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Stereochemical Study of Phosphonothioate Cleavage by a Metallomicelle

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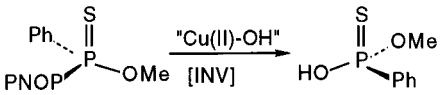
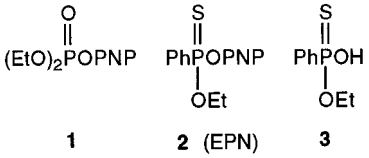
ABSTRACT



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 phosphotriesterases[4] 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



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

(1) (a) Moss, R. A.; Kanamathareddy, S.; Vijayaraghavan, S. *Langmuir* **2001**, *17*, 6108. (b) Moss, R. A.; Morales-Rojas, H. *Langmuir* **2000**, *16*, 6485. (c) Moss, R. A.; Morales-Rojas, H.; Zhang, H.; Park, B.-D. *Langmuir* **1999**, *15*, 2738.

(2) Reviews: (a) Yang, Y.-C. *Acc. Chem. Res.* **1999**, *32*, 109. (b) Séguès, B.; Pérez, E.; Rico-Lattes, I.; Rivière, M.; Lattes, A. *Bull. Soc. Chim. Fr.* **1996**, *133*, 925 (c) Yang, Y.-C. *Chem. Ind. (London)* **1995**, May 1, 334. (d) Yang, Y.-C.; Baker, J. A.; Ward, J. R. *Chem. Rev.* **1992**, *92*, 1729.

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

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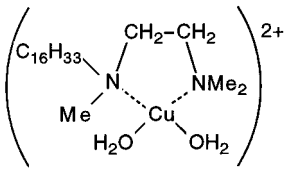
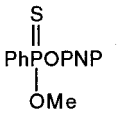
cleave by a two-step addition−elimination mechanism that transits a trigonal bipyramidal intermediate.[7] Nerve agents sarin and soman belong to the latter group.

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**,



4 (Cu(II)-HTMED)

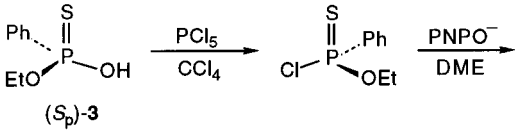


5 (Me-EPN)

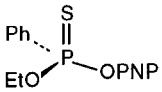
the methyl analogue of EPN, with $k = 0.012\ s^{-1}$, an acceleration of ~2400 over (CTA)Cl, pH 8 hydrolysis.[1b,10]

In the phosphoryltic 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]



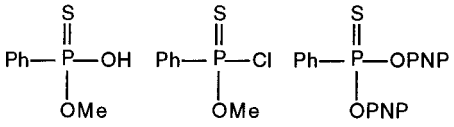
(S_p)-**3**



(1)

(R_p)-EPN (**2**)

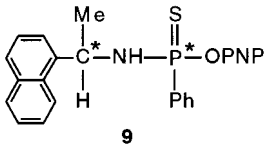
We attempted to apply this methodology to enantiospecific syntheses of Me-EPN. Resolution of *rac*-*O*-methyl phenylphosphonothioic acid (**6**) was achieved by procedures similar to those of Raushel[4a] and DeBruin,[12] affording both (R_P)-**6** and (S_P)-**6** with >90% ee. In our hands, however, conversion



6 **7** **8**

of **6** to chloridothioate **7** with PCl_5 in CH_2Cl_2 afforded **7** with only 37% ee; similar results were reported by DeBruin (20-44% ee).[12] Successive reactions of **6** with SO_2Cl_2 and PCl_5 in benzene[13] 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 Purnanand[17] 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 DBU[19] in dry CH_2Cl_2 to afford a mixture of the four diastereomers of **9**, *N*-1-(1-naphthyl)ethylamino-*O*-(4-nitrophenyl) phenylphosphonothioate.



9

Chromatography (silica gel, CH_2Cl_2 eluent), followed by fractional crystallization from diethyl ether/petroleum ether, gave 18.5% of (S_PS_C)/(R_PR_C)-**9** (mp 127−128 °C). The structure and absolute configuration were verified by X-ray diffraction; cf. Figure 1, in which the (S_PS_C) enantiomer is

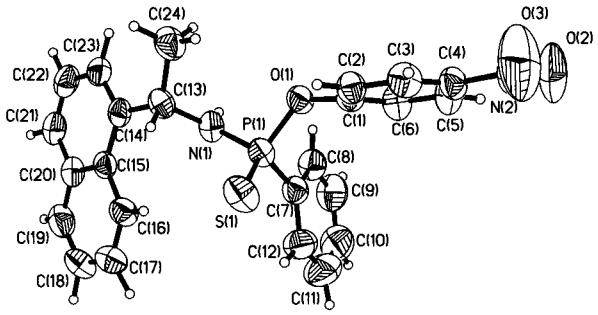


Figure 1. ORTEP rendering of (S_PS_C)-**9**. Crystallographic information has been submitted as Supporting Information.

illustrated. (S_PS_C)/(R_PR_C)-**9** exhibited a ^{31}P NMR resonance in $CDCl_3$ at δ 73.89 (vs external 85% H_3PO_4) and was 98.2% pure (from the NMR analysis). From the mother liquor, we

(7) Zheng, F.; Zhan, C.-G.; Ornstein, R. L. *J. Chem. Soc. Perkin Trans. 2* **2001**, 2355.

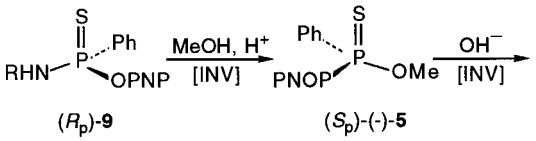
(8) This reagent was introduced as the tetradecyl derivative: Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 2800.

(9) (a) Scrimin, P.; Ghirlanda, G.; Tecilla, P.; Moss, R. A. *Langmuir* **1996**, *12*, 6235. (b) Hay, R. W.; Govan, N.; Parchment, K. E. *Inorg. Chem. Commun.* **1998**, *1*, 228.

subsequently crystallized 15% of pure (S_PR_C)/(R_PS_C)-**9** (mp 105−107 °C) that exhibited a ^{31}P NMR resonance at δ 75.26 ($CDCl_3$).

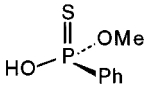
The preparation of **9** from **8** was then repeated using (*S*)-(−)-1-(1-naphthyl)ethylamine,[20] now affording only (S_PS_C)-**9** and (R_PS_C)-**9**. These diastereomers were separated by extensive repetitive spinning TLC on a Harrison Research chromatotron, fitted with a 4 mm preparative silica 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 ^{31}P NMR; the retention times and ^{31}P 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 H_2SO_4 in MeOH, 45 °C, 4 d), followed by chomatotron 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



(R_p)-**9** (S_p)-(-)-**5**

[R = (S_C)-1-(1-naphthyl)-ethylamine]



(2)

(S_p)-(-)-**6**

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 phenylphosphonothioic 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 ^{1}H 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

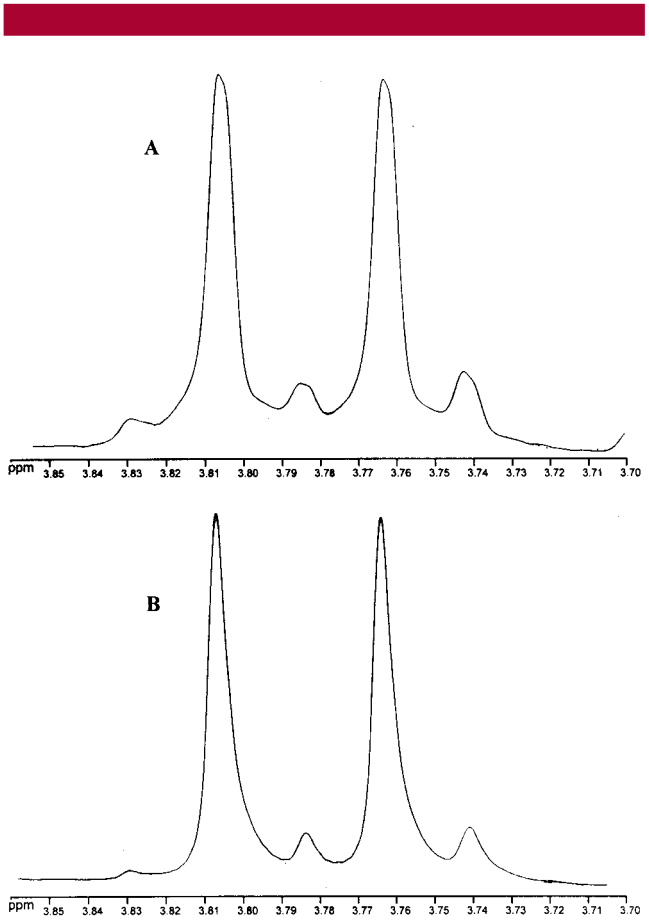


Figure 2. (A) "Raw" ^{1}H NMR O-Me signals of 86% (S_P)-**6** from the KOH cleavage of (S_P)-**5**; signals due to ∼14% of (R_P)-**6** are also visible. The spectrum was determined in the presence of 3 equiv of (*S*)-α-methylbenzylamine in pyridine-d_5. (B) "Raw" ^{1}H NMR O-Me signals of (S_P)-**6** 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.

(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).

(12) DeBruin, K. E.; Tang, C.-I. W.; Johnson, D. M.; Wilde, R. L. *J. Am. Chem. Soc.* **1989**, *111*, 5871. Specific rotations and configurational correlations for **6** and **7** appear in this article.

(13) Corriu, R. J. P.; Lanneau, G. F.; Leclerq, D. *Tetrahedron* **1980**, *36*, 1617.

(14) Details appear in Morales-Rojas, H. Ph.D. Dissertation, Rutgers University, New Brunswick, NJ, 2001; pp 44−52, 65−68.

(15) Hall, C. R.; Inch, T. D. *Tetrahedron Lett.* **1977**, *18*, 3761, 3765. Hall, C. R.; Inch, T. D. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1646

(16) Koizumi, T.; Takagi, H.; Yoshii, E. *Chem. Lett.* **1980**, 1403.

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

(18) Purnanand; Danikel, R. K. *Synthesis* **1983**, 731.

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

(20) For related usage of this chiral auxiliary, see: Uznanski, B.; Grajkowski, A.; Krzyzanowska, B.; Kazmierkowska, A.; Stec, W. J.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 10197.

(21) $[\alpha]^{20}_D$ +65.2° (*c* = 4.0, $CHCl_3$, 95% S_PS_C, 5% R_PS_C by ^{31}P NMR). An appropriate ^{1}H NMR and satisfactory C, H, N microanalysis were obtained.

(22) $[\alpha]^{20}_D$ +55.2° (*c* = 4.0, $CHCl_3$, 92% R_PS_C, 8% S_PS_C by ^{31}P NMR). An appropriate ^{1}H NMR and satisfactory C, H, N microanalysis were obtained.

(23) $[\alpha]^{20}_D$ −30.8° (*c* = 4.0, $CHCl_3$); ^{31}P NMR δ = 87.8 ($CDCl_3$).

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 ^{1}H 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 $NaClO_4$, Cu(II) was precipitated with Na_2S, unreacted **5** was extracted with $CHCl_3$, and hydrolysis product **6** (80%) was recovered by ethereal extraction of the acidified (H_2SO_4, 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 consistent with previously demonstrated large rate accelerations for the Cu(II)-HTMED mediated hydrolysis of **5**[1b] and related phosphorus-based substrates.[9]

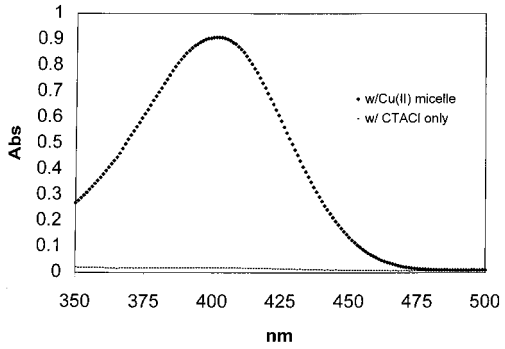


Figure 3. UV−vis spectra recorded after 1.1 h for the hydrolysis of Me-EPN (**5**) in (CTA)Cl micellar solution with or without added Cu(II)-HTMED (**4**). *p*-Nitrophenolate absorbance is apparent (and quantitative) at ~400 nm when **4** is present. See text for hydrolytic conditions.

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]

Acknowledgment. P.K.G. acknowledges support from a NIH Biotechnology Training Grant (GM 08339). We are grateful to Dr. Thomas Emge for the X-ray structure determination of (S_PS_C)/(R_PR_C)-**9**. We thank the U.S. Army Research Office for financial support.

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

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(24) Allahyari, R.; Hollinghaus, J. G.; Lapp, R. L.; Timm, E., Jacobson, R. A.; Fukuto, T. R. *J. Agric. Food Chem.* **1980**, *28*, 594.

(25) Allahyari, R.; Lee, P. W.; Lin, G. H. Y.; Wing, R. M.; Fukuto, T. R. *J. Agric. Food Chem.* **1977**, *25*, 471.

(26) ^{31}P NMR δ = 83.3 ($CDCl_3$); 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.; Omelañczuk, 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) ^{31}P NMR δ = 87.8 ($CDCl_3$). 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.

(30) Bunton, C. A.; Scrimin, P.; Tecilla, P. *J. Chem. Soc., Perkin Trans. 2* **1996**, 419.