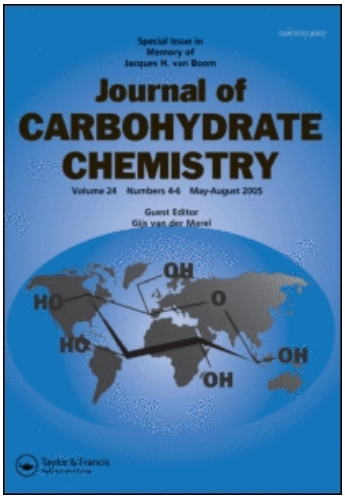


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

1-DEOXY-D-XYLULOSE SYNTHESIZED FROM THE (*S*)-CYANOHYDRIN OF ACROLEIN

Martin H. Fechter[a]; Richard Gaisberger[a]; Herfried Griengl[a]
[a] Technische Universität Graz, SFB Biokatalyse, Institut für Organische Chemie, Graz, Austria

Online publication date: 02 July 2002

To cite this Article Fechter, Martin H. , Gaisberger, Richard and Griengl, Herfried(2001) '1-DEOXY-D-XYLULOSE SYNTHESIZED FROM THE (*S*)-CYANOHYDRIN OF ACROLEIN', Journal of Carbohydrate Chemistry, 20: 9, 833 — 839
To link to this Article: DOI: 10.1081/CAR-100108660
URL: http://dx.doi.org/10.1081/CAR-100108660

J. CARBOHYDRATE CHEMISTRY, 20(9), 833–839 (2001)

1-DEOXY-D-XYLULOSE SYNTHESIZED FROM THE (*S*)-CYANOHYDRIN OF ACROLEIN

Martin H. Fechter, Richard Gaisberger, and Herfried Griengl

Institut für Organische Chemie, SFB Biokatalyse,
Technische Universität Graz, Stremayrgasse 16,
A-8010 Graz, Austria

ABSTRACT

The biocatalytic transformation of acrolein into (*S*)-2-hydroxybut-3-enenitrile using the (*S*)-hydroxynitrile lyase from *Hevea brasiliensis* followed by Grignard C-elongation, asymmetric epoxidation and nucleophilic ring-opening afforded 1-deoxy-D-xylulose (**1**) in 47% overall yield.

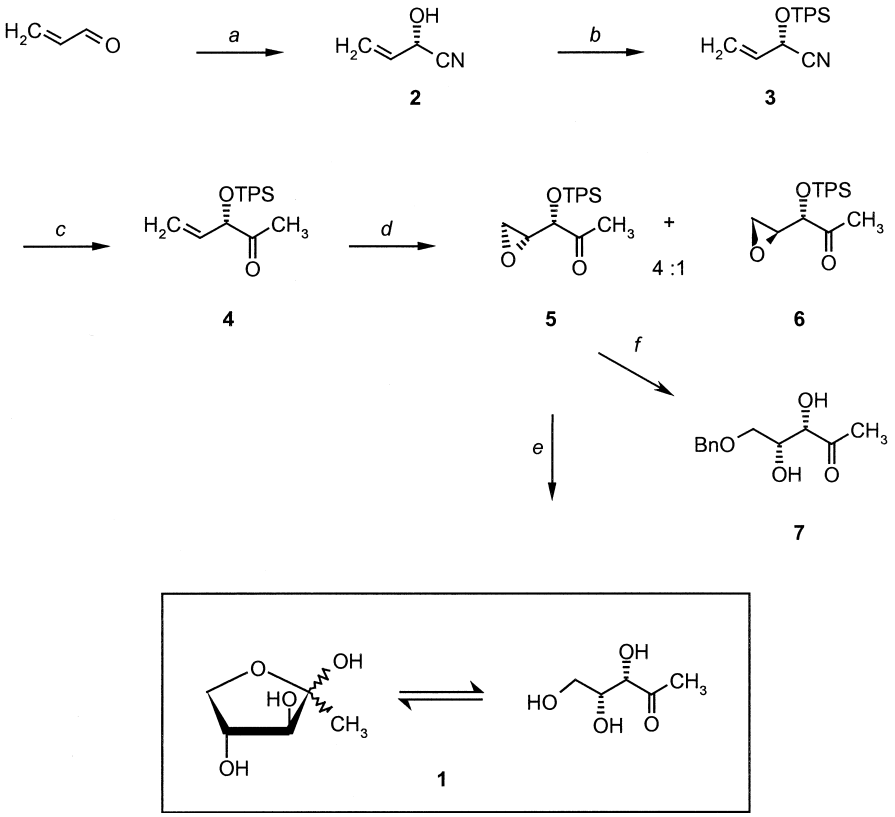
INTRODUCTION

1-Deoxy-D-xylulose (**1**),[1] first isolated in 1976 from *Streptomyces hygroscopicus* (UC-5601),[2] represents an important intermediate in several biochemical pathways of procaryotes and eucaryotes. In bacteria, for example, *E. coli* incorporates **1** into the thiazole nucleus of thiamine (vitamin B_1)[3] and pyridoxine (vitamin B_6).[4–6] In the chloroplasts of green algae[7] and higher plants this sugar has been found to be a substrate for enzymes involved in the alternate non-mevalonate biosynthesis of terpenoid building blocks.[8–11] Due to the high biological importance of 1-deoxy-D-xylulose, syntheses of this compound[12–16] and of isotopically labelled derivatives,[3,14,17–20] either by chiral pool techniques or by asymmetric *de novo* strategies, have been developed. Following our concept to apply the enzyme catalysed cyanohydrin reaction to the synthesis of compounds with biological relevance, we report here a *de novo* approach to 1-deoxy-D-xylulose starting from the (*S*)-cyanohydrin of acrolein.

RESULTS AND DISCUSSION

The biocatalytic transformation of acrolein and hydrocyanic acid in a two-phase system (methyl *tert*-butyl ether/buffer pH 5.5) with the (*S*)-hydroxynitrile lyase from *Hevea brasiliensis*, cloned and overexpressed in *Pichia pastoris*,[21] led to (*S*)-2-hydroxybut-3-enenitrile (**2**) in 95% yield. The enantiomer excess achieved by this reaction varies between 90 and 99%[22] and the material used in this synthesis had an *ee* of 91%. The cyanohydrin **2** was protected by treatment with *tert*-butyldiphenylsilyl chloride and imidazole following Brussee's procedure[23] to give product **3**, the *ee* of 91% being retained (Scheme 1). As reference material for HPLC measurements racemic cyanohydrin **3** was synthesised according to Gassmann[24] employing trimethylsilyl chloride followed by acidic hydrolysis and *tert*-butyldiphenylsilyl protection.

Grignard reaction of nitrile **3** with 10 mole equivalents of methylmagnesium iodide gave, in 81% yield, (*S*)-3-*tert*-butyldiphenylsilyloxypent-4-en-2-one (**4**). The enantiomeric excess slightly decreased to 87%. Previous attempts with 1.5 to



Scheme 1. *Reagents and conditions*: (*a*) (*S*)-hydroxynitrile lyase from *Hevea brasiliensis,* 95%; (*b*) TPSCl, imidazole, 99%; (*c*) MeMgI, 81%; (*d*) *m*CPBA, 4°C, 87%; (*e*) aq $HClO_4$, 93%; (*f*) 1. BnOH, BF_3. Et_2O, 2. TBAF, 33%.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

6 equivalents of methylmagnesium iodide resulted in lower yields. Grignard addition with commercially available methylmagnesium chloride (3 M solution in THF) led to the formation of side products.

Under optimised conditions ketone **4** was exposed to 3 mole equivalents of 3-chloroperoxybenzoic acid over 5 to 7 days at 4°C to give **5** and **6** in a 4 to 1 ratio, the desired *threo* epoxide **5** as the main product, in 87% combined yield. After column chromatography on silica gel, the *ee* of 4,5-anhydro-1-deoxy-D-xylulose (**5**) was ascertained to be 86%. Epoxide ring opening and hydrolysis of the *tert*-butyldiphenylsilyl group with perchloric acid led to the desired ketose **1** as an equilibrium mixture consisting of the open chain and the α/β furanoid form. The absolute configuration of compound **5** was determined by boron trifluoride etherate catalyzed epoxide ring opening with benzyl alcohol, followed by standard cleavage of the silyl protective group, to give known derivative **7**.[14] Unfortunately, this compound was afforded in only 33% yield and additional, more polar products were not isolated.

By the sequence described, **1** could be obtained in five steps starting from acrolein in 47% overall yield. This method is also suitable for large scale production. Recently an approach to **1** was published giving 69% overall yield starting from 2,3-isopropylidene-D-threitol.[16] However, the high price of this starting material is prohibitive for large scale synthesis. Our synthetic route is sufficiently versatile to incorporate isotopes of carbon or hydrogen. In addition the enantiomer of **1**, 1-deoxy-L-xylulose, should also be available by this method simply employing the (*R*)-hydroxynitrile lyase from *Linum usitatissimum*[25] or *Prunus amygdalus*. Further investigations have also been performed on the nucleophilic ring opening of epoxide **5** by phosphate. In contrast to relevant literature, the preparation of the desired 5-phosphate could not be achieved. Attempts to perform a nucleophilic epoxide opening with dipotassium hydrogenphosphate,[26] disodium hydrogenphosphate,[27] phosphoric acid[28] or dibenzyl hydrogenphosphate[29] in different solvents were also to no avail. In all cases no reaction took place or, after raising the reaction temperature, only decomposition of the starting material could be observed.

EXPERIMENTAL

General Methods. Optical rotations were measured using a Perkin Elmer 341 instrument at 589 nm at ambient temperature. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 200 MHz and Bruker MSL 300 MHz instruments. Residual non-deuterated solvent was used as an internal standard for determination of chemical shifts. The signals of protecting groups were in the expected regions and have not been listed explicitly. HPLC enantiomeric separations were performed using a Jasco 880-PU intelligent pump and a Jasco 875-UV intelligent UV/VIS detector connected to a CHIRACEL OD-H chiral HPLC column (25 cm × 0.46 cm at 20°C) as the chiral selector. The mobile phase was a mixture of *n*-heptane/2-propanol (99.75:0.25), 0.6 mL/min, with detection at 254 nm. TLC was performed on precoated aluminium plates (Merck 5554) employing 5% vanillin/

sulfuric acid as well as ceric ammonium molybdate as staining agents. For column chromatography, silica gel 60, 230–400 mesh (Merck 9385), was used. All reagents were obtained from Sigma-Aldrich and were used as purchased, except acrolein which was distilled under reduced pressure prior to use. A recombinant (*S*)-hydroxynitrile lyase was prepared by overexpression in *Pichia pastoris*. A crude cytosolic extract[30] was used for the biotransformation of acrolein.

(*S*)-2-*tert*-Butyldiphenylsilyloxybut-3-enenitrile (3). To a 10% solution of cyanohydrin **2** (4.43 g, 53.3 mmol) in DMF at 0°C, *tert*-butyldiphenylsilyl chloride (16.3 g, 58.0 mmol, 1.1 equiv) and imidazole (4.36 g, 64.0 mmol 1.2 equiv) were added. The mixture was allowed to warm to room temperature and stirred until TLC indicated completed conversion (12 to 18 h). The solution was concentrated under reduced pressure. The residue was partitioned between dichloromethane and 3% aq HCl, and the organic layer was washed with water and dried over sodium sulfate. After filtration, the filtrate was removed under reduced pressure and the remaining yellow oil was purified on silica gel, employing cyclohexane/ethyl acetate 20:1 v/v as the eluent, to give (**3**) (16.9 g, 99%) as a colourless oil: *ee* 91% (HPLC), $[\alpha]_D^{20}$ −29.0° (*c* 3.8, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.92 (ddd, 1 H, $J_{2,3}$ 5.5 Hz, $J_{3,4}$ 16.9 Hz, $J_{3,4'}$ 10.1 Hz, H-3), 5.52 (dd, 1 H, $J_{4,4'}$ 1.3 Hz, H-4), 5.37 (dd, 1 H, H-4′), 4.89 (m, 1 H, H-2); ^{13}C NMR δ 132.6 (C-3), 118.9 (C-4), 117.8 (C-1), 63.5 (C-2).

Anal. Calcd for $C_{20}H_{23}NOSi$ (321.50): C, 74.72; H, 7.21; N, 4.36. Found: C, 74.41; H, 7.03; N, 4.52.

(*S*)-3-*tert*-Butyldiphenylsilyloxypent-4-en-2-one (4). To a suspension of freshly prepared methylmagnesium iodide (Mg: 5.11 g, 210 mmol, 11 equiv; MeI: 26.5 g, 190 mmol, 10 equiv) in 150 mL diethyl ether, nitrile **3** (6.16 g, 19.2 mmol) in 70 mL of diethyl ether was added dropwise. The mixture was stirred and refluxed for 2 h and poured onto 200 g of ice, containing 10 mL of conc. HCl, and stirred for 10 min. The biphasic mixture was separated and the organic layer washed with water, dried, filtered and concentrated. The crude product was purified by column chromatography using a gradient mixture of cyclohexane/ethyl acetate (100:1–20:1 v/v) as the eluent which yielded methylketone **4** (5.24 g, 81%) as a colourless oil: *ee* 87% (HPLC), $[\alpha]_D^{20}$ −65.1° (*c* 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.84 (ddd, 1 H, $J_{3,4}$ 4.9 Hz, $J_{4,5}$ 17.1 Hz, $J_{4,5'}$ 10.5 Hz, H-4), 5.52 (dd, 1 H, $J_{5,5'}$ 1.7 Hz, H-5), 5.26 (dd, 1 H, H-5′), 4.62 (m, 1 H, H-3), 2.07 (s, 3 H, H-1); ^{13}C NMR δ 208.3 (C-2), 135.1 (C-4), 117.5 (C-5), 81.2 (C-3), 24.7 (C-1).

Anal. Calcd for $C_{21}H_{26}O_2Si$ (338.53): C, 74.51; H, 7.74. Found: C, 74.33; H, 7.92.

4,5-Anhydro-3-*tert*-butyldiphenylsilyl-1-deoxy-D-xylulose (5) and **4,5-anhydro-3-*tert*-butyldiphenylsilyl-1-deoxy-D-ribulose (6).** To a solution of **4** (1.21 g, 3.6 mmol) in 40 mL of dichloromethane at 0°C, 3-chloroperoxybenzoic acid ~ 70% (2.64 g, 10.7 mmol, 3 equiv) was added. The solution was placed in a

refrigerator at 4°C. After complete conversion, monitored by TLC (7–9 days), the pale white suspension was warmed to room temperature and sodium sulfite was added with stirring until the foaming subsided. The solids were filtered off, the filtrate was washed consecutively with 5% aq HCl and water. The organic phase was dried, filtered and concentrated. The crude mixture of *threo*- and *erythro*-epoxides (4:1, as detected by ^{1}H NMR) was purified and separated by column chromatography employing cyclohexane/ethyl acetate (250:1 v/v) as the eluent. Compound **5** *threo*-epoxide (830 mg, 66%) as a colourless oil: *ee* 86% (HPLC) $[\alpha]_D^{20}$ −18.3° (*c* 0.9, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.80 (d, 1 H, $J_{3,4}$ 4.7 Hz, H-3), 3.23 (m, 1 H, H-4), 2.77 (bd, 2 H, *J* 2.9 Hz, H-5,5′), 1.77 (s, 3 H, H-1); ^{13}C NMR δ 169.1 (C-2), 92.2 (C-3), 52.8 (C-4), 44.3 (C-5), 26.7 (C-1).

Compound **6** *erythro*-epoxide (250 mg, 21%) as a slightly yellow oil: $[\alpha]_D^{20}$−9.3° (*c* 0.7, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.85 (d, 1 H, $J_{3,4}$ 4.5 Hz, H-3), 3.17 (m, 1 H, H-4), 2.70 (m, 2 H, H-5,5′), 1.88 (s, 3 H, H-1); ^{13}C NMR δ 169.2 (C-2), 91.4 (C-3), 52.3 (C-4), 43.9 (C-5), 26.7 (C-1).

Anal. Calcd for $C_{21}H_{26}O_3Si$ (354.53): C, 71.15; H, 7.39. Found: **5** C, 69.88; H, 7.17; **6** C, 70.02, H, 7.34.

5-*O*-Benzyl-1-deoxy-D-xylulose (7). To a solution of epoxide **5** (300 mg, 0.846 mmol) in 15 mL of dichloromethane at 0°C, freshly distilled benzyl alcohol (0.18 mL, 1.74 mmol, 2.1 equiv), a catalytic amount of boron trifluoride etherate and molecular sieve 3Å (300 mg) were added. The solution was placed in a refrigerator at 4°C overnight. The solids were filtered off and the filtrate was partitioned between dichloromethane and 3% aq HCl. The organic layer was washed with water, dried and concentrated under reduced pressure. Conventional silyl deprotection with tetrabutylammonium fluoride yielded, after removal of solvent under reduced pressure, a yellow oil. This was purified on silica gel, employing a gradient mixture of cyclohexane/ethyl acetate (50:1–5:1 v/v) as the eluent, to give the benzyl protected xylulose **7** (127 mg, 33%) as a colourless oil: $[\alpha]_D^{20}$ +51.1° (*c* 0.7, chloroform). Lit:[14] $[\alpha]_D^{20}$ +52.5° (*c* 1.2, dichloromethane); ^{1}H NMR and ^{13}C NMR spectra were identical.

1-Deoxy-D-xylulose (1). Epoxide **5** (360 mg, 1.02 mmol) was disssolved in a mixture of 70% perchloric acid, water and acetonitrile (15 mL, 1:10:4) and was stirred at 50°C for 14–16 h. The mixture was cooled, neutralised with solid Na_2CO_3, the solvent removed in vacuo and purified on silica gel using chloroform/methanol 10:1 v/v as the eluent, to give **1** (126 mg, 93%) as a colourless syrupy mixture of the furanoid anomers and open chain form: ^{1}H NMR and ^{13}C NMR spectra were identical to those published in literature.[16]

ACKNOWLEDGMENT

We are indebted to DSM Fine Chemicals Austria for financial support.

REFERENCES

1. The systematic name according to the IUPAC Recommendations 1996 is 1-deoxy-D-*threo*-pent-2-ulose.
2. Slechta, L.; Johnson, L.; Le Roy, E. A New Metabolite *from Streptomyces hygroscpicus*. J. Antibiot. **1976**, *29*, 685–687.
3. David, S.; Estramareix, B.; Fischer, J.-C.; Therisod, M. The Biosynthesis of Thiamine. J. Chem. Soc., Perkin Trans. 1 **1982**, 2131–2137.
4. Hill, R.E.; Sayer, B.G.; Spenser, I.D. Biosynthesis of Vitamin B_6: Incorporation of D-1-Deoxyxylulose. J. Am. Chem. Soc. **1989**, *111*, 1916–1917.
5. Himmeldirk, K.; Kennedy, I.A.; Hill, R.E.; Sayer, B.G.; Spencer, I.D. Biosynthesis of vitamins B_1 and B_6 in *Escherichia coli*: concurrent incorporation of 1-deoxy-D-xylulose into thiamin (B_1) and pyridoxol (B_6). J. Chem. Soc., Chem. Commun. **1996**, 1187–1188.
6. Cane, D.E.; Du, S.; Robinson, J.K.; Hsiung, Y.; Spencer, I.D. Biosynthesis of Vitamin B_6: Enzymatic Conversion of 1-Deoxy-D-xylulose-5-phosphate to Pyridoxol Phosphate. J. Am. Chem. Soc. **1999**, *121*, 7722–7723.
7. Schwender, J.; Seemann, M.; Lichtenthaler, H.K.; Rhomer, M. Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophylls and plastoquinone) via a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga *Scenedesmus obliquus*. Biochem. J. **1996,** *316*, 73–80.
8. Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. Glyceraldehyde 3-Phosphate and Pyruvate as Precursors of Isoprenic Units in an Alternative Non-mevalonate Pathway for Terpenoid Biosynthesis. J. Am. Chem. Soc. **1996**, *118*, 2564–2566.
9. Piel, J.; Donath, J.; Bandemer, K.; Boland, W. Induzierte und konstitutiv emittierte Pflanzendüfte: Mevalonat-unabhängige Biosynthese terpenoider Duftstoffe. Angew. Chem. **1998**, *110* (18), 2622–2625; Angew. Chem. Int. Ed. Engl. **1998**, *37*, 2478–2481.
10. Lichtenthaler, H.K. The plants' 1-deoxy-D-xylulose-5-phosphate pathway for biosynthesis of isoprenoids. Fett Lipid **1998**, *100* (4–5), 128–138.
11. Lichtenthaler, H.K. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **1999**, *50*, 47–65.
12. Yokota, A.; Sasajima, K. Formation of 1-Deoxy-D-threo-pentulose and 1-Deoxy-L-threo-pentulose by Cell-free Extracts of Microorganisms. Agric. Biol. Chem. **1984**, *48* (1), 149–158.
13. Backstrom, A.D.; McMordie, R.A.S.; Begley, T.P. A New Synthesis of 1-Deoxy-D-threo-2-pentulose, a Biosynthetic Precursor to the Thiazole Moiety of Thiamin. J. Carbohydr. Chem. **1995**, *14* (1), 171–175.
14. Giner, J.L. New and Efficient Synthetic Routes to 1-Deoxy-D-xylulose. Tetrahedron Lett. **1998**, *39*, 2479–2482.
15. L-enantiomer: Shabat, D.; List, B.; Lerner, R.A.; Barbas III; C.F. A Short Enantioselective Synthesis of 1-Deoxy-L-xylulose by Antibody Catalysis. Tetrahedron Lett. **1999**, *40*, 1437–1440.
16. Blagg, B.S.J.; Poulter, C.D. Synthesis of 1-Deoxy-D-xylulose and 1-Deoxy-D-xylulose-5-phosphate. J. Org. Chem. **1999**, *64*, 1508–1511.
17. Piel, J.; Boland, W. Highly Efficient and Versatile Synthesis of Isotopically Labelled 1-Deoxy-D-xylulose. Tetrahedron Lett. **1997**, *38* (36), 6387–6390.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

18. Zeidler, J.G.; Lichtenthaler, H.K.; May, H.U.; Lichtenthaler, F.W. Is Isoprene Emitted by Plants Synthesized via the Novel Isopentenyl Pyrophosphate Pathway? Z. Naturforsch., C: J. Biosci. **1997**, *52* (1/2), 15–23.
19. Putra, S.R.; Lois, L.M.; Campos, N.; Boronat, A.; Rohmer, M. Incorporation of [2,3-$^{13}C_2$]- and [2,4-$^{13}C_2$]-D-1-Deoxyxylulose into Ubiquinone of *Escherichia coli* via the Mevalonate-Independent Pathway for Isoprenoid Biosynthesis. Tetrahedron Lett. **1998**, *39*, 23–26.
20. Jux, A.; Boland, W. Improved Protocol towards Isotopically Labelled 1-Deoxy-D-xylulose. Tetrahedron Lett. **1999**, *40*, 6913–6914.
21. Hasslacher, M.; Schall, M.; Hayn, M.; Griengl, H.; Kohlwein, S.D.; Schwab, H. Molecular Cloning of the Full Length cDNA of (*S*)-Hydroxynitrile Lyase from *Hevea brasiliensis*, Functional Expression in *E. coli* and Identification of an Active Site Residue. J. Biol. Chem. **1996**, *271* (10), 5884–5891.
22. Klempier, N.; Pichler, U.; Griengl, H. Synthesis of α,β-unsaturated (*S*)-Cyanohydrins using the Oxynitrilase from *Hevea Brasiliensis*. Tetrahedron: Asymmetry **1995**, *6* (4), 845–848.
23. Brussee, J.; Loos, W.T.; Kruse, C.G.; Van der Gen, A. Synthesis of Optically Active Silyl Protected Cyanohydrins. Tetrahedron **1990**, *46* (3), 979–986.
24. Gassman, P.G.; Talley, J.J. Cyanohydrins–A General Synthesis. Tetrahedron Lett. **1978**, *40*, 3773–3776.
25. Trummler, K.; Roos, J.; Schwaneberg, U.; Effenberger, F.; Förster, S.; Pfizenmaier, K.; Wajant, H. Expression of the Zn^{2+}-containing hydroxynitrile lyase from flax (*Linum usitatissimum*) in *Pichia pastoris*–utilization of the recombinant enzyme for enzymatic analysis and site-directed mutagenesis. Plant Sci. **1998**, *139* (1), 19–27.
26. a) Lampson, G.P.; Lardy, H.A. Phosphoric Esters of Biological Importance. J. Biol. Chem. **1949**, *181*, 693–696; b) Pitsch, S.; Pombo-Villar, E; Eschenmoser, A. Über die Bildung von 2-Oxoethyl-phosphaten ('Glycolaldehyd-phosphaten') aus *rac*-Oxirancarbonitril und anorganischem Phosphat und über (formale) konstitutionelle Zusammenhänge zwischen 2-Oxoethyl-phosphaten und Oligo(hexo- und pentopyranosyl) nucleotid-Rückgraten. Helv. Chim. Acta **1994**, *77*, 2251–2285; c) Guerard, C.; Alphand, V.; Archelas, A.; Demuynck, C.; Hecquet, L.; Furstoss, R.; Bolte, J. Transketolase-Mediated Synthesis of 4-Deoxy-D-fructose-6-Phosphate by Epoxide Hydrolase-Catalysed Resolution of 1,1-Diethoxy-3,4-epoxybutane. Eur. J. Org. Chem. **1999**, 3399–3402.
27. Barbas, C.F.; Wang, Y.-F.; Wong, C.-H. Deoxyribose-5-phosphate Aldolase as a Synthetic Catalyst. J. Am. Chem. Soc. **1990**, *112*, 2013–2014.
28. Eidebenz, E.; Depner, M.; Jodhaltige aliphatische Phosphorsäureester. Arch. Pharm. **1942**, 227–231.
29. Harvey, W.E.; Michalski, J.J.; Todd, A.R. Studies on Phosphorylation. J. Chem. Soc. **1951**, 2271–2278.
30. Hasslacher, M.; Schall, M.; Hayn, M.; Kohlwein, S.D.; Griengl, H. High-level intracellular expression of hydroxynitrile lyase from the tropical rubber *tree Hevea brasiliensis* in microbial hosts. Protein Express. Purif. **1997**, *11* (1), 61–71.

Received October 17, 2000
Accepted September 24, 2001

Request Permission or Order Reprints Instantly!