A Method for Chiral Thiophosphonate Synthesis and Its Use in Elucidating the Phosphorus Stereochemistry of the Hydrolytic C-P Bond Cleavage Reaction of Thiophosphonoacetaldehyde

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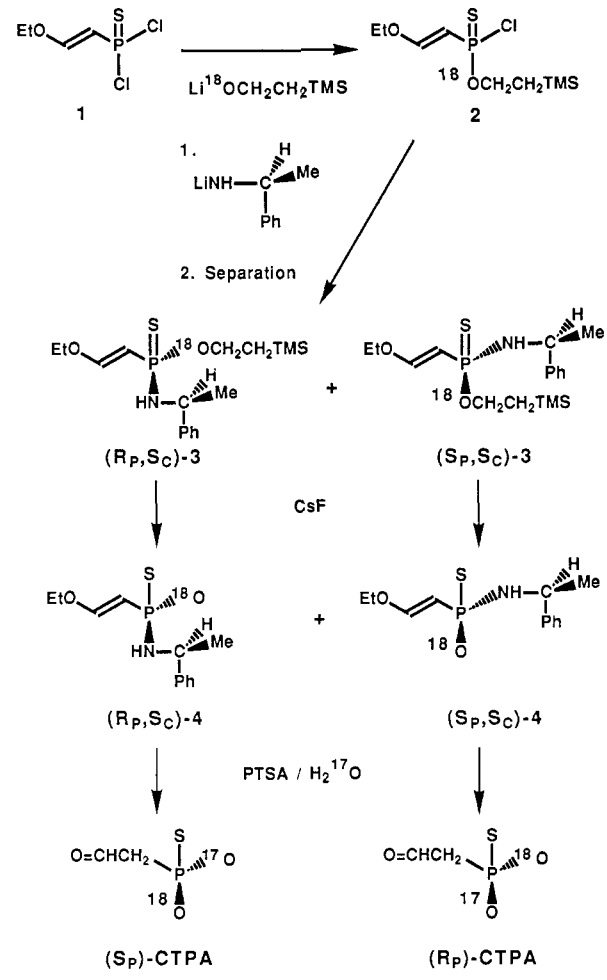
Received August 7, 1990

Summary: The enzyme phosphonoacetaldehyde hydrolase (phosphonatase) catalyzes the conversion of phosphonoacetaldehyde to phosphate and acetaldehyde. Previous studies have demonstrated that phosphonatase labilizes the C-P bond in this process by forming a protonated Schiff base between an active site lysine and the carbonyl group of phosphonoacetaldehyde. The synthesis of potential stereochemical probes of this C-P bond cleaving reaction, the chiral [^{18}O,^{17}O]thiophosphonoacetaldehyde enantiomers, and the stereochemical course of their aniline-catalyzed hydrolyses to yield acetaldehyde and [^{18}O,^{17}O,^{16}O]thiophosphate are described.

The advent of C-P bond containing herbicides, pesticides, antibiotics, and neurotoxins, together with the discovery of naturally occurring phosphonates, have promoted interest in the biological degradation of these compounds.[1] To date, two fundamentally different C-P bond cleaving pathways in bacteria have been uncovered. C-P Lyase[2] transforms a number of alkylphosphonates into their respective hydrocarbons and orthophosphate via radical mechanisms.[3] In contrast phosphonatase, a component of the (2-aminoethyl)phosphonate metabolizing pathway,[4,5] catalyzes the cleavage of phosphonoacetaldehyde to acetaldehyde and orthophosphate via a polar Schiff base mechanism.[5]

Central to delineating the mechanisms of phosphoryl transfer from organophosphates has been the determination of the stereochemical course of the reactions at phosphorus.[6] We now report a method for elucidating the phosphorus stereochemistry in the hydrolytic C-P bond cleavage reactions of phosphonates which is based on the preparation of chiral [^{18}O,^{17}O]thiophosphonates.[7] Herein, we describe the syntheses and configuration assignments of the enantiomers of chiral [^{18}O,^{17}O]thiophosphonoacetaldehyde (CTPA) and the stereochemical course of their aniline-catalyzed hydrolysis to form chiral [^{18}O,^{17}O,^{16}O]-thiophosphate (CTP).

Scheme I



The antipodes of CTPA were prepared by the sequence shown in Scheme I beginning with the (ethoxyvinyl)thiophosphoric dichloride **1**. Treatment of **1** with 1 equiv of Li[^{18}O]-β-(trimethylsilyl)ethoxide[9] gave the monoester **2** (74%). Reaction of the phosphonyl chloride **2** with the lithium (*S*)-α-methylbenzylamide yielded a mixture of the diastereomeric thiophosphonamides **3** (76%). The individual diastereomers of **3** (separated by HPLC) were treated with cesium fluoride to induce TMS-ethyl ester C-O bond cleavage yielding (ca. 100%) the cesium salts (R_P,S_C)-**4** and (S_P,S_C)-**4**. The absolute configuration at phosphorus in these substances was determined by X-ray analysis[10] of the crystalline 9-anthracenylmethyl thioester, (S_P,S_C)-**5**, formed from the ^{16}O analogue of S_P,S_C diastereomer.

(1) Hildebrand, R. L., Ed. *The Role of Phosphonates in Living Systems*; CRC Press: Boca Raton, 1983.

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(3) Cordeno, M. L.; Pompliano, D. L.; Frost, J. W. *J. Am. Chem. Soc.* **1986**, *108*, 332. Frost, J. W.; Loo, S.; Cordeiro, M. L.; Li, D. *J. Am. Chem. Soc.* **1987**, *109*, 2166. Avila, L. Z.; Loo, S. H.; Frost, J. W. *J. Am. Chem. Soc.* **1987**, *109*, 6758. Avila, L. Z.; Frost, J. W. *J. Am. Chem. Soc.* **1988**, *110*, 7904. Shames, S. L.; Wackett, L. P.; LaBarge, M. S.; Kuczkowski, R. L.; Walsh, C. T. *Bioorg. Chem.* **1987**, *15*, 366.

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(5) La Nauze, J. M.; Rosenberg, H.; Shaw, D. C. *Biochim. Biophys. Acta* **1970**, *212*, 332. LaNauze, J. M.; Coggins, J. R.; Dixon, H. B. F. *Biochem. J.* **1977**, *165*, 409. Olsen, D. B.; Hepburn, T. W.; Moos, M.; Mariano, P. S.; Dunaway-Mariano, D. *Biochemistry* **1988**, *27*, 2229. Dumora, C.; Lacoste, A. M.; Cassaigne, A. *Biochim. Biophys. Acta* **1989**, *997*, 193.

(6) For a review, see: Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877.

(7) Thiophosphonoacetaldehyde is an alternate substrate for phosphonatase (k_{cat} = 50 min^{-1}; K_m = 25 μM).[8] We are optimistic that thiophosphonates will substitute for phosphonates in other enzymic hydrolytic C-P bond cleavage reactions.

(8) Hepburn, T. W. Ph.D. Dissertation, University of Maryland, 1988.

(9) (a) The [^{18}O](trimethylsilyl)ethanol was prepared (83%) by oxymercuration of vinyltrimethylsilane in H$_2$^{18}O/THF by use of the method of Soderquist (ref 9b). Both GC/MS and ^{13}C NMR analysis indicated that this alcohol had >85% ^{18}O enrichment. (b) Soderquist, J. A.; Thompson, K. L. *J. Organomet. Chem.* **1978**, *159*, 237.

(10) The crystallographic data will be reported in a full paper on this subject.

0022-3263/90/1955-5435$02.50/0

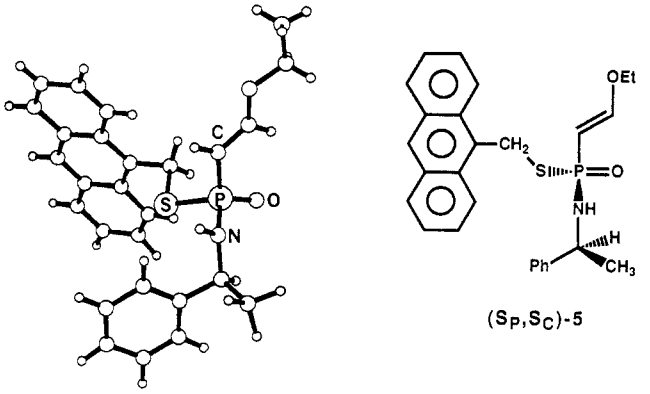


Figure 1. Computer-generated drawing resulting from X-ray crystallographic analysis of (S_P,S_C)-**5** formed by reaction of the ^{16}O analogue of (S_PS_C)-**4** with 9-(chloromethyl)anthracene.

reomer of **4** (Figure 1). Since acid hydrolysis of phosphonamidic acids is known to proceed with predominant inversion of phosphorus configuration,[11] treatment of the diasteromers of **4** with 1.5 M PTSA in $THF/H_2{}^{17}O$[12] yielded the respective enantiomers of [^{18}O,^{17}O]thiophosphonoacetaldehyde, (S_P)- and (R_P)-CTPA.

The phosphonatase-catalyzed C–P bond cleavage reaction is known to occur on a protonated, active-site lysine and phosphonoacetaldehyde (or thiophosphonoacetaldehyde)[7,8] Schiff base. Thus, the aniline-catalyzed dethiophosphonylation of CTPA was investigated as a chemical model of the enzymatic process. Reactions of the CTPA enantiomers with a large excess of aniline were run at pH 8, one found to be optimal for phosphonatase activity.[13] Whereas thiophosphonoacetaldehyde is stable in pH 8 buffer for several days, in the presence of 0.22 M aniline the CTPA enantiomers (12 mM) are quantitatively converted to CTP within 3 h at 25 °C.

The absolute configuration of the [^{18}O,^{17}O,^{16}O]thiophosphate generated in each reaction was assigned by using a procedure adapted from those described by Webb and Trentham[14] and Tsai.[15] Accordingly, the thiophosphate product was transformed into (S_P)-[β-^{18}O,^{17}O,^{16}O]ATPβS (65% yield) by an enzymatic reaction sequence with overall retention of the phosphorus configuration. The P_β regions of the ^{31}P NMR spectra of the ATPβS isomers produced from (R_P)-CTPA and (S_P)-CTPA by this sequence are shown in Figure 2, parts a and b, respectively. The major resonance displayed in Figure 2a arises from ATPβS containing ^{18}O in the nonbridging position, thus indicating that the (S_P)-CTP enantiomer results from dephosphonylation of (R_P)-CTPA predominantly. The major resonance seen in Figure 2b arises from ATPβS containing ^{18}O in the bridging position thus indicating that (R_P)-CTP derives from (S_P)-CTPA predominantly. After taking into account the percent of ^{17}O enrichment in the starting CTPA enantiomers, the extent of isotope washout (ca. 10%) occurring in the hydrolysis and or ensuing enzymic reactions, and the enantiomeric purity (ca. 60% ee) of the CTPA isomers used,[16] these data

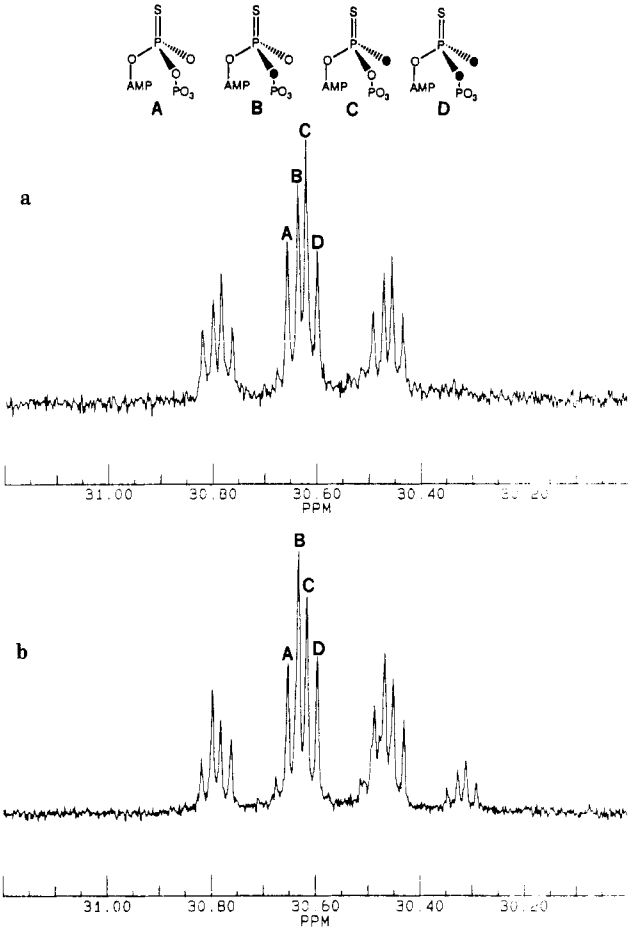
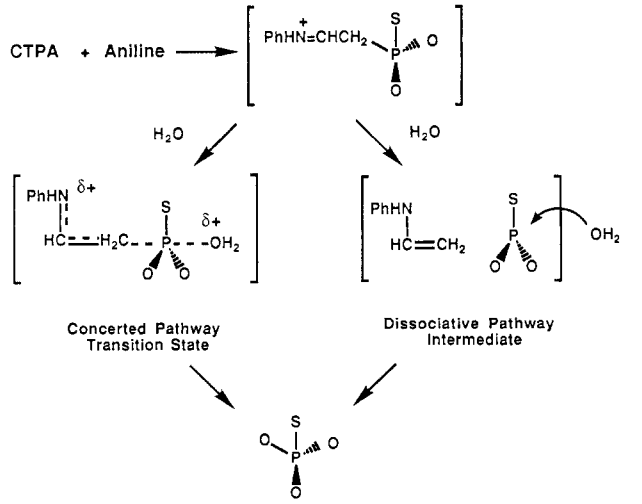


Figure 2. The β-P region of the ^{31}P NMR spectrum of (S_P)-[β-^{18}O,^{17}O]ATPβS derived from the CTP enantiomer arising from (R_P)-CTPA (a) and (S_P)-CTPA (b). Chemical shifts are in ppm relative to 85% H_3PO_4 in D_2O (external standard). The spectra are from a Brucker AM-400 spectrometer (162.04 Hz, 25 °C, deuterium field lock, spectral width 8333 Hz, aquisition time 3.9 μs, pulse width of 12.0 μs). The signals centered at 30.3 ppm in spectrum (b) are for the β-P resonances for the R_P isomer formed from ADPβS in the pyruvate kinase reaction.

Scheme II



demonstrate that the aniline-catalyzed dethiophosphonylation of CTPA occurs with ca. 90% inversion

(11) Cooper, D. B.; Harrison; J. M.; Inch, T. D. *Tetrahedron Lett.* **1974**, *31*, 2697. Harrison, J. M.; Inch, T. D.; Lewis, G. I. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1982. Because P–N bond cleavage precedes hydrolysis of the enol ether moiety (by NMR) the possibility of intramolecular assistance of phosphonamide hydrolysis by the hydrate form of the aldehyde seems unlikely.

(12) Isotope distribution was 48.6% $H_2{}^{17}O$, 30.7% $H_2{}^{18}O$, 20.7% $H_2{}^{16}O$.

(13) Olsen, D. B. Ph.D. Dissertation, University of Maryland, 1988.

(14) Webb, M. R.; Trentham, P. R. *J. Biol. Chem.* **1980**, *255*, 1775. Webb, M. R. In *Methods Enzymol.* **1982**, *87*, 301.

(15) Tsai, M. D. *Biochemistry* **1980**, *19*, 5310.

(16) (a) The enantiomer purities of the CTPA antipodes were determined by using the method of Cullis et al. (ref 16b) to be ca. 60% ee; Cullis, P. M.; Iagrossi, A.; Rous, A. J. *J. Am. Chem. Soc.* **1986**, *108*, 7869.

of configuration at phosphorus.

These results suggest that the mechanism for C–P bond cleavage in the protonated, aniline–CTPA Shiff base intermediate involves either a concerted displacement with an in-line arrangement of nucleophile (H_2O) and leaving group ($PhNHCH{=}CH_2$) or a dissociative process via a tightly paired metathiophosphate intermediate (Scheme II).[17] The stereochemical course of the enzyme catalyzed CTPA reaction is now being probed by use of this potentially general methodology.

Acknowledgment. Financial assistance was provided by NIH Grants GM-28688 (D.D.M.) and GM-27251 (P. S.M) and a grant from the Center of Agricultural Biotechnology at the University of Maryland. We are indebted to Dr. Joseph Ferrara (Molecular Structure Corporation) and Professor Herman Ammon (University of Maryland) for carrying out the X-ray analysis. William H. Swartz provided expert technical assistance during the course of this research.

(17) Hydrolyses of chiral thiophosphate esters occur with inversion of configuration accompanied, to varying degrees, by racemization.[18] Pressure effects on the rate of hydrolysis of 2,4-dinitrophenyl thiophosphate dianion gives evidence for a dissociation mechanism.[19]

(18) Cullis, P. M.; Misra, R.; Wilkins, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 1594.

(19) Burgess, J.; Blundell, N.; Cullis, P. M.; Hubbard, C. D.; Misra, R. *J. Am. Chem. Soc.* **1988**, *110*, 7900.

Stereoselective Synthesis of α-Alkyl α-Amino Acids. Alkylation of 3-Substituted 5*H*,10b*H*-Oxazolo[3,2-*c*][1,3]benzoxazine-2(3*H*),5-diones

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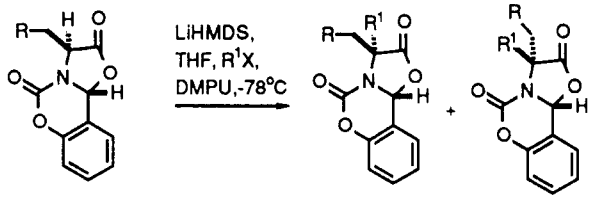
Received May 16, 1990

Summary: The alkylation of 3-methyl-, 3-benzyl-, or 3-isobutyl-5*H*,10b*H*-oxazolo[3,2-*c*][1,3]benzoxazine-2-(3*H*),5-dione proceeded with retention of configuration (83 to >97% ds), and the resulting products were hydrolyzed to afford α-alkyl α-amino acids.

In connection with our recent synthesis of dipeptide isosteres containing γ- or δ-lactams, we needed an expedient route to multigram quantities of optically pure (*S*)-α-allylphenylalanine 1.[3] Schollkopf reported a synthesis of 1 [90% ee (*S*)] in 1978 and to our knowledge no other synthesis has been reported.[4] Since that initial report of Schollkopf, a number of routes to α-alkyl α-amino acids have appeared in the literature.[5] However, a smaller number of these routes have addressed the synthesis of α-allylated amino acids.[6] In this paper, we report a general and efficient synthesis of α-alkyl α-amino acids which is also suitable for the preparation of α-allylated amino acids.

The starting material for our synthesis was reported in 1971 by Block and Faulkner as part of their work on peptide coupling reactions.[7] They showed that the condensation of various amino acids with salicylaldehyde and phosgene produced oxazolidinones **2a–c** in 65–70% yield (Scheme I). These compounds are tricyclic versions of the oxazolidinones that Seebach and others have used for the synthesis of α-alkyl α-amino acids.[5a–e] Block and Faulkner found that treatment of **2a** with 1 equiv of *n*-hexylamine resulted in the immediate precipitation of the corresponding carboxamide–urea **3** in 47% yield. None of the expected product **4** was isolated, and this approach to peptide synthesis was abandoned. Upon seeing this result, we reasoned that the addition of hydroxide ion to an alkylated derivative of **2a–c** should also occur and that this would directly lead to the desired amino acid (eq 1).

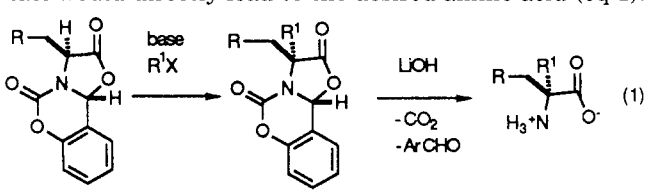
Table I. Survey of Electrophiles



2a-c **5** major **5'** minor

entry	R	R^1X	ratio[a] 5/5'	% yield[b]
1	Ph	allyl bromide	>31:1	94
2	Ph	ethyl iodide	31:1	74
3	Ph	methyl iodide	8:1	66
4	*i*-Pr	allyl bromide	>31:1	81
5	*i*-Pr	methyl iodide	5:1	82
6	H	allyl bromide	11:1	74

[a] Ratios determined by analysis of 300-MHz NMR spectrum. [b] Yield refers to single isomers except as noted in text.



(1)

(1) Cardiovascular Research Division.

(2) Analytical Research Division. Author to whom inquiries regarding the crystal structure should be sent.

(3) Zydowsky, T. M.; Dellaria, J. F.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607.

(4) Schollkopf, U.; Hausberg, H. H.; Hoppe, I.; Segal, M.; Reiter, U. *Angew. Chem., Intl. Ed. Engl.* **1978**, *17*(2), 117.

(5) (a) Fitzi, R.; Seebach, D. *Tetrahedron* **1988**, *44*(17), 1988. (b) Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243. (c) Fadel, A.; Salaun, J. *Tetrahedron Lett.* **1987**, *28*(20), 2243. (d) Ojima, I.; Chen, H.-J. C. *Tetrahedron* **1988**, 5307. (e) Karady, S.; Amato, J. S.; Weinstock, L. M. *Tetrahedron Lett.* **1984**, *25*(39), 4337. (f) Kruizinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. *J. Org. Chem.* **1988**, *53*(8), 1826. (g) Bajgrowicz, J. A.; Cossec, B.; Pigiere, C.; Jacquier, R.; Villefont, P. *Tetrahedron. Lett.* **1983**, *24*(35), 3721. (h) Schollkopf, U.; Groth, U.; Westphalen, K.-O.; Deng, C. *Synthesis* **1981**, 969. (i) Schollkopf, U.; Busse, U.; Kilger, R.; Lehr, P. *Synthesis* **1984**, 271. (j) Belokon, Y. N.; Cheronoglazova, N. I.; Kochetkov, C. A.; Garblinskaya, N. S.; Belikov, V. M. *J. Chem. Soc., Chem. Commun.* **1985**, 171. (k) Ihara, I.; Takahashi, M.; Niitsuma, H.; Taniguchi, N.; Yasui, K.; Fukumoto, K. *J. Org. Chem.* **1989**, *54*, 5413. (l) Schmidt, U.; Respondek, M.; Lieberkncht, A.; Werner, J.; Fischer, P. *Synthesis* **1989**, 256.

(6) The oxazolidinone and imidazolidinone-based methods listed in ref 5 require strong acid or catalytic hydrogenolysis for final deprotection of alkylated intermediates. The reactivity of the allyl group under these conditions precludes their use. An oxazolidinone-based method which employs mild basic conditions for final deprotection would expand the scope of this methodology.

(7) Block, H.; Faulkner, P. J. *J. Chem. Soc. C* **1971**, 329.

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