A Comparison of Phosphonothioic Acids with Phosphonic Acids as Phosphatase Inhibitors

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Phosphorothioates, analogues of phosphate esters in which a sulfur replaces an oxygen atom in the phosphoryl group, are competent surrogate substrates for a number of phosphatases. In some cases the thio analogues show similar binding (as estimated by K_m) while other phosphatases show quite different K_m values for phosphate compared to phosphorothioate esters. On this basis it was hypothesized that there might be different inhibitory tendencies by the nonhydrolyzable analogues, phosphonothioic acids compared with phosphonic acids. A series of phosphonothioic acids and corresponding phosphonic acids were synthesized and their inhibitory properties were compared toward human placental and *E. coli* alkaline phosphatases, the protein-tyrosine phosphatase from *Yersinia*, and the serine/threonine protein phosphatases PP2C and lambda. Sulfur substitution for oxygen gives the phosphonothioic acids pK_a values that are close to those of phosphate esters, in contrast to the higher pK_a values typical of phosphonic acids. Despite different steric requirements and differences in charge distribution in the anions of phosphonothioic acids compared with phosphonic acids, it was found that, with some exceptions, differences in inhibitory properties were modest.

Introduction

Protein phosphorylation plays a crucial role in the regulation of many biochemical processes, including the regulation of metabolism and signal transduction.[1−5] The phosphorylation levels are controlled by the balancing actions of kinases and phosphatases. The crucial roles played by phosphatases have led to considerable interest in designing inhibitors. Phosphonic acids have formed the basis for a number of nonhydrolyzable phosphate ester substrate mimics. Such compounds have a carbon−phosphorus bond in place of the scissile P−O ester bond of the natural substrates of phosphatases. As a result of this replacement, phosphonic acids have higher first and second pK_a values than phosphate esters. This has relevance due to the fact that protein-tyrosine phosphatases and serine/threonine phosphatases that have been studied utilize the dianion forms of their substrates in catalysis. Thus phosphonic acids bearing one or two fluorine atoms at the α-methylene position, which lowers the pK_a values[6] (Table 1), have been widely explored as the basis for phosphate mimetics in inhibitor design. However, their superior inhibitory properties compared to simple phosphonic acids have been attributed, at least in some instances, to direct interactions between the fluorine atoms and active site resides rather than their reduced pK_a.[7]

The substitution of a sulfur atom for one of the nonbridging oxygen atoms in a phosphonic acid, yielding a phosphonothioic acid, will also reduce the pK_a values. The resulting phosphonothioic acids are also mimics of phosphate esters and are nonhydrolyzable under physiological conditions. We have synthesized several such compounds and evaluated their inhibitory properties toward a number of phosphatases.

The corresponding sulfur analogues of phosphate esters, phosphorothioates ($RO-PSO_2H_2$), have found use as surrogate substrates for phosphate esters in the study of a number of enzymatic phosphoryl transfer reactions. Phosphorothioates may be made chiral by the use of a sulfur atom and two isotopes of oxygen. Such chiral phosphorothioates have been used to study a number of enzyme-catalyzed phosphoryl transfer reactions, the results of which have been reviewed.[8] Aside from stereochemical studies, phosphorothioates have also been used in a number of other mechanistic investigations of enzymatic phosphoryl transfer reactions of monoesters.[9−11] In some kinetics studies, phosphorothioates exhibit K_m values that are similar to those of corresponding phosphate esters, while in other cases, K_m values are more disparate. For example, in a study of several protein-tyrosine phosphatases (PTPases) using phosphate and phosphorothioate ester substrates it was found that K_m values for the two types of compounds differed by 3-fold or less; for the *Yersinia* PTPase the values were the same within experimental uncertainty.[9] In contrast, alkaline phosphatase from *E. coli* exhibits a 10-fold higher K_m for *p*-nitrophenyl phosphorothioate than for *p*-nitrophenyl phosphate.[10]

These results imply that the active sites of phosphatases vary in their abilities to accommodate phosphorothioates, which differ in the larger radius of sulfur and longer P−S bond length. In addition to the different steric demands of the thiophosphoryl group compared to the phosphoryl group, there is also a difference in charge distribution in their respective dianions. Experimental and computational evidence indicates that one negative charge in phosphorothioate dianions is borne

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Table 1. Comparison of the Second pK_a Values for Phenyl Phosphate and Related Phosphonic Acids[6]

Compound	pK_a
X = O	6.2
X = CH_2	7.7
X = CHF	6.6
X = CF_2	5.7

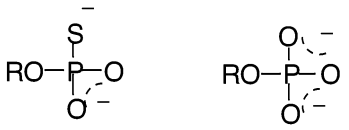

Figure 1. In a phosphorothioate dianion, one negative charge resides on the sulfur atom and the other is delocalized between the two nonbridging oxygen atoms. In a phosphate ester the charge is equally distributed among the three nonbridging atoms.

by the sulfur atom while the second is delocalized between the two oxygen atoms[12,13] (Figure 1). Thus there is an unequal distribution of charge among the three nonbridging atoms of the thiophosphoryl group in contrast to the phosphoryl dianion. Experimental evidence indicates that the dianion is the substrate for alkaline phosphatases as well as protein phosphatases.

The differences in geometry and in charge distribution between the anions of phosphonothioic and phosphonic acids could potentially be exploited, in that phosphonothioic acids might display different relative specificity in inhibition of phosphatases than corresponding phosphonic acids. To evaluate the potential inhibitory properties of phosphonothioic acids and to compare these properties with those of the corresponding phosphonic acids, we have synthesized a number of simple analogues of both compounds (Table 2) and tested them as inhibitors. Inhibition by these compounds was measured with human placental alkaline phosphatase (PLAP) and *E. coli* alkaline phosphatase, protein-tyrosine phosphatase from *Yersinia* (YOP), and the serine/threonine protein phosphatases PP2C and lambda. These phosphatases represent three different classes of phosphatase active sites and catalytic mechanisms. The alkaline phosphatases have a dinuclear zinc active site and catalyze phosphoryl transfer by a two-step mechanism with a phosphoserine intermediate.[14,15] The *Yersinia* enzyme has the active site typical of PTPases, which lack a metal cofactor and also catalyze phosphoryl transfer by a two-step mechanism, with a phosphocysteine intermediate.[16] The serine/threonine protein phosphatases utilize a dinuclear metal center, but catalyze direct phosphoryl transfer to a metal-bound water molecule.[3]

The general phosphonothioic acid structure shown at the top of Table 2 is shown in the thiono form. Neutral phosphonothioic acids have two tautomeric structures (Figure 2a). Studies indicate that the thiono−thiolo equilibrium lies far on the side of the thiono form in phosphonothioic acids (in which a carbon−phosphorus bond is present). In compounds where only phosphorus−oxygen bonds are present (Figure 2b) more of the thiolo form is typically present, depending upon the solvent.[17−19]

The preparation of some of the compounds shown required the development of some new synthetic methodology. The majority of the thiophosphonic acids (**1S**−**6S**) were synthesized as previously described.[20] However this method was not amenable for the synthesis of compounds **7** and **8**, with heteroatoms in the alkyl group. Therefore, an alternative method was devised that is based on modifications of a series of known

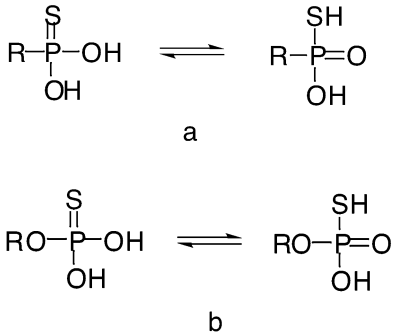

Figure 2. The thiono and thiolo tautomers of phosphonothioic acids (a) and phosphorothioic acids (b). The thiono−thiolo equilibrium lies far to the left phosphonothioic acids (a). In phosphorothioic acids (b) more of the thiolo form is typically present, depending upon the solvent.

Table 2. Phosphonothioic and Phosphonic Acids Compared for Inhibitory Properties in This Study

R	R−P(=S)(OH)−OH	R−P(=O)(OH)−OH
H_3C—	**1S**	**1O**
$CH_3CH_2CH_2$–	**2S**	**2O**
Ph	**3S**	**3O**
HO	**4S**	**4O**
H_3CO	**5S**	Not prepared
COOH	**6S**	**6O**
CH_3SCH_2–	**7**	
Ph−S−CH_2–	**8**	

Scheme 1

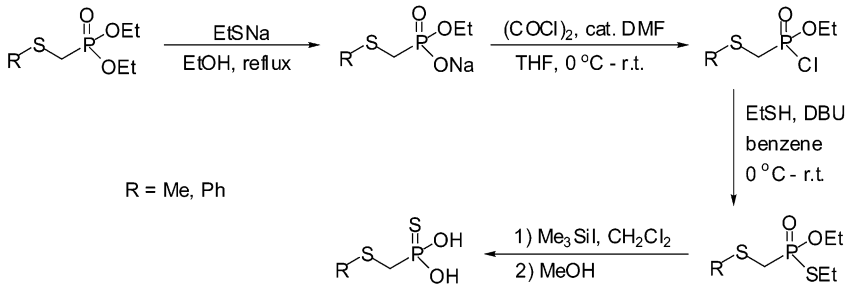

Table 3. Millimolar Inhibition Constants (K_i) for the Compounds in Table 2 toward Human Placental Alkaline Phosphatase (PLAP) and *E. coli* Alkaline Phosphatase (AP), PTPase from *Yersinia* (YOP), and the Serine/Threonine Protein Phosphatases PP2C and Lambda[a]

	K_i values (mM)				
	alkaline phosphatases		PTPase	Ser/Thr phosphatases	
inhibitor	PLAP	*E. Coli* AP	YOP	PP2C	lambda
1S	19 ± 3	no inhibition	7.0 ± 0.8	2.7 ± 0.3	17 ± 10
2S	0.60 ± 0.06	no inhibition	no inhibition	0.33 ± 0.02	20 ± 6
3S	0.20 ± 0.01	no inhibition	no inhibition	0.187 ± 0.008	0.57 ± 0.06
4S	1.5 ± 0.3	no inhibition	29 ± 4	0.210 ± 0.006	1.5 ± 0.2
5S	0.33 ± 0.03	no inhibition	no inhibition	0.22 ± 0.02	1.1 ± 0.1
6S	1.5 ± 0.2	no inhibition	0.20 ± 0.01	0.015 ± 0.001	0.17 ± 0.01
7	0.40 ± 0.06	no inhibition	no inhibition	0.70 ± 0.07	1.2 ± 0.2
8	0.46 ± 0.04	no inhibition	15.8 ± 0.7	0.20 ± 0.02	0.47 ± 0.03
1O	11 ± 1	no inhibition	30 ± 1	0.67 ± 0.08	1.01 ± 0.07
2O	1.55 ± 0.06	0.61 ± 0.06	no inhibition	precipitation	precipitation
3O	0.58 ± 0.02	no inhibition	no inhibition	precipitation	precipitation
4O	7.0 ± 0.7	no inhibition	42 ± 4	0.41 ± 0.04	0.43 ± 0.01
6O	0.39 ± 0.01	0.30 ± 0.02	no inhibition	0.048 ± 0.003	0.13 ± 0.02

[a] The entry "no inhibition" designates cases where no reduction in rate was observed at 5 mM concentration of the inhibitor. Both PP2C and lambda phosphatases are routinely assayed in the presence of Mn^{2+}; in some cases phosphonates precipitate under these conditions, precluding kinetic measurements.

reactions. The reaction of easily accessible diethyl esters of phosphonic acid with an equimolar amount of sodium ethanethiolate in refluxing ethanol gives high yields of monodealkylation products in the form of their sodium salts.[21] Such salts react with oxalyl chloride in the presence of a catalytic amount of dimethylformamide to give monochlorides of phosphonic acid monoesters.[22,23] These monochlorides subsequently undergo nucleophilic substitution of chlorine by the ethanethiolate anion.[24] The *O,S*-diethyl esters thus obtained can be easily dealkylated with iodotrimethylsilane to the free acids, which may be precipitated as their anilinium salts. This approach, used to prepare compounds **7** and **8**, is outlined in Scheme 1.

This approach affords a route to pure phosphonothioates without the need for purification of intermediates. Moreover, we successfully applied this new approach for synthesis of the previously reported propyl- and benzylphosponothioic acids (**2S** and **3S**),[20] suggesting this method is suitable for the general synthesis of phosphonothioic acids.

Results and Discussion

The second pK_a of propyl phosphonothioic acid **2S** was determined to be 6.64, while the reported pK_a of the oxygen analogue **2O** is 8.18;[25] we obtained an experimentally indistinguishable value of 8.17. The second pK_a of the benzyl compound **3S** was determined to be 6.41, compared to 7.55[26] for the oxygen analogue **3O**. Thus, the net effect of sulfur substitution is to depress the second pK_a value of phosphonates to values similar to phosphate esters. The first pK_a is expected to be lowered as well, but was not measured in this study. This effect of sulfur substitution for oxygen is similar to that reported in esters,[27] where the second pK_a for *p*-nitrophenyl phosphorothioate (3.68 ± 0.08) is reduced compared to that of *p*-nitrophenyl phosphate (5.0).

The Ser/Thr phosphatases PP2C and lambda have been most often studied kinetically in the presence of Mn^{2+}. Unfortunately, phosphonates are known to form insoluble polymeric complexes with a number of divalent cations. Complexes of the propyl and benzyl phosphonates **2O** and **3O** precipitated from the Mn^{2+}-containing buffer solutions used with these two phosphatases, precluding K_i measurements. Phosphonates bearing a carboxylate moiety (**4O** and **6O**) were soluble under the same conditions. No precipitation occurred with any of the phosphonothioic acids. Their superior solubility in the presence of Mn^{2+}, if found to extend to other divalent metal ions, would make phosphonothioic acids more amenable than phosphonic acids to kinetics studies requiring divalent metal ions to be supplied in solution.

Inhibition Results. The inhibitory properties reported in Table 3 were evaluated using *p*-nitrophenyl phosphate as the substrate. All of the inhibitors were initially screened using a 5 mM concentration of the inhibitor. Instances where no reduction of rate was observed in this preliminary screen are designated as "no inhibition" in Table 3. In all other cases, a value for K_i is given with the standard error. The inhibition was found to be competitive in all cases.

Alkaline Phosphatases. No significant inhibition of the alkaline phosphatase from *E. coli* by any of the

phosphonothioic acids was observed. The phosphonic acids **2O** and **6O** showed moderate inhibition, with no detectable inhibition from the others when screened at 5 mM concentration. These results are consistent with previous reports of only weak inhibition of *E. coli* AP by phosphonates.[28]

Much better inhibition was observed by both phosphonothioic and phosphonic acids of the human placental alkaline phosphatase (PLAP). PLAP shares the active site residues and dinuclear zinc center of the *E. coli* AP. However, in the *E. coli* enzyme active site is close to the surface and the ester group of the substrate is solvent exposed, accounting for the lack of substrate specificity of this enzyme. In contrast, the active site in PLAP lies at the bottom of a large cleft,[14] providing a means for discrimination of both substrates and of inhibitors. The phosphonothioic acids **2S**−**4S** have 2.5 to 4.7-fold lower K_i values than their oxygen analogues. However, this trend is reversed for the smallest (**1**) and bulkiest (**6**) pendant groups, where the oxygen analogue is the superior inhibitor. The compounds **7** and **8** incorporate a second sulfur atom with the potential to coordinate to the dinuclear metal center. The approximately isosteric compounds **7** and **2S** exhibit very similar K_i values indicating the second sulfur atom does not contribute to binding.

***Yersinia* PTPase.** The only compound to show appreciable inhibition of the *Yersinia* PTPase is **6S**. The naphthyl ring system has previously been shown to be superior to phenyl in phosphonic acid-derived inhibitors of another PTPase, PTP-1B.[29] The 200 μM K_i of **6S** stands in contrast to the absence of any observed inhibition of the oxygen analogue **6O** at 5 mM concentration. Thus, in the case of the *Yersinia* PTPase the combination of the naphthyl ring and the thiophosphonate moiety is much more effective than either functionality alone. The difference in K_i between **6S** and **6O** is also interesting in light of the similar K_m values reported for *p*-nitrophenyl phosphorothioate (2.7 ± 0.6 mM) and *p*-nitrophenyl phosphate (2.6 ± 0.1 mM).[9] Inorganic phosphate is a weaker inhibitor of *Yersinia* PTPase (K_i = 15 ± 1 mM) than inorganic thiophosphate (6.4 ± 0.3 mM).[9]

Serine/Threonine Phosphatases. The phosphatases PP2C and lambda are members of the serine/threonine phosphoprotein phosphatase family, which share the utilization of a dinuclear metal center for catalysis. Unlike the alkaline phosphatases, evidence indicates that the metal centers in these enzymes catalyze the direct phosphoryl transfer to water with no phosphoenzyme intermediate.[3]

The serine/threonine phosphoprotein phosphatases fall into two structurally distinct gene families, designated as PPP and PPM. X-ray structures of the PPP subfamily members PP1,[30,31] calcineurin,[32,33] and lambda[34] reveal the same secondary structural characteristics providing ligands for the dinuclear metal complex. The PPM subfamily has a different secondary structure and no significant sequence homology with the PPP phosphatases. PP2C is regarded as its defining member, and members of the PP2C subfamily show strong dependence on Mg^{2+} or Mn^{2+} for activity. The X-ray structure of PP2C shows an active site containing several carboxylates that serve as metal-binding residues.[35] The metal ions in PP2C have fewer amino acid ligands than in the PPP family, and the resulting looser coordination is evidenced by kinetic analyses that reveal K_{metal} values in the millimolar range.[36] Mn^{2+} and Fe^{2+} result in the highest activities; saturation kinetics reveals concentrations of 5 mM or greater of Mn^{2+} are required for maximum activity to be observed.[36] In contrast lambda phosphatase has a greater affinity for Mn^{2+}, with dissociation constants in the micromolar range.[37]

Inhibition of PP2C by methyl phosphonic acid **1O** was ∼4-fold better than by the thio analogue, but this preference is reversed for compounds **4** and **6**. The small size of the methyl group gives compounds **1O** and **1S** much more freedom to adopt a geometry to make hydrogen bonds to the (thio)phosphonyl group, although the methyl group does not provide any interactions to directly assist or otherwise affect binding.

Experiments with lambda show the same preferential inhibition by the oxygen analogues in the methyl derivatives, which disappears in compounds with larger substituents. Of the longer alkyl chain derivatives, **4S** is a slightly better inhibitor than **4O**. The carboxynaphthyl **6S** and **6O** have very similar K_i values.

In general, PP2C was inhibited somewhat better than lambda by the phosphonothioic acids. Compound **7**, incorporating a potential second metal ligand in comparison with the approximately isosteric **2S**, shows approximately a 17-fold better inhibition of lambda. In contrast, the difference is about 2-fold with PP2C. Only minor differences were observed in the inhibition by **8** and **3S** of PP2C and of lambda.

Summary

Phosphonothioic acids have second pK_a values lower than those of phosphonic acids and very similar to those of phosphate esters. In general, only moderate differences are observed between the inhibitory properties of phosphonothioic acids and phosphonic acids toward the phosphatases tested in this study. One notable exception is the observation that the combination of the phosphonothioic acid and a naphthyl moiety (**6S**) yielded a much better inhibitor of the *Yersinia* PTPase than any other compound tested. It was also noted that the carboxynaphthyl moiety (**6S** and **6O**) conveys fairly good inhibitory properties toward PP2C, which has not been previously reported. The phosphonothioic acid **6S** was superior to the phosphonic acid **6O**. It was also found that the phosphonothioic acids show markedly better solubility in the presence of manganese ion than the phosphonic acids, a useful property when working with metallophosphatases that require metal ions supplied in the buffer for full activity.

Experimental Section

All chemicals and solvents were purchased from commercial sources and used without purification. All melting points are uncorrected. All reactions with phosphorus compounds were carried out under a nitrogen atmosphere. Elemental analyses were performed by Atlantic Microlabs. All analyses agreed within ±0.4% of the calculated values. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{31}P NMR (162 MHz) spectra were recorded in D_2O solutions using 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt as an internal standard for ^{1}H and ^{13}C NMR and 85% phosphoric acid as an external standard for ^{31}P NMR.

The phosphatases were commercial products with the exception of lambda, which was provided by the laboratory of Frank Rusnak (Mayo Clinic and Foundation), and PP2C, which was overexpressed and purified as previously described.[36] BL21DE3 strain of *E. coli* were transformed with the expression plasmid pCW−PP2C α and grown in 2xYT medium containing 140 μg /mL ampicillin. The supernatant obtained after centrifugation of the cells lysed by French press was precipitated between 30% and 55% saturation with $(NH_4)_2SO_4$. The pellet was resuspended in buffer and loaded onto a Q-Sepharose anion exchange column; fractions showing high activity with *p*NPP (*p*-nitrophenyl phosphate) were pooled and loaded on a Phenyl Sepharose hydrophobic column. Fractions were analyzed for purity by SDS-gel electrophoresis, pooled, concentrated to ∼40 mg/mL, and stored at −20° C in Tris-HCl, pH 7.0, DTT, and glycerol.

Measurement of Inhibition Constants. Inhibition experiments were conducted using *p*-nitrophenyl phosphate as the substrate at 25 °C. Rates were measured with a range of substrate and inhibitor concentrations. Lineweaver−Burke plots were used to assess the type of inhibition. The K_i values in Table 3 were obtained by fitting the data to eq 1, using Microcal Origin 5.0 software.

$$\frac{v}{V_{max}} = \frac{[S]}{K_m\left(1 + \frac{[I]}{K_i}\right) + [S]} \quad (1)$$

Conditions for inhibition studies were: PLAP and *E. coli* AP: 250 mM TRIS buffer, pH = 9.0, 1 mM $ZnCl_2$ and 1 mM $MgCl_2$. For YOP: 100 mM succinic acid buffer, pH = 6.6. For PP2C: 100 mM TRIS buffer, pH = 7.5, 5 mM $MnCl_2$ and 1 mM DTT. For lambda phosphatase: 100 mM MOPS buffer, pH = 7.3, 1 mM $MnCl_2$ and 1 mM DTT.

Determination of pK_a Values. Samples were dissolved in water to obtain ∼0.01 M solutions. These solutions were titrated with 0.1 M sodium hydroxide as pH was monitored during the titration using a glass electrode at 25 °C. The pK_a values were calculated directly from plots of titrant volume vs pH.

Syntheses of Inhibitors. Compounds **1O** and **2O** and **3O** were commercial products. Salts of the acids **2S**, **3S**, **4S**, **5S**, and **6S** were synthesized according our previously published method.[20] The bis(cyclohexylammonium) salt of methylphosphonothioic acid (**1S**) was obtained by hydrolysis of the commercial dichloride in aqueous KOH. After titration to pH 9.0 with cyclohexylamine, and the bis(cyclohexylammonium) salt of **1S** was recrystallized from aqueous ethanol. ^{1}H NMR δ 3.20−3.30 (m, 2H), 2.0−2.1 (m, 4H), 1.8−1.9 (m, 4H), 1.68−1.75 (m, 2H), 1.56 (d, *J* = 14.1 Hz, 3H), 1.35−1.49 (m, 8H), 1.20−1.31 (m, 2H). ^{31}P NMR δ 57.4.

6-Phosphonohexanoic Acid (4O), Anilinium Salt. A solution of 6-bromohexanoic acid (20.0 g, 0.103 mol) and *p*-toluenesulfonic acid (2 g) in methanol (200 mL) was refluxed overnight. The residue after evaporation of solvent was dissolved in benzene (100 mL), washed with water, with a sodium hydrogen carbonate solution, and again with water, and then dried with anhydrous magnesium sulfate. Solvent was removed by rotary evaporation to give the methyl ester of 6-bromohexanoic acid (19.77 g, 92%) as a colorless oil. This material (19.77 g, 95 mmol) was mixed with triethyl phosphite (29.1 g, 0.175 mol) and heated to 160 °C for 48 h. The reaction mixture was then cooled, dissolved in water (300 mL), and heated to boiling for several minutes. After cooling, the mixture was extracted with benzene (4 × 50 mL). The benzene extracts were washed with water, dried with anhydrous magnesium sulfate, and evaporated to give 6-(diethoxyphosphoryl)hexanoic acid methyl ester (21.26 g, 84%) as a colorless oil. This material (21.26 g, 80 mmol) was refluxed overnight with concentrated hydrochloric acid (100 mL). The reaction mixture was then evaporated under reduced pressure, dissolved in water (100 mL), and then evaporated to dryness (this procedure was repeated three times) to give crude 6-phosphonohexanoic acid as a sticky white mass (15.71 g, 100%). This material (5 g, 25.5 mmol) was dissolved in acetone (50 mL), and excess aniline was added to precipitate the white 6-phosphonohexanoic acid anilinium salt (6.13 g, 83%). Mp 141−142 °C (2-butanone−ethanol). Anal. ($C_{12}H_{20}NO_5P$) C, H, N. ^{1}H NMR δ 7.30−7.55 (m, 5H), 2.37 (t, *J* = 7.6 Hz, 2H), 1.32−1.67 (m, 8H). ^{13}C NMR (D_2O) δ 182.7 (s, C═O), 133.0 (s, $CH_{aromatic}$), 130.5 (s, $CH_{aromatic}$), 124.7 (s, $CH_{aromatic}$), 37.1 (s, CH_2), 32.6 (d, *J* = 17.5 Hz, CH_2), 30.5 (d, *J* = 132.8 Hz, P−CH_2), 27.0 (s, CH_2), 25.6 (d, *J* = 4.6 Hz, CH_2). ^{31}P NMR δ 27.5.

7-Phosphonomethylnaphthalene-1-carboxylic Acid (6O), Trimethylammonium Salt. To a suspension of sodium hydride (95%, 3.88 g, 0.150 mol)) in tetrahydrofuran (100 mL) was added (foaming) a solution of diethyl phosphite (94%, 21.52 g, 0.146 mol) in tetrahydrofuran (50 mL) at 0 °C. After the mixture was stirred for 1 h, a solution of 7-bromomethylnaphthalene-1-carboxylic acid *tert*-butyl ester[20] (44.8 g, 0.139 mol) in tetrahydrofuran (100 mL) was added. The mixture was left to slowly reach room temperature and stirred overnight. Methanol (100 mL) was added, and solvents were removed by rotary evaporation. The residue was dissolved in dichloromethane (250 mL), washed with sodium hydrogen carbonate solution and brine, and then dried with anhydrous magnesium sulfate. Solvent was evaporated under reduced pressure to give crude 7-(diethoxyphosphorylmethyl)naphthalene-1-carboxylic acid *tert*-butyl ester as an orange oil (45.4 g, 86%). This material (6.0 g, 15.9 mmol) was refluxed with concentrated hydrochloric acid (150 mL) overnight, evaporated under reduced pressure, dissolved in water (100 mL), and evaporated to dryness (this procedure was repeated three times). The residue was dissolved in ethanol (150 mL) and saturated with gaseous trimethylamine. Then diethyl ether (800 mL) was added, and the precipitate was filtered off, washed with ether, and dried to give the trimethylammonium salt of 7-phosphonomethylnaphthalene-1-carboxylic acid as white powder (3.77 g, 73%). Mp 245−250 °C (ethanol−diethyl ether, with decomposition). Anal. ($C_{15}H_{20}NO_5P$) C, H, N. ^{1}H NMR δ 8.22−8.26 (m, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.82 (d, *J* = 7.1 Hz, 1H), 7.48−7.60 (m, 2H), 3.23 (d, *J* = 20.9 Hz, 2H), 2.86 (s, 9H). ^{13}C NMR (D_2O) δ 177.0 (s, C═O), 137.7 (d, *J* = 9.2 Hz, $C_{naphthyl}$), 134.9 (d, *J* = 2.3 Hz, $C_{naphthyl}$), 134.7 (d, *J* = 1.5 Hz, $CH_{naphthyl}$), 133.1 (s, $C_{naphthyl}$), 132.9 (d, *J* = 3.1 Hz $C_{naphthyl}$), 131.5 (d, *J* = 6.1 Hz CH_{phenyl}), 131.4 (d, *J* = 3.8 Hz, $CH_{naphthyl}$), 131.2 (s, $CH_{naphthyl}$), 128.1 (d, *J* = 7.6 Hz, $CH_{naphthyl}$), 127.4 (d, *J* = 1.5 Hz, $CH_{naphthyl}$), 47.6 (s, N−CH_3), 39.5 (d, *J* = 127.4 Hz, P−CH_2). ^{31}P NMR δ 17.2.

Methylsulfanylmethylphosphonothioic Acid (7), Anilinium Salt. Commercially available methylsulfanylmethylphosphonic acid diethyl ester (19.89 g, 0.1 mol) and sodium ethanethiolate (8.41 g, 0.1 mol; prepared in situ prior to reaction from sodium and ethanethiol) were refluxed for 24 h in ethanol (250 mL). Solvent was then removed by rotary evaporation, and the remaining yellowish precipitate was washed with diethyl ether and dried in vacuo to yield the crude sodium salt of methylsulfanylmethylphosphonic acid monoethyl ester (17.39 g, 91%). This salt was suspended in tetrahydrofuran (250 mL) and a catalytic amount of DMF (0.1 mL) and cooled using an ice bath, and oxalyl chloride (13.21 g, 0.104 mol) was added (foaming). The resulting mixture was stirred at 0 °C for 1 h and then for 3 h at room temperature. At this time, a precipitate was filtered off, and the solvent was removed by rotary evaporation to give the crude methylsulfanylmethylphosphonic acid monoethyl ester monochloride as a yellow oil (13.02 g, 76%). This material (13.02 g, 0.069 mol) was added at 0 °C to a solution of ethanethiol (4.29 g, 0.069 mol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (10.51 g, 0.069 mol) in benzene (250 mL). The resulting suspension was stirred for 1 h, and then the ice bath was removed and stirring was continued overnight. The mixture was washed carefully first with diluted (1:9) hydrochloric acid and then with a saturated solution of sodium bicarbonate and finally dried with anhydrous magnesium sulfate. The solvent was removed by rotary evaporation to give crude methylsulfanylmethylphosphonothioic acid *O,S*-diethyl ester as a yellow oil (8.27 g, 56%). The *O,S*-diethyl ester was converted to the free phosphono-

thioic acid according to our previously reported method[20] (2.1 equiv of iodotrimethylsilane in dichloromethane followed by hydrolysis) and precipitated as the white anilinium salt (8.53 g, 88%) after washing with ether. Mp 135.5−137 °C (with decomposition). Anal. ($C_8H_{14}NO_2PS_2$) C, H, N. ^{1}H NMR δ 7.27−7.54 (m, 5H), 2.90 (d, J = 10.2 Hz, 2H), 2.24 (s, 3H). ^{13}C NMR (D_2O) δ 132.9 (s, $CH_{aromatic}$), 128.8 (s, $CH_{aromatic}$), 123.6 (s, $CH_{aromatic}$), 41.7 (d, J = 102.2 Hz, P−CH_2), 19.7 (d, J = 4.6 Hz, CH_3). ^{31}P NMR δ 62.0.

Phenylsulfanylmethylphosphonothioic Acid (8), Anilinium Salt. This was prepared from commercially available phenylsulfanylmethylphosphonic acid diethyl ester (27.99 g, 0.108 mol) following the same procedures described above for **7**. The yields of crude intermediates were as follows: sodium salt of phenylsulfanylmethylphosphonic acid monoethyl ester (26.03 g, 95%), phenylsulfanylmethylphosphonic acid monoethyl ester monochloride (22.29 g, 87%), phenylsulfanylmethylphosphonothioic acid *O,S*-diethyl ester (14.52 g, 59%). The yield on the final step was 94%, yielding 15.53 g. Mp 165−166 °C (with decomposition). Anal. ($C_{13}H_{16}NO_2PS_2$) C, H, N. ^{1}H NMR δ 7.24−7.51 (m, 5H), 3.41 (d, J = 11.7 Hz, 2H). ^{13}C NMR (D_2O) δ 132.9 (s, $CH_{aromatic}$), 132.2 (s, $CH_{aromatic}$), 131.4 (s, $CH_{aromatic}$), 129.3 (s, $CH_{aromatic}$), 129.2 (s, $CH_{aromatic}$), 123.8 (s, $CH_{aromatic}$), 41.6 (d, J = 99.9 Hz, P−CH_2). ^{31}P NMR δ 58.0.

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