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Phosphoenolpyruvate as a Dual Purpose Reagent for Integrated Nucleotide/Nicotinamide Cofactor Recycling

Wolf-Dieter Fessner §* and Gudrun Sinerius

Department of Organic Chemistry and Biochemistry, University of Freiburg, Albertstr. 21, D-79104 Freiburg, Germany

Abstract—An efficient technique is presented which integrates cofactor dependent enzymic phosphorylation and dehydrogenation into a single, closed-loop system by employing phosphoenolpyruvate as the sacrificial reagent for sequential ATP and NAD^+ recycling steps. Exemplary applications are developed for the synthesis of 6-phosphogluconate from glucose, and that of dihydroxyacetone phosphate from glycerol. The latter system is combined with exergonic diastereoselective aldol additions for the one-flask synthesis of a ketosugar (D-sorbose), thiosugar (L-*threo*-5-thiopentulose), or a sugar acid (L-*threo*-pent-4-ulosonic acid) starting from a mixture of glycerol and simple aldehydes.

Introduction

Enzymic methods are now broadly accepted as a valuable addition to the armamentarium of asymmetric synthesis,[1] and even cofactor-requiring enzymes are increasingly considered as preparative tools.[2,3] In particular, costly ATP and NAD(P)H are routinely required to mediate phosphorylation and asymmetric reduction reactions, respectively. To make such enzymic processes technologically and economically feasible, practical procedures have been devised for an *in situ* cofactor regeneration.[4] Recycling of the oxidized nicotinamide(s) $NAD(P)^+$ used for dehydrogenations, however, is less well developed.

The best procedure at present for ATP regeneration, e.g. routinely applied in the enzymic preparation of sugar phosphates, is that based on the pyruvate kinase–phosphoenolpyruvate (PEP) system because of the reagent's superior hydrolytic stability and phosphoryl donor capacity.[5,6] An inherent disadvantage of this procedure is the necessity for purification of the phosphorylated products from pyruvate which is formed stoichiometrically as a byproduct. Due to its high chemical reactivity, pyruvate gives rise to a number of secondary contaminants such as products stemming from self aldolization[7] or additions of other nucleophiles;[8] thus, pyruvate caused complications have been noted frequently in preparative cofactor regenerations.[9–11] While, in our experience, ion exchange techniques are often inconvenient for separation of the major components of the product mixtures because of the similar retention behavior of both pyruvate and organophosphate esters, there are alternative options to simplify purification. Crystallization of the phosphorylated product[11] for example, is often feasible with alkyl ammonium counterions because pyruvate remains in alcoholic solution or forms soluble condensation products in the presence of amines.[12] Chemically, pyruvate can be removed by oxidative decomposition into volatile acetaldehyde and CO_2 using pyruvate decarboxylase[11,13] or by aldol reaction with a pyruvate specific aldolase.[14]

Another strategy would be the reductive conversion of pyruvate to less complicating lactate, however, chemical reducing agents cannot be used for this purpose if reducible functionalities are present in the desired product. *In situ* combination of the PEP based cofactor recycling scheme with a specific enzymic reduction catalyzed by lactic dehydrogenase (LDH), although long established for assay purposes,[15] to the best of our knowledge has not previously been employed in one-flask preparative applications, plausibly because of the implicit requirement for an extra NADH regeneration; only a singular case of a stepwise operation, where the isolated product solution containing the accumulated pyruvate from an ATP regeneration had been utilized further to serve in a separate LDH catalyzed NAD^+ regeneration, has been reported by the Whitesides group.[16] On the other hand, with recent advances in the development of multiple enzyme-catalyzed reaction cascades for the synthesis of complex compounds, an integration of several cofactor-dependent steps within 'artificial metabolisms' may be a necessity.[3,10] One of the advantages of such integrated processes is that inhibitory intermediates are maintained at a minimum concentration level. This fact should be particularly useful in case of reactions generating pyruvate since this compound frequently acts as an inhibitor for a variety of enzymes.[17]

Here we propose a practical solution to these problems with the use of PEP as a dual purpose reagent in those types of conversions that in consecutive (or separate) steps depend on recycling schemes for both ATP and NADH cofactors. To demonstrate the scope and practicality of this system, exemplary procedures are presented that include the one-flask conversion of glucose into 6-phosphogluconate and that of glycerol into dihydroxyacetone phosphate followed by its *in situ* consumption in enzymatic aldol reactions.

#Enzymes in Organic Synthesis, Part 8. For Part 7 see ref 30.

§New address: RWTH Aachen, Institute of Organic Chemistry, Professor-Pirlet-Str. 1, D-52056 Aachen, Germany

Results and Discussion

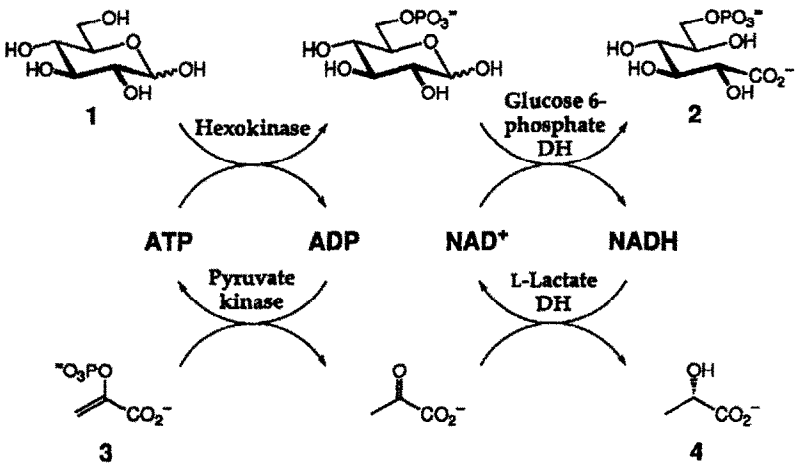
Integrated synthesis of 6-phospho-D-gluconate from D-glucose

Phosphogluconate (**2**), a compound which because of its strong tendency for complex formation is used as a food additive in the meat and beverage industries, may be obtained by fermentative or enzymic conversion of glucose (**1**). In principle, two biosynthetic pathways can be assembled with the use of commercially available enzymes: (1) phosphorylation of **1** to glucose 6-phosphate (G6P) by hexokinase (EC 2.7.1.1)[18] followed by oxidation to **2** by glucose 6-phosphate dehydrogenase (EC 1.1.1.49),[19] and a reversed sequence (2) by oxidizing **1** first to gluconate by glucose dehydrogenase (EC 1.1.1.47)[20] and subsequent phosphorylation by gluconate kinase (EC 2.7.1.12). Integrated cofactor regeneration was provided by the coupled two-step conversion of PEP (**3**) into L-lactate (**4**) catalyzed by pyruvate kinase (EC 2.7.1.40) and L-lactate dehydrogenase (EC 1.1.1.27). Route (1) was chosen for this study (Scheme I) primarily because it involves inexpensive enzymes (in fact, this sequence has also been followed in the early development and demonstration of practicality of cofactor regeneration schemes,[18,19] although simpler chemoenzymic routes have since been devised[21]), but also because difficulties had to be envisioned for system (2) from adverse kinetic effects due to the counter-current operation of productive and regenerative branches.

Scheme I illustrates the total closed-loop synthetic scheme employed. According to thermodynamic considerations, complete overall conversion had to be expected for several reasons. Firstly, phosphoryl transfer from **3** to **1** is associated with a gain of 48 kJ mol^{-1} in free energy.[22] Secondly, the phosphogluconate/G6P redox pair (E'_0 = –0.43 V) has a considerably higher oxidation potential than the pyruvate/lactate pair (E'_0 = –0.185 V),[4] and the reaction becomes essentially irreversible by the spontaneous chemical hydrolysis of the initially formed δ-lactone. In practice, this system proved highly efficient when carried out at the 10 mmol scale. To avoid the build-up of the inhibitor pyruvate,[23] those enzymes acting later in the sequence were applied at a relatively higher dosage. Indeed, according to enzymic assays pyruvate did not accumulate at any stage of the reaction, but was effectively pulled through to the reduction product. Also, no remaining G6P was detectable at the end of the reaction. The stability of the enzymes allowed a repeated batch operation with intervening recovery of the proteins by ultrafiltration. Straightforward separation of the products phosphogluconate (**2**, 73 % isolated yield) and L-lactate (**4**) from unreacted glucose was performed by anion exchange chromatography followed by crystallization. Separation is also possible by fractional precipitation as the barium and zinc salts, respectively, though the compounds thus obtained are of inferior purity.[19] It should be pointed out that a D-lactate dehydrogenase (EC 1.1.1.28) is available with opposite enantiomeric specificity. Thus, this procedure (and those below) may be altered by demand to furnish the valued by-product lactic acid in both of its optically active forms.

Integrated synthesis of dihydroxyacetone phosphate from glycerol and its utilization in situ *for diastereoselective aldol additions*

A second type of application concerns the formation of dihydroxyacetone phosphate (DHAP, **6**) from the inexpensive source glycerol (**5**). DHAP is the essential substrate of a set of four stereochemically distinct aldolases which catalyze highly stereoselective C–C bond formations between **6** and a broad range of aldehydic electrophiles.[24] This technique has recently received considerable attention because of its potential in the building-block type asymmetric synthesis[25] of sugars and related poly-hydroxylated compounds such as glycosidase



Scheme I. Closed-loop ATP/NAD^+ cofactor regeneration by using phosphoenolpyruvate, applied to the multi-enzymic synthesis of 6-phosphogluconate from D-glucose.

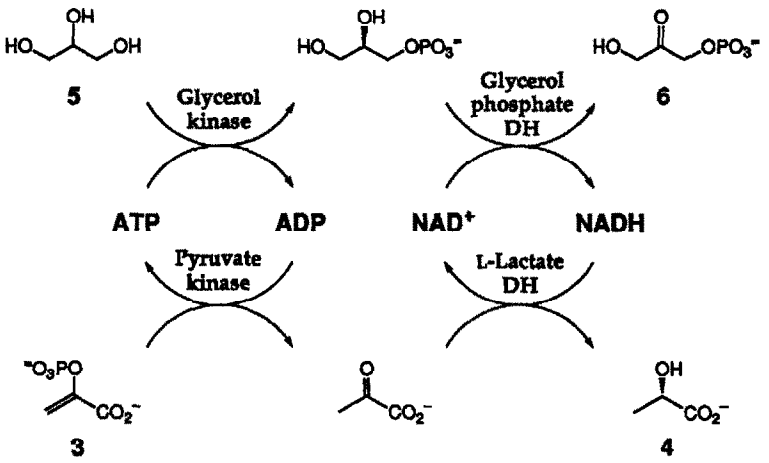
inhibiting alkaloids.[26] Compound **6** can be synthesized by multi-step chemical routes in protected form from which it must be liberated before use.[27] Because of its sensitive nature, however, **6** is best generated enzymically and consumed *in situ* by enzymic aldol additions to avoid high stationary concentrations. Thus, **6** may be generated by cleavage of D-fructose 1,6-bisphosphate[28] (which may be produced *in situ* from inexpensive fructose, glucose, or sucrose by a multi-enzymic cascade reaction[10]) using fructose 1,6-bisphosphate aldolase in combination with triose phosphate isomerase, by phosphorylation of dihydroxyacetone using glycerol kinase,[29] or by oxidation of L-glycerol 3-phosphate (G3P) using a microbial glycerol phosphate oxidase.[30] Neither of these methods is generally applicable since each is either restricted to a single type of aldolase (specific for fructose stereochemistry), produces large amounts of inhibitory byproducts (e.g. pyruvate or inorganic phosphate), or demands strongly oxygenating conditions, respectively. The combination of glycerol phosphorylation to G3P by glycerol kinase (EC 2.7.1.30) and oxidation of G3P to **6** by glycerol phosphate dehydrogenase (EC 1.1.1.8) seemed to offer an advantage, particularly for oxygen sensitive aldehydes, as both enzymes prefer anaerobic conditions. When combined with the PEP to lactate double cofactor regeneration (Scheme II), however, overall conversion to **6** would be hampered by the very similar redox potentials of pyruvate (E'_0 = –0.185 V) and **6** (E'_0 = –0.192 V).[4] Synthetic usefulness would thus strongly rely on an efficient removal of **6** from the equilibrium; this was expected to be accomplished by coupling to an energetically favorable aldol addition, especially if it would result in the formation of stable furanoid or pyranoid products.

In the event, the system smoothly generated a sugar phosphate when supplemented by a microbial rhamnulose 1-phosphate aldolase (RhuA; EC 4.1.2.19)[31] and a suitable aldehyde (Scheme III). From the reaction with D-glyceraldehyde (**7**) the pyranoid D-sorbose 1-phosphate (**8**) was obtained in fair yield only (48%; unoptimized), possibly hampered by the less satisfactory redox equilibrium. Pure D-sorbose (**9**) was obtained in free form by enzymic dephosphorylation employing alkaline phosphatase (EC 3.1.3.1). Engagement of the oxygen sensitive mercaptoacetaldehyde (**10**) in the combined enzymic process gave rise to the expected L-*threo*-configurated thiopentulofuranose 1-phosphate **11**. In this case a slight precipitate was formed upon addition of **10**, either due to the compound's low solubility or to partial protein denaturation. Nevertheless, **11** (enantiomeric to the compound obtainable with rabbit muscle aldolase[32]) was isolated in good yield. Its sensitivity required acid phosphatase (EC 3.1.3.2) for mild hydrolysis of the phosphate ester to provide the free thiosugar **12** which is of interest as a potential glycosidase inhibitor.

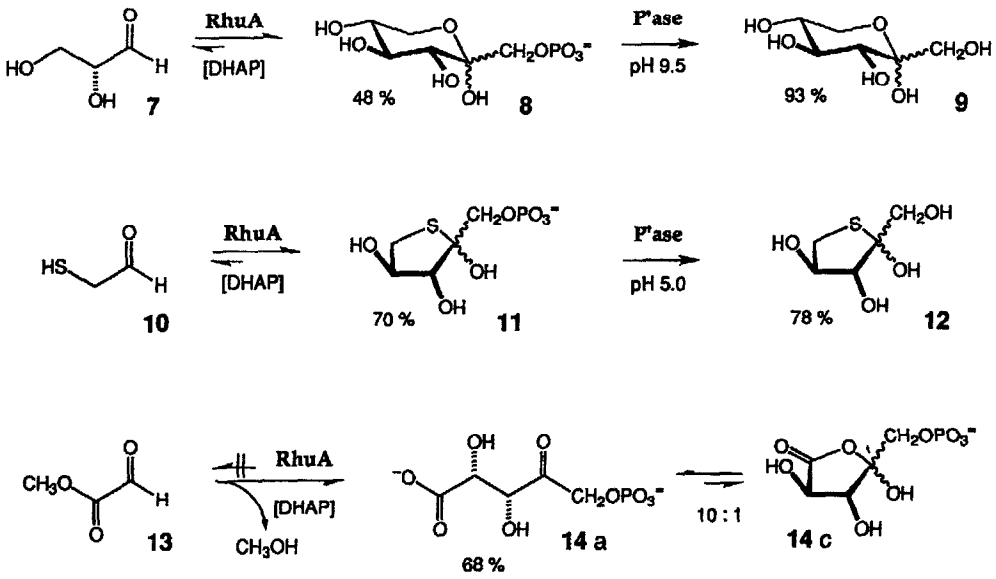
In a third example, methyl glyoxylate (**13**) was applied as the aldol acceptor when it was discovered that the ester function became hydrolyzed during (or after) C–C bond formation. Since free glyoxylic acid and the carboxylic acid **14** formed upon aldol addition were not recognized and converted by the RhuA enzyme, complete conversion of the DHAP equivalent to the phosphorylated L-*threo*-pent-4-ulosonic acid (10:1 equilibrium of the acyclic form **14a** and a cyclic lactone hemiacetal **14c** according to ^{1}H NMR) resulted through practically irreversible adduct formation.

Summary

We have demonstrated that facile integration of parallel ATP and NAD$^+$ cofactor dependent reactions into a closed-loop scheme can be achieved when employing PEP as a sacrificial dual purpose regenerant in cooperation with pyruvate kinase and lactic dehydrogenase. This multi-enzymic protocol provides a straightforward access to phosphorylated compounds of a relatively high complexity that would make their chemical preparation difficult. By obviating the need for isolation of intermediates these schemes provide high overall yields of final product. The technique greatly alleviates difficulties which otherwise



Scheme II. Closed-loop ATP/NAD$^+$ cofactor regeneration by using phosphoenolpyruvate, applied to the multi-enzymic synthesis of dihydroxyacetone phosphate from glycerol.



Scheme III. Consumption of dihydroxyacetone phosphate (**6**, generated *in situ* according to Scheme II) in diastereoselective aldol additions. Abbreviations: RhuA = rhamnulose 1-phosphate aldolase, P'ase = phosphatase, DHAP = dihydroxyacetone phosphate.

may be encountered during product isolation from reaction mixtures which contain large quantities of pyruvate from cofactor regeneration. Thus, while relying on the current status of development of cofactor regeneration systems in general,[4] this technique may be seen as a significant improvement in efficiency for the preparation of a range of phosphorylated compounds.

The protocol for a one-flask preparation of 6-phosphogluconate from glucose with the closed-loop cofactor regeneration, as adapted from published work,[18,19] provides a product of at least comparable yield and purity. At the same time it also proves more practical because no intermediate has to be isolated and the very low stationary concentration of the transient inhibitor pyruvate assures a high overall conversion rate which is considerably higher than that observed for a batchwise two-step procedure.

A more realistic assessment of preparative utility, however, is possible for the case of DHAP generation for which several chemical[27] and enzymic[29] literature methods are existent. All procedures suffer from the relatively delicate nature of DHAP in aqueous solution due to its propensity for phosphate elimination, particularly at pH values ≥ 7, or hydrolytic ester cleavage to form methyl glyoxal or dihydroxyacetone, respectively. Hence, the method of choice obviously is an enzymic generation of DHAP *in situ*, ideally in combination with a consumptive reaction to avoid accumulation. Relative to the direct enzymic phosphorylation of dihydroxyacetone using glycerol kinase[29] the route to DHAP described in this paper contains an extra oxidative transformation that imposes the need for one additional enzyme and cofactor, if only catalytic quantities, a fact that is hardly offset by the relative advantage in cost of starting materials (glycerol:dihydroxyacetone = 1:6). However, this extra effort allows, by the lactic dehydrogenase interface, for the ready *in situ* removal of pyruvate, which is produced upon each cofactor cycle, within a closed-loop regeneration scheme. Although the former method is certainly more practical for standard applications, the method presented here will give superior results in respect to product yield and purity because retardation of conversion due to product inhibition of pyruvate kinase is lifted and purification of subsequent products is greatly facilitated if separation of the phosphorylated material from pyruvate presents a difficulty. This advantage is only slightly offset by the extra costs of a more intricate catalyst system since the additional enzyme and nicotinamide cofactor are stable and fairly inexpensive 'reagents'. Likewise, a disadvantage may be seen in the fact that the enzymic phosphorylations rely on the availability of PEP which is marketed as an expensive fine chemical. However, both chemical or enzymic DHAP syntheses require a phosphorylation step, and PEP is indeed readily obtained in high purity on a multi-molar scale in just two fairly simple routine manipulations from pyruvate.[6] In any case, the inconvenience of PEP synthesis compares quite favorably to the requirement in labor, time, and cost of a multi-step synthesis of DHAP along any of the published chemical routes.[27]

Thus, we believe the technique outlined here will prove a valuable and practical extension to current enzymic methodology.

Experimental

General

^1H and ^{13}C NMR spectra were recorded in D_2O with a Bruker WM-400 instrument operating at 400 and 100.6 MHz, respectively, against sodium (2,2,3,3-$^2H_4$)-3-(trimethylsilyl)-propionate and acetonitrile (0.00 and 1.3 ppm) as internal standards. The pH of reaction mixtures was controlled with a Metrohm Model 665 Dosimat.

Reactions were monitored by TLC on silica gel 60 F_{254} (Merck), with detection by staining with anisaldehyde. Photometric measurements were made with a Pharmacia-LKB Ultrospec Plus spectrophotometer. Commercial enzymes were purchased from Sigma. Media for ion exchange chromatography were obtained from Bio-Rad. Ultrafiltration equipment was from Amicon. PEP monopotassium salt was prepared by the procedure of Hirschbein *et al.*[6]

6-Phospho-D-gluconate (2)

To a deoxygenated aqueous solution (20 mL) containing D-glucose (2.00 g, 11.1 mmol), PEP-K (2.22 g, 10.8 mmol), $MgCl_2$ (142 mg), EDTA (12 mg), and β-mercaptoethanol (50 μL) was added catalytic quantities of ATP-Na_3 (60 mg, 0.1 mmol) and NAD^+ (30 mg, 0.04 mmol). After adjusting the pH to 7.5, the soluble enzymes hexokinase (80 U; bakers yeast), pyruvate kinase (225 U; rabbit muscle), L-lactate dehydrogenase (170 U; rabbit muscle), and glucose 6-phosphate dehydrogenase (100 U; *Leuconostoc mesenteroides*) were added, and the mixture was incubated under nitrogen at room temperature with gentle stirring. The pH was maintained at 7.5 by adding 1.0 M KOH solution by using an automatic pH controller, and conversion was monitored by TLC and by enzymic assay for PEP and pyruvate. After 12 h proteins were separated by ultrafiltration (YM-10 membrane), and the recovered enzyme solution was used repeatedly to charge subsequent batches which were processed as above. For purification, the combined product solutions of two consecutive runs were applied to an ion exchange column (Dowex AG1-X8, HCO_3^- form; 100 mL). After washing with deionized water, products were recovered by elution with triethylammonium bicarbonate (TEAB) buffer. L-Lactate (**4**) was eluted with 0.1 M buffer (600 mL) while (**2**) was eluted with 0.3 M buffer (1 L). After removal of excessive buffer by repeated co-evaporation with water, the sugar phosphate was converted to the tris(cyclohexylammonium) salt by ion exchange to the free acid and addition of cyclohexylamine to alkalinity. Concentration provided pure **2** as a colorless solid (8.4 g, 73 %).

D-Sorbose 1-phosphate (8)

To a deoxygenated aqueous solution (6 mL) containing glycerol (140 mg, 1.5 mmol), PEP-K (206 mg, 1.0 mmol), $MgCl_2$ (8 mg), and β-mercaptoethanol (2 μL) was added catalytic quantities of ATP-Na_3 (8 mg, 10 μmol) and NAD^+ (6 mg, 10 μmol). After adjusting the pH to 8.3, glycerol kinase (50 U; *Candida mycoderma*), pyruvate kinase (90 U; rabbit muscle), L-lactate dehydrogenase (70 U; rabbit muscle), and glycerol phosphate dehydrogenase (50 U; rabbit muscle) were added. The mixture was incubated under nitrogen at room temperature for 2 h, after which D-glyceraldehyde (**7**; 100 mg, 1.1 mmol) and rhamnulose 1-phosphate aldolase (100 U; *Escherichia coli*) were added. The solution was allowed to stand at room temperature until monitoring by enzymic assay for PEP and pyruvate indicated that conversion was complete (2 d). Purification by ion exchange chromatography (Dowex AG1-X8, HCO_3^- form; 10 mL) was performed by elution with a stepwise gradient of TEAB buffer (0, 0.1, 0.2 M). The sugar phosphate **8** was eluted with 0.2 M buffer (120 mL). Conversion into the K^+ form by conventional ion exchange techniques furnished pure **8** as a colorless solid (300 mg, 48 %), indistinguishable by ^1H and ^{13}C NMR from an authentic sample.[31,33]

5-Thio-L-threo-pentulose 1-phosphate (11)

Similar to the reaction above, a flask was charged with glycerol (286 mg, 3 mmol), PEP-K (412 mg, 2.0 mmol), $MgCl_2$ (14 mg), ATP-Na_3 (16 mg, 20 μmol), NAD^+ (12 mg, 20 μmol), and β-mercaptoethanol (6 μL) in deoxygenated water (10 mL) and the pH adjusted to 8.3. Glycerol kinase (50 U; *Candida mycoderma*), pyruvate kinase (90 U; rabbit muscle), L-lactate dehydrogenase (70 U; rabbit muscle), and glycerol phosphate dehydrogenase (50 U; rabbit muscle) were added and the mixture was pre-incubated under nitrogen at room temperature for 2 h. Rhamnulose 1-phosphate aldolase (200 U; *Escherichia coli*) was introduced followed by the portionwise addition of a solution of thioglycolaldehyde (**10**; 152 mg, 2.0 mmol) in warm water (1 mL every 2 h, total of 5 mL). Progress of the reaction was monitored by TLC and enzymic assay for PEP/pyruvate. A small precipitate which accompanied the addition of **10** was removed by filtration and the product solution was purified by ion exchange (Dowex AG1-X8, HCO_3^- form; 20 mL). Fractions which eluted with 0.1 M buffer were discarded while the product was isolated with 0.2 M buffer (300 mL). Repeated evaporation from aqueous solution yielded **11** as the oily bis-(triethylammonium) salt (631 mg, 70 %); ^1H NMR δ 2.67 (dd, 5-H_a β), 3.05 (dd, 5-H_a α), 3.10 (dd, 5-H_b β), 3.20 (dd, 5-H_b α), 3.86 (dd, 1-H_a β), 3.90 (d, 3-H β), 3.93 (dd, 1-H_a α), 4.00 (dd, 1-H_b β), 4.01 (dd, 1-H_b α), 4.15 (d, 3-H α), 4.34 (ddd, 4-H β) 4.40 (q, 4-H α), $J_{1a,P}$ (α) = $J_{1b,P}$ (α) = 7.9, $J_{1a,1b}$ (α) = 11.2, $J_{3,4}$ (α) = 4.9, $J_{4,5a}$ (α) = $J_{4,5b}$ (α) = 5.2, $J_{5a,5b}$ (α) = 11.2, $J_{1a,P}$ (β) = 7.5, $J_{1b,P}$ (β) = 6.3, $J_{1a,1b}$ (β) =11.5, $J_{3,4}$ (β) = 9.4, $J_{4,5a}$ (β) = 9.7, $J_{4,5b}$ (β) = 7.2, $J_{5a,5b}$ (β) = 10.5 Hz, anomeric composition α:β = 1:4; ^{13}C NMR δ 29.29 (C-5 β), 35.03 (C-5 α), 67.43 (C-1 α), 69.92 (C-1 β, $J_{C,P}$ = 4.6 Hz), 75.16, 79.34, (C-3, -4 β), 77.08, 82.69 (C-3, -4 α), 89.05 (C-2 β).

L-Threo-pent-4-ulosonic acid 5-phosphate (14)

A solution was prepared containing glycerol (143 mg, 1.5 mmol), PEP-K (206 mg, 1.0 mmol), $MgCl_2$ (7 mg), ATP-Na_3 (8 mg, 10 μmol), NAD^+ (6 mg, 10 μmol), and β-mercaptoethanol (3 μL) in deoxygenated water (5 mL), and the pH was adjusted to 8.3. Glycerol kinase (50 U; *Candida mycoderma*), pyruvate kinase (90 U; rabbit muscle), L-lactate dehydrogenase (70 U; rabbit muscle), and glycerol phosphate dehydrogenase (50 U; rabbit muscle) were added and the mixture was pre-incubated under nitrogen at room temperature for 2 h. Rhamnulose 1-phosphate aldolase (100 U; *Escherichia coli*) was added followed by methyl glyoxylate (**13**; 152 mg, 2.0 mmol), previously dissolved in water (60 mL) by heating the mixture at 100 °C for 10 min. Progress of the reaction was monitored by TLC and enzymic assay for PEP/pyruvate,

none of which could be detected at the end of the reaction. Product was isolated by ion exchange (Dowex AG1-X8, HCO_3^- form; 20 mL). After washing with 0.1 M buffer **14** eluted with 0.3 M buffer (200 mL). Removal of excessive buffer by repeated co-evaporation with water and ion exchange provided the syrupy sodium form (211 mg, 68 %); ^{1}H NMR δ 3.85 (dd, 5-H_a c), 4.02 (dd, 5-H_b c), 4.06 (d, 2-H c), 4.59 (d, 3-H c), 4.60 (d, 3-H a), 4.73 (dd, 5-H_a a), 4.78 (d, 2-H a), 4.80 (dd, 5-H_b a), $J_{2,3}$ (c) = 1.5, $J_{5a,P}$ (c) = 5.2, $J_{5b,P}$ (c) = 6.0, $J_{5a,5b}$ (c) = 10.8, $J_{2,3}$ (a) = 2.5, $J_{5a,P}$ (a) = 7.5, $J_{5b,P}$ (a) = 7.2, $J_{5a,5b}$ (a) = 16.5, ratio of isomeric forms cyclic (c):acyclic (a) = 1:10; ^{13}C NMR δ 70.38 (C-5 a, $J_{C,P}$ = 3.6 Hz), 75.02 (C-2 a), 79.33 (C-3 a), 179.62 (C-1 a), 213.55 (C-4 a, $J_{C,P}$ = 7.5 Hz).

D-Sorbose (9)

An aqueous solution (20 mL) of **8** (250 mg, 0.6 mmol) was adjusted to pH 9.5 by addition of 1 M NaOH. Alkaline phosphatase (100 U; calf intestine) was added, and the solution was allowed to stand at room temperature for 12 h. The mixture was desalted by ion exchange and the solvent removed *in vacuo* to provide pure **9** (98 mg; 93 %).

*5-Thio-L-*threo*-pentulose (12)*

A solution (10 mL) of **11** (340 mg, 0.8 mmol) was adjusted to pH 5.0, acid phosphatase (100 U; sweet potato) was added, and reaction progress was monitored by TLC. Desalting furnished free **12** (128 mg; 78 %). ^{1}H NMR δ 2.66 (dd, 5-H_a), 3.11 (dd, 5-H_b), 3.65 (s, 1-H), 3.80 (d, 3-H), 4.35 (ddd, 4-H), $J_{3,4}$ = 9.0, $J_{4,5a}$ = 9.8, $J_{4,5b}$ = 7.5, $J_{5a,5b}$ = 10.5 Hz; ^{13}C NMR δ 29.31 (C-5), 65.99 (C-1), 75.17, 78.29 (C-3, -4), 87.89 (C-2).

Acknowledgments

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