, E.; Fessner, W.-D.; Aguilar, J. *J.Bacteriol.* **1991**, *173*, 5144-5150.
24. Boulter, J.R.; Gielow, W.O. *J. Bacteriol.* **1973**, *113*, 687-696.
25. Eyrisch, O.; Fessner, W.-D., unpublished results.
26. Chenault, H.K.; Simon, E.S.; Whitesides, G.M. *Biotechnol. Genet. Eng. Rev.* **1988**, *6*, 221-270.
27. Zagalak, B.; Frey, P.A.; Karabatsos, G.L.; Abeles, R.H. *J. Biol. Chem.* **1966**, *241*, 3028-3035.
28. Bednarski, M.D.; Simon, E.S.; Bischofberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G.M. *J. Am. Chem. Soc.* **1989**, *111*, 627-635.
29. Durrwachter, J.R.; Drueckhammer, D.G.; Nozaki, K.; Sweers, H.M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812-7818.
30. Hirschbein, B.L.; Mazenod, F.P.; Whitesides, G.M. *J. Org. Chem.* **1982**, *47*, 3765-3766.
31. Pederson, R.L.; Esker, J.; Wong, C.-H. *Tetrahedron* **1991**, *47*, 2643-2648.
32. Sinerius, G.; Fessner, W.-D., manuscript in preparation.
33. Dische, Z.; Borenfreund, E. *J. Biol. Chem.* **1951**, *192*, 583-587.