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Phosphonothioate Hydrolysis by Molybdocene Dichlorides: Importance of Metal Interaction with the Sulfur of the Thiolate Leaving Group

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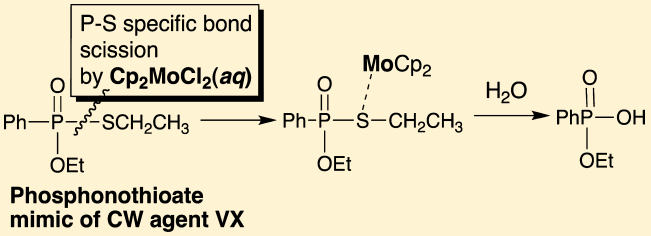
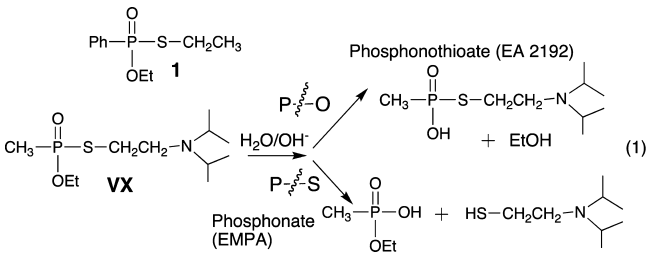
Supporting Information

ABSTRACT: The metallocene bis(cyclopentadienyl)-molybdenum(IV) dichloride Cp_2MoCl_2 hydrolyzes *O*,*S*-diethyl phenylphosphonothioate (**1**) with only P−S scission to yield a phosphonate under mild aqueous conditions. In terms of degrading phosphonothioate neurotoxins, exclusive cleavage of the P−S linkage is preferred, for P−O scission yields another toxic phosphonothioate. Structure−activity relationship studies were undertaken with various phosphonothioates to test the hypothesis for the exclusive P−S scission by $Cp_2Mo(aq)$. Kinetics data show that the rates of phosphonothioate hydrolysis correspond to the size of the alkanethiolate leaving group on treatment with Cp_2MoCl_2. This suggests that the exclusive P−S scission of **1** is due to the interaction between the thiophilic Cp_2Mo with the sulfur site of the P−SR linkage.



INTRODUCTION

Phosphonothioates with the tetrahedral $RP(O)(SR^1)(OR^2)$ structure are acetylcholine esterase inhibitors,[1,2] which function as pesticides and chemical warfare agents.[3,4] In this regard, the compound *O*,*S*-diethyl phenylphosphonothioate[5] (**1**) is an analogue of the chemical warfare agent VX due to its P−O and P−S linkages that have similar hydrolytic labilities.[5] In the hydrolytic degradation of phosphonothioates, P−S scission is the preferred avenue, as it yields a less toxic phosphonate.[5] For example, alkaline hydrolysis of VX (and **1**) in 0.10 M NaOH proceeds 15−18% of the time through P−O scission to yield another phosphonothioate (EA 2192) that is as toxic as the parent VX (eq 1).[4,5] Moreover, in alkaline solution the toxic EA 2192 product is an anion that is further recalcitrant to hydrolysis by the hydroxide nucleophile.

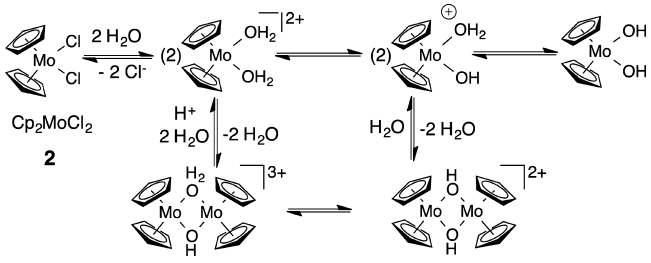


As such, in the degradation of phosphonothioates, there is a need for compounds that proceed exclusively through P−S scission. Methanolysis catalyzed by lanthanide(III) is an effective tool for degrading phosphonothioates,[6,7] and the rapid nature of this protocol has been capitalized for live nerve agent (i.e., VX) destruction.[7b] In addition, iodosobenzoate (IBA) promotes exclusive P−S scission of **1** in water, albeit with no turnover.[8] The fluoride anion also hydrolyzes VX to form an intermediate phosphonofluoridate that subsequently undergoes hydrolysis to form a phosphonate.[9]

In terms of organometallic compounds that degrade organophosphates, the metallocenes have been investigated, specifically, the compound bis(cyclopentadienyl)molybdenum-(IV) dichloride[10] (**2**, Cp_2MoCl_2; Cp = η^5-C_5H_5). In water, the Cp rings remain bound to the diamagnetic Mo(IV) center even at neutral pH while both chlorides rapidly hydrolyze ($\tau_{1/2} \approx 20$ min) to form several aquated species (Scheme 1).[11]

The molybdenum metallocene has strategic aqueous properties that have yielded many transformations in water. These include aqueous C−H activation (catalytic),[12] and nitrile and ester hydrolysis.[13] Its aqueous chemistry is dominated by a equilibrium between the monomer and the μ-OH dimer (Scheme 1), in which the latter is significantly favored.[14]

Scheme 1



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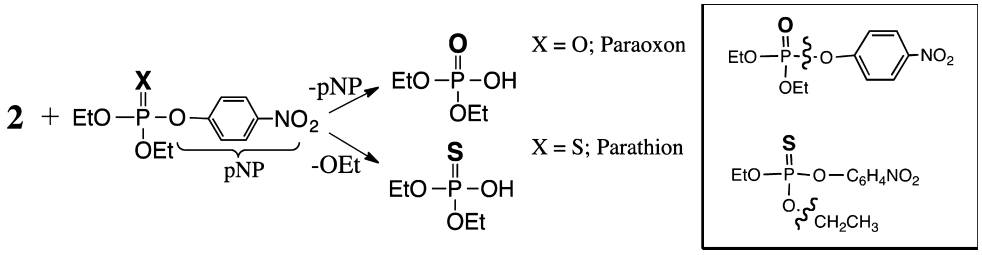
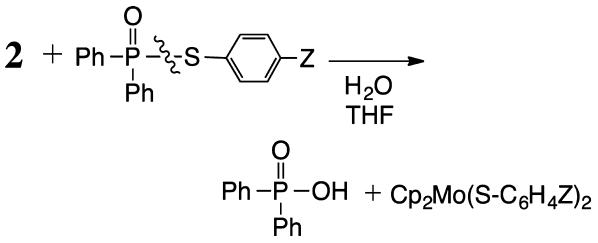
Scheme 2



We reported that **2** was the first organometallic compound that hydrolyzed organophosphates. This included phosphate diesters with activated (*p*-nitrophenolate)[15] and nonactivated (methoxide)[16] leaving groups. The phosphate hydrolytic studies by **2** were extended toward the pesticides parathion and paraoxon, where the Cp_2Mo moiety served as a Lewis acid to activate these triesters to effect a ~3000-fold rate acceleration.[17] Although the two pesticides differ by only one atom in the P═X functionality, **2** effected very different hydrolytic pathways, as summarized in Scheme 2. In one case ethoxide was the leaving group for parathion hydrolysis by **2** that resulted from C–O scission. However, the leaving group for paraoxon hydrolysis was *p*-nitrophenolate that resulted from P–O cleavage.

In terms of sulfur-containing organophosphates, we reported **2** hydrolyzed two types of thiophosphates. In the first class, a series of thioaryl leaving groups were installed on diphenylphosphinates. This yielded a convenient handle to probe the mechanism of hydrolysis by the aquated Cp_2Mo (eq 2).[18]



(2)

Structure–activity relationships on the hydrolysis of various thioaryl diphenylphosphinate by **2** indicated a pathway that was not $S_N2(P)$.[18] In addition, there were large negative entropies of activation of −49 and −63 J/(mol K) in Scheme 2[17] and eq 2,[18] respectively. This indicated triester phosphate hydrolysis by **2** proceeds with a bimolecular mechanism or one that goes through an ordered transition state.

In terms of the second class of sulfur-containing phosphate esters, we reported the first case of an organometallic compound that hydrolyzes phosphonothioate **1** under extremely mild conditions (pH 7, 30 °C) with specific P–S scission (Figure 1).[19]

This work was complemented by a report showing that cyclopentadienyl or solvent variations altered the monomer–dimer equilibrium which subsequently controlled the rate of DEPP hydrolysis.[20] While phosphonothioate hydrolysis of **1** by **2** was only stoichiometric, the addition of thiophilic materials such as borohydride-capped silver nanoparticles did promote modest turnover.[21]

One pressing question is as follows: what is the basis for the P–S specific hydrolysis of **1** in the presence of **2**? A reasonable hypothesis revolves around the known thiophilicity of the Mo(IV) center, wherein several Cp_2Mo–thiolate complexes are

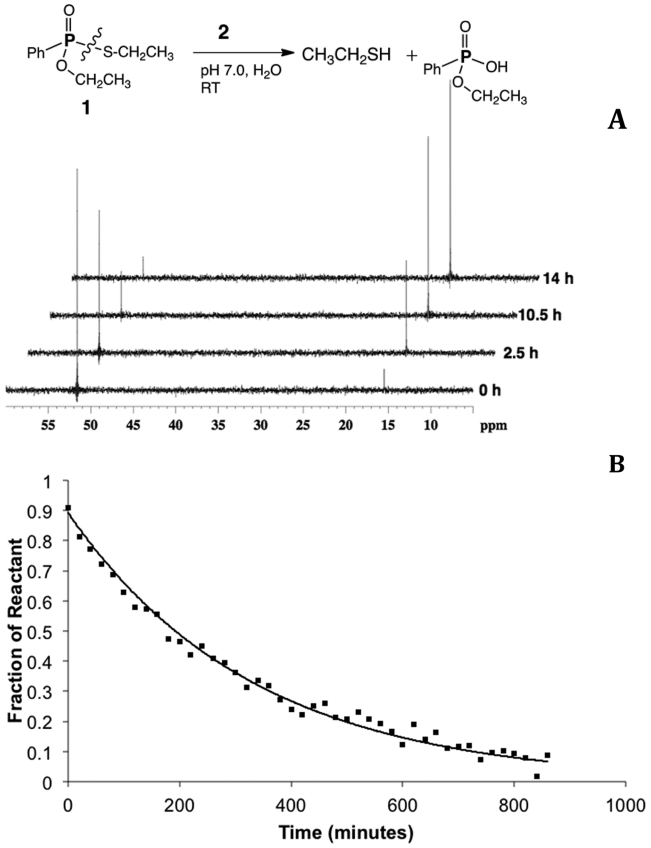
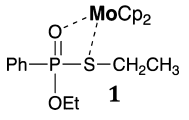


Figure 1. (A) ^{31}P NMR of the hydrolysis of **1** by **2** (1:7 ratio) at room temperature in pH 7 MOPS (300 mM) buffer. Starting **1** is at 51.5 ppm and only one resulting phosphonate product (P–S scission only) appears at 15.5 ppm. (B) Plot showing the fraction of **1** remaining with time that was used to obtain first-order rate constants for subsequent correlation plots (k_{obs} = 2.89 × 10^{-3} min^{-1}).

readily made from **2**.[22] The $Cp_2Mo–(SPr)_2$ bond of 243 kJ/mol[23] makes the Cp_2Mo–S bond as strong or stronger than typical transition-metal bonds to carbon or dialkylamino groups.[24] Indeed, the formation of $Cp_2Mo(SCH_2CH_3)_2$ from the reaction of **2** and **1** is proposed to be the reason the molybdenum metallocene alone does not hydrolyze the phosphonothioate with turnover. The strong thiophilicity of **2** promotes the desired P–S scission, but it also yields an inert $Cp_2Mo(SR)_2$ product that is unable to hydrolyze additional equivalents of **1**.



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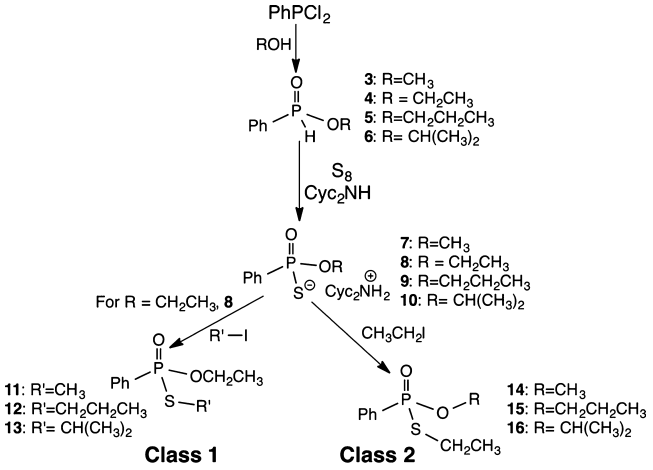
In this connection, we examine if Cp_2Mo binding to the thiolate leaving group is important for P−S scission through a structure−activity investigation. Accordingly, several alkyl phenylphosphonothioates were made in which the alkanethiolate and alkoxide groups are varied. If Cp_2Mo accessibility to the sulfur linkage is important to phosphonothioate hydrolysis, then alkanethiolate variations would affect the rates of phosphonothioate hydrolysis. Conversely, variations of the alkoxide would minimally affect the rates of phosphonothioate hydrolysis by **2**. We therefore report the syntheses of these phosphonothioate derivatives and their corresponding hydrolytic rates by **2** that support this hypothesis. In addition, an ancillary finding in phosphorus chemistry is made. Hydrogen phenylphosphinates (PhP(O)(H)(OR)) are key intermediates in making phosphonothioates, and we demonstrate a facile H/D exchange in D_2O that underscores the lability of the P−H functionality.

RESULTS AND DISCUSSION

Phosphonothioate Synthesis. Phosphonothioate derivatives of **1** were made according to Scheme 3, developed by

Scheme 3

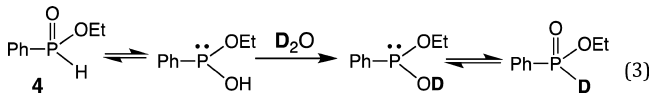


DeBruin and co-workers.[25] This begins with the addition of dichlorophenylphosphine to the appropriate alcohol to afford the *O*-alkyl phenylphosphinate intermediate PhP(O)(H)(OR) (**3**−**6**). These phosphinates were converted to the corresponding phosphonothioic acids with the addition of sulfur and stored as the dicyclohexylammonium salts **7**−**10** for stability purposes.

From this point, there are two classes of phosphonothioates. The first class, **11**−**13**, was made by nucleophilic substitution on the appropriate alkyl iodide (R′-I) with *O*-ethyl phenylphosphonothioate (**8**) to yield analogues containing variations on the alkanethiolate group. The second class of compounds, **14**−**16**, has the same variations on the alkoxide functionality and uses the *O*-alkyl phenylphosphonothioates **7**−**10** in the substitution reaction with ethyl iodide. This route installs methyl, ethyl, propyl, and isopropyl groups (corresponding steric Taft parameter: 0.00, −0.07, −0.36, −0.47)[26] on the bridging sulfur or oxygen atoms of the phosphonothioate. These Taft parameters have been used to gauge how pharmaceutical effects depend on steric effects for medicinal compounds.[27] Steric factors such as the Taft parameter are part of the Hansch equation[28] used in quantitative structure analysis relationship (QSAR) for drug development.[29] Attempts to append halogenated alkyl groups off the oxygen to alter the electronic environment were unsuccessful. The intermediate *O*-haloethyl phenylphosphinates (i.e., 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, and 2-chloroethyl groups) were unstable for subsequent sulfur insertion to form the phosphonothioate salt.

H/D Exchange of Phenyl Phosphinate in D_2O. A key intermediate in the synthesis of these phosphonothioates was the phosphinate containing the P−H functionality. These phosphinates are critical for making disubstituted phosphinic acid esters, $R^1R^2P(O)(OR)$, which have medicinal and ligation properties.[30] We were interested in the fundamental aqueous P−H chemistry of these phosphinates, since this functionality is the launching point toward many useful organophosphorus compounds. For example, Montchamp and co-workers, who used phosphinates in several useful coupling transformations, proposed a mechanism that involved a P(V) → P(III) tautomerization to generate the key P(III) nucleophile.[31] In this connection, we found a facile H/D exchange of the P−H group that goes through this tautomerization.[32] When **4** is added to D_2O (pH 7, 300 mM MOPS buffer) the ^{31}P NMR spectroscopic (^1H decoupled) singlet at 31.5 ppm gradually changes to a 1:1:1 triplet, as shown in Figure 2.

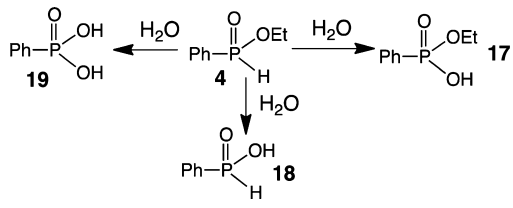
Initially, this signal has a J_{PH} of 570 Hz (doublet) with the ^1H decoupler off, but upon incubation in D_2O, a 1:1:1 triplet grows in with a coupling constant of 89 Hz. The ratio of these two coupling constants (6.40) is close to the gyromagnetic ratio of hydrogen to deuterium (γ_H/γ_D = 6.51),[33] which underscores the P−H → P−D transformation. This H/D exchange in pH 7 D_2O was sufficiently slow enough that a temperature dependence study was made to give a E_a value of 22.7 ± 0.5 kcal/mol (Figure S1, Supporting Information). We propose that the P−H → P−D transformation proceeds via a tautomerization (eq 3) similar to that noted by Montchamp.[31]



(3)

Prior theoretical work on the phosphonate $HP(O)(OMe)_2$[34] suggested this tautomerization was a 1,2-hydride shift. In our particular case the tautomerization process for **4** occurs under aqueous and aerobic conditions.

In addition to forming the deuterated phosphinate in eq 3, ^{31}P NMR spectra (Figure S2, Supporting Information) shows that the hydrolysis of **4** (pH 7) readily yields three other phosphonates (eq 4), as confirmed by authentic addition. They



(4)

are ethyl phenylphosphonate (**17**), phenylphosphinate (**18**), and phenyl phosphonate (**19**) (eq 3). Unfortunately the formation of these species did not follow clean first-order kinetics and there was also a fourth unidentified species. The facile aqueous "decomposition" of **4** to multiple products precludes the use of this type of phosphinate as a possible hydride[35] source in water.

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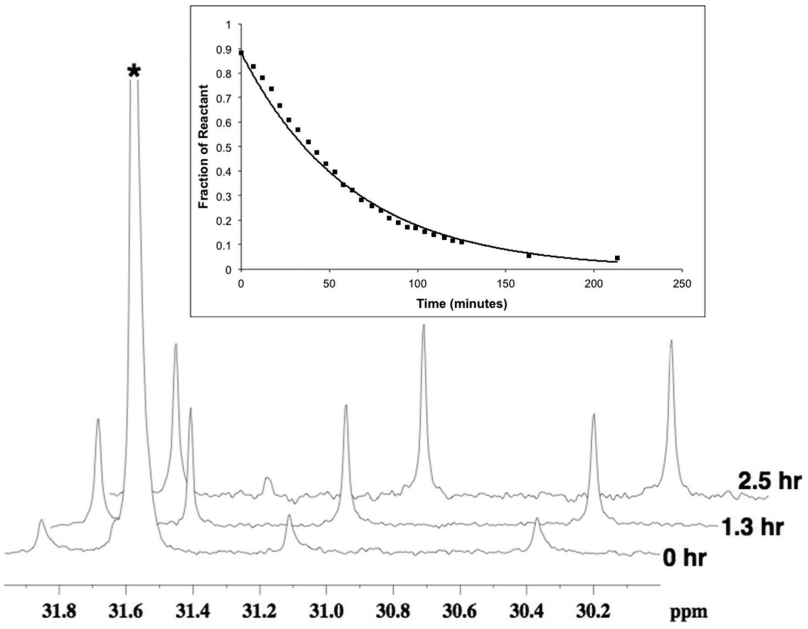

Figure 2. ^{31}P NMR spectrum (^{1}H-decoupled) showing H/D exchange in the P−H bond of phosphinate **4** in D_2O (pH 7, 300 mM MOPS buffer) at 15 °C. The signal marked with an asterisk (*) represents the starting **4** that gradually fades away to be replaced by a 1:1:1 triplet with a coupling constant of 89 Hz. The starting **4** signal is a 570 Hz doublet with the ^{1}H decoupler off. Inset: time plot showing the decreasing fraction of the starting signal for **4** that has a first-order decay rate of 16.9×10^{-3} min^{-1}.

Structure−Activity Relationship of Phosphonothioate Hydrolysis. The title compound in hydrolytic rate comparisons is compound **1**, and compounds **11−13** have steric variations on only the alkanethiolate group, while **14−16** contain the same variations on the alkoxide. Both phosphonothioate classes were subject to anaerobic hydrolysis by **2** under identical aqueous conditions (pH 7, 300 mM MOPS, 25 °C) where the molybdenum metallocene was in ∼7-fold excess. To a first approximation, the ^{31}P NMR spectroscopic signal (∼51 ppm) for the phosphonothioates decayed with first-order kinetics in the presence of **2** (Figure S3, Supporting Information). Moreover, in all these cases only one ^{31}P NMR spectral product signal (∼15 ppm) was seen that was consistent with phosphonate production (i.e., only P−S scission).

The hydrolysis rates of the four phosphonothioates **1** and **11−13** by **2** correlate well with the Taft parameter (E_s) of the alkanethiolate leaving group (Figure 3). It should be noted the alkoxide group (OCH_2CH_3) for these four phosphonothioates was kept constant, and the Taft parameter, a quantitative steric parameter, is derived from hydrolysis rates of alkyl benzoates.[26] The data in Figure 3 show that as the size of the alkanethiolate leaving group increases, the rates of phosphonothioate hydrolysis by **2** decreases. This structure−rate relationship supports the hypothesis that interaction between the thiophilic molybdenum center and the sulfur leaving group is an important part of phosphonothioate hydrolysis by **2**. This is consistent with the observed P−S specific scission of **1** upon hydrolysis by **2**. IBA (iodosobenzoate)-promoted hydrolysis of phosphonothioates proceeds with P−S specific bond scission (vide supra). Interestingly, the correlation between phosphonothioate hydrolysis rates and the size of the thiolate leaving group was also seen in IBA-promoted degradation (6-fold excess of IBA) (Figure S4, Supporting Information).

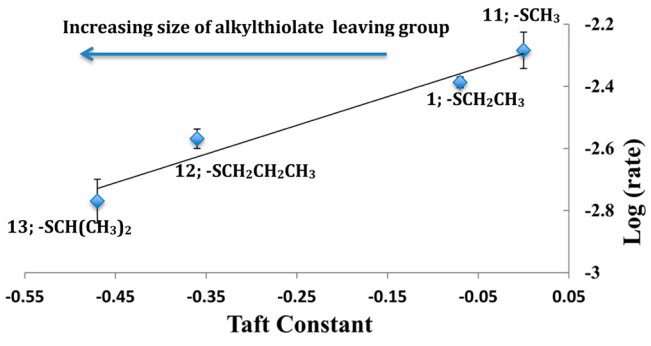

Figure 3. Hydrolyses of various *S*-alkyl *O*-ethyl phenylphosphonothioates by **2** (25 °C, pH 7, 300 mM MOPS) that show a correlation between the size of the alkanethiolate leaving group (E_s) and the hydrolysis rates. This is consistent with the hypothesis that accessibility to the sulfur linkage by Cp_2Mo (**2**) plays a critical role in the P−S specific scission of phosphonothioates by **2**. The slope was 0.925.

We were interested in examining this structure−rate correlation for the *alkaline* hydrolysis of phosphonothioates, because this transformation yields both P−S and P−O scission (eq 1). It therefore provides the platform to ask the converse question of whether a nonspecific P−S scission is connected with the absence of a structure−rate correlation. A concentration of 0.020 M NaOH allowed a sufficiently slow hydrolysis that could be monitored at room temperature over a reasonable time span for phosphonothioates **1** and **11−13**. As shown in Figure 4, there is little or no correlation in the structure−rate relationship for these phosphonothioates where the alkanethioate group was varied. In fact, the compound with the smallest leaving group, methanethiolate in **11**, had the slowest rate of alkaline hydrolysis. This result underscores the importance of accessibility to the bridging sulfur of phosphonothioates for selective P−S scission.

The final structure−rate correlation is with a phosphonothioate series containing variations on the alkoxide where the ethanethiolate leaving group was unchanged. If accessibility to the sulfur leaving group by Cp_2Mo is critical for P−S scission,

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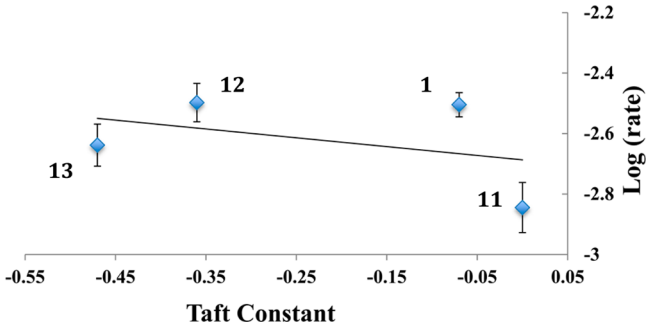

Figure 4. Alkaline hydrolysis (25 °C, 0.020 M NaOH) of the *S*-alkyl *O*-ethyl phenylphosphonothioates that yields both P−O and P−S scission. Hydrolysis rates measured the fraction of starting phosphonothioate with respect to time. This shows no correlation with the size of the alkanethiolate leaving group (E_s) for a nonspecific hydrolysis process (i.e., alkaline hydrolysis).

then alterations on the alkoxide would have a negligible effect on the rates of phosphonothioate hydrolysis by **2**. This is borne out in the structure−rate graph in Figure 5, which shows a

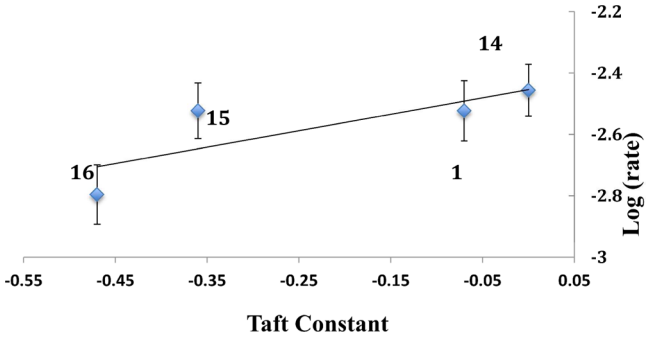

Figure 5. Hydrolysis of various *O*-alkyl-*S*-ethyl phenylphosphonothioates by **2** (25 °C, pH 7) where steric alteration (E_s) is on the alkoxide group. The particular phosphonothioates were **1** and **14**−**16** where the alkanethiolate leaving group was not altered. A weaker correlation with the steric size on the alkoxide and hydrolysis rates by **2** vis a vis Figure 3 suggests little interaction between the Cp_2Mo and the oxygen linkage. The slope is 0.536.

weaker correlation between the size of the alkoxide group and the rates of phosphonothioate hydrolysis. In comparison to Figure 3, the structure−rate plot for alkoxide variation has half the slope and is flat if the most sterically encumbered isopropoxide phosphonothioate (**16**) is not considered.

CONCLUSION

The metallocene bis(cyclopentadienyl)molybdenum(IV) dichloride promotes the specific and desired P−S scission in the hydrolysis of phenyl phosphonothioates. This represents the first organometallic compound to accomplish the degradation of this class of neurotoxin under benign conditions. The structure−activity results support the hypothesis that this P−S specific scission is due in part to an interaction between the thiophilic Cp_2Mo metal center and the sulfur site of the alkanethiolate leaving group. A similar interaction may be possible with the alkoxide functionality of the phosphonothioate, but it has at best a marginal effect toward phosphonothioate hydrolytic activity. These findings are also consistent with the rationale for why **2** is unable to promote turnover in the hydrolysis of **1**. The thiophilicity of the molybdenum center, which promotes the desired P−S specific scission, also precludes it from hydrolyzing additional equivalents of **1** because the Cp_2Mo moiety is bound to the alkanethiolate leaving group.

EXPERIMENTAL SECTION

Caution! The phosphonothioates are known neurotoxins, and all experimental work was done with gloves in well-ventilated hoods. In addition, all glassware exposed to the phosphonothioates was bleached overnight prior to base bath cleaning to oxidize any of the phosphonothioates. It is highly recommended that these phosphonothioates be synthesized on a semi-microscale as described below to minimize release and exposure.

Equipment. All NMR spectra (^{13}C, ^{31}P, and ^{1}H) were acquired using a Bruker Advance-300 MHz NMR spectrometer at 75, 121, and 300 MHz, respectively. High-resolution mass spectra were measured by the University of Illinois at Champaign-Urbana Spectrometry Lab Services. Molybdocene dichloride (**2**) was purchased from Strem Chemical Co. (Newburg, MA) and was used as received. This metallocene was stored in an Innovative Technology System One Glovebox, and experiments outside of the glovebox were done with standard Schlenk line techniques using argon gas to protect **2**. The IBA, dichlorophenylphosphine, and organophosphorus compounds **17**−**19** were purchased from Tokyo Chemical Industrial Company (Portland, OR). Before use, the IBA was first converted to an IBA sodium salt with NaOH. The D_2O and alcohols were purchased from Aldrich (Milwaukee, WI) and used as is. MOPS (3-(*N*-morpholino)-propanesulfonic acid) and the MOPS salt were purchased from Sigma.

Phosphonothioate Synthesis. The phosphonothioate syntheses utilized the procedure by DeBruin and co-workers[25] where the initial phenylphosphinate (**3**−**5**) was made from dichlorophenylphosphine and the appropriate alcohol. For example, in the synthesis of ethyl phenylphosphinate (**4**) used to make **1**, a solution of ethanol (22.1 mL, 540 mmol) and pyridine (26.2 mL, 325 mmol) in 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 give 29.9 g (190 mmol) of *O*-ethyl hydrogen phenylphosphinate (**4**) in 76% yield. The spectroscopic properties were similar to those reported previously.[25]

The other alkyl phenylphosphinates were made in the same manner, but in place of ethanol, the alcohols methanol, propanol, and 2-propanol were used to make **3**, **5**, and **6**, respectively.

*Methyl Phenylphosphinate (**3**).* ^{1}H NMR ($CDCl_3$, ppm): δ 3.80 (d, $^{3}J_{PH}$ = 12.9 Hz, O−CH_3, 3H), 7.79 (d, ortho, 2H), 7.57 (m, meta and para, 3H), 7.55 (d, J_{PH} = 565 Hz, P*H*, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 52.5 (d, $^{2}J_{PC}$ = 6.7 Hz, O−CH_3), 131.4 (d, $^{2}J_{PC}$ = 11.8 Hz, ortho), 129.2 (d, $^{3}J_{PC}$ = 13.8 Hz, meta), 133.6 (d, $^{4}J_{PC}$ = 2.8 Hz, para). ^{31}P NMR ($CDCl_3$, ppm): δ 27.1.

*Propyl Phenylphosphinate (**5**).* ^{1}H NMR ($CDCl_3$, ppm): δ 4.04 (m, $^{3}J_{HH}$ = 7.1 Hz, O−CH_2−CH_2CH_3, 2H), 1.75 (m, O−CH_2−CH_2−CH_3, 2H), 0.97 (t, $^{3}J_{HH}$ = 7.1 Hz, O−CH_2CH_2−CH_3, 3H), 7.79 (t, $^{3}J_{PH}$ = 14.8, Hz, ortho, 2H), 7.56 (m, meta and para, 3H), 7.61 (d, J_{PH} = 565 Hz, P*H*, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 67.8 (d, $^{2}J_{PC}$ = 6.4 Hz, O−CH_2−CH_2CH_3), 24.1 (d, $^{3}J_{PC}$ = 6.5 Hz, O−CH_2−CH_2−CH_3), 10.4 (O−CH_2CH_2−CH_3), 131.2 (d, $^{2}J_{PC}$ = 11.9 Hz, ortho), 129.1 (d. $^{3}J_{PC}$ = 13.6 Hz, meta), 133.4 (d, $^{4}J_{PC}$ = 2.7 Hz, para). ^{31}P NMR ($CDCl_3$, ppm): δ 24.9.

*Isopropyl Phenylphosphinate (**6**).* ^{1}H NMR ($CDCl_3$, ppm): δ 4.72 (spt, O−C*H*−$(CH_3)_2$, 1H), 1.42 and 1.34 (dd, $^{3}J_{HH}$ = 6.2 Hz, O−CH−$(CH_3)_2$, 6H), 7.78 (t, $^{3}J_{PH}$ = 14.5 ortho, 2H), 7.55 (m, meta and para, 3H), 7.25 (d, J_{PH} = 564 Hz, P*H*, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 71.7 (d, $^{2}J_{PC}$ = 6.5 Hz, O−CH−$(CH_3)_2$), 24.5 and 24.2 (dd, $^{3}J_{PC}$ = 4.5 Hz, O−CH−$(CH_3)_2$), 131.2 (d, $^{2}J_{PC}$ = 11.6 Hz, ortho), 129.0 (d.

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$^3J_{PC}$ = 14.4 Hz, meta), 133.2 (d, $^4J_{PC}$ = 3.2 Hz, para). ^{31}P NMR ($CDCl_3$, ppm): δ 22.3.

These phosphinates containing the P−H functionality were converted to the dicyclohexylammonium salts of the corresponding phosphonothioates (**7**−**10**) through a procedure described by Glazier and co-workers for **8**.[6b] This involved the addition of an equimolar amount of sulfur to an ether (300 mL) solution of the phosphinate (**3**−**6**) containing 1 equiv of dicyclohexylamine. The resulting solid was collected by filtration, dried, and recrystallized with ethyl acetate.

A semi-microscale synthesis of the final phosphonothioates **11**−**16** was developed that began with the dicyclohexylammonium salt of the phosphonothioate **8**. The synthesis of *O*-ethyl *S*-methyl phenylphosphonothioate (**11**) began with dissolving 0.50 g of **8** (1.3 mmol) in 3.5 mL of methyl iodide (0.056 mol). This solution was stirred constantly for 24 h, while being monitored by ^{31}P NMR. Once the formation of the final phosphonothioate (∼52 ppm) was confirmed, the dicyclohexylammonium iodide salt was then filtered and purified by several washes with HPLC-grade hexane. The hexane washes were concentrated using high-vacuum Kügelrohr distillation, giving a colorless oil (0.09 g, 0.42 mmol) with a 32% yield. Later syntheses done *without* Kügelrohr distillation gave a 50% yield. ^{1}H NMR ($CDCl_3$, ppm): δ 4.31 (dq, O−CH_2−CH_3, 2H), 1.44 (t, O−CH_2−CH_3, 3H), 2.20 (d, S−CH_3, 3H), 7.89 (t, ortho, 2H), 7.52 (m, meta 2H), 7.59 (t, para 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 62.61 (d, $^2J_{PC}$ = 6.9 Hz, O−CH_2−CH_3), 12.38 (d, $^3J_{PC}$ = 3.3 Hz, O−CH_2−CH_3), 16.77 (d, $^2J_{PC}$ = 6.9 Hz, S−CH_3), 131.63 (d, $^2J_{PC}$ = 10.8 Hz, ortho), 128.8 (d, $^3J_{PC}$ = 14.5 Hz, meta), 133.0 (d, $^4J_{PC}$ = 3.2 Hz, para), 132.5 (d, J_{PC} = 150.5 Hz, ipso). ^{31}P NMR ($CDCl_3$, ppm): δ 52.5. HRMS: calcd for $C_9H_{13}O_2SP$ 216.03739, found 216.03835.

***O*-Ethyl *S*-Propyl Phenylphosphonothioate (12).** The dicyclohexylammonium salt **8** (0.500 g, 1.3 mmol) was dissolved in 2 mL (20.4 mmol) of 1-iodopropane and stirred for 1 day. An additional 0.25 mL (0.975 mmol) of 1-iodoproane was added to the solution with continued stirring. The reaction was monitored with ^{31}P NMR, and once a single peak at around 52 ppm was obtained, the reaction mixture could be purified. HPLC-grade hexane was added to the final solution to precipitate out the dicyclohexylammonium iodide salt. The clear hexane layer above was extracted, and further hexane washes were done with the remaining dicyclohexylammonium iodide salt. Combination of the hexane washes followed by drying in vacuo gave **12** as a yellow oil (0.15 g, 0.64 mmol, 50% yield). ^{1}H NMR ($CDCl_3$, ppm): δ 4.22 (dq, O−CH_2−CH_3, 2H), 1.41 (t, O−CH_2−CH_3, 3H), 2.72 (m, S−CH_2−CH_2−CH_3, 4H), 0.93 (t, S−CH_2−CH_2−CH_3, 3H), 7.89 (t, ortho, 2H), 7.51 (m, meta, 2H), 7.56 (t, para, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 62.4 (d, $^2J_{PC}$ = 6.8 Hz, O−CH_2−CH_3), 16.7 (d, $^3J_{PC}$ = 7.1 Hz, O−CH_2−CH_3), 32.7 (d, $^2J_{PC}$ = 3.0 Hz, S−CH_2−CH_2−CH_3), 24.4 (d, $^3J_{PC}$ =5.4 Hz, S−CH_2−CH_2−CH_3), 14.5 (s, S−CH_2−CH_2−CH_3), 131.5 (d, $^2J_{PC}$ = 10.9 Hz, ortho), 128.8 (d, $^3J_{PC}$ = 14.6 Hz, meta), 132.9 (d, $^4J_{PC}$ = 3.4 Hz, para). ^{31}P NMR ($CDCl_3$, ppm): δ 51.8. HRMS: calcd for $C_{11}H_{17}O_2SP$ 244.06869, found 244.06822.

O-Ethyl *S*-isopropyl phenylphosphonothioate (**13**) was synthesized in a similar manner with a slight variation. First 0.75 g of the **8** salt (1.95 mmol) was stirred in 2 mL of 2-iodopropane (20.4 mmol). This solution was stirred for approximately 2 days before an additional 1 mL of 2-iodopropane (10.2 mmol) was added. After the mixture was stirred for another day, ^{31}P NMR revealed the phosphonothioate signal at ∼50 ppm; the dicyclohexylammonium salt was then gravity-filtered and washed with HPLC-grade hexane followed by removal of the solvent in vacuo. The iso-PEPP product **13** was formed (0.103 g, 0.422 mmol, 22%) as an orange oil. ^{1}H ($CDCl_3$, ppm): δ 4.22 (dq, O−CH_2−CH_3, 2H), 1.39 (t, O−CH_2−CH_3, 3H), 3.38 (sep, S−CH−$(CH_3)_2$, 1H), 1.28 and 1.41 (d, S−CH−(CH_3)$_2$, 6H), 7.9 (t, ortho, 2H), 7.50 (m, meta, 2H), 7.54 (t, para, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 62.3 (d, $^2J_{PC}$ = 6.9 Hz, O−CH_2−CH_3), 16.7 (d, $^3J_{PC}$ = 7.0 Hz, O−CH_2−CH_3), 37.8 (d, $^2J_{PC}$ = 2.5 Hz, S−CH−$(CH_3)_2$), 25.9 and 25.8 (d, $^3J_{PC}$ = 6.0 Hz, S−CH−$(CH_3)_2$), 131.4 (d, $^2J_{PC}$ = 11.8 Hz, ortho), 128.9 (d, $^3J_{PC}$ = 14.0 Hz, meta), 132.7 (d, $^4J_{PC}$ = 3.2 Hz, para). ^{31}P NMR ($CDCl_3$, ppm): δ 50.7. HRMS: calcd for $C_{11}H_{17}O_2SP$ 244.06869, found 244.06936.

***O*-Alkyl *S*-Ethyl Phenylphosphonothioates (14−16).** Compounds **7**, **9**, and **10** were converted to the corresponding *S*-ethyl phenylphosphonothioates by stirring 5.0 g (∼13 mmol) of the dicyclohexylammonium salt in 100 mL of distilled toluene and 2.4 mL (30 mmol) of iodoethane. The mixture was stirred for 3 days, and the resulting suspension was filtered, washed with anhydrous hexanes, and concentrated under reduced pressure. This oil was washed repeatedly with anhydrous hexanes to remove the residual solid salt. The hexane washes were concentrated down to the resulting oil (∼1.5 g, 6.3 mmol, 50% yield). Kügelrohr distillation was used if the hexane extractions did not remove impurities.

*O-Methyl S-Ethyl Phenylphosphonothioate (**14**).* ^{1}H NMR ($CDCl_3$, ppm): δ 3.89 (d, $^3J_{PH}$ = 12.6 Hz, O−CH_3, 3H), 2.77 (dq, S−CH_2−CH_3, 2H), 1.28 (t, $^3J_{HH}$ = 7.1 Hz, S−CH_2−CH_3, 3H), 7.89 (t, ortho, 2H), 7.50 (m, meta, 2H), 7.58 (t, para, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 53.2 (d, $^2J_{PC}$ = 7.1 Hz, O−CH_3), 25.3 (d, $^2J_{PC}$ = 3.1 Hz, S−CH_2−CH_3), 15.4 (d, $^3J_{PC}$ = 5.7 Hz, S−CH_2−CH_3), 131.3 (d, $^2J_{PC}$ = 10.7 Hz, ortho), 129.4 (d. $^3J_{PC}$ = 14.9 Hz, meta), 134.1 (d, $^4J_{PC}$ = 3.3 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$, ppm): δ 46.7. HRMS: calcd for $C_9H_{13}O_2SP$ 216.03739, found 216.03806.

*O-Propyl S-Ethyl Phenylphosphonothioate (**15**).* ^{1}H NMR ($CDCl_3$, ppm): δ 4.16 (m, O−CH_2−CH_2CH_3, 2H), 1.79 (m, O−CH_2−CH_2−CH_3, 2H), 1.01 (t, $^3J_{HH}$ = 8 Hz, O−CH_2CH_2−CH_3, 3H), 2.76 (m, S−CH_2−CH_3, 2H), 1.27 (t, $^3J_{HH}$ = 7.5 Hz, S−CH_2−CH_3, 3H), 7.89 (t, ortho, 2H), 7.49 (m, meta, 2H), 7.56 (t, para, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 67.8 (d, $^2J_{PC}$ = 7.2 Hz, O−CH_2−CH_2CH_3), 24.1 (d, $^3J_{PC}$ = 7.1 Hz, O−CH_2−CH_2−CH_3), 10.5 (O−CH_2CH_2−CH_3), 131.5 (d, $^2J_{PC}$ = 10.9 Hz, ortho), 128.8 (d. $^3J_{PC}$ = 14.5 Hz, meta), 132.7 (d, $^4J_{PC}$ = 3.1 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$, ppm): δ 44.6. HRMS: calcd for $C_{11}H_{17}O_2SP$ 244.06869, found 244.06902.

*O-Isopropyl S-Ethyl Phenylphosphonothioate (**16**).* ^{1}H NMR ($CDCl_3$, ppm): δ 4.93 (sept, O−CH−$(CH_3)_2$, 1H), 1.40 (d, O−CH−(CH_3)$_2$, 6H), 2.75 (dq, S−CH_2−CH_3, 2H), 1.25 (t, S−CH_2−CH_3, 3H), 7.88 (t, ortho, 2H), 7.50 (m, meta, 2H), 7.54 (t, para, 1H). ^{13}C NMR ($CDCl_3$, ppm): δ 71.9 (d, $^2J_{PC}$ = 7.0 Hz, O−CH−$(CH_3)_2$), 24.6 and 24.4 (dd, $^3J_{HH}$ = 6.3 Hz, O−CH−$(CH_3)_2$), 131.5 (d, $^2J_{PC}$ = 10.6 Hz, ortho), 128.8 (d. $^3J_{PC}$ = 14.9 Hz, meta), 132.6 (d, $^4J_{PC}$ = 3.1 Hz, para), 132.3 (d, J_{PC} = 150 Hz, ipso). ^{31}P NMR ($CDCl_3$, ppm): δ 43.3. HRMS: calcd for $C_{11}H_{17}O_2SP$ 244.06869, found 244.06824.

Kinetics Experiment. The hydrolysis of phosphonothioates **1** and **11**−**16** with **2** was carried out in a pH 7 MOPS (300 mM in D_2O) buffer. The MOPS buffer was sparged with argon for 10 min. First, 30 mg (0.102 mmol) of **2** was transferred into an inlet reaction tube. Outside the box, the degassed MOPS buffer (0.700 mL) was syringed under argon flush into the reaction inlet tube containing **2**. Compound **2** was allowed to dissolve in the D_2O MOPS buffer for about 90−120 min to form a dark green solution. This solution was syringed into a screw-cap NMR tube purged with argon for about 10 min. Finally, 2 μL (0.016 mmol) of the phenyl phosphonothioate was added using a microliter syringe. This gave a 7:1 molar ratio of **2** to phenyl phosphonothioate.

NMR spectra were taken at 20 min time intervals over approximately 13 h in order to examine the kinetics of the reaction. ^{31}P NMR spectra were taken with 64 scans and a delay of 1 s between each scan. The ^{31}P NMR peaks of the starting phosphonothioate and the product phosphonate were integrated for all spectra taken and used to determine the rate of the reaction. It was found that rate constants of the phosphonothioate hydrolysis reactions were independent of the delay time (i.e., 5 s vs 15 s). Rate constants and error bars shown in Figures 3−5 represent at least triplicate independent runs.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures giving details of the analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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