A Carbon-13 Nuclear Magnetic Resonance Study on an Organophosphate. Formation and Characterization of Methamidophos (*O,S*-Dimethyl Phosphoramidothioate) *S*-Oxide

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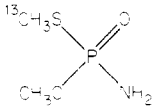
Oxidation of [SCH_3-^{13}C]*O,S*-dimethyl phosphoramidothioate (methamidophos) provided the diastereomeric *S*-oxides as characterized by ^{13}C NMR. A large difference in the chemical shift for the ^{13}C doublets was attributed to intramolecular hydrogen bonding between the amino and sulfoxide moieties. An interesting geminal coupling phenomena between phosphorus and $CH_3S(O)$ was also observed. Treatment of the *S*-oxides with an oxygen scavenger returned starting material.

Methamidophos or *O,S*-dimethyl phosphoramidothioate (1) is highly toxic to insects and mammals but is a relatively poor inhibitor of either insect or mammalian acetylcholinesterase.[1,2] Animals exposed to 1, however, exhibit typical signs of cholinergic poisoning. Owing to the discrepancy between in vitro anticholinesterase activity and high toxicity, it was suggested that 1 is activated in vivo to a metabolic intermediate of high inhibitory potency that is responsible for intoxication.[3] Further, on the basis of indirect evidence obtained from a study on the oxidation of 1, methamidophos *S*-oxide (2) was proposed as the structure of the intermediate.

Recent studies[4] in this laboratory have demonstrated that the P–S bond is cleaved during the inhibition of electric eel acetylcholinesterase by 1. In continuing the study on the mechanism of action of this compound, we have examined the oxidation of 1 in a model system by means of ^{13}C NMR using ^{13}C-enriched (SCH_3) material.



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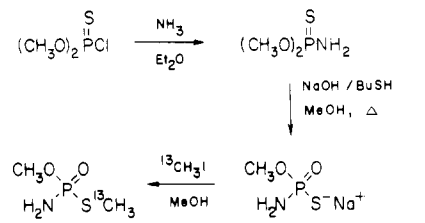
This paper reports on the characterization of products obtained following oxidation of 1 by *m*-chloroperbenzoic acid (*m*-CPBA).

Results and Discussion

Synthesis. The reaction sequence employed for the preparation of $^{13}CH_3S$-1 is given in Scheme I. *O,O*-Dimethyl phosphorochloridothioate was converted to the corresponding phosphoramidothioate[5] in 83% yield by reaction with anhydrous ammonia. Demethylation with *n*-butylthiolate anion and remethylation with ^{13}C-methyl iodide provided isotopically enriched 1 (90.25% by EIMS) in 84% yield.

Oxidation of Methamidophos (1) by *m*-CPBA. The oxidation of phosphoramidothioates by *m*-CPBA has been achieved under a variety of conditions.[3] Because of the suspected labile nature of the expected product, a dilute solution of chilled *m*-CPBA in $CDCl_3$ was added to 1 and the reaction was allowed to proceed overnight. Even with

Scheme I. Synthetic Route to Enriched 1



2 equiv of *m*-CPBA the reaction did not proceed to completion and there was partial recovery of 1. When CD_2Cl_2 was the solvent, the reaction occurred rapidly and numerous side products were observed, i.e., oxidation products in which the P–S bond was cleaved. Addition of greater than 2 equiv of *m*-CPBA did not afford any of the desired product under the conditions outlined in the Experimental Section. Bellet and Casida[3b] reported similar results, getting primarily rearranged sulfoxide products.

Carbon-13 Nuclear Magnetic Resonance Studies. The ^{13}C NMR (^{1}H decoupled, broad band) spectrum of ^{13}C-enriched 1 is shown in Figure 1a. 1 gives rise to an upfield doublet (δ = 12.46 ppm, J = 4.27 Hz) attributable to the ^{13}C–S–^{31}P coupling. The chemical shift reveals a highly shielded moiety imparted predominantly by the sulfur atom and is in agreement with reported thioalkyl C-13 chemical shifts.[6] Typical $J(^{13}C–^{31}P)$ values (two-bond interaction) have been recorded in this laboratory that vary from about 4.0 to 11.0 Hz. No P–OCH_3 resonance was observed at the natural abundance in 1.

The ^{13}C NMR spectrum of products obtained in 16 h following treatment of 1 with 2 equiv of *m*-CPBA in $CDCl_3$ is given in Figure 1b. All peaks occurring downfield (not shown, see Table I) from the $CDCl_3$ triplet (75.6, 77.0, 74.8 ppm) are attributed to aromatic and carbonyl resonances of *m*-CPBA and *m*-chlorobenzoic acid and are noninteractive with the product peaks. Significant amounts of 1 persisted even after the 16.0-h oxidation conditions and was confirmed by TLC and HPLC. The peaks for the major products **2a** and **2b** were observed as two distinct doublets centered at 36.37 and 47.11 ppm with coupling constants of J = 75.7 and 82.4 Hz, respectively. A T_1 experiment demonstrated that the four resonances modulated as two distinct doublets and not as four singlets. Other minor side products were observed at 18.25 ppm and at 42–43 ppm (Figure 1b). The peak at 18.25 ppm probably represents a cleaved thiomethyl moiety owing to its chemical shift and absence of coupling to phosphorus. The

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(3) (a) Eto, M.; Okabe, S.; Ozoe, Y.; Maekawa, K. *Pest. Biochem. Physiol.* **1977**, *7*, 367. (b) Bellet, E. M.; Casida, J. E. *J. Agric. Food Chem.* **1974**, *22*, 207.
(4) Thompson, C. M.; Fukuto, T. R. *J. Agric. Food Chem.* **1982**, *30*, 282.
(5) Mel'nikov, N. N.; Zen'kovich, A. G. *J. Gen. Chem. USSR* **1955**, *25*, 793.
(6) The chemical shift of dimethyl sulfide is 18 ppm, dimethyl sulfoxide 40.6 ppm, and dimethyl sulfone 42.0 ppm. See: Abraham, R. J.; Loftus, P. "Proton and Carbon-13 NMR Spectroscopy; An Integrated Approach"; Heyden and Sons, Ltd.: Philadelphia, PA, 1980.

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Table I. Carbon NMR Data for *m*-CPBA Oxidation Products of Methamidophos

compound	no.	chemical shift (δ)	J (Hz) ^{13}C-X-^{31}P
$(^{13}CH_3S)(CH_3O)P(O)NH_2$	1	12.465, 54.1	4.27 [X = S] 6.20 [X = O]
m-CPBA		128.2, 129.7, 130.2, 131.3, 133.6, 134.6, 169.3	
$P(OCH_3)_3$		48.85	11.0 [X = O]
$(CH_3O)_2(CH_3S)P(O)$	4	11.88, 53.40	4.88 [X = S] 6.11 [X = O]
methamidophos *S*-oxide	2a, 2b	36.37, 47.11	75.7 [X = S(O)] 82.4 [X = S(O)]

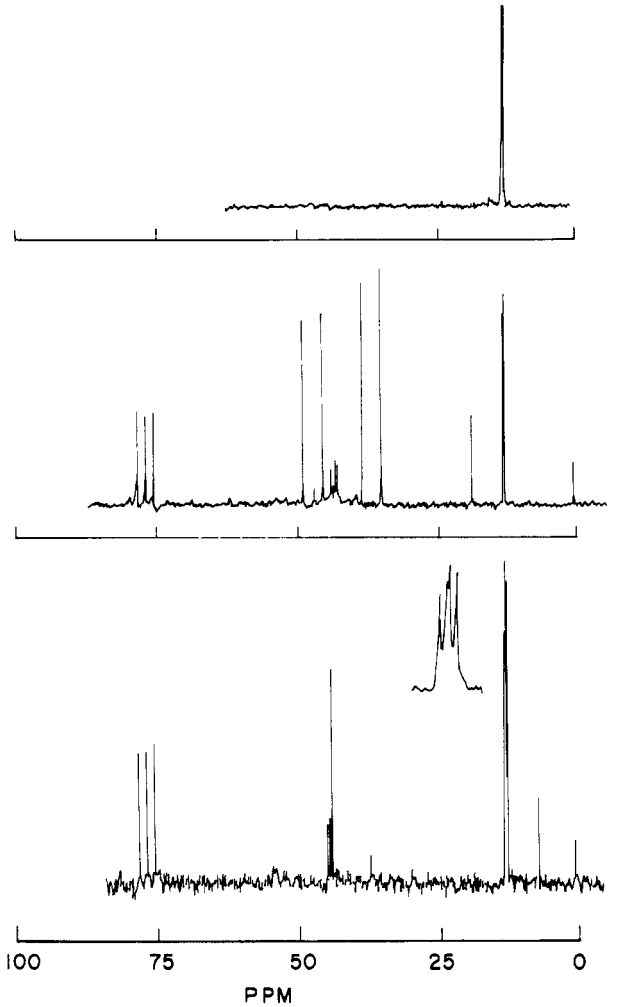

Figure 1. (a) (top) ^{13}C NMR of [$^{13}CH_3S$]methamidophos (*O,S*-dimethyl phosphoramidothioate); (b) (middle) ^{13}C NMR of the oxidation reaction mixture after 16 h; (c) (bottom) ^{13}C NMR of the reaction mixture after addition of trimethyl phosphite.

smaller group of peaks at 42–43 ppm correspond to methyl sulfoxide and/or methyl sulfone moieties.[6,7]

The major oxidation products **2a** and **2b** are doubtless the diastereomers of methamidophos *S*-oxide as indicated in Figure 2. This conclusion is supported by the following considerations. First, the doublets appear in the known sulfoxide chemical shift region (Table I). Also, the doublets observed for **2a** and **2b**, attributable to ^{13}C and ^{31}P coupling, show that the C–S–P linkage is still intact. Cleaved oxidation products would give rise to singlets.[8] Evidently, the system involving the C–S–P linkage has been perturbed to such an extent as to cause a large change in the coupling constant and formation of two doublets.

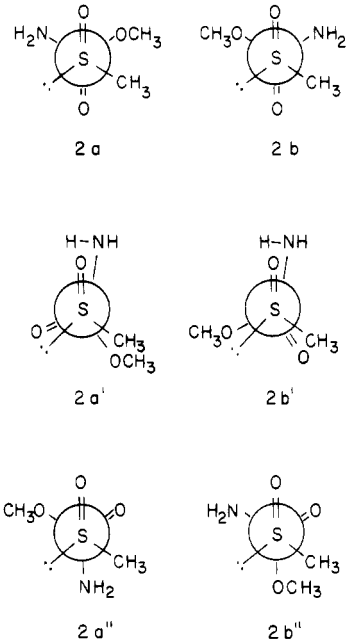

Figure 2. Possible Newman rotamers for diastereomers of methamidophos *S*-oxide: **2a** and **2b** with dipolar oxygens anti; **2a′** and **2b′** with hydrogen-bonding eclipsed forms; **2a″** and **2b″** with gauche effect enforced.

The phosphorus center in **1** is tetrahedral and, therefore, chiral. The oxidation of unsymmetrical sulfides to tetrahedral sulfoxides is well-known[9] and in the case of **1** would introduce a second chiral center in the molecule. The sulfoxide moiety in either **2a** or **2b** is characterized by the presence of four stereoelectronic groups, i.e., the lone pair of electrons, the oxygen atom, the methyl group, and the (methoxy)amidophosphinyl moiety. The diastereomers **2a** and **2b** would clearly explain the production of two doublets of approximately equal intensity and coupling constant.

The unusually large C–S–P coupling constants (75.7 and 82.4 Hz) warrants further explanation. C-13 to P-31 two-bond coupling constants have not been examined in detail, but most *J* values reported in this laboratory were on the order of 4.0–11.0 Hz. Geminal coupling constants of $^2J_{FCP}$ = 82 Hz have been reported for the system F_2H-CF_2CPCl_2.[10] For ^{13}C–S–^{31}P we would expect the *J* value to be less than the corresponding ^{13}C–^{31}P coupling[11] but would expect the former to resemble geminal proton ($^2J_{HH^{gem}}$) splitting where spin = $^1/_2$ nuclear interactions are

(7) Bass, S. W.; Evans, S. A., Jr. *J. Org. Chem.* **1980**, *45*, 710.
(8) That is, formation of CH_3SOH, CH_3SO_2H, and related oxidation products are possible, giving rise to singlets only.

(9) (a) For a review, see: Morrison, J. D.; Mosher, H. S. "Asymmetric Organic Reactions"; Prentice Hall, Inc.: Washington, DC, 1971; p 355. (b) Rayner, D. R., Gordon, A. J.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4854. (c) Mislow, K., Simmons, T., Melillo, J. T.; Ternay, A. L., Jr. *Ibid.* **1964**, *86*, 1452.
(10) Crutchfield, M. M.; Dungan, C. H.; Van Wazer, J. R. *Top. Phos. Chem.* **1967**, *5*, 1.
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involved. Therefore, the parameters that govern geminal coupling constants may be applied here. The magnitude of geminal coupling constants, ranging from −20 to 40 Hz in proton spectra, is governed largely by electronegative (inductive) effects and angle dependence.[12] The influence of electronegative substituents is the major contributor to geminal coupling constants in either saturated or unsaturated systems. In ordinary cases disubstituted methylene protons vary in coupling constant by about 0 to 6 Hz, while in the extreme case of formaldehyde a geminal coupling constant of 40.0 Hz is observed.[6] In the case of formaldehyde, in addition to the increase in s character of the carbon and therefore increased bond angles, there is also a back donation of the oxygen orbitals in the CH_2 plane. The inductive effect is somewhat enhanced, and hence the large coupling constant. In **2a** or **2b**, the equivalent d^{π}–p^{π} interaction between sulfur and oxygen is responsible for the electronic effect and should be quite substantial.[13] The large coupling constants are explained on this basis.

It is necessary also to explain the large differences in chemical shifts between **2a** and **2b**. The diastereomers must contain a substantial difference in their chemical environment about the enriched methyl moiety. Preliminary analysis of the isomers with the aid of Dreiding bond angle corrected models resulted in the Newman projections shown in Figure 2 in which the dipolar oxygens assume the anti conformation. Placement of the oxygens in this anti conformation, however, provides only a subtle difference in the steric constraints involving the ^{13}C-methy moiety. Gauche interactions and their effect on the ^{13}C chemical shifts have been reported[14] and their resulting shielding effect rationalized. On the basis of the work of Grant et al.,[14a,c,d] the electronegative nitrogen is expected to impart a more drastic effect on the gauche conformer **2b** as a field effect than would the corresponding methoxy in **2a**. However, from a steric standpoint Taft's E_s values[15] do not offer an appreciable difference between NH_2 (E_s = −0.61) and CH_3O (E_s = −0.55).

The field effect would seem to provide the only anisotropic effect large enough to impart such a difference. Unfortunately, there have been no reports of shifts of this magnitude, especially when both amino and methoxy moieties are expected to impart an upfield shift on the methyl group. Eggert and Djerassi[16] have reported a steric upfield shift for acyclic aliphatic amines and have rationalized 1–4 nonbonded steric gauche interactions to be about the same for CH_3/NH_2 and CH_3/CH_3. This reasoning led us to believe that other factors must be involved to account for the large difference in chemical shifts. For one doublet to be upfield and one downfield in the known sulfoxide region would suggest that opposite forces are competing in the two conformations.

The tendency of sulfoxides to enter into hydrogen bonding is well established[17] and can ultimately result in the cis pyrolytic elimination mechanism suggested by Kingsbury and Cram.[18] Applying these two established observations to the diastereomeric gauche rotamers **2a** and **2b** would then suggest that the phosphate amino group could form an intramolecular hydrogen bond with the newly formed sulfoxide, giving rise to the eclipsed Newman projections illustrated in Figure 2 (**2a′** and **2b′**). In **2b′** the labeled methyl group is eclipsing the oxygen and the electronegative environment would produce an anisotropic effect substantial enough to induce an upfield shift in accordance with previous reports.[14a,c,e,f,16] The analogous shift relative to the other diastereomer should not be as important, and thus the large difference in shift. Prashad et al.[19] have observed 7–8 ppm chemical shift differences due to steric compression in diastereomers.

A third possibility which also requires attention would be to rotate the projections **2a** and **2b** by 120° and produce the favorable dipolar gauche effect,[20] giving rise to the conformers **2a″** and **2b″** as shown in Figure 2. But, as in the first case, there is not sufficient steric or anisotropic effects to warrant a chemical shift difference of 10 ppm. From the information presented the conformers **2a′** and **2b′** would best explain the large chemical shift difference.

Reaction of Methamidophos *S*-Oxide with Trimethyl Phosphite. Further evidence for the formation of methamidophos *S*-oxide was needed to support the spectroscopic evidence. Oxygen is readily removed from sulfoxides and sulfones by trivalent phosphorus compounds.[3a,21] It seemed practical to apply this known reaction to the *S*-oxides formed in situ because it would avoid exposure of the compounds to air or moisture and yet provide chemical information. If the reaction were to proceed as documented, the product of the scavenger reaction should then be parent material and show unequivocally that the *S*-oxide is formed upon oxidation of methamidophos by *m*-CPBA. Trimethyl phosphite was selected as the oxygen scavenger, which in natural abundance gives rise to a doublet at 48.8 ppm with J = 11.0 Hz. Addition of 4 equiv of trimethyl phosphite to the reaction mixture and allowing it to stir at room temperature for 72 h gave rise to the spectrum shown in Figure 1c. Inspection of the spectrum reveals complete loss of both sulfoxide resonances at 36.37 and 47.11 ppm and the appearance of a new upfield doublet at 12.23 ppm in addition to the doublet for **1**. An increase in the amount of **1** present is the most significant finding, proving, at least in part, that the *S*-oxides were generated from starting material. The minor doublet at 44.24 ppm was assigned to a CH_3O–P moiety from an undetermined species, although trimethyl phosphate or *O*-methyl phosphoramidic acid are possible candidates. The new doublet at 12.23 ppm (J = 4.2 Hz), upfield from methamidophos **1**, suggests the presence of *O,O,S*-trimethyl phosphorothioate while the singlet at 43 ppm is suggestive of dimethyl sulfone. The generation of these two compounds from **1** under the experimental conditions described has been previously noted in the literature.[3a,22]

Conclusions

Evidence has been provided for the formation of a sulfoxide moiety α to a phosphorus atom. The diaste-

(12) An increase in the HCH bond angle increases the "s" character of the orbitals and makes the coupling more positive. See: Roberts, J. D. "Nuclear Magnetic Resonance, Applications to Organic Chemistry"; McGraw Hill: New York, 1969.

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(15) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley-Interscience: New York, 1979.

(16) Eggert, H.; Djerassi, C. *J. Am. Chem. Soc.* **1973**, *95*, 3710.

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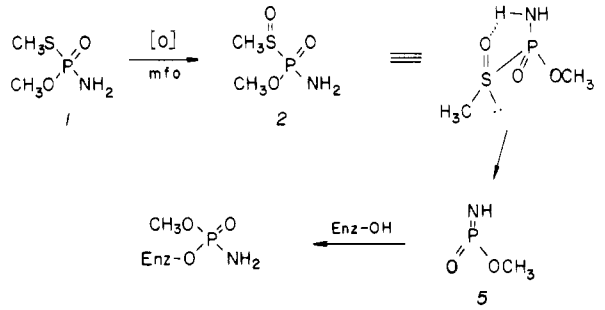
Scheme II. Possible Mechanism for Inhibition of Acetylcholinesterase by Methamidophos *S*-Oxide



reomeric *S*-oxides, characterized by ^{13}C NMR, were further examined by conformational analysis. Intramolecular hydrogen bonding between the amino and sulfoxide groups provided a plausible explanation for the large chemical shift difference. An intramolecular H-bond distance of 2.15 Å was calculated with the aid of Dreiding bond angle corrected models. Cis elimination[18] of the sulfoxide would result in the formation of a metaphosphorimidate (**5**). It was previously suggested[23] that base-catalyzed hydrolysis of methamidophos in either ethanol or propanol proceeds through a highly reactive metaphosphorimidate. Therefore, formation of **5** is one possibility in explaining the transitory nature of the *S*-oxides and the proposed increased inhibitory potential[3a] when **2** is generated (Scheme II).

The observation that spin = $^1/_2$ nuclei, other than protons, follows traditional geminal coupling interactions is a significant finding. The large coupling constants obtained for **2a** and **2b** are due to p^π–d^π back-bonding phenomena analogous to the p-orbital interactions in formaldehyde. Similar observations would be expected in the entire class of phosphorothioate esters and should be examined further. Substituents on phosphorus could play a significant role on the coupling constant and furnish information regarding the sulfoxide linkage.

As mentioned, attempts to isolate these intermediates were unsuccessful due to their sensitivity to workup. Elimination of methyl sulfenic acid or subsequent related products could also explain the singlet occurring upfield at 18 ppm.[7]

Experimental Section

General Methods. Dry ether refers to solvent freshly distilled under nitrogen from $LiAlH_4$. Dry chloroform refers to solvent stirred with 10 g/L of phosphorus pentoxide, filtered, and distilled under nitrogen. It can be assumed that reactions involving air- or moisture-sensitive compounds were handled under a blanket of nitrogen.

Silica gel 60F-254 Sheets (E.M. Reagents) of 0.25-mm thickness were used for analytical TLC. Solvent systems employed were ethyl acetate–methanol (1:1) or dichloromethane–acetone (1:5) and were visualized with 0.5% 2,6-dibromoquinone-4-chloroimide (DBQ) in ether as a spray reagent[24] or 0.5% palladium chloride in 1.0 N HCl as a spray reagent. Analytical HPLC was conducted on a Waters 6000A (Waters Assoc., MA) solvent delivery system equipped with a Rheodyne injector (Model 7125) and dual detection system (UV, 2100 Å, and refractive index detector). A Waters reversed-phase KC18 10 column Radial Compression Module (RCM-100) was employed. Solvent systems were water–methanol (85:15) at 1 mL/min or water–methanol (1:1) at 1 mL/min. Solvents and solvent combinations for the Waters 6000A system were routinely vacuum filtered before use.

Carbon-13 NMR spectra were recorded on a Bruker WH90D-18 multinuclear FTNMR spectrometer broad-band decoupled. Tetramethylsilane (Me_4Si) vs. dioxane was used as an internal standard for aqueous samples and deuteriochloroform served as the internal standard for all others. Chemical shifts are in δ values and coupling constants (J) in Hz.

Sodium *O*-Methyl Phosphoramidothioate. To a suspension of 12.76 g of *n*-butanethiol (0.14 mol) and 5.68 g of sodium hydroxide (0.14 mol) in 40 mL of methanol was added 20.0 g (0.14 mol) of *O,O*-dimethyl phosphoramidothioate[1] dropwise at 0 °C. The reaction mixture was then refluxed for 6.0 h, the resultant turbid solution was filtered and dried, and the solvent was removed by rotary evaporation. The residual white solid was recrystallized from 1-propanol and acetone to yield 84% of sodium *O*-methyl phosphoramidothioate.

***O*-Methyl *S*-[^{13}C]Methyl Phosphoramidothioate (1).** To a solution of 1.036 g of sodium *O*-methyl phosphoramidothioate (7.05 mmol) in 10 mL of anhydrous methanol was added 1.0 g of [^{13}C]methyl iodide (7.07 mmol, 90% enriched, Merck and Co., Teterboro, NJ) dropwise at 10 °C over a period of 4.0 h. The reaction mixture was stirred an additional 2.0 h, the solvent removed by rotary evaporation, and the yellowish oil taken up in a minimum of ethyl acetate and dried with magnesium sulfate overnight. Recrystallization from ethyl acetate–diethyl ether afforded 0.980 g (97.8%) of chromatographically and spectrally pure *O*-methyl *S*-[^{13}C]methyl phosphoramidothioate as white crystalline needles.

Chemical Oxidation. In a nitrogen atmosphere 0.018 g of **1** (0.126 mmol) was placed in 2.0 mL of dry $CDCl_3$ at 0 °C. To this mixture was added a 0.5 mL solution of *m*-chloroperbenzoic acid (0.043 g, 0.252 mmol) in $CDCl_3$ over a period of 2.0 h. The reaction mixture was allowed to come to room temperature and stirred an additional 14.0 h. The crude reaction mixture was submitted directly for ^{13}C NMR analysis.

Nonlabeled methamidophos was reacted similarly to the enriched material but subjected to neutralization with saturated sodium bicarbonate and concomitant workup. The resulting organic solution was not stable enough for TLC analysis or reversed-phase LC analysis.

Reaction with the Oxygen Scavenger.[21] To the crude reaction mixture obtained from the labeled oxidation study was added 0.068 g (0.55 mmol) of trimethyl phosphite. The solution was stirred at room temperature for 48.0 h in a sealed tube and resubmitted for ^{13}C NMR analysis.

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Registry No. **1**, 10265-92-6; ^{13}CHS-1, 89618-80-4; **2a**, 89618-81-5; **2b**, 89618-82-6; **4**, 152-20-5; sodium *O*-methyl phosphoramidothioate, 14261-33-7; *n*-butanethiol, 109-79-5; *O,O*-dimethyl phosphoramidothioate, 17321-47-0; [^{13}C]methyl iodide, 4227-95-6; trimethyl phosphite, 121-45-9.

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