

Stereochemical Study of Phosphonothioate Cleavage by a Metallomicelle

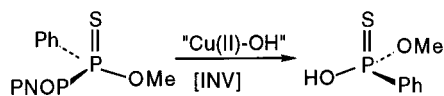
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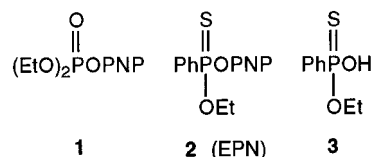
ABSTRACT



The copper metallomicellar hydrolysis of *O*-methyl *O*-(4-nitrophenyl) phenylphosphonothioate to *O*-methyl phenylphosphonothioic acid takes place with effectively complete inversion at phosphorus.

The recent anthrax attack in the United States greatly intensified interest in biological and chemical defense research. In this regard, efficient chemical destruction of phosphonate and phosphonothioate nerve agents and related toxins remains a matter of urgency.^{1,2} Not only relatively simple decontamination reagents, such as (micellar) *o*-iodosobenzoate,^{1,3} but also biological catalysts, including phosphotriesterases⁴ and tailored antibodies,⁵ have been effectively deployed.

The zinc phosphotriesterase from *Pseudomonas diminuta*, for example, catalyzes the hydrolyses of paraoxon (**1**),⁶ EPN (**2**),⁶ other phosphorus-based insecticides and fluorophosphonate nerve agents such as sarin and soman.⁴ Enzymatic



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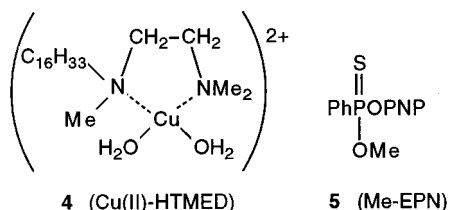
hydrolysis of EPN to *O*-ethyl phenylphosphonothioic acid (**3**) occurs with *inversion* at phosphorus, considered a consequence of “in-line displacement” by a zinc-activated water molecule at the backside of the chiral P; i.e., an S_N2(P) mechanism. Simultaneously, the phosphoryl oxygen coordinates to a second Zn in the enzyme’s active site.^{4a,d,g} The phosphotriesterase hydrolysis, moreover, is stereospecific; only the (*S*_P) enantiomer of EPN is hydrolyzed at an appreciable rate.^{4a}

Cleavages of phosphonates **1** or **2**, which carry the good *p*-nitrophenolate leaving group, are computed to occur by a single-step, S_N2(P) process, whereas phosphonates with poor leaving groups (e.g., fluoride, alkoxide) are computed to

(6) PNP = *p*-nitrophenyl; EPN = *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate.

cleave by a two-step addition–elimination mechanism that transits a trigonal bipyramidal intermediate.⁷ Nerve agents sarin and soman belong to the latter group.

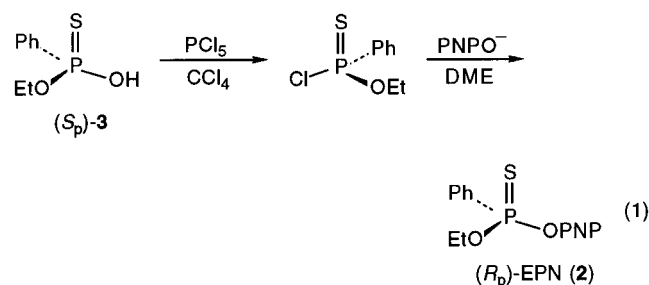
Metallomicelles can serve as models for metalloenzymes. The simple Cu(II) complex of *N*-*n*-hexadecyl-*N,N',N'*-trimethylethylenediamine (**4**, Cu(II)-HTMED)⁸ in cetyltrimethylammonium (CTA) ion micelles is highly reactive and catalytic for the hydrolysis of a variety of *p*-nitrophenyl phosphate substrates,^{9a} 2,4-dinitrophenyl phosphate and phosphonate substrates, and sarin.^{9b} More recently, we found that excess **4** in CTA micellar solution at pH 8 cleaves **5**,



the methyl analogue of EPN, with $k = 0.012 \text{ s}^{-1}$, an acceleration of ~ 2400 over (CTA)Cl, pH 8 hydrolysis.^{1b,10}

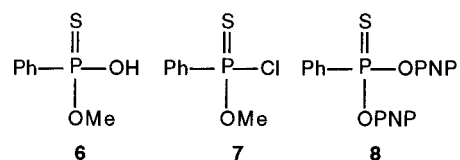
In the phosphoryltyc reactions of micellar **4**, the actual nucleophile is a Cu-bound hydroxide.^{9a} Thus, there is some mechanistic parallel between the nucleophilic hydrolysis of **2** or **5** by the phosphotriesterase (Zn-OH) and metallomicellar **4** (Cu-OH). Will this parallel persist in the stereochemical course of the hydrolysis of Me-EPN (**5**) by **4**? Here we present the initial stereochemical study of phosphonothioate cleavage by a metallomicelle.

Our first task was the preparation of the enantiomers of Me-EPN.¹¹ Raushel et al. resolved racemic phosphonothioic acid **3** by fractional crystallization of its diastereomeric salts with (*R*)- or (*S*)- α -methylbenzylamine.^{4a} Then, (*R*_P)- or (*S*_P)-**3** was converted to EPN by the sequence of eq 1. Both steps in eq 1 are considered to occur with inversion at P.^{4a}



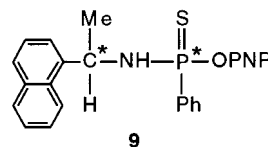
We attempted to apply this methodology to enantiospecific syntheses of Me-EPN. Resolution of *rac*-*O*-methyl phenylphosphonothioic acid (**6**) was achieved by procedures similar to those of Raushel^{4a} and DeBruin,¹² affording both (*R*_P)-**6**

and (*S*_P)-**6** with >90% ee. In our hands, however, conversion



of **6** to chloridithioate **7** with PCl₅ in CH₂Cl₂ afforded **7** with only 37% ee; similar results were reported by DeBruin (20–44% ee).¹² Successive reactions of **6** with SO₂Cl₂ and PCl₅ in benzene¹³ gave **7** with 26.4% ee. Although we could complete the sequence of eq 1, converting **7** to Me-EPN by reaction with sodium *p*-nitrophenylate in DME,¹⁴ we opted to develop an alternative, more stereoselective synthesis of Me-EPN.

Our strategy derived from work of Inch,¹⁵ Koizumi,¹⁶ and Purnanand¹⁷ and involved the synthesis and separation of diastereomeric phosphonamidothioates followed by stereoselective acidic methanolysis to Me-EPN. Thus, bis-*O*-(4-nitrophenyl) phenylphosphonothioate (**8**)¹⁸ reacted (25 °C, 3 d) with excess racemic 1-(1-naphthyl)ethylamine and DBU¹⁹ in dry CH₂Cl₂ to afford a mixture of the four diastereomers of **9**, *N*-1-(1-naphthyl)ethylamino-*O*-(4-nitrophenyl) phenylphosphonothioate.



Chromatography (silica gel, CH₂Cl₂ eluent), followed by fractional crystallization from diethyl ether/petroleum ether, gave 18.5% of (*S*_P*S*_C)/(*R*_P*R*_C)-**9** (mp 127–128 °C). The structure and absolute configuration were verified by X-ray diffraction; cf. Figure 1, in which the (*S*_P*S*_C) enantiomer is

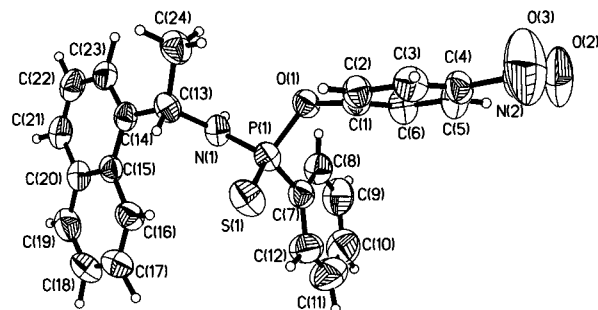


Figure 1. ORTEP rendering of (*S*_P*S*_C)-**9**. Crystallographic information has been submitted as Supporting Information.

illustrated. (*S*_P*S*_C)/(*R*_P*R*_C)-**9** exhibited a ³¹P NMR resonance in CDCl₃ at δ 73.89 (vs external 85% H₃PO₄) and was 98.2% pure (from the NMR analysis). From the mother liquor, we

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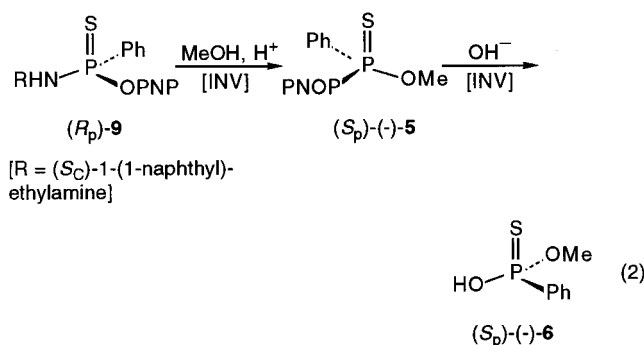
(8) This reagent was introduced as the tetradecyl derivative: Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 2800.

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subsequently crystallized 15% of pure (*S_PR_C*)/(*R_PS_C*)-**9** (mp 105–107 °C) that exhibited a ³¹P NMR resonance at δ 75.26 (CDCl₃).

The preparation of **9** from **8** was then repeated using (*S*)-(-)-1-(1-naphthyl)ethylamine,²⁰ now affording only (*S_PS_C*)-**9** and (*R_PS_C*)-**9**. These diastereomers were separated by extensive repetitive spinning TLC on a Harrison Research chromatotron, fitted with a 4 mm preparative silica plate and using 30:70 ether/hexanes as eluent. The *S_PS_C* diastereomer eluted first (*R_f* = 0.38, vs *R_f* = 0.32 for the *R_PS_C* diastereomer). Diastereomer compositions were monitored via TLC, HPLC, and ³¹P NMR; the retention times and ³¹P NMR chemical shifts of the isolated *S_PS_C*²¹ and *R_PS_C*²² stereoisomers of **9** were identical to those of the previously examined (*S_PS_C*)/(*R_PR_C*) and (*S_PR_C*)/(*R_PS_C*) racemic diastereomers.

Acid-catalyzed methanolysis of 150 mg of (*R_PS_C*)-**9** (92% *R_PS_C*, 8% *S_PS_C*; 0.5 M H₂SO₄ in MeOH, 45 °C, 4 d), followed by chromatotron purification, gave 74 mg (72%) of (-)-Me-EPN (**5**).²³ Acidic methanolysis of **9** should proceed with inversion at P,¹⁵ so we represent this transformation by the first step of eq 2. The levorotatory sense of (*S_P*)-**5** corresponds



to that of related *O*-aryl *O*-alkyl phenylphosphonothioates.²⁴

Hydrolysis of (*S_P*)-**5** with 0.5 M KOH in 1:1 aqueous MeCN (25 °C, 1 h) gave (*S_P*)-(-)-*O*-methyl phenylphos-

phonothioic acid (**6**).^{1b,12,14} The hydroxide cleavage of Me-EPN occurs with inversion,^{4a,24,25} so we illustrate the formation of (*S_P*)-(-)-**6**²⁶ as shown in the second step of eq 2. The enantiomeric purity of (*S_P*)-**6** was determined by proton NMR in the presence of 3 equiv of (*S*)-α-methylbenzylamine in pyridine-*d*₅.^{12,14,27} Under these conditions, the ¹H NMR *O*-Me resonance of the (*R_P*)-**6**/(*S_C*)-amine salt appears as a doublet (*J_{P-H}* = 12.9 Hz) at δ 3.783 and 3.740, whereas the *O*-Me doublet of the (*S_P*)-**6**/(*S_C*)-amine salt appears at δ 3.804 and 3.761; see Figure 2A. From NMR integration, product

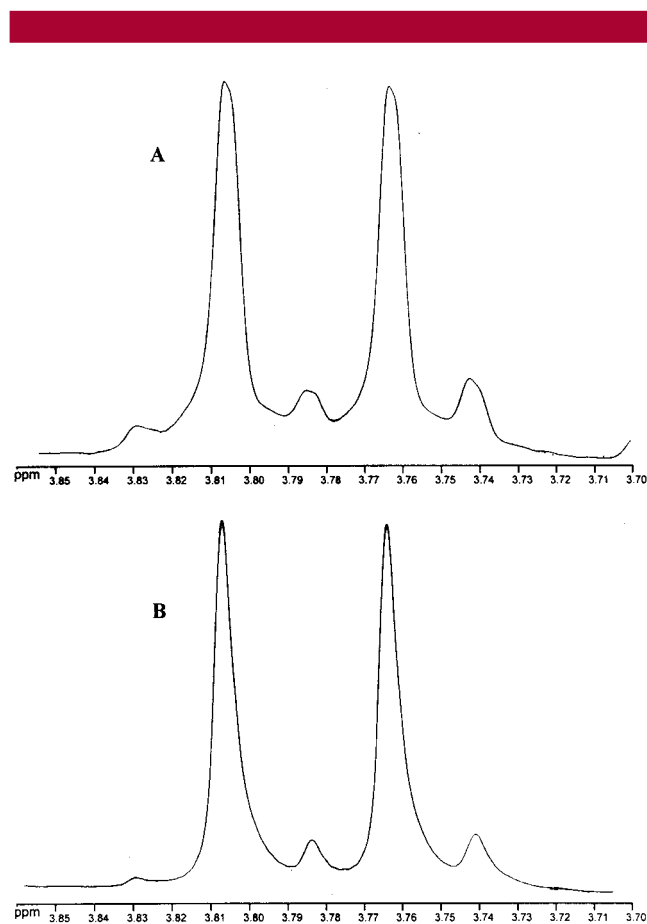


Figure 2. (A) “Raw” ¹H NMR *O*-Me signals of 86% (*S_P*)-**6** from the KOH cleavage of (*S_P*)-**5**; signals due to ~14% of (*R_P*)-**6** are also visible. The spectrum was determined in the presence of 3 equiv of (*S*)-α-methylbenzylamine in pyridine-*d*₅. (B) “Raw” ¹H NMR *O*-Me signals of (*S_P*)-**6** from the cleavage of (*S_P*)-**5** by metallomicellar **4** (under the same NMR conditions). The deconvoluted *S_P/R_P* distribution (87/13) is identical to the deconvoluted distribution from the KOH cleavage in (A).

(*S_P*)-**6** was 86% enantiomerically pure, i.e., 14% of (*R_P*)-**6** was also present. Thus, starting from 92% diastereomerically pure (*R_P*)-**9**, the reactions of eq 2 afforded 86% enantiomerically pure (*S_P*)-**6**; ~6% of racemization occurred, presumably during methanolysis.¹⁵

In parallel experiments, (*S_PS_C*)-**9** (86–90% *S_P*) was converted with acidic methanol to (*R_P*)-**5**²⁸ in 70% yield.

(23) [α]_D²⁰ -30.8° (*c* = 4.0, CHCl₃); ³¹P NMR δ = 87.8 (CDCl₃).

(10) The rate acceleration in the cleavage of, e.g., Me-EPN by micellar **4** is largely due to concentration of the substrate and the metal complex into the condensed aggregate pseudophase, as well as the enhanced acidity of the Cu-bound water molecules in the cationic micelles.^{9a}

(11) We chose Me-EPN (**5**) rather than EPN (**2**)^{4a} because of the simpler proton NMR spectrum of the former; the OMe resonance is ideal for tracking the stereochemistry (see below).

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(17) Purnanand; Batra, B. S.; Lal, G. *Tetrahedron Lett.* **1994**, *35*, 4641.

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(19) Cf., Tawfik, D. S.; Eshar, Z.; Bentolila, A.; Green, B. S. *Synthesis* **1993**, 968.

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(21) [α]_D²⁰ +65.2° (*c* = 4.0, CHCl₃); 95% *S_PS_C*, 5% *R_PS_C* by ³¹P NMR). An appropriate ¹H NMR and satisfactory C, H, N microanalysis were obtained.

(22) [α]_D²⁰ +55.2° (*c* = 4.0, CHCl₃); 92% *R_PS_C*, 8% *S_PS_C* by ³¹P NMR). An appropriate ¹H NMR and satisfactory C, H, N microanalysis were obtained.

Cleavage with aqueous KOH then afforded (*R_P*)-**6** which was 86% *R_P* and 14% *S_P*, indicating ~1–4% of racemization in the course of the reaction sequence. The ¹H NMR analysis of the (*R_P*)-**6** was done in the presence of (*S*)- α -methylbenzylamine, as described for (*S_P*)-**6**, affording results (not shown) similar to those in Figure 2A, except of course for a reversal of the major and minor *O*-Me doublets.

With highly enantiomerically enriched (*S_P*)- and (*R_P*)-Me-EPN in hand, we turned our attention to the metallomicellar cleavage reaction. Thus, 25 mg (0.081 mmol) of 86–92% enantiomerically pure (*S_P*)-Me-EPN (**5**)²⁹ in 1 mL of MeCN was added to a solution of 5 mM **4** and 50 mM (CTA)Cl in 25 mL of 25 mM, pH 8 aqueous HEPES buffer. After 1 h at 25 °C, (CTA)Cl was precipitated with NaClO₄, Cu(II) was precipitated with Na₂S, unreacted **5** was extracted with CHCl₃, and hydrolysis product **6** (80%) was recovered by ethereal extraction of the acidified (H₂SO₄, pH <2) aqueous phase.

NMR analysis (Figure 2B) demonstrated the formation of 87% (*S_P*)-**6** and 13% (*R_P*)-**6**. Comparison with Figure 2A for the KOH cleavage of (*S_P*)-**5** shows that both the Cu(II)-HTMED and KOH cleavages of **5** follow experimentally identical stereochemical courses, which correspond to effectively complete inversion.

Parallel results (not illustrated) were obtained from the KOH and Cu(II)-HTMED cleavages of (*R_P*)-Me-EPN to (*R_P*)-**6**. In particular, the Cu(II)-mediated hydrolysis of 90–95% (*R_P*)/5–10% (*S_P*)-**5**²⁹ afforded 92% (*R_P*)/8% (*S_P*)-**6**, again denoting complete inversion for this reaction.

It is crucial to demonstrate that the inverting hydrolysis of Me-EPN to **6** is mediated by Cu-supplied hydroxide, and not simply hydroxide ions associated with the (CTA)Cl micelles. Figure 3 demonstrates that, under the Cu(II)-HTMED/(CTA)Cl micellar conditions described above, Me-EPN is completely hydrolyzed in 1 h (as measured spectroscopically by released *p*-nitrophenolate), whereas less than 2% of hydrolysis occurs in the absence of Cu(II)-HTMED, when only (CTA)Cl micelles are present. These results are

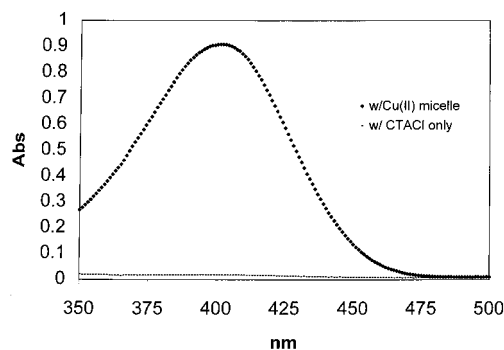


Figure 3. UV–vis spectra recorded after 1.1 h for the hydrolysis of Me-EPN (**5**) in (CTA)Cl micellar solution with or without added Cu(II)-HTMED (**4**). *p*-Nitrophenolate absorbance is apparent (and quantitative) at ~400 nm when **4** is present. See text for hydrolytic conditions.

consistent with previously demonstrated large rate accelerations for the Cu(II)-HTMED mediated hydrolysis of **5**^{1b} and related phosphorus-based substrates.⁹

The inverting Cu(II)-metallomicellar hydrolysis of **5** to **6** accords with an S_N2(P) mechanism in which copper delivers a bound hydroxide to the rear of the substrate's chiral P atom, displacing *p*-nitrophenolate, and forming product **6** in a single step.⁷ The stereochemical course of the metallomicellar hydrolysis of **5** parallels that of the phosphotriesterase cleavage of **3**.^{4a}

An “in-line” S_N2(P) mechanism requires a linear array of hydroxide nucleophile, substrate P atom, and leaving group oxygen. For the phosphotriesterase, this array is supported by 2 zinc atoms; one which supplies the hydroxide and a second which appears to electrophilically activate the (paraoxon) substrate's P=O by binding at oxygen.^{4d} In the present case, however, micellar Cu(II)-HTMED most likely operates only via the Cu-OH nucleophilic mode; it has been shown (in related micelle-catalyzed phosphorolytic reactions) that “electrophilic assistance is only present in the monomeric [copper] complex and vanishes in the metallomicelles.”³⁰

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Supporting Information Available: Cif file. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(26) ³¹P NMR δ = 83.3 (CDCl₃); the enantiomeric purity was determined by NMR, see text. The crystal structure of the (*S_P*)-(-)-**6**/(*S_C*)-(-)- α -methylbenzylamine salt has been determined,²⁴ ensuring the (*R_P*)-(+)- and (*S_P*)-(-) correlations for **6**.

(27) Mikołajczyk, M.; Omelańczuk, J.; Leitloff, M.; Drabowicz, J.; Ejchart, A.; Jurczak, J. *J. Am. Chem. Soc.* **1978**, *100*, 7003. We find that pyridine-*d*₅ supports a better resolution of the *O*-Me signals of (*R_P*)- and (*S_P*)-**6** than does benzene-*d*₆.

(28) ³¹P NMR δ = 87.8 (CDCl₃). The specific rotation of either pure enantiomer of **5** is ~[44–46°]. However, a small but variable degree of racemization in the **9** → **5** conversion makes a precise value difficult to obtain.

(29) The uncertainty in enantiomeric composition reflects racemization in the methanolysis step of eq 2, which provides the Me-EPN; see above.