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Stereochemical Inversion of Phosphonothioate Methanolysis by La(III) and Zn(II): Mechanistic Implications for the Degradation of Organophosphate Neurotoxins

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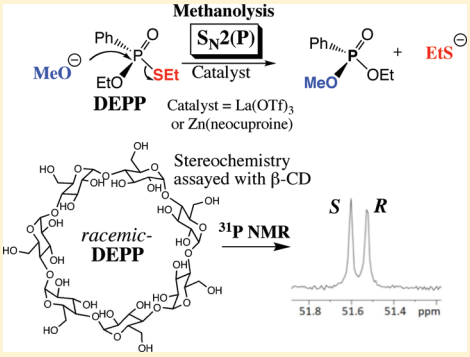
Supporting Information

ABSTRACT: The utility of phosphonothioate methanolysis to degrade organophosphate neurotoxins has prompted the stereochemical investigation of this useful transformation. The methanolysis of enantiomerically pure *O*,*S*-diethyl phenylphosphonothioate (**5**) was studied both in the presence and in the absence of metal ions known to catalyze the phosphonothioate → phosphonate transformation. This report outlines the syntheses of enantiomerically pure **5** and its methanolysis product *O*-ethyl *O*-methyl phenylphosphonate (**7**). Compound **7** results from exclusive P−S scission of **5**, which is the desired mode of phosphonothioate methanolysis (E_a = 14.5 ± 0.5 kcal/mol). The stereochemical analysis of the phosphonothioate methanolysis was done for the first time with *β*-cyclodextrin, and it shows complete inversion on the phosphorus center upon methoxide displacement of ethanethiolate. The presence of La(III) or Zn(II) complexes do not alter this S_N2-like substitution which sheds new light on the mechanism of methanolysis of phosphonothioates.

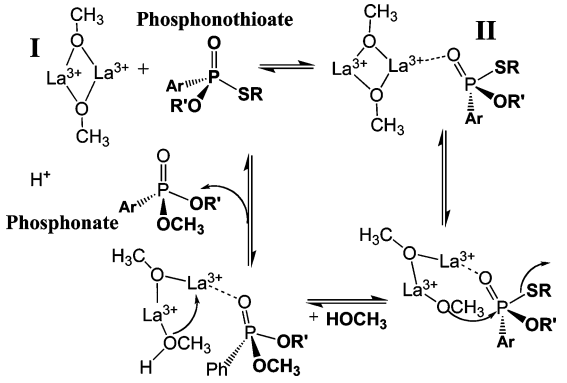


INTRODUCTION

Phosphonothioates are neurotoxins, and there are societal benefits for degrading them through methanolysis.[1,2] As acetylcholine esterase inhibitors,[3,4] phosphonothioates are used as pesticides for agricultural use,[5] but their greatest notoriety is found as chemical warfare agents.[3] As such, a major effort has been devoted toward the chemical degradation of these compounds through oxidative[6−13] and hydrolytic transformations.[14] A problem with alkaline hydrolysis of phosphonothioates is the production of both phosphonothioate and phosphonate anions[3] that are resistant to further nucleophilic attack. Frustratingly, in some cases the phosphonothioate product is just as toxic as the parent neurotoxin.[15] To that end, alcoholysis is a promising route especially when coupled with metal complexes that serve as catalysts. Brown and co-workers[16,17] have used many metal complexes to catalyze phosphonothioate methanolysis reactions with up to 10^9-fold rate enhancements. The methoxide-bridged lanthanum(III) system[18] has been used to degrade live nerve agents such as the infamous VX. The proposed mechanism (Scheme 1) starts from the La-μ-$(OMe)_2$-La dimer[16,17] (**I**), and it involves delivery of the La(III)-bound methoxide onto a coordinated phosphonothioate (**II**) leading to displacement of the alkythiolate. The methoxide-bridged lanthanum dimer is regenerated with the addition of the methanol solvent that also releases the phosphonate product. Subsequent alkoxide displacement was also found to occur from this released phosphonate.

The P−S bond scission is the desired route, for the phosphonothioate → phosphonate transformation leads to a less toxic product.[16−18] In terms of the alcoholysis mechanism

Scheme 1



there are two possible routes that yield exclusive P−S scission of a phosphonothioate (Scheme 2).[19] One is a concerted S_N2(P) in which the nucleophile has a facial selectivity to attack opposite the alkylthiolate leaving group (SR). In the other route the nucleophile has the opposite facial selectivity as it attacks opposite the alkoxide (OR′) to initially yield a trigonal bipyramid intermediate (**A**). This is followed by a low-energy-barrier pseudorotation (Ψ)[20,21] to intermediate **B** that places the departing ethanethiolate leaving group in the axial (apical) position.

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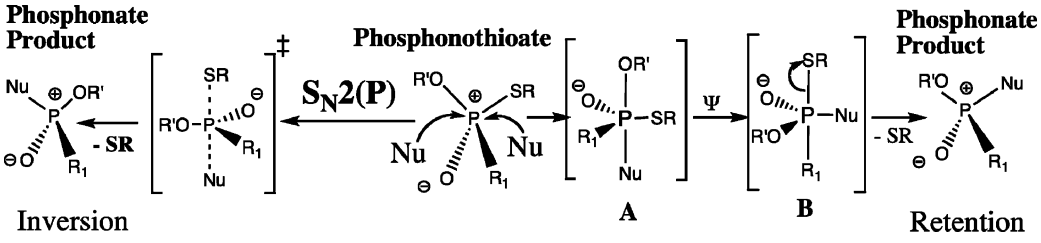
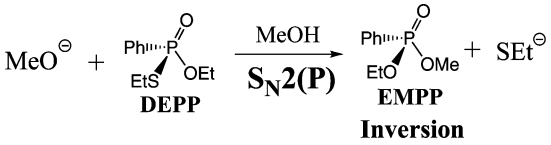
Scheme 2



The phosphorus stereocenter of chiral phosphonothioates allows one to interrogate the stereochemistry of alcoholysis with enantiomerically pure substrates. Therefore, a phosphonate product with inverted stereochemistry results from a $S_N2(P)$ pathway in which the nucleophile has a facial selectivity opposite the alkythiol group. A phosphonate product with retention of the stereocenter proceeds through the trigonal bipyramid intermediate that undergoes the low-energy **A** → **B** isomerization (Ψ). DeBruin and co-workers[19] showed that nucleophilic attack of ethoxide on *O*,*S*-dimethyl phenylphosphonothioate has a facial selectivity that places the methylthiolate ligand axial and opposite the alkoxide (EtO) nucleophile. This leads to inversion of configuration ($S_N2(P)$) that is consistent with earlier findings which used alkoxide nucleophiles.[22−32] Interestingly, Grignard nucleophiles resulted in retention of configuration.[27,33−39]

Patterson and co-workers[20,40] have carried out several computational studies on the hydrolysis and perhydrolysis on model phosphonothioates. Initial attack by HO^- or HOO^- had a facial selectivity where the nucleophile attacked the face opposite the alkoxy ligand. This resulted in a trigonal bipyramid intermediate (**A**) in which the alkoxide group occupies the apical position. Accordingly, P−O scission would result which contradicts experimental results that favor P−S over P−O bond scission by 87:13 for alkaline hydrolysis[41] and 100:0 for perhydrolysis.[10] Their computational work revealed a low-energy isomerization (Ψ) that interchanged the apical alkoxide for the alkylthiolate (**A** → **B** in Scheme 2) which rationalized the preferential P−S bond scission. This facial selectivity for attack opposite the alkoxy ligand followed by the pseudorotation was also seen in the calculation of ethoxide attack on *O*,*S*-dimethyl phenylphosphonothioate.[21] In this work, Patterson and Menke found that the pseudorotation of the initial trigonal bipyramind (**A**) will lead to both P−O and P−S scission, but P−O cleavage was reversible and slightly endothermic while the P−S cleavage pathway was irreversible and highly exothermic. Their calculation work was in disagreement with the results of DeBruin in terms of the facial selectivity of the ethoxide nucleophilic attack. As the authors concluded, "Further studies may be required to resolve this discrepancy."[21]

In line with prior alcoholysis work, we report methanolysis results on the chiral substrate *O*,*S*-diethyl phenylphosphonothioate (DEPP) that show a $S_N2(P)$ route (eq 1) during the initial reaction period wherein exclusive P−S scission yields *O*-ethyl *O*-methyl phenylphosphonate (EMPP). Evidence for this result comes from 100% inversion of the starting DEPP configuration. In addition, we also show that the La(III) and Zn(II)-promoted methanolysis (yielding P−S scission) proceed through this $S_N2(P)$ pathway.



(1)

RESULTS AND DISCUSSION

The investigation on the stereochemistry of DEPP methanolysis (P−S scission) proceeds through the following three points: (1) the synthesis of enantiomerically-pure starting phosphonothioate and product phosphonate, (2) enantiomeric resolution with ^{31}P NMR using a chiral encapsulating host, and (3) the stereochemical outcome of the methoxide attack on the chiral phosphonothioate with and without metal complexes.

The two title compounds involved in this study are the starting *O*,*S*-diethyl phenylphosphonothioate (**5**) and the product, *O*-ethyl *O*-methyl phenylphosphonate (**7**). The enantiomeric synthesis of these key compounds has been described by DeBruin and co-workers.[19]

In terms of making the (*S*)-*O*,*S*-diethyl phenylphosphonothioate ((*S*)-**5**), the enantiomeric resolution occurs with *O*-ethyl phenylphosphonothioic acid (**3**). This was made from sulfur addition to *O*-ethyl phenylhydrogenphosphinate (**1**) in the presence of dicyclohexylamine followed by HCl acidification (Scheme 3). The racemic phosphonothioic acid **3** was resolved with the addition of brucine that formed the phosphonothioate anion **4**. The crystals isolated from the acetone solution of this brucine salt were confirmed by single crystal X-ray crystallography to be the *S* enantiomer of *O*-ethyl phenylphosphonothioate ((*S*)-**4**) as shown in Figure 1.

Acidification of this brucinium salt gave the optically pure thioic acid (*S*)-**3**, which was converted to and stored as the dicyclohexylammonium salt ((*S*)-**2**). Finally, addition of ethyl iodide to this (*S*)-**2** salt gave the enantiomerically pure (*S*)-*O*,*S*-diethyl phenylphosphonothioate ((*S*)-**5**) starting compound (Scheme 3).

The methanolysis of (*S*)-**5** would give either the *S* or *R* enantiomer of *O*-ethyl *O*-methylphosphonate ((*S*)-**7** or (*R*)-**7**). We modified a prior literature protocol[19] for making (*S*)-**7** shown in Scheme 4b that starts from the dicyclohexylammonium salt of (*S*)-*O*-ethyl phenylphosphonothioate ((*S*)-**2**) made in Scheme 3. Addition of methyl iodide (Scheme 4b) to this salt yielded the corresponding S-methylated phosphonothioate, (*S*)-**6**. The final conversion to the phosphonate was done in methanol with $AgNO_3$, which is known to promote the replacement of alkylthiolates with the alkoxide (CH_3O) with inversion.[29] The (*S*)-**6** → (*S*)-**7** conversion represents an inversion, due to a priority-assignment change (circled numbers in Schemes 4a and 4b) when the leaving group (MeS; priority 1) was replaced by MeO (priority 2) in (*S*)-**7**.

Therefore, if (*S*)-**5** underwent nucleophilic substitution with inversion, (*S*)-**7** would be formed as shown in Scheme 4a. This

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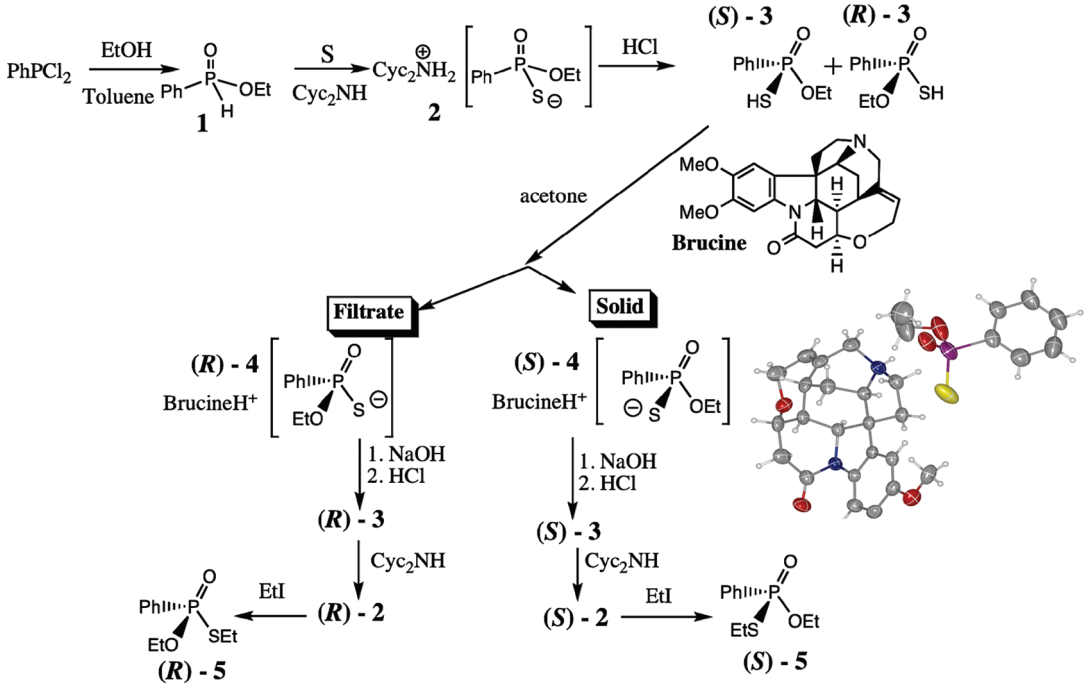
Scheme 3



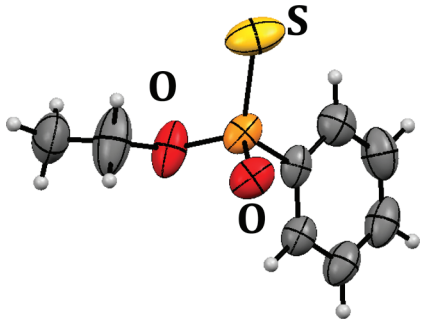
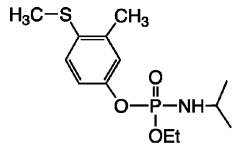


Figure 1. Crystal structure of (*S*)-*O*-ethyl phenylphosphonothioate ((*S*)-**4**) isolated in Scheme 3 showing just the phosphonothioate without the brucine cation for clarity purposes. The phenyl group is placed pointing away to show the (*S*) configuration with the following priority ranking in decreasing order: S (yellow) → OEt → O (red) → Ph. R = 4.28%.

would be confirmed by addition of the authentic optically pure (*S*)-*O*-ethyl *O*-methyl phenylphosphonate enantiomer ((*S*)-**7**) made in Scheme 4a. Likewise if methanolysis of (*S*)-**5** underwent retention, then the (*R*)-**7** product would be formed, which upon enantiomeric resolution would be distinct from the synthesized (*S*)-**7**.

Enantiomeric resolution by NMR spectroscopy was done with a cyclodextrin (CD) encapsulating agent. Inclusion complexation of small molecules into the CD cavity (Figure 2) in aqueous media is driven by a combination of chiral recognition and hydrophobic interactions,[42] and they have been proposed as potential chiral shift reagents for NMR spectroscopy.[43]

Recently, Talebpour and Molaabasi[44] showed that cyclodextrins successfully resolve enantiomeric mixtures of chiral organophosphates that possess a hydrophobic group. Specifically when the phenyl-containing pesticide fenamiphos (racemic) was added to various β-CDs, there were two distinct and equal ^{31}P NMR signals, and this chemical shift non-



Fenamifos

equivalence was attributed to enantiomeric discrimination by the host CDs.

We applied the same enantiomeric discrimination strategy to discern the stereochemistry of DEPP methanolysis. Initially, a racemic mixture of DEPP yielded two distinct and equal ^{31}P NMR signals in a D_2O solution when β-CD was added. We identified the upfield ^{31}P signal to be the *R* enantiomer when authentic (*R*)-DEPP ((*R*)-**5**) was added (Figure 3). This chemical shift nonequivalence is consistent with a guest–host interaction between DEPP and β-CD that results from an optimal fit of the phenyl group of DEPP into the β-CD cavity. In this connection, the addition of α- or γ-CD to racemic DEPP yielded no enantiomeric discrimination as seen by ^{31}P NMR; the smaller and larger CDs did not have the optimal fit for an effective guest–host interaction. Moreover, when isopropanol was added to the β-CD + racemic-DEPP mixture, both ^{31}P signals merged back to one (Figure 3d). Confirmation that only one enantiomer (*S*) of **5** (DEPP) and **7** was made in Schemes 3 and 4b comes from the observation that both compounds yielded only one ^{31}P signal in the presence aqueous β-CD.

The methanolysis of (*R*)-DEPP ((*R*)-**5**) was monitored with ^{31}P NMR until the reaction reached completion (~30 min at room temperature in 0.6 M NaOMe) with only P–SEt scission. This was evident with the production of only one product ^{31}P NMR signal at ~22 ppm attributed to *O*-ethyl *O*-methyl phosphonate (**7**). We rule out ethoxide displacement during this time period as there was no 46.7 ppm signal due to *O*-methyl-*S*-ethyl phenylphosphonothioate (**8**), which was independently made. Interestingly, the measured activation energy barrier for this process (Supporting Information, S1) was 14.5 ± 0.5 kcal/mol,

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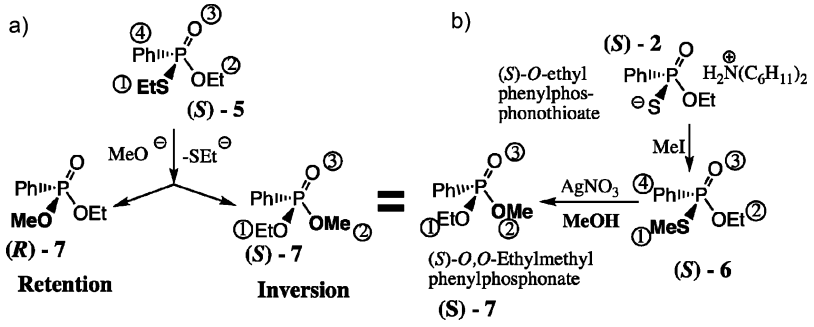
Scheme 4



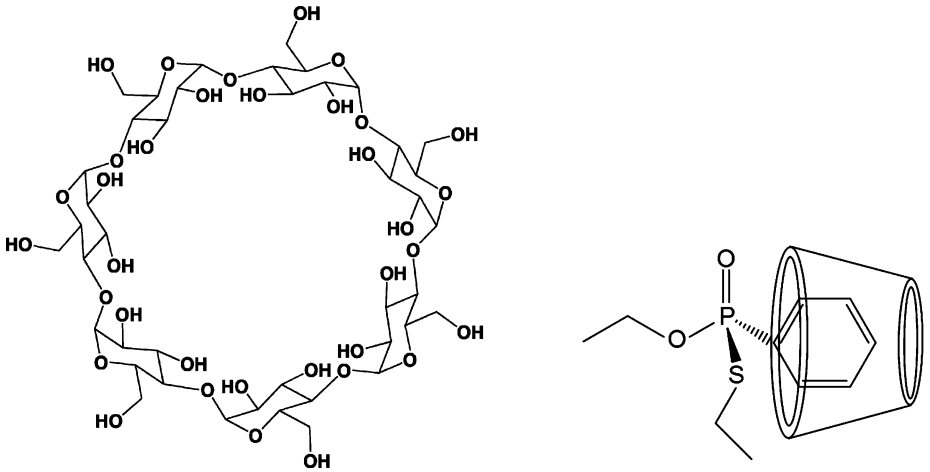


Figure 2. Structure of β-CD showing the cyclic polysaccharide that forms a conical shape with hydrophobic cavity. β-CD forms a guest−host interaction with the phenyl groups of DEPP (*R* enantiomer of DEPP shown docked).

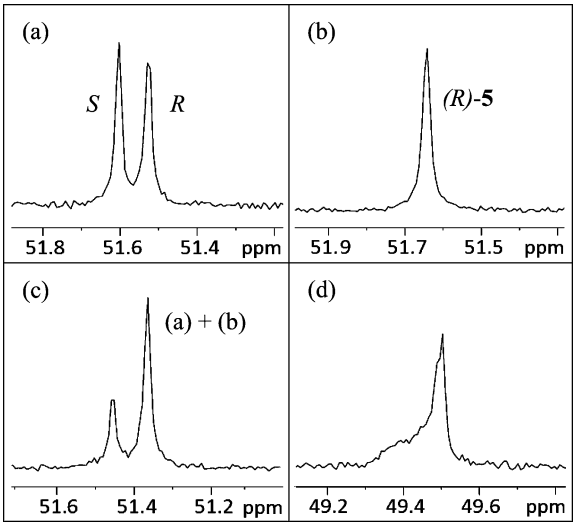


Figure 3. ^{31}P NMR spectra of β-cyclodextrin in D_2O (a) with 3 μL of racemic DEPP (**5**), (b) with 3 μL of (*R*)-DEPP ((*R*)-**5**), (c) with 3 μL of racemic DEPP and (*R*)-DEPP added, and (d) with the addition of propanol (the varying chemical shifts are due to changes in concentrations and environments).

which was almost identical to the calculated enthalpy (14.4 kcal/mol) of activation for ethoxide attack on *O*,*S*-dimethyl methylphosphonothioate.[21]

Upon completion of the (*R*)-**5** methanolysis, the methanol was removed in vacuo and replaced with D_2O and β-CD. The ^{31}P NMR showed only one product signal at 22.2 ppm which indicated a stereospecific process. If this product was (*S*)-7 phosphonate (retention, in Scheme 4a), then the addition of authentic (*S*)-7 yields only one enantiomer that would show up as *one* ^{31}P signal in the presence of β-CD. Instead (Figure 4a−d), addition of the independently made (*S*)-7 to this methanolysis product yielded a second ^{31}P signal in the presence of β-CD; the original *O*-ethyl *O*-methyl phenylphosphonate (7) product was the *R* enantiomer. This indicated a (*R*)-**5** → (*R*)-7 methanolysis.

Further confirmation of this stereochemical transformation was undertaken for the methanolysis of (*S*)-**5**. This enantiomer was made from the (*S*)-**4** in Scheme 3, and it contained a small amount of the *R* enantiomer. Therefore, the starting (*S*)-**5** was not optically pure, for it contained a minor amount of the (*R*)-**5** enantiomer when assayed with β-CD (Supporting Information, S2). Nevertheless, when the (*S*) -**5** phosphonothioate underwent methanolysis as described above, the (*S*)-7 product was formed; addition of authentic (*S*)-7 (containing some (*R*)-7 impurity) to the methanolysis-product/β-CD mixture yielded mainly one ^{31}P signal as shown in Figure 4e−h. Both the aforementioned (*S*)-**5** → (*S*)-7 and the (*R*)-**5** →(*R*)-7 conversion indicate an inversion (Scheme 4b) for the methanolysis process.

This methanolysis result is consistent with prior stereochemical findings of alkoxide attack on phosphonothioates.[22−32] Therefore, this analysis was extended to two metal-catalyzed methanolyses of phosphonothioates. Specifically, $La(OTf)_3$ and Zn/2,9-dimethylphenanthroline were investigated to see how the stereochemical outcome of methanolysis is influenced by these metal ions/complexes. Brown and co-workers found a 10^9-fold rate enhancement for the methanolysis of aryl phonosphonothioates in the presence of La(III),[16] and a 10^6-fold rate

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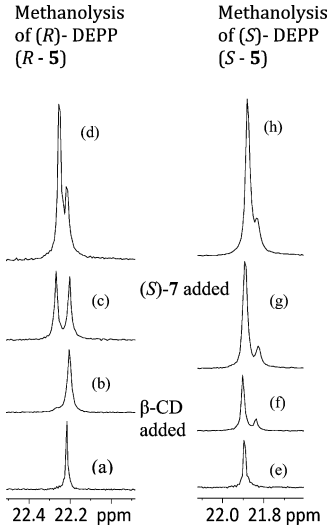
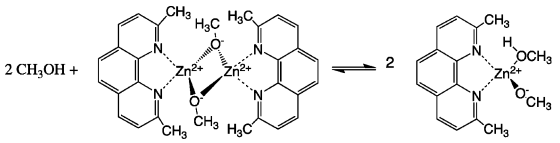


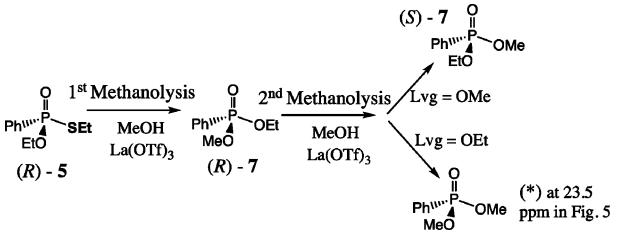
Figure 4. ^{31}P NMR spectra of the product of the methanolysis of (*S*)-DEPP and (*R*)-DEPP. The methanolysis of (*R*)-DEPP: (a) the methanolysis product, (b) methanolysis product + *β*-CD, (c) 1.3 *μ*L of (*S*)-7 added, (d) another 1.3 *μ*L of (*S*)-7 added. ^{31}P NMR solvent was D_2O. The methanolysis of (*S*)-DEPP: (e) the methanolysis product, (f) the methanolysis product + *β*-CD, (g) 1.3 *μ*L of (*S*)-7 added, (h) another 1.3 *μ*L of (*S*)-7 added. The small upfield signals in (f) and (g) result from the optical impurity of the starting (*S*)-DEPP. The authentic (*S*)-7 contained some *R* enantiomer impurity.

enhancement when the Zn/2,9-dimethylphenanthroline system was applied to catalytically degrade (i.e., methanolysis) phosphate triesters.[45] The Zn(II) coordination system resulted from the complexation of $Zn(OTf)_2$ and one equivalent of 2,9-dimethylphenanthroline (neocuproine) to form a monomer–dimer mixture (eq 2).[45]



(2)

The methanolysis of **5** by stoichiometric $La(OTf)_3$ was complete after one hour (room temperature), and this isolate contained no *O*-methyl-*S*-ethyl phenylphosphonothioate (**8**) at 46.7 ppm (vide supra) that would result from ethoxide release. The measured activation energy barrier of 15.6 ± 1.1 kcal/mol (Supporting Information, S3) for the La(III)-catalyzed methanolysis of **5** is close to the E_a for the methanolysis without any metal ions (14.5 ± 0.5 kcal/mol in the Supporting Information, S1). However, these two processes were done under radically different solvent conditions wherein the former was buffered with *N*-ethylmorpholine (pH 9.80^{HOMe}) and the latter was done in 0.6 M NaOMe. This precludes making reasonable assessments on the activation effects the La(III) ion makes on the methanolysis. Removal of $La(OTf)_3$ from the *N*-ethylmorpholine and NaOMe solution yielded a methanolysis process too slow to measure.



(3)

Moreover, we saw 100% stereospecificity when (*R*)-**5** was used in the presence of $La(OTf)_3$. Specifically, when *β*-CD was added to the product (after replacement of MeOH with D_2O) in Figure 5 (time = 1 h), we saw the identical behavior as in Figure 4a–d (Supporting Information, S5). This indicated complete inversion ($S_N2(P)$) for the (*R*)-**5** → (*R*)-7 methanolysis. At room temperature, the 7 product showed no (*S*)-7 enantiomer at time 60 min. Only the (*R*)-**5** was used as the starting material, for it was 100% optically pure (vide supra).

Over time, the initial (*R*) − 7 product undergoes racemization to yield the (*S*)-7 enantiomer which is seen in Figure 5. This is due to a second methanolysis on the initial (*R*)-7 product as shown in eq 3. This secondary transformation is further substantiated with the appearance of *O*,*O*-dimethyl phenylphosphonate (i.e., ethoxide leaving group) with a ^{31}P signal at 23.5 ppm (* in Figure 5).

Interestingly, the Zn(II)–neocuproine system (eq 2) yielded the same stereochemical results when (*R*)-**5** underwent methanolysis. Complete conversion to the phosphonate (7)

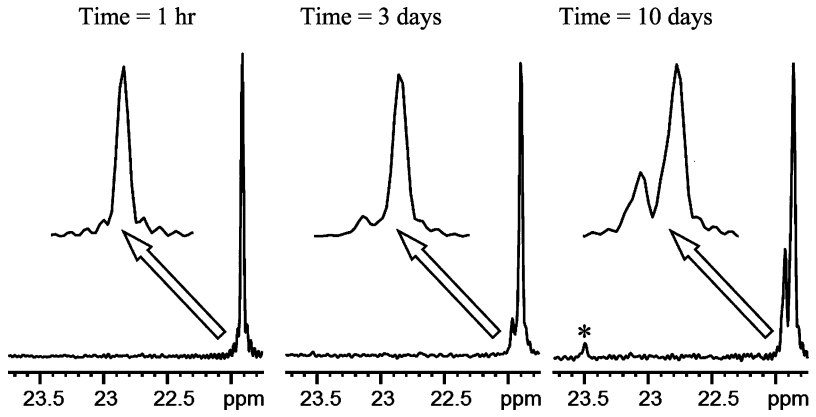


Figure 5. Methanolysis (^{31}P NMR) of of (*R*)-**5** with $La(OTF)_3$ catalyst at room temperature over extended time to show production of both *O*,*O*-dimethyl phenylphosphonate (* at 23.5 ppm) and (*S*)-7. Methanolysis product was dried and combined with aqueous *β*-CD. Verification of these two products was done with authentic addition (Supporting Information, S4). The production of both compounds indicates a second methanolysis on the initial (*R*)-7 product as shown in eq 3.

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at room temperature took six hours with no sign of **8** (i.e., ethoxide leaving group). β-Cyclodextrin addition to this product followed by authentic (*S*)-**7** (Supporting Information, S6) gave the same results as $La(OTf)_3$-catalyzed methanolysis and sodium methoxide degradation (Figure 4a−d). Like the case with $La(OTf)_3$-catalyzed methanolysis, this showed that the Zn(II)−neocuprine coordination complex promoted a (*R*)-**5** → (*R*)-**7** conversion with inversion on the phosphorus center.

CONCLUSION

These results indicate that methanolysis either with or without La(III) or Zn(II) catalysis proceeds with inversion of stereochemistry that is consistent with an "S_N2-like" pathway (Scheme 2). In the methanolysis of diethyl *S*-aryl phosphonothioates catalyzed by La(III),[17] Brønsted plot measurements suggest that the alkoxide nucleophile attacks opposite the leaving group in most likely a concerted process. Interestingly the metal ions did not stabilize the hypothesized intermediate/transition state to promote a stepwise pathway. Recent calculation studies[46] show that an inversion of stereochemistry could occur through either a concerted or stepwise mechanism. In the case for the latter pathway, a poor leaving group (pK_a > 8) favors a nonconcerted route that proceeds through a phosphorane (trigonal bipyramid) intermediate with no pseudorotation. As this is the first case of employing cyclodextrins to probe the stereochemical outcome of methanolysis, future work is focused on using these oligosaccharides to promote hydrolysis of phosphonothioates.

EXPERIMENTAL SECTION

Materials and Methods. ^{31}P, ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance-300 at 121 MHz, 300 MHz, and 75 MHz, respectively. High resolution mass spectral analyses were done by U. Illinois-Champaign Urbana Mass Spectrometry Lab Services. All cyclodextrins and reagents for the synthesis of phosphonothioates were purchased from TCI (Portland, OR) and used without further purification. In a typical methanolysis reaction without the La(III) catalyst, 2.6 μL of DEPP (**5**) was added to a 1 mL 0.6 M NaOMe/MeOH solution at room temperature. Samples were periodically removed and monitored by ^{31}P NMR in $CDCl_3$ until the reaction reached completion (~30 min). Upon completion, the methanolic/methoxide solution was neutralized with HCl, dried in vacuo and then redissolved in 800 μL of D_2O. Cyclodextrin (70 mg) was added to this aqueous solution for enantiomeric resolution with ^{31}P NMR. For temperature dependent studies the methanolysis of DEPP was monitored (^{31}P) in a 0.6 M NaOMe/CD_3OD solution. Methanolysis with La(III) was done according to the procedure of Tsang and co-workers[16] except three times more $La(OTf)_3$ (40 mg versus 12.9 mg) was used. The methanolysis of DEPP by Zn(II) complexes followed the procedure of Desloges and co-workers[45] wherein the reaction took place in 1.0 mL of dry MeOH (with 20% CD_3OD as a NMR lock) containing 1 mM each of $Zn(OTf)_2$, neocuproine, NaOMe and **5**. The methanolysis was buffered in 0.5 mM tetrabutylammonium hydroxide.

Synthesis of *O*-Methyl or *O*-Ethyl Phenylphosphinate (PhP(O)(H)OEt) or PhP(O)(H)OMe) (1 or 1b). A solution of ethanol (22.1 mL, 540 mmol) (or appropriate alcohol), pyridine (26.2 mL, 325 mmol), and toluene (36 mL) was added dropwise over 30 min to a solution of dichlorophenylphosphine (34 mL, 250 mmol) in toluene (175 mL). The mixture was stirred for 1.5 h and allowed to sit without stirring for 1 day. The solution and resulting white solid were washed with saturated sodium bicarbonate (80 mL), and the aqueous layer was back extracted with methylene chloride (70 mL). The toluene and methylene chloride layers were combined, dried over magnesium sulfate, filtered and then concentrated down to the *O*-ethyl (**1**) oil product (29.94 g, 190 mol) with a 76% yield. The *O*-methyl product (**1b**) was made with a similar procedure with methanol as the alcohol. ^{1}H NMR ($CDCl_3$): δ 1.4 (t, 3H, O−CH_2−CH_3), 4.2 (q, 2H, O−CH_2−CH_3), 7.2 (m, 2H, meta) 7.5 (d, 2H, ortho), 7.6 (s, 1H, P−*H*), 7.8(d, 1H, para). ^{13}C NMR ($CDCl_3$): δ 16.7 (d, J_{PC} =7.1 Hz, O−CH_2−CH_3), 62.4 (d, J_{PC} = 6.4 Hz, O−CH_2−CH_3), 129.2 (d, J_{PC} = 13.7 Hz, meta), 131.3 (d, J_{PC} = 12 Hz, ortho), 133.5 (d, J_{PC} = 3 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$): δ 24.6.

Synthesis of *O*-Methyl and *O*-Ethyl Phenylphosphonothioate Dicyclohexylammonium Salt (2). Elemental sulfur (6.15 g, 190 mmol) was added to a solution of **1** (29.94 g, 190 mmol) and dicyclohexylamine (34.45 g, 190 mmol) in diethyl ether (300 mL) slowly over 30 min and then stirred for 4 h. The resulting solid was filtered off, dried and recrystallized with ethyl acetate. The resulting salt crystals of **2** were obtained (44.3 g, 120 mmol) with a 63% yield. The same protocol was used to make the dicyclohexylammonium salt of *O*-methyl phenylphosphonothioate (**2b**). ^{31}P NMR ($CDCl_3$): δ 67.43.

Interconversion of *O*-Alkyl Phenylphosphonothioate Dicyclohexylammonium Salt with Corresponding Thioacid (3). The **2** salt (44.3 g, 0.12 mol) was added to a stirring solution of sodium hydroxide (1.5 M, 230 mL), stirred for 45 min and then washed with toluene (3 × 100 mL). The aqueous layer was then acidified with sulfuric acid (6 N, 80 mL), which was then saturated with sodium chloride. The organic layer that formed was extracted with ether (4 × 200 mL), and the ether layer was dried over sodium sulfate and filtered. The ether solution was finally concentrated under reduced pressure to the resulting **3** thioacid oil (22.42 g, 120 mmol) with a 100% yield. ^{1}H NMR ($CDCl_3$): δ 1.4 (t, 3H, O−CH_2−CH_3), 4.2 (q, 2H, O−CH_2−CH_3), 7.5 (m, 3H, meta and para), 7.9 (d, 2H, ortho), 4.5 (br, 1H, S*H*). ^{31}P NMR ($CDCl_3$): δ 80.2.

Resolution of *O*-Ethyl Hydrogen Phenylphosphonothioate (3) with Brucine. A solution of the racemic **3** thioacid (20 g, 104 mmol) in acetone (50 mL) was added dropwise over 30 min to a boiling and stirring solution of acetone (550 mL) and brucine (41 g, 96 mmol). The volume was reduced below 600 mL with boiling and then cooled to room temperature and allowed to sit for 5 days. The resulting solid was then filtered from solution and recrystallized from methanol (300 mL) giving the white (*S*)-**4** brucine salt (26.04 g, 41.7 mmol; mp 198−210 °C). The remaining acetone solution was concentrated to an oil, which was converted to a solid upon the addition of methanol (120 mL). The mixture was then heated to boiling and filtered, giving the (*R*)-**4** brucine salt (22.75 g, 36.4 mmol; mp 110−115 °C). The combined yield of the brucine salts ((*S*)-**4** and (*R*)-**4**) was 75%. ^{31}P NMR ($CDCl_3$): δ 70.9.

The (*S*)-**4** brucine salt (25.0 g, 40.0 mmol) was dissolved in a solution of sodium hydroxide in 35% v/v methanol (0.900 M, 50 mL) and upon the addition of water (65 mL) it solidified. The resulting solid was then washed with methylene chloride (4 × 40 mL). The aqueous layer was then acidified with hydrochloric acid (6 M, 8 mL), and the resulting organic layer was extracted with methylene chloride (4 × 15 mL) and concentrated under reduced pressure to give the (*S*)-**3** thioacid oil (7.02 g, 34.6 mmol). The thioacid products are unstable and require transformation into the corresponding dicyclohexylammonium salt for long-term storage. Dicyclohexylamine (7.23 mL, 34.6 mmol) was added to a solution of this (*S*)-**3** thioacid (7.02 g, 34.6 mmol) in ether (70 mL), stirred for 2.5 h and filtered. The resulting (*S*)-**2** salt was recrystallized from ethyl acetate. Starting from the 25.0 g of brucine (*S*)-**4** salt, the recovered (*S*)-**2** cyclohexylammonium salt (5.49 g, 0143 mol) represented a 34% yield (mp 153−154 °C; $[\alpha]_D$ = +9.3°, methanol)

The (*R*)-**4** brucinium salt was converted to the thioacid ((*R*)-**3**) and then to the dicyclohexylammonium salt, (*R*)-**2**, with the same procedure (4.01 g, 11 mmol; 30% yield from the brucine salt; mp 152−153 °C; $[\alpha]_D$ = −7.7°, methanol).

Synthesis of *O,S*-Diethyl Phenylphosphonothioate, DEPP (5) and *O*-Methyl-*S*-Ethyl Phenylphosphonothioate (8). The dicyclohexylammonium salt of compound **2** (5.0 g, 13 mmol) was slowly added to a stirring solution of distilled toluene (100 mL) and ethyl iodide (2.4 mL, 30 mmol). The mixture was stirred for 3 days (but less time may be used). The resulting suspension was filtered and washed with anhydrous hexanes, which were concentrated under reduced pressure. This oil was washed repeatedly with a minimal amount of

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anhydrous hexanes to remove residual salt. The hexane washes were concentrated down to the resulting **5** oil (1.5 g, 6.3 mmol). Kugelrohr distillation was used if extraction with hexanes did not remove impurities. The enantiomerically pure (*S*)- and (*R*)-**5** were obtained from the resolved dicyclohexylammonium salts with a similar procedure with 53% and 61% yields respectively. ^{1}H NMR ($CDCl_3$): δ 1.3 (t, 3H, O−CH_2−CH_3), 1.4 (t, 3H, S−CH_2−CH_3), 2.8 (q, 2H, S−CH_2−CH_3), 4.2 (q, 2H, O−CH_2−CH_3), 7.5 (m, 3H, meta and para), 7.9 (d, 2H, ortho). ^{13}C NMR ($CDCl_3$): δ 16.6 (d, J_{PC} = 5.9 Hz, S−CH_2−CH_3), 16.8 (d, J_{PC} = 6.9 Hz, O−CH_2−CH_3), 25.3 (d, J_{PC} = 3.1 Hz, S−CH_2−CH_3), 62.5 (d, J_{PC} = 6.9 Hz, O−CH_2−CH_3), 128.9 (d, J_{PC} = 14.75 Hz, meta), 131.6 (d, J_{PC} = 10.6 Hz, ortho), 132.9 (d, J_{PC} = 3.1 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$): δ 44.65. HRMS: calculated for $C_{10}H_{15}O_2SP$ 230.05304 (EI^+); found 230.05364.

The *O*-methyl-*S*-ethyl phenylphosphonothioate (**8**) was made in the same manner starting with the dicyclohexylammonium *O*-methyl phenylphosphonothioate salt, **2b**. ^{1}H NMR ($CDCl_3$): δ 3.89 (s 3H, O−CH_3), 2.77 (q, 2H, S−CH_2−CH_3), 1.29 (t, 3H, S−CH_2−CH_3), 7.9 (d, ortho, 2H), 7.6 (m, meta and para, 3H). ^{13}C NMR ($CDCl_3$): δ 16.6 (d, J_{PC} = 5.6 Hz, S−CH_2−CH_3), 25.3 (d, J_{PC} = 3.0 Hz, S−CH_2−CH_3), 52.6 (d, J_{PC} = 7.2 Hz, O−CH_3), 128.9 (d, J_{PC} = 14.9 Hz, meta), 131.6 (d, J_{PC} = 10.7 Hz, ortho), 132.9 (d, J_{PC} = 3.3 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$, ppm): δ 46.7.

Synthesis of *S*-Methyl *O*-Ethyl Phenylphosphonothioate (6). The dicylcohexylammonium salt of racemic *O*-ethyl phenylphosphonothioate (**2**) (500 mg, 1.3 mmol) was added to stirring distilled methyl iodide (3.5 mL, 56 mmol). The solution was stirred for 24 h and filtered, and the precipitate was washed with anhydrous hexanes. The filtrate and hexane washes were concentrated under reduced pressure and then Kugelrohr distilled at 70−85 °C (15−20 μm) giving the resulting colorless **6** oil (90 mg, 0.40 mmol) with a 32% yield. ^{1}H NMR ($CDCl_3$): δ 1.4 (t, 3H, O−CH_2−CH_3), 2.2 (s, 3H, S−CH_3), 4.2 (m, 2H, O−CH_2−CH_3), 7.5 (m, 3H, meta and para), 7.9 (d, 2H, ortho). ^{13}C NMR ($CDCl_3$): δ 12.4 (d, J_{PC} = 3.43 Hz, S−CH_3), 16.8 (d, J_{PC} = 6.7 Hz, O−CH_2−CH_3), 62.6 (d, J_{PC} = 7.2 Hz, O−CH_2−CH_3), 128.9 (d, J_{PC} = 13.35 Hz, meta), 131.6 (d, J_{PC} = 11.5 Hz, ortho), 132.9 (d, J_{PC} = 3.3 Hz, para), 133.2 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$): δ 45.5. HRMS: calculated for $C_9H_{13}O_2PS$ (EI^+) 216.03739; found 216.03835.

Synthesis of Enantiomerically Pure (*S*)-*O*-Ethyl *O*-Methyl Phenylphosphonothioate (7). (*S*)-**6** (1.17 g, 5.0 mmol), made from (*S*)-**2** as indicated above, was added to a suspension of silver nitrate (1.68 g, 10 mmol) in 10 mL of methanol at 0 °C. The mixture was then stirred for 24 h. This suspension was filtered, reduced down to 5 mL, and then diluted with methylene chloride (50 mL). The solution was washed with saturated sodium bicarbonate (4 × 25 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure to the **7** oil (625 mg, 3.0 mmol) with a 62.5% yield. No further purification was performed. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, O−CH_2−CH_3), 3.7 (s, 3H, O−CH_3), 4.15 (m, 2H, O−CH_2−CH_3), 7.53 (m, 3H, meta and para), 7.8 (d, 2H, ortho). ^{13}C NMR ($CDCl_3$): δ 16.8 (d, J_{PC} = 6.5 Hz, O−CH_2−CH_3), 52.9 (d, J_{PC} = 5.2 Hz, O−CH_3), 62.7 (d, J_{PC} = 5.5 Hz, O−CH_2−CH_3), 128.3 (d, J_{PC} = 14.9 Hz, meta), 131.6 (d, J_{PC} = 10.9 Hz, ortho), 132.3 (d, J_{PC} =3.3 Hz, para), 133.2 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$): δ 20.23. HRMS: calculated for $C_9H_{13}O_3P$ (EI^+) 200.05898; found 200.06024.

X-ray Structure Analysis of Brucinium Salt of (*S*)-*O*-Ethyl Phenylphosphonothioate. A colorless platelike crystal of dimensions 0.080 mm × 0.300 mm × 0.400 mm was used for X-ray crystallographic analysis on a Bruker SMART X2S benchtop diffractometer equipped with a Mo Kα microfocus source (λ = 0.7107 Å). A total of 2160 frames were collected that were integrated (Bruker SAINT v7.68) using a monoclinic unit cell in the range of $2.52° \leq \theta \leq 25.02°$. The structure was solved and refined using the Bruker SHELXTL Software Package with space group *P*1 21 1 and *Z* = 2. The final anistropic full-matrix least-squares refinement converged at R1 = 4.28%. Table 1 summarizes the crystal data and final residuals, and more extensive tables including specific details of data collection and structure refinement are in the Supporting Information.

Table 1. Crystallographic Data for Compound 4

chemical formula	$C_{31}H_{37}N_2O_6PS$	
formula weight	596.66	
temperature	199(2) K	
wavelength	0.71073 Å, Mo Kα	
crystal size	0.080 × 0.300 × 0.400 mm	
crystal habit	colorless plate	
crystal system	monoclinic	
space group	*P* 1 21 1	
unit cell dimensions	*a* = 8.608(2) Å	α = 90°
	b = 14.461(3) Å	β = 109.748°
	c = 12.669(4) Å	γ = 90°
volume	1484.3(7) Å^3	
Z	2	
density (calcd)	1.335 Mg/cm^3	
adsorption coefficient	0.210 mm^{-1}	
F(000)	632	
theta range	2.52 to 25.02°	
reflections collected	23505	
independent reflections	5180 [*R*(int) = 0.0706]	
max. and min transmission	0.9834 and 0.9208	
function minimized	$\sum w(F_o^2 - F_c^2)^2$	
data/restraints/parameters	5180/1/377	
final *R* indices[a]	4240 data; $I > 2\sigma(I)$ R1 = 0.0428, wR2 = 0.1023	
	all data R1 = 0.0581, wR2 = 0.1098	

[a] $w = 1/[\sigma^2(F_o^2) + (0.0508P)^2 + 0.2209P]$ where $P = (F_o^2 + 2F_c^2)/3$.

■ ASSOCIATED CONTENT

Supporting Information

Detailed ^{31}P NMR spectra, activation measurements, and crystal structure report for LewClark_brucine. This material is available free of charge via the Internet at http://pubs.acs.org.

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