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Practical synthesis of 4-hydroxy-3-oxobutylphosphonic acid and its evaluation as a bio-isosteric substrate of DHAP aldolase [1,2]

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Abstract

An efficient four step synthesis of the title compound 4-hydroxy-3-oxobutylphosphonate (**2**) has been developed based on inexpensive 4-ethoxy-1-hydroxybutane-2-one using an Arbusow reaction (59% overall yield). Several dihydroxyacetone-dependent aldolases having different stereospecificities were tested for their acceptance of this phosphonomethyl substrate mimic as the aldol donor. Individual enzymes belonging to both type I (Schiff base formation) and type II (Zn^{2+} catalysis) mechanistic classes were found to catalyze the stereoselective addition of **2** to simple aldehydes to provide bio-isosteric analogs of sugar 1-phosphates in high yields. The lack of acceptance by specific enzymes is discussed with regard to recent protein X-ray data. © 1998 Elsevier Science Ltd.

Keywords: Dihydroxyacetone phosphate mimic; Asymmetric synthesis; Enzyme catalysis; Sugar phosphonates; D-Fructose 1,6-bisphosphate aldolase

1. Introduction

Phosphonic acids isosteric to naturally occurring phosphate esters are attractive targets for synthesis because of their potential bioactivity, because replacement of the ester oxygen atom by a methylene group renders the corresponding phosphonates incapable of hydrolytic cleavage. Owing to their geometrical and polar similarity to the natural metabolites or effectors, they can act as stable inhibitors or regulators of metabolic processes, as has been amply demonstrated for a large structural variety of isosteric phosphonate analogs of sugar phosphates in their interaction with relevant enzymes [1–3]. Analogs corresponding to glycolysis intermediates were of particular interest because this metabolic pathway is

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[1] Enzymes in Organic Synthesis, Part 13. For Part 12, see Ref. [10].

[2] Professor George A. Olah on the occasion of his 70th birthday.

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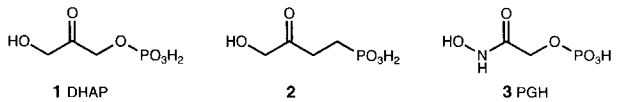
the only source of energy for some human parasites such as the trypanosomes [4].

Enzymatic techniques are well developed for the stereocontrolled synthesis of carbohydrate backbones by making use of dihydroxyacetone phosphate-dependent aldolases [5–7]. Detailed mechanistic [8–10] and structural [11–17] knowledge about, and the recent commercial availability of, several aldolases having different stereospecificities further underscore the practicality of this rather flexible and stereodivergent scheme for the asymmetric synthesis of carbohydrates and related compounds [5–7]. Thus, we became interested in extending this scheme to the synthesis of sugar phosphonates [18,19], as an alternative to the lengthy conventional routes which typically involve the chain elongation of parent sugars by a phosphonomethyl unit and, hence, suffer from the need for extensive protective group manipulations.

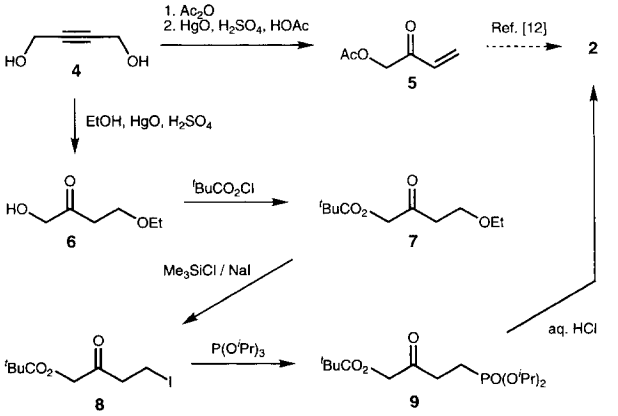
4-Hydroxy-3-oxobutylphosphonate **2** as the requisite phosphonate analog to the aldolase substrate dihydroxyacetone phosphate (DHAP, **1**) had been prepared previously only in small quantity by multistep routes of rather low efficiency [20–24]. Recently, we have investigated the synthesis of (*S*)-3,4-dihydroxybutylphosphonic acid (**12**) starting from L-malic acid and its enzymatic oxidation by an enantiospecific glycerol phosphate oxidase (EC 1.1.3.21) as a means for the in situ preparation of **2** [19] Scheme 1. This route was primarily designed for determining respective kinetic parameters and also proved suitable for preparing small amounts of **2** but in view of the non-asymmetric product, of course, an enantiomerically pure starting material was an inappropriate burden. With a perspective for broader preparative applications, we had to search for a more practical access to **2**.



2. Results and discussion

Synthesis of 4-hydroxy-3-oxobutylphosphonate **2**. —The phosphonate **2** was synthesized first in 1972 based on the expensive 3-butenol (4 steps, 16% yield) by Engel et al. [20] who later published an improved synthesis based on 2-butyne-1,4-diol (**4**; 3 steps, 50% overall yield) [21] while Dixon and Sparkes [22] concurrently reported a more laborious preparation from acrylic acid (5 steps, 16% yield). The corresponding diethyl ester had also been described in 1975 by Paulsen and Bartsch [23] in the context of unsaturated sugar phosphonates. Only after conclusion of the present study, a more recent protocol was detailed by Page et al. [24] who started from acrolein acetal (4 steps, 34% yield).

For our studies, the Engel route from **4** occurred to be the most promising and economical. Contrary to literature reports [21,25] and despite considerable experimentation, however, we found that acid-catalyzed isomerization of its diacetate to 1-acetoxybutenone in the presence of mercuric salts [25] gave only erratic results because of the strong propensity of this material to polymerize [25,26]; in addition, further elaboration towards **2** required a labor-intensive purification [21]. On the other hand, in situ capture of the Michael system or related intermediates during the isomerization in the presence of solvent alcohols provided a convenient access to 4-alkoxy-1-hydroxybutanones (e.g., **6**) as an alternative, but shelf-stable building block with a similar level of differential functionalization [25,26]. Such an intercept could not be effected, however, by adding phosphites which would have provided a more direct access to **2**. As became apparent subsequently, not only were the best results obtained by using ethanol as solvent (isopropanol gave slightly lower yields of adduct, whereas methanol gives rise to 1,4-dioxanes by dimerization [26]) but also the ethoxy group in **6** matched best the requirements for the ensuing substitution for phosphonate. The latter was anticipated to be most effectively conducted by an Arbusow displacement which required the prior change of the alkoxy to a leaving group, suitably by iodide. This operation in turn would have to be effected by a regioselective ether cleavage using iodotrimethylsilane.



In order to preserve the free 1-hydroxy functionality of **6** under such conditions, we had to find a protecting group which ought to be stable to chlorotrimethylsilane–sodium iodide, a convenient in situ iodotrimethylsilane reagent for ether cleavage [27,28], but would be labile to the conditions of the prospective hydrolysis of phosphonic esters. Ideally, this could be served by a carboxylic ester function but it is known that iodotrimethylsilane also can rapidly cleave simple alkanoic esters [27–29]. Indeed, none of the acetyl, butyryl, or 3,5-dinitrobenzoyl groups tested survived treatment of the ethoxy ethers with the chlorotrimethylsilane–sodium iodide system. On the contrary, a pivaloyl ester group (i.e., **7**) exhibited complete stability towards optimized reaction conditions (vide infra).

Another issue concerned the regioselectivity of ether cleavage with consequences for the choice of amounts of reagent and reaction time. In fact, we found that not only was replacement of the ethoxy group by halogen quite rapid without employing a large excess of silane reagent but it was also highly selective in the desired sense: only the iodide **8** and ethyl trimethylsilyl ether, but no appreciable amounts of ethyl iodide or the trimethylsilyl ether corresponding to **7** were detected among the products. Thus, whereas the original procedure for dealkylation of dialkyl ethers by chlorotrimethylsilane–sodium iodide [27,28] recommends the reaction to be carried out in refluxing acetonitrile over several hours, the cleavage of **7** could actually be carried out in dichloromethane at room temperature within 15 min under vigorous stirring.

Because of its presumed lability, the iodide **8** without further purification was directly subjected to Arbusow conditions to furnish the corresponding phosphonic ester **9**. Triisopropyl phosphite was chosen rather than primary alkyl phosphites to facilitate hydrolysis of the respective phosphonate esters. Indeed, hydrolysis of the diisopropyl ester **9** was complete after treatment with refluxing 3.5 M hydrochloric acid for 3 h whereas that of the respective diethyl phosphonate, made for comparison, in refluxing 10 M hydrochloric acid was still incomplete after 20 h. Azeotropic removal of the volatiles provided the free phosphonic acid **2** in pure form.

Thus, by this 4-step sequence, **2** was made readily available in 59% overall yield in multi-gram quantities, starting from stable 4-ethoxy-1-hydroxybutanone **6** and using only economical reagents.

Synthesis of sugar phosphonates.—It has been reported from biochemical assays (Scheme 1) that **2** is accepted as a substrate analog by redox enzymes such as the *sn*-glycerol 3-phosphate dehydrogenase (GDH; EC 1.1.1.8) from rabbit muscle or *E. coli* [22,30–32], but not for triose phosphate isomerases (TPI; EC 5.3.1.1) from chicken [22] or rabbit muscle [19]. In addition, it has been shown [22,30] that **2** can act as a substrate replacement for the D-fructose 1,6-bisphosphate aldolase from rabbit muscle ($FruA_{rab}$; EC 4.1.2.13) which enabled the synthesis of the 1-phosphonomethyl isostere **11** ($X = CH_2$) of fructose 1,6-bisphosphate (FBP; **11**, X = O) by the $FruA_{rab}$ catalyzed aldol addition of **2** to D-glyceraldehyde 3-phosphate (**10**, X = O) [30]. More recently, we have demonstrated in a preliminary study [18] that **2** is also accepted by the L-rhamnulose 1-phosphate aldolase from *E. coli* ($RhuA_{eco}$; EC 4.1.2.19) by its addition to L-glyceraldehyde to furnish 2-deoxy-L-*arabino*-hept-3-ulose 1-phosphonate (**17**), the corresponding analog to L-fructose 1-phosphate.

In an extended search for a comparable donor tolerance among DHAP-dependent aldolases, in addition to the two enzymes previously investigated ($FruA_{rab}$ [18,31–34], $RhuA_{eco}$ [18]) we now have included four other aldolases covering both mechanistic classes as well as differing stereospecificities. Thus, we have used three further enzymes with FruA specificity from different origins: (1) the enzyme from *Staphylococcus carnosus* ($FruA_{sca}$) [35,36] is a class I enzyme similar to the $FruA_{rab}$ which binds the aldol donor through Schiff base formation at an active site lysine residue, whereas (2) the commercial enzyme from *Saccharomyces cerevisiae* ($FruA_{yst}$) as well as (3) the recombinant aldolase from *E. coli* ($FruA_{eco}$) [37] belong to the class II aldolases which activate the substrate by Zn^{2+}-dependent enediol chelation [10]. In addition, a recombinant L-fuculose 1-phosphate aldolase from *E. coli* ($FucA_{eco}$; EC 4.1.2.17) [38] was investigated as the first class II enzyme to be structurally [11–13] and mechanistically [10] characterized at the atomic level.

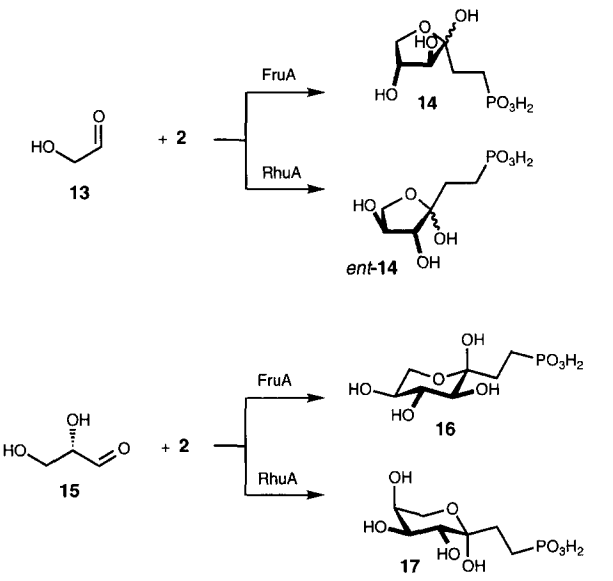
Contrary to our expectations, we found that **2** was not accepted by all of the aldolases tested (Table 1). In fact, out of the six enzymes, only three showed the capacity for product formation when incubated with **2** in the presence of an acceptor aldehyde. Positive results were obtained with both Schiff base forming enzymes having FruA specificity, which is not surprising given their sequence homology [36] and hence their likely similar structure and function. For comparison, none of the corresponding class II enzymes with FruA specificity tested positive. Out of the two

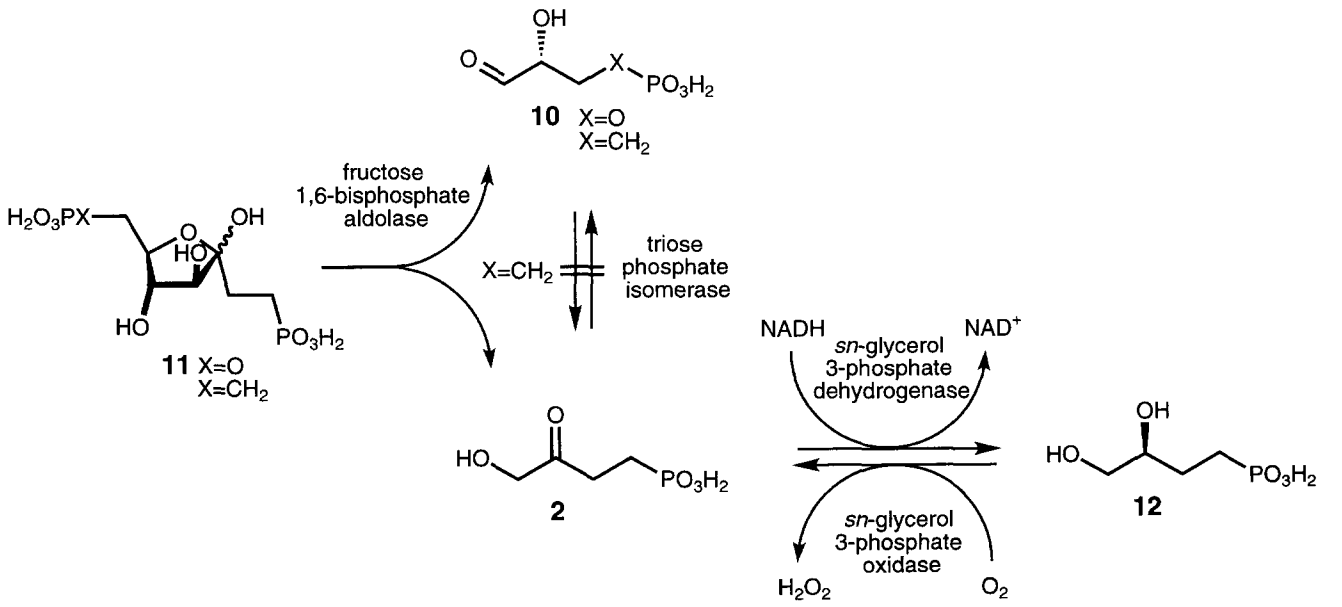
Table 1
Acceptance of **2** by different DHAP-dependent aldolases in the presence of hydroxyaldehyde electrophiles

Enzyme	Type	Substrate	Result
$FruA_{rab}$	Class I	**13**	+
$FruA_{rab}$	Class I	L-**15**	+
$FruA_{sca}$	Class I	**13**	+
$FruA_{sca}$	Class I	L-**15**	+
$FruA_{yst}$	Class II	**13**	–
$FruA_{yst}$	Class II	L-**15**	–
$FruA_{eco}$	Class II	**13**	–
$FruA_{eco}$	Class II	L-**15**	–
$RhuA_{eco}$	Class II	**13**	+
$RhuA_{eco}$	Class II	L-**15**	+
$FucA_{eco}$	Class II	**13**	–
$FucA_{eco}$	Class II	L-**15**	–

microbial deoxysugar aldolases, only that with RhuA, but not that with FucA specificity was active.

Preparative applications of DHAP (**1**), the natural donor substrate of the aldolases, suffer from the fact that this compound rapidly fragments with loss of phosphate at increasing pH values [39,40] which limits its use to neutral or slightly acidic conditions [6,7]. Advantageously, enzymatic aldolizations involving **2** can be conducted at alkaline pH, where the biocatalysts usually have their pH optima (ca. pH 8.0 to 8.5), because of its stability against base-catalyzed elimination. The successful examples of preparative reactions (Table 1) conducted at pH 8.0 with 1.5 equiv. of glycolaldehyde (**13**) and L-glyceraldehyde (**15**) all reached practically complete conversion according to ^{1}H NMR analysis of the crude mixtures which demonstrates the thermodynamic advantage of C–C bond formation at equilibrium [6–8]. The expected furanoid (**14**, *ent*-**14**) and pyranoid ketose phosphonates (**16**, **17**) were isolated by ion-exchange separation in high yield and high stereochemical purity, as generally is observed for 2-hydroxyaldehydes [6,7].





Scheme 1. In vitro biocatalytic conversions involving **2**.

Kinetic determinations.—The effectiveness of **2** as a substrate for aldolases is difficult to assess in the synthetic direction since no continuous spectrophotometric assay is available, and kinetic analysis is complicated from equilibration effects even at low levels of conversion (Scheme 1). Thus, kinetic evaluations with $FruA_{rab}$ were conducted by coupling the retro-aldolization of **14** and **16** with NADH-dependent removal of **2** from equilibrium which can be effected by using GDH due to its known capacity for reduction of **2** [22,30–32]. An apparent Michaelis constant (K_m) of 1.6 mM was determined for the furanoid phosphonate **14** at pH 7.5 while for the pyranoid **16** this amounted to 19.0 mM. Relative maximum rates for cleavage of both phosphonates reach ca. 45% of the v_{max} determined in parallel for the natural substrate fructose 1,6-bisphosphate (**11**, X = O). When the kinetic data for **14** and **16** are compared to reference values of substrate binding for **11**, having α/β-anomeric furanoid structures prevailing in solution, at a K_m in the range of 0.002–0.06 mM [41] or to fructose 1-phosphate, with a pyranoid structure dominating, at a K_m value of 7–10 mM [42,43], it becomes obvious that the (mono)phosphonates are recognized at a level mainly dominated by the anionic charge, and that the influence from the lower pK values of phosphonic acids relative to phosphoric monoesters seem likely to be only marginal. Clearly, the lower K_m value measured for the phosphonate **14** as compared to that of fructose 1-phosphate may be due to the closer structural relationship with the sugar portion of FBP but likely also may reflect to some extent the higher equilibrium fraction of an acyclic structural isomer displaying an unmasked carbonyl group which should improve the apparent K_m.

As had to be expected from the failure to catalyze the forward reaction by the class II $FruA_{eco}$ and $FruA_{yst}$ enzymes, assays using these catalysts for cleavage of compound **14**, as obtained from the synthetic reaction with the class I aldolases of identical stereopreference, also proved negative.

For an assessment of the possible causes of the failure of the $FruA_{yst}$, $FruA_{eco}$, and $FucA_{eco}$ as well as the TPI enzymes to recognize **2** as compared to the corresponding active $RhuA_{eco}$ catalyst, only limited structural data are available at present. Structural data published for the $FruA_{eco}$ [17] have been deposited in the Brookhaven PDB but have not been publicly released yet. From an analysis of the crystal structure of the chicken TPI in complex with phosphoglycolohydroxamate (PGH; **3**), a compound almost isostructural to DHAP (**1**), it has been pointed out that all its phosphate oxygens, including the carbon bound bridging oxygen, are tightly connected to the protein backbone by a network of eight hydrogen bonding contacts [44], so that anchoring of the substrate will be severely interrupted upon replacement of the ester oxygen by methylene in shifting to the formally isosteric (but not isopolar) phosphonate mimic **2**.

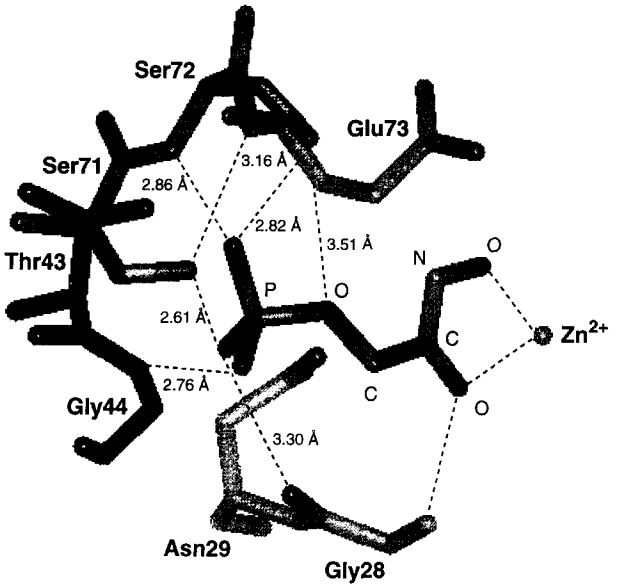


Fig. 1. Binding of the inhibitor phosphoglycolohydroxamate (**3**) in the active site of $FucA_{eco}$ as determined by X-ray analysis [12] showing the relevant hydrogen bonding interactions within the phosphate binding pocket.

Inspection of the X-ray structural model for the FucA enzyme in complex with **3** [12], bound in a fashion that resembles a transition state or reaction intermediate of the aldolization cycle, on the other hand does not offer a similarly conclusive picture as for the TPI interpretation since no dominant interactions seem to exist for the relevant bridging phosphate oxygen, except for one relatively weak contact to the side chain hydroxyl of serine 72 at 3.51 Å (Fig. 1). Thus, from structural data, it remains unclear at this point as why the methylene replacement could completely interrupt, or induce an impassable deviation from, the correct binding mode.

For a possible cause, there still remains the fact that phosphonates differ in their respective dissociation constants from the corresponding natural phosphate ester substrates, although the kinetic data obtained with the rabbit aldolase are prima facie not

supportive. The second pK_a of **1** is ca. 6.0 [45], whereas that of **2** is ca. 7.1 [22]. The second dissociation constant of the CH_2–phosphonate is clearly lower which may thus diminish substrate binding significantly under physiological conditions. Additionally, the difference in binding may be due to small differences in geometry, including the possible greater preference of the methylene group for the staggered conformation [46], interfering potentially at one or several intermediate stages of catalysis. In fact, certain other cases are known where substitution of methylene for oxygen prevents enzymic action even though this group is not directly involved in the reaction [47].

3. Experimental

General methods.—Moisture sensitive reactions were performed in oven dried glassware under nitrogen; solvents were distilled and when necessary dried prior to use. Analytical TLC was carried out on Merck $60F_{254}$ Silica Gel plates; visualization was done by dipping into 5% phosphomolybdic acid or *p*-anisaldehyde stain followed by heating. Column chromatography was performed on Merck 60 Silica Gel (0.063–0.200 mesh). Optical rotations were determined on a Perkin-Elmer P241 polarimeter. ^1H and ^{13}C NMR spectra were recorded on Varian VXR 300 or Gemini 300 spectrometers; chemical shifts are referenced to internal Me_4Si or TSP (0.00 ppm). IR spectra were recorded with a Perkin-Elmer 842 or Perkin-Elmer 1420 spectrometer. GC analysis was performed with a Hewlett Packard 5890 Series II gas chromatograph on an SE-30 column. Mass spectra were recorded on a Finnigan MAT 212 system and elemental analyses were performed on a Heraeus CHN–O–Rapid system. Commercial enzymes (D-fructose 1,6-bisphosphate aldolase, glycerol phosphate dehydrogenase, triose phosphate isomerase, all from rabbit muscle, and D-fructose 1,6-bisphosphate aldolase from yeast) were purchased from Sigma or were provided by Boehringer Mannheim. L-Rhamnulose 1-phosphate aldolase, L-fuculose 1-phosphate aldolase (both from *E. coli*) [38], and D-fructose 1,6-bisphosphate aldolase (from *E. coli* [37] or *S. carnosus* [35,36]) were overproduced from recombinant strains and purified according to published procedures. Analytical grade ion-exchange media were purchased from Bio-Rad.

Enzyme assay.—The fructose 1,6-bisphosphate aldolase reaction was assayed by a method based on the routine procedure for determination of DHAP [48]. The GDH assay solution (500 μL) contained reduced nicotinamide adenine dinucleotide (NADH, 0.02 mM), potassium chloride (6.0 mM), and a variable concentration of substrate in Tris buffer (6.0 mM, pH 7.5) to which was added glycerol phosphate dehydrogenase (3.6 U). After addition of fructose 1,6-bisphosphate aldolase (1.8 U), the disappearance of NADH was followed photometrically at 340 nm. The kinetic parameters of FruA for **11**, **14** and **17** were determined by measuring the reaction rate at substrate concentrations ranging from 0.1 K_m to 10 K_m. Data processing was performed according to standard linearization methods (Lineweaver-Burk, Eadie-Hofstee, Hanes-Woolf) as implemented in the program *EnzymeKinetics* (Trinity Software).

4 - Ethoxy - 1 - hydroxybutan - 2 - one (**6**).—This compound was obtained following the procedure of Hennion and Kupiecki [25]. The routine yield of **6** was 55%, containing some contamination by unreacted **4**. This material was usually prepared prior to use without further purification.

2,2-Dimethylpropionic acid (4'-ethoxy-2'-oxobutyl) ester (**7**).—Crude **6** (10.2 g, 77 mmol) was dissolved in dry Et_2O (200 mL) under nitrogen and cooled in an ice bath to 0 °C. Et_3N (42 mL, 300 mmol) was then added, followed by the addition of pivaloyl chloride (25.3 g, 210 mmol) over a period of 20 min. The mixture was stirred overnight at room temperature, then poured into ice water. The organic layer was washed with 1 M HCl, aqueous 5% $NaHCO_3$, and water before being dried with $MgSO_4$. The product **7** was isolated by column chromatography (1:9 Et_2O–petroleum ether) to yield a colorless liquid (16.0 g, 96%); bp 87–90 °C/0.5 mbar; IR (film): ν 1733, 1162, and 1135 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.18 (t, 3 H, ^2J 7.1 Hz, OCH_2CH_3), 1.27 (s, 9 H, Me_3C), 2.67 (t, 2 H, $J_{3',4'}$ 6.4 Hz, H-3'), 3.49 (q, 2 H, ^2J 7.1 Hz, OCH_2CH_3), 3.70 (t, 2 H, H-4'), 4.69 (s, 2 H, H-1'); ^{13}C NMR ($CDCl_3$): δ 15.1 (OCH_2CH_3), 27.1 (Me_3C), 38.7 (Me_3C), 39.5 (C-3'), 64.9 (OCH_2CH_3), 66.5 (C-4'), 68.4 (OCH_2), 177.8 (COO), 202.6 (C-2'); MS: *m/z* 216 (M^+). Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.32. Found: C, 60.82; H, 9.17.

2,2-Dimethylpropionic acid (4'-iodo-2'-oxobutyl) ester (**8**).—To a solution of **7** (16.0 g, 74 mmol) and sodium iodide (12.2 g, 81 mmol) in dry CH_2Cl_2 (250 mL) flushed with dry nitrogen was added at 0 °C Me_3SiCl (10.3 mL, 81 mmol) during 15 min under vigorous stirring. Stirring was continued at room

temperature for complete conversion (15 min, TLC). The brown mixture was poured into ice water and washed several times with aqueous 10% $Na_2S_2O_3$, brine, and water, and dried over $MgSO_4$. The iodide **8** was prepared immediately prior to use without further purification. ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 9 H, Me_3C), 3.10 (t, 2 H, $J_{3',4'}$ 7.1 Hz, H-4'), 3.30 (t, 2 H, H-3'), 4.63 (s, 2 H, H-1'); ^{13}C NMR ($CDCl_3$): δ −6.1 (C-4'), 27.1 (*Me*$_3$C), 38.7 (Me$_3$*C*), 42.7 (C-3'), 67.8 (C-1'), 177.7 (COO), 201.8 (C-2').

3 - Oxo - 4 - (2, 2 - dimethylpropionyl)oxybutylphosphonic acid diisopropyl ester (**9**).—A solution of **8** (21.2 g, 71 mmol) in triisopropyl phosphite (175 mL, 710 mmol) was heated at 120 °C under nitrogen for 4 h. The phosphite was removed under reduced pressure and the remaining residue was purified by silica chromatography (EtOAc) to give **9** (16.7 g, 70%) as a colorless liquid; IR (film): ν 1734, 1284, 1244, 1159, 1108, 1093 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 9 H, Me_3C), 1.33 (2 d, 12 H, ^2J 6.1 Hz, OCH*Me*$_2$), 2.00 (m, 2 H, H-1), 2.72 (m, 2 H, H-2), 4.68 (s, 2 H, H-4), 4.70 (m, 2 H, ^2J 6.1 Hz, OC*H*Me$_2$); ^{13}C NMR ($CDCl_3$): δ 20.2 (d, $J_{C,P}$ 146.4 Hz, C-1), 23.9 (2 d, $J_{C,P}$ 3.7, $J_{C',P}$ 5.5 Hz, OCH*Me*$_2$), 27.1 (*Me*$_3$C), 32.2 (d, $J_{C,P}$ 3.7 Hz, C-2), 38.7 (Me$_3$*C*), 67.7 (C-4), 70.4 (2 s, O*C*HMe$_2$), 177.8 (COO), 202.2 (d, $J_{C,P}$ 15.9 Hz, C-2); MS: *m/z* 336 (M^+), 235 ($M^+ - Me_3CCO_2$), 221 ($M^+ - Me_3CCO_2CH_2$), 179 ($M^+ - Me_3CCO_2C_3H_4O$). Anal. Calcd for $C_{15}H_{29}O_6P$: C, 53.56; H, 8.69. Found: C, 53.54; H, 8.76.

4 - Hydroxy - 3 - oxobutylphosphonic acid (**2**).—A stirred mixture of **9** (16.7 g, 50 mmol), water (120 mL), and conc. HCl (50 mL) was boiled under reflux for 3 h. After diluting with water (100 mL), the solution was once extracted with Et_2O. The aqueous layer was evaporated and the remaining residue was dissolved in water (ca. 120 mL). The solvent was again evaporated, and the remaining pale yellow oil (9.7 g, 92%) was diluted with water to give a final 0.5 M solution of **2**. Spectral data corresponded to the literature values [18,24].

2 - Deoxy - D - threo - *hex - 3 - ulose 1 - phosphonate disodium salt* (**14**).—A solution containing glycolaldehyde (180 mg, 3 mmol; previously monomerized by warming to 50 °C at pH 1.5 for 3 h) and **2** (424 mg, 2 mmol) was adjusted to pH 8.0 with 0.2 M NaOH and purged with nitrogen. After addition of fructose 1,6-bisphosphate aldolase from rabbit muscle or from *S. carnosus* (40 U) the reaction mixture was incubated at room temperature for 2 days, then filtered through charcoal and concentrated in vacuo. The residue was purified by ion-exchange chromatography to yield the disodium salt of **14** (446 mg, 82%); $[\alpha]_D^{23}$ +11.9° (*c* 1.0, H_2O); IR (KBr): ν 2374, 2345, 1715, 1609 cm^{-1}; ^{1}H NMR (D_2O; major diastéreomer): δ 1.51 (m, 2 H, $J_{1,2}$ 8.4, $J_{1,P}$ 15.8 Hz, H-1), 2.66 (m, 2 H, $J_{2,P}$ 16.8 Hz, H-2), 3.49 (dd, 1 H, $J_{6a,5}$ 6.7, $J_{6a,6b}$ 11.4 Hz, H-6a), 3.56 (dd, 1 H, $J_{6b,5}$ 5.7 Hz, H-6b), 4.06 (m, 2 H, H-4,5); ^{13}C NMR (D_2O): δ 25.2 (d, $J_{C,P}$ 128.7 Hz, C-1), 36.6 (C-2), 64.9 (C-6), 74.1 and 79.6 (C-4,5), C-3 not obsd. Anal. Calcd. for $C_6H_{11}O_7PNa_2$: C, 26.49; H, 4.08. Found: C, 26.80; H, 4.38.

2 - Deoxy - L - threo - *hex - 3 - ulose 1 - phosphonate disodium salt* (ent − **14**).—Compound **2** and glycolaldehyde were reacted following the same procedure as above, but employing L-rhamnulose 1-phosphate aldolase to give after purification *ent*-**14** (463 mg, 85%); $[\alpha]_D^{23}$ −13.2° (*c* 1.1, H_2O); spectroscopic data matched those of the enantiomer given above. Anal. Calcd. for $C_6H_{11}O_7PNa_2$: C, 26.49; H, 4.08. Found: C, 26.67; H, 4.41.

2 - Deoxy - L - arabino - *hept - 3 - ulose 1 - phosphonate disodium salt* (**16**).—A solution containing monomeric L-glyceraldehyde (270 mg, 3 mmol) and **2** (424 mg, 2 mmol) was treated at pH 8.0 with fructose 1,6-bisphosphate aldolase from rabbit muscle or from *S. carnosus* (40 U), and the reaction mixture was incubated and processed as described for compound **14** to furnish 526 mg of **16** as colorless crystals (87%; Na^+ salt); $[\alpha]_D^{23}$ −72.3° (*c* 2.0, H_2O); IR (KBr): ν 2345, 1233, 1188, 1055 cm^{-1}; ^{1}H NMR (D_2O): δ 1.36 (m, 2 H, H-2), 1.78 (m, 2 H, H-1), 3.36 (d, 1 H, $J_{4,5}$ 8.8 Hz, H-4), 3.62 (d, 1 H, $J_{7a,7b}$ 12.1 Hz, H-7a), 3.66 (d, 1 H, $J_{5,6}$ 11.8 Hz, H-5), 3.68 (dd, 1 H, $J_{6,7b}$ 3.8 Hz, H-6), 3.70 (dd, 1 H, H-7b); ^{13}C NMR (D_2O): δ 28.5 (d, $J_{C,P}$ 132.9 Hz, C-1), 38.8 (C-2), 68.8 (C-7), 76.6, 80.3 and 80.8 (C-4,5,6), 105.6 (d, $J_{C,P}$ 15.5 Hz, C-3). Anal. Calcd. for $C_7H_{13}O_8PNa_2$: C, 27.83; H, 4.34. Found: C, 27.36; H, 4.47.

2 - Deoxy - L - xylo - *hept - 3 - ulose 1 - phosphonate disodium salt* (**17**).—Following the same procedure as above, but employing L-rhamnulose 1-phosphate aldolase, compound **17** was obtained (556 mg, 92%) as colorless crystals; $[\alpha]_D^{23}$ +61.6° (*c* 1.0, H_2O); IR (KBr): ν 2378, 2346, 1055 cm^{-1}; ^{1}H NMR (D_2O): δ 1.66 (m, 2 H, H-2), 1.98 (m, 2 H, H-1), 3.65 (dd, 1 H, $J_{7a,6}$ 2.0, $J_{7a,7b}$ 12.1 Hz, H-7a), 3.67 (d, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.87 (dd, 1 H, $J_{5,6}$ 3.5, $J_{5,4}$ 9.7 Hz, H-5), 3.97 (d, 1 H, $J_{7b,7a}$ 12.1 Hz, H-7b), 4.01 (dd, 1 H, $J_{6,7a}$ 2.0, $J_{6,5}$ 3.5 Hz, H-6); ^{13}C NMR (D_2O): δ

23.4 (d, $J_{C,P}$ 132.4 Hz, C-1), 34.8 (C-2), 66.2 (C-7), 77.5, 81.2 and 83.1 (C-4,5,6), 101.9 (d, $J_{C,P}$ 15.2 Hz, C-3). Anal. Calcd. for $C_7H_{13}O_8PNa_2$: C, 27.83; H, 4.34. Found: C, 27.45; H, 4.75.

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References

[1] R. Engel, *Chem. Rev.*, 77 (1977) 349–367.
[2] R.L. Hilderbrand (Ed.), *The Role of Phosphonates in Living Systems*, CRC Press, Boca Raton, 1988.
[3] R. Engel, *The Use of Carbon–Phosphorus Analog Compounds in the Regulation of Biological Processes* in R. Engel (Ed.), *Handbook of Organophosphorus Chemistry*, Dekker, New York, 1992, pp 559–600.
[4] F.R. Opperdoes, *Ann. Rev. Microbiol.*, 41 (1987) 127–151.
[5] W.-D. Fessner, *A Building Block Strategy for Asymmetric Synthesis: The DHAP Aldolases*, in S. Servi (Ed.), *Microbial Reagents in Organic Synthesis*, Kluwer Academic Publishers, Dordrecht, 1992, pp 43–55
[6] W.-D. Fessner and C. Walter, *Top. Curr. Chem.*, 184 (1996) 97–194.
[7] C.-H. Wong and G.M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Pergamon, Oxford, 1994, pp 195–251
[8] T. Gefflaut, C. Blonski, J. Perie, and M. Willson, *Prog. Biophys. Mol. Biol.*, 63 (1995) 301–340.
[9] J.A. Littlechild and H.C. Watson, *Trends Biochem. Sci.*, 18 (1993) 36–39.
[10] W.-D. Fessner, A. Schneider, H. Held, G. Sinerius, C. Walter, M. Hixon, and J.V. Schloss, *Angew. Chem. Int. Ed. Engl.*, 35 (1996) 2219–2221.
[11] M.K. Dreyer and G.E. Schulz, *J. Mol. Biol.*, 231 (1993) 549–553.
[12] M.K. Dreyer and G.E. Schulz, *J. Mol. Biol.*, 259 (1996) 458–466.
[13] M.K. Dreyer and G.E. Schulz, *Acta Cryst.*, D52 (1996) 1082–1091.
[14] J. Sygusch, D. Beaudry, and M. Allaire, *Proc. Natl. Acad. Sci. USA*, 84 (1987) 7846–7850.
[15] S.J. Gamblin, G.J. Davies, J.M. Grimes, R.M. Jackson, J.A. Littlechild, and H.C. Watson, *J. Mol. Biol.*, 219 (1991) 573–576.
[16] G. Hester, O. Brenner-Holzach, F.A. Rossi, M. Struck-Donatz, K.H. Winterhalter, J.D.G. Smit, and K. Piontek, *FEBS Lett.*, 292 (1991) 237–242.
[17] S.J. Cooper, G.A. Leonard, S.M. McSweeney, A.W. Thompson, J.H. Naismith, S. Qamar, A. Plater, A. Berry, and W.N. Hunter, *Structure*, 4 (1996) 1303.
[18] W.-D. Fessner and G. Sinerius, *Angew. Chem. Int. Ed. Engl.*, 33 (1994) 209–212.
[19] H.-L. Arth, G. Sinerius, and W.-D. Fessner, *Liebigs Ann.*, (1995) 2037–2042.
[20] J. Kabak, L. DeFilippe, R. Engel, and B.E. Tropp, *J. Med. Chem.*, 15 (1972) 1074–1075.
[21] S.L. Goldstein, D. Braksmayer, B.E. Tropp, and R. Engel, *J. Med. Chem.*, 17 (1974) 363–364.
[22] H.B.F. Dixon and M.J. Sparkes, *Biochem. J.*, 141 (1974) 715–719.
[23] H. Paulsen and W. Bartsch, *Chem. Ber.*, 108 (1975) 1745–1755.
[24] P. Page, C. Blonski, and J. Périé, *Tetrahedron Lett.*, 36 (1995) 8027–8030.
[25] G.F. Hennion and F.P. Kupiecki, *J. Org. Chem.*, 18 (1953) 1601–1609.
[26] W. Reppe, *Liebigs Ann. Chem.*, 596 (1955) 44–77.
[27] M.E. Jung and M.A. Lyster, *J. Org. Chem.*, 42 (1977) 3761–3764.
[28] G.A. Olah, S.C. Narang, B.G.B. Gupta, and R. Malhotra, *J. Org. Chem.*, 44 (1979) 1247–1251.
[29] M.E. Jung and M.A. Lyster, *J. Am. Chem. Soc.*, 99 (1977) 968–969.
[30] D. Stribling, *Biochem. J.*, 141 (1974) 725–728.
[31] P.-J. Cheng, R. Hickey, R. Engel, and B.E. Tropp, *Biochim. Biophys. Acta*, 341 (1974) 85–92.
[32] P.R. Adams, R. Harrison, and T.D. Inch, *Biochem. J.*, 141 (1974) 729–732.
[33] N. Bischofberger, H. Waldmann, T. Saito, E.S. Simon, W. Lees, M.D. Bednarski, and G.M. Whitesides, *J. Org. Chem.*, 53 (1988) 3457–3465.
[34] M.D. Bednarski, E.S. Simon, N. Bischofberger, W.-D. Fessner, M.-J. Kim, W. Lees, T. Saito, H. Waldmann, and G.M. Whitesides, *J. Am. Chem. Soc.*, 111 (1989) 627–635.
[35] H.P. Brockamp and M.R. Kula, *Appl. Biochem. Biotechnol.*, 34 (1990) 287–291.
[36] C. Witke and F. Götz, *J. Bacteriol.*, 175 (1993) 7495–7499.
[37] C.H. von der Osten, A.J. Sinskey, C.F. Barbas, R.L. Pederson, Y.F. Wang, and C.-H. Wong, *J. Am. Chem. Soc.*, 111 (1989) 3924–3927.
[38] W.-D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G.E. Schulz, J. Badía, and J. Aguilar, *Angew. Chem. Int. Ed. Engl.*, 30 (1991) 555–558.
[39] J.P. Richard, *J. Am. Chem. Soc.*, 106 (1984) 4926–4936.
[40] J.P. Richard, *Biochem. Soc. Trans.*, 21 (1993) 549–553.
[41] D. Schomburg and M. Salzmann (Eds.), *Enzyme Handbook*, Vol. 1, Springer, Berlin, 1990.
[42] W.J. Rutter, *Fed. Proc.*, 23 (1964) 1248–1257.
[43] P.D. Spolter, R.C. Adelman, and S. Weinhouse, *J. Biol. Chem.*, 240 (1965) 1327–1331.
[44] Z.D. Zhang, S. Sugio, E.A. Komives, K.D. Liu, J.R. Knowles, G.A. Petsko, and D. Ringe, *Biochemistry*, 33 (1994) 2830–2837.

[45] B. Plaut and J.R. Knowles, *Biochem. J.*, 129 (1972) 311–320.
[46] J.-M. Lehn, in G. Chiurdoglu (Ed.), *Conformational Analysis*, Academic Press, New York, 1971, pp 129–155
[47] T.S. Papas and R.V. Case, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 29 (1970) 915–918.
[48] H.U. Bergmeyer, *Methods of Enzymatic Analysis*, 3rd edn., Vol. 2, Verlag Chemie, Deerfield, 1984, pp 146–147