of $NaIO_4$ (1.39 g, 6.5 mmol) in water (30 mL) was added portionwise to a stirrd solution of the diol **29** (1.20 g, 6.5 mmol) in dilute MeOH (H_2O:MeOH, 3:5, 80 mL) at 0 °C, and the mixture was stirred for 5 min at 0 °C. To this mixture was added $NaBH_4$ (0.24 g, 6.5 mmol) portionwise at the same temperature, and the reaction was quenched with 6 N HCl. The mixture was extracted with CH_2Cl_2 and the extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (40 g) by using EtOAc:*n*-hexane (1:8) as eluent to give the primary alcohol **31** (0.81 g, 80.9%): bp (Kugelrohr) ~120 °C (350 mmHg); $[\alpha]_D$ -67.81° (*c* 2.908, $CHCl_3$); IR (film) 3350, 3080, 1645 cm^{-1}; MS, *m/e* (relative intensity) 154 (M^+), 109 (100); ^1H NMR δ 1.43 (br, 1 H, disappeared with D_2O, -OH), 1.67 (d, 3 H, J = 1 Hz, =C(CH_3)—), 1.7 (br s, 6 H, =C(CH_3)$_2$), 1.53-2.1 (m, 2 H, -CH_2CH_2CH<), 2.7-3.42 (m, 1 H, -CH_2CH<), 3.65 (br t, 2 H, J = 7 Hz, -CH_2OH), 4.72 (s, 2 H, =CH_2), 5.05 (br d, 1 H, J = 9 Hz, >CHCH=). Anal. Calcd for $C_{10}H_{18}O$: 154.1356 (M^+). Found: 154.1330 (M^+).

(3*R*)-3-Isopropenyl-5-methyl-4-hexenyl 2-Nitrophenyl Selenide (32). To a stirred solution of the primary alcohol **31** (740 mg, 4.8 mmol) and 2-nitrophenyl selenocyanate (1.92 g, 7.2 mmol) in THF (10 mL) was added tri-*n*-butylphosphine (1.79 mL, 7.2 mL) dropwise at room temperature, and the mixture was stirred at the same temperature for 30 min. The mixture was concentrated under reduced pressure, and the residue was chromatographed on silica gel (70 g) by using *n*-hexane as eluent to give the selenide **32** (1.51 g, 93%): IR (film) 3080, 1640 cm^{-1}; MS, *m/e* (relative intensity) 339 (M^+), 109 (100); ^1H NMR δ 1.52-2.1 (m, 2 H, >CHCH_2CH_2-), 1.67 (br s, 6 H, =C(CH_3)$_2$), 1.73 (d, 3 H, J = 1 Hz, =C(CH_3)—), 2.73-3.3 (m, 1 H, >$CH$$CH_2$-), 2.87 (t, 2 H, J = 7.5 Hz, -CH_2CH_2Se-), 4.75 (s, 2 H, >C=CH_2), 5.05 (br d, 1 H, J = 9 Hz, >CHCH=), 7.08-7.62 (m, 3 H, Ar H), 8.25 (br d, 1 H, J = 8 Hz, Ar H). Anal. Calcd for $C_{16}H_{21}NO_2Se$: 339.0736 (M^+). Found: 339.0718 (M^+).

(-)-(*R*)-Santolinatriene (33). To a stirred solution of the selenide **32** (2.03 g, 6.0 mmol) in THF (5 mL) was added 30% H_2O_2 (5.21 mL, 60 mmol) dropwise at 0 °C, and the mixture was stirred for 5 h at the same temperature. Water (10 mL) was added to the reaction mixture, and the mixture was extracted with *n*-hexane. The extract was washed with saturated sodium carbonate and then with brine, dried ($MgSO_4$), and evaporated to leave crude santolinatriene (**33**) (750 mg, 92%). This was distilled immediately by using a Kugelrohr tube to minimize decomposition to give pure (*R*)-santolina triene (**33**) (120 mg, 15%): bp (Kugelrohr) 45° (14 mmHg) (lit.[20] bp_{20} 54.5 °C); $[\alpha]_D$ -55.58° (*c* 0.806, $CHCl_3$) (lit.[20] $[\alpha]_D$ +64°); IR (film) 3080, 1665, 1640, 1630 cm^{-1}; MS, *m/e* (relative intensity) 136 (M^+), 93, 79 (100); ^1H NMR δ 1.62 (s, 3 H, =C(CH_3)—), 1.72 (br s, 6 H, =C(CH_3)$_2$), 3.55 (m, 1 H, >CH-), 4.72 (br s, 2 H, =CH_2), 4.78-4.95 (m, 1 H, -CH=CH_2), 5.0-5.33 (m, 2 H, >C=CH—, -CH=CH_2), 5.5-6.1 (m, 1 H, -CH=CH_2). Anal. Calcd for $C_{10}H_{16}$: 136.1252 (M^+). Found: 136.1252 (M^+).

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Synthesis and Rearrangements of Alkyl Phosphorothioates

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Reaction of $PSCl_3$ with benzyl alcohol in triethyl phosphate followed by aqueous workup produces *S*-benzyl phosphorothioate, **2**, while similar reaction of cyclohexanol produces *O*-cyclohexyl phosphorothioate. The *S*-benzyl ester is postulated to arise from rearrangement of either *O*-benzyl phosphorodichloridate, **5**, or a hydrolysis product. Reaction of P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate, **3**, with BrCN in aqueous solutions buffered at pH 7.2 with lutidine or at pH 10.3 with triethylamine produces P^1-cyclohexyl P^2-*n*-propyl diphosphate, **4**, in essentially quantitative yield within 10 min. Similar reaction in $H_2{}^{18}O$ produces exclusively [P^1-^{18}O]**4** with no indication of the presence of ^{18}O at P^2. The reaction is postulated to involve the intermediate formation of P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiocyanatodiphosphate, **9**, by reaction of **3** with BrCN. **9** undergoes hydrolysis with displacement of SCN^- by H_2O, producing **4**. Similar reaction of P^1-*O*-cyclohexyl 1-thiodiphosphate in $H_2{}^{18}O$ produces cyclohexyl [1-^{18}O,2-^{18}O]diphosphate in high yield, with approximately 50% ^{18}O-enrichment at each position. This labeling pattern is postulated to arise from neighboring group participation by the terminal phosphoryl group. Initial reaction of P^1-*O*-cyclohexyl 1-thiodiphosphate produces the intermediate P^1-*O*-cyclohexyl 1-thiocyanatodiphosphate, **10**. The latter is partitioned between two pathways, direct displacement of SCN^- by water to form cyclohexyl diphosphate and internal displacement of SCN^- by the neighboring phosphoryl group to form cyclohexyl cyclodiphosphate, **11**, a highly reactive species which undergoes immediate hydrolysis to cyclohexyl diphosphate.

Reactions of adenosine 5′-*O*-[1-thiodiphosphate], ADPαS, and adenosine 5′-*O*-[2-thiotriphosphate], ATPβS, with BrCN in aqueous solutions produce ADP and ATP, respectively.[1a,b] These reactions carried out in $H_2{}^{18}O$ lead to the incorporation of ^{18}O into two positions within the di- or triphosphate moieties of ADP and ATP, demonstrating the operation of a complex mechanism. The mechanism involves a novel, spontaneous rearrangement of the polyphosphate moiety subsequent to the reaction of sulfur in ADPαS or ATPβS with BrCN to form the corresponding thiocyanato derivatives. The rearrangements lead to randomization of oxygens in the di- and triphosphate systems, as well as incorporation of oxygen from solvent at two positions[1a,b], and are thought to involve neighboring group participation by the terminal phosphoryl group leading to the formation of cyclodiphosphates as intermediates (see eq 1).

Factors governing the propensities of polyphosphates to form cyclodiphosphates are poorly understood. To

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(1) (a) Iyengar, R.; Ho, H.-T.; Sammons, R. D.; Frey, P. A. *J. Am. Chem. Soc.* **1984**, *106*, 6038-6049. (b) Sammons, R. D., Ho, H.-T.; Frey, P. A. *J. Am. Chem. Soc.* **1982**, *104*, 5841-5842.

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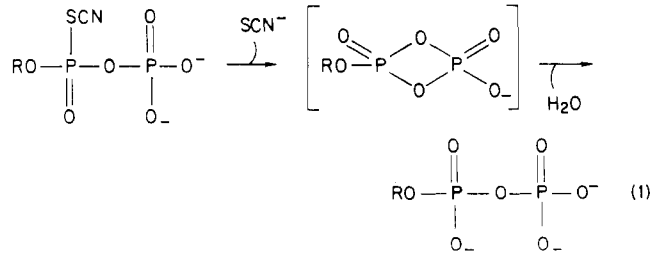


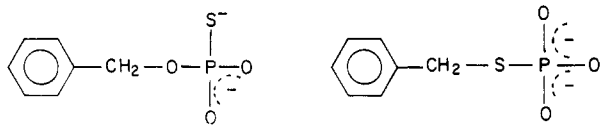
(1)

determine the effect of the absence of a terminal unesterified phosphoryl group, we have investigated the reaction of P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate with BrCN and other electrophilic reagents in aqueous solutions. We observed no indication of rearrangements involving cyclodiphosphates as intermediates. Reaction with BrCN in water resulted in quantitative conversion to P^1-cyclohexyl P^2-*n*-propyl diphosphate with incorporation of ^{18}O from $H_2{}^{18}O$ exclusively at the P^1 position.

We also describe an attempted synthesis of *O*-benzyl phosphorothioate by a standard procedure that leads instead to *S*-benzyl phosphorothioate. The unanticipated course of this reaction presumably involves an unusual but chemically reasonable rearrangement of the primary thiophosphorylation product.

Results

O-Alkyl phosphorothioates and *O*-alkyl thiophosphoanhydrides have generally been synthesized by procedures outlined in Scheme I. We have found, however, that reaction of benzyl alcohol with $PSCl_3$ followed by aqueous workup does not produce *O*-benzyl phosphorothioate, **1**, but rather *S*-benzyl phosphorothioate, **2**. The compound would not react in neutral aqueous solutions with DTNB.[2]

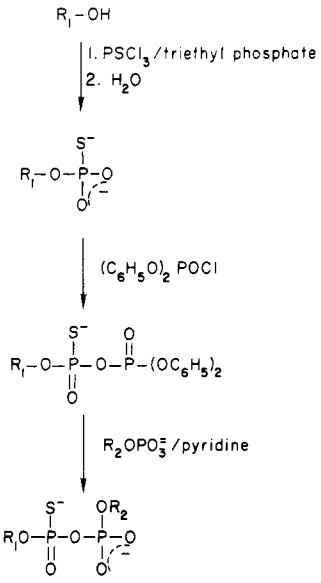


1 **2**

Moreover, the ^{31}P NMR spectrum of the compound was also inconsistent with **1**, in that the chemical shift was only 15.7 ppm downfield from H_3PO_4, whereas the chemical shifts for *O*-alkyl phosphorothioates are typically 40 to 50 ppm downfield. We confirmed the structure as **2** by showing that the compound is a substrate for *E. coli* alkaline phosphatase, which is known to catalyze hydrolysis of *S*-alkyl phosphorothioates but not *O*-alkyl phosphorothioates.[3]

Reaction of cyclohexanol with $PSCl_3$ and aqueous workup followed the usual course, producing a high yield of *O*-cyclohexyl phosphorothioate. The compound was characterized by its elemental analysis, positive reactivity with DTNB, and its ^{31}P NMR signal at 41.35 ppm downfield from the signal of H_3PO_4. Coupling of *O*-cyclohexyl phosphorothioate with *n*-propyl phosphate to P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate by the Michelson phosphoanhydride synthesis procedure[4a,b] was also uneventful. These transformations followed the course outlined in Scheme I, in which R_1 represents the cyclohexyl and R_2 the *n*-propyl group.

Scheme I



Reaction of a P^1,P^2-*O*-Dialkyl 1-Thiodiphosphate with BrCN. P^1-*O*-Cyclohexyl P^2-*n*-propyl 1-thiodiphosphate, **3**, reacts smoothly with BrCN in aqueous solutions, producing P^1-cyclohexyl P^2-*n*-propyl diphosphate, **4**. This transformation is essentially quantitative as evaluated by ^{31}P NMR analysis when the molar ratio of BrCN to **3** is equal to or greater than 2. The reaction is complete within 10 min at millimolar concentrations of reactants, maintained either at pH 7.2 by the presence of excess lutidine or at pH 10.3 by excess triethylamine.

The reaction carried out in an NMR tube proceeds with the disappearance of the phosphorothioate signal at 41.04 ppm assigned to P^1 of **3** and the appearance of a new signal in the −10 to −12 ppm region.

The proton spin-decoupled ^{31}P NMR spectrum of the product was consistent with an unsymetrical P^1,P^2-dialkyl pyrophosphate, with doublet signals centered at −10.8 and −11.7 ppm for P^1 and P^2 and a coupling constant $J_{1,2}$ = 22 Hz. The phosphorus resonances were assigned to P^1 and P^2 on the basis of the phosphorus–proton couplings, with the doublet of doublets at δ −11.7 ppm, resulting from coupling to one proton assigned to P^1, and the doublet of triplets at δ −10.8, representing coupling to two protons assigned to P^2. Within the limits of the sensitivity of ^{31}P NMR analysis, desulfurization of **3** to **4** was quantitative.

We screened a few other compounds as prospective electrophilic agents for promoting the displacement of sulfur from **3** by water. Aldehydes could be expected to form thiohemiacetals with **3** and thereby promote the displacement. 2,4-Dinitrobenzaldehyde (60–80 mM) in 1:1 H_2O–dioxane with 10–20 mM **3** induced a very slow conversion of **3** to **4**. The reaction required days to produce significant amounts of **4** at 25° and was, moreover, accompanied by substantial hydrolysis to phosphomonoesters. Carbodiimides might also react preferentially with sulfur in phosphorothioate; however, reaction of **3** with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide in water produced an intractable mixture of products.

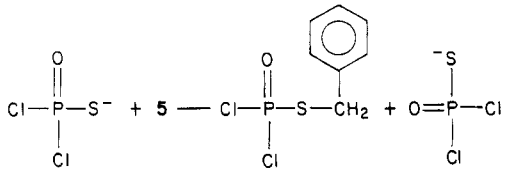
Desulfurization of 3 in $H_2{}^{18}O$. Reaction of **3** with BrCN in $H_2{}^{18}O$ produces **4** with ^{18}O enrichment exclusively at P^1. The ^{31}P NMR spectrum of ^{18}O-enriched **4** is identical with that of the **4** at the resonance position of P^2, but the P^1 resonance is shifted 0.027 ppm upfield due to the presence of ^{18}O. This difference is clearly revealed in the spectra of mixed samples of labeled and unlabeled **4**, whereas there is no sign of ^{18}O at P^2. Therefore, within

(2) The abbreviations are: DTNB, 5,5′-dithiobis(2-nitrobenzoate); ADPαS, adenosine 5′-*O*-[1-thiodiphosphate]; ATPβS, adenosine 5′-*O*-[2-thiotriphosphate]; β-(cyanoethyl)-ADPαS, P^1-*O*-(5′-adenosine) P^2-(2-cyanoethyl) 1-thiodiphosphate.

(3) Neumann, H.; Boross, L.; Katchalski, E. *J. Biol. Chem.* **1967**, *242*, 3142–3147.

(4) (a) Michelson, A. M. *Biochim. Biophys. Acta* **1964**, *91*, 1–13. (b) Moffat, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1958**, *80*, 3756–3761.

Scheme II



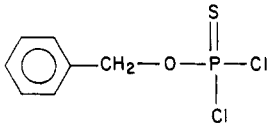
6

the sensitivity limits of ^{31}P NMR analysis, the conversion of **3** to **4** by reaction with BrCN in water proceeds by a mechanism that involves the replacement of sulfur at P^1 by oxygen from water.

Desulfurization of P^1-*O*-Cyclohexyl 1-Thiodiphosphate in $H_2{}^{18}O$. The reaction of P^1-*O*-cyclohexyl 1-thiodiphosphate with BrCN in $H_2{}^{18}O$ follows a more complex course than that of P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate. The reaction is not quantitative, though the yield of cyclohexyl diphosphate is high (78%); and ^{18}O is incorporated in comparable amounts at both P^1 and P^2. The presence of ^{18}O at both positions was demonstrated by the ^{31}P NMR spectrum of the product, with ^{18}O-enrichment of about 50% at each position, similar to the results obtained in the reactions of ADPαS and ATPβS with BrCN in $H_2{}^{18}O$.[1a,b]

Discussion

The production of *S*-benzyl phosphorothioate, **2**, rather than the *O*-benzyl ester in the thiophosphorylation of benzyl alcohol, presumably results from a rearrangement of the initial thiophosphorylation product *O*-benzyl thiophosphorodichloridate, **5**, or of intermediates produced

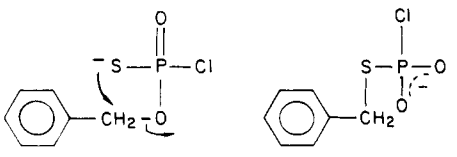


5

from it in the course of its hydrolytic workup. Compound **5** should be a moderately active alkylating agent; however, direct alkylation of $PSCl_3$ by **5** could not account for the formation of **2** because such a process would consume 2 mol of $PSCl_3$/mol of product formed. Since the crude product yield was 93% using only 20% excess $PSCl_3$, alkylation of $PSCl_3$ by **5** cannot account for our results.

The rearrangement may occur during hydrolytic workup. Partial hydrolysis products of unreacted $PSCl_3$ such as thiophosphorodichloridate should be nucleophilic. Alkylation of thiophosphorodichloridate anion by **5** would produce *S*-benzyl thiophosphorodichloridate, **6**, and regenerate thiophosphorodichloridate (see Scheme II). Hydrolysis of **6** would lead to **2**. In this pathway thiophosphorodichloridate anion plays a catalytic role.

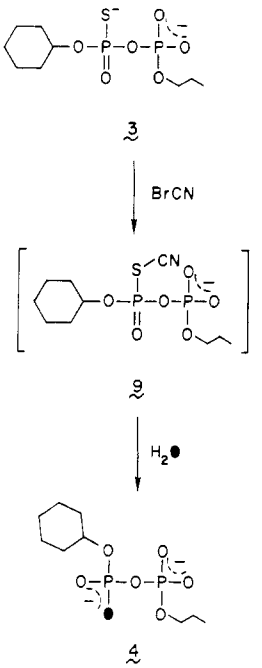
It is also conceivable that the rearrangement is an intramolecular process involving a four-center cyclic transition state. Such a rearrangement might involve the direct transformation of **5** to **6** or perhaps the rearrangement of a partial hydrolysis product such as that of **7** to **8**.



7 8

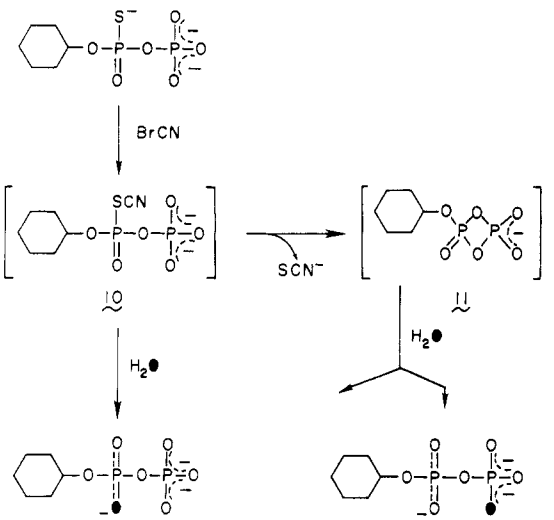
The reaction of **3** with BrCN in aqueous solution apparently follows the uncomplicated course outlined in

Scheme III[a]



[a] • = ^{18}O.

Scheme IV



Scheme III. Displacement of Br^- from BrCN by sulfur in **3** produces an intermediate, **9**, which has high electrophilic reactivity. The reactivity of **9** can be understood on the basis that neutralization of negative charge and electron withdrawal by the cyano group makes P^1 reactive with nucleophiles, including water. In the hydrolysis of **9** the most stable leaving group, SCN^-, is displaced by water.

There is no evidence of internal displacement by the adjacent *n*-propyl phosphoryl group in **9** which, had it occurred, would have resulted in the incorporation of ^{18}O at both P^1 and P^2 of **4**. The reaction is in this respect simpler than that of P^1-*O*-cyclohexyl 1-thiodiphosphate, which proceeds with neighboring group participation and incorporation of ^{18}O at both P^1 and P^2.

Initial reaction with BrCN produces Br^- and the thiocyanato intermediate **10**, which can react with $H_2{}^{18}O$ by several routes outlined in Scheme IV. Direct displacement of SCN^- by $H_2{}^{18}O$ produces P^1-cyclohexyl [1-^{18}O]diphosphate. Internal displacement of SCN^- by the P^2-phosphoryl group produces cyclohexyl cyclodiphosphate

11, a highly reactive intermediate. Hydrolysis of 11 by $H_2{}^{18}O$ probably proceeds with incorporation of ^{18}O into both phosphoryl groups. Incorporation into P^2 is expected on the basis that reaction at P^2 would generate the more stable leaving group, the hydrolytic ring opening of alkyl cyclotriphosphates is known to be governed by the stability of the leaving group.[5] In the case of the cyclodiphosphate 11, it is possible that the reaction is less selective.

The absence of neighboring group participation in the reaction of 3 must be attributed to the presence of the *n*-propyl group. Electronic and steric effects both tend to reduce participation.

The reaction of BrCN with P^1,P^2-dialkyl 1-thiodiphosphates has been applied to the synthesis of (S_p)-[α-^{18}O]ADP and (R_p)-[α-^{18}O]ADP and the corresponding ^{17}O-enriched nucleotides. These compounds were efficiently produced by reaction of (R_p)-β-(cyanoethyl)-ADPαS and its S_p epimer with BrCN in $H_2{}^{18}O$.[6a] The R_p and S_p epimers of β-(cyanoethyl)-ADPαS were synthesized as efficiently and conveniently as the corresponding epimers of ADPαS; removal of the β-cyanoethyl protecting group in the desulfurized product proceeded essentially quantitatively in dilute base. The desulfurization process followed the same course as that in Scheme III and with at least 97% inversion of configuration.[6b]

Desulfurization of (R_p)-ADPαS and (S_p)-ADPαS by reaction with brominating agents in aqueous acidic solutions also produces (S_p)-[α-^{18}O]ADP and (R_p)-[α-^{18}O]ADP in $H_2{}^{18}O$ with inversion of configuration.[7a,b,c] These reactions also proceed with a degree of rearrangement when carried out in neutral solutions. It is likely that rearrangements in these cases would also be prevented by esterification of the β-phosphoryl group.

Experimental Section

Materials. Tri-*n*-octylamine, tri-*n*-butylamine, lutidine, dithiothreitol, 5,5′-dithiobis(2-nitrobenzoic acid), cysteine, *E. coli* alkaline phosphatase (36 units/mg), DEAE Sephadex A-25, 2,4-dinitrobenzaldehyde, cyanogen bromide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide-HCl, thiophosphoryl trichloride Dowex AG-50, Dowex AG-1, deuterium oxide (99.7%), and $H_2{}^{18}O$ were commercial products.

Pyridine was refluxed over BaO for 12 h and then distilled. 1,4-Dioxane and triethylphosphate were dried over CaH_2 for three days and then distilled. Each were placed in sealed flasks over Linde 4-A molecular sieves or KOH pellets. Thiophosphoryl trichloride was redistilled just prior to being used. Tri-*n*-butylamine was distilled over KOH pellets and stored in a flask darkened with aluminum foil. Cyclohexanol, benzyl alcohol, *n*-propanol, and methanol were refluxed over CaH_2 for 8 h and then distilled. Alumina was dried at 100 °C for 1 h before use. Tri-*n*-octylamine was distilled at reduced pressure and stored in a sealed flask. Diethyl ether was used only from newly unsealed containers. 1,4-Dioxane (previously dried and distilled) and high boiling petroleum ether were passed through a column of alumina (40.0 g/100 mL) just prior to being used.

Methods. Thiophosphates and pyrophosphates were purified by chromatography through columns of Dowex AG-1 and DEAE Sephadex A-25 eluted with linear gradients of trimethylammonium bicarbonate. The concentrations of the monothiophosphates were determined by reaction with DTNB. Sample aliquots (100 μL) were placed in test tubes with 100 μL of 11 mM DTNB at pH 8. The samples were diluted to 1 mL, the A_{412} were measured, and concentrations were calculated by using the extinction coefficient 11×10^3 M^{-1} cm^{-1}. Total phosphates were measured as inorganic phosphate in ashed samples as described by Ames and Dubin.[8]

^{31}P NMR spectra were obtained by using 200-MHz spectrometers operating at 80 MHz in the Fourier transform mode. The spectrometer was field frequency locked to the deuterium resonance of D_2O in the solvent. The sweep width varied from 500 to 10 000 Hz. Proton spins were decoupled at a power of 2.0 or 2.5 W. The pulse width was 8 or 12 μs and the number of scans ranged from 50 to 3000, depending on the concentrations of the samples. The temperatures of the samples were maintained at 25 °C. Chemical shifts upfield from an external 85% orthophosphoric acid standard were assigned negative values.

Synthesis of *S*-Benzyl Phosphorothioate. Triethyl phosphate (10 mL) and 1.04 mL (10 mmol) of benzyl alcohol were combined in a dry 50-mL round-bottom flask containing a magnetic stirring bar and cooled to 0 °C. After 15 min 1.25 mL (12 mmol) of thiophosphoryl trichloride was added with stirring and the reaction mixture maintained at 0 °C for 30 min and at room temperature for an additional 8 h. The flask was then evacuated to 0.2 torr at 30 °C for 2 h to remove the HCl and excess $PSCl_3$. A 50/50 solution (v/v) of triethylamine/water was added to the stirred reaction during 2 h in sufficient volume to maintain the pH above 8. The pH was then adjusted to 11.5 by addition of 2 M LiOH and the solvent removed by rotary evaporation. The residue was twice dissolved in 95% ethanol and again evaporated. The white precipitate was dissolved in a minimum of methanol and combined with 20 volumes of anhydrous diethyl ether. After centrifugation of the precipitate, the process was repeated three times.

The lithium salt of the crude product (1.97 g) was dissolved in water and purified by chromatography through a 3.5 × 25 cm column of Dowex AG-1 eluted with a 4-L linear gradient of triethylammonium bicarbonate increasing from 0.4 to 0.8 M. Analysis of fractions by A_{260} measurements and colorimetric analysis for DTNB-sensitive phosphorothioates revealed two major products, thiophosphate emerging at 0.48 M salt and S-benzyl phosphorothioate emerging at 0.64 M salt. Pooled fractions of the latter were evaporated to dryness and the triethylammonium salt was converted to the lithium salt as described above for the crude product. Anal. ($C_7H_7O_3PSLi_2$) Calcd: C, 38.71; H, 3.69; O, 22.12; P, 14.29; S, 14.75. Found: C, 38.70; H, 3.81; O, 22.18; P, 14.11; S, 14.84. ^{31}P NMR δ 15.66 (triplet, J_{P-H} = 6.7 Hz).

Synthesis of *O*-Cyclohexyl Phosphorothioate. To a 50-mL round-bottom flask was added 2.08 mL (20 mmol) of previously dried and distilled cyclohexanol along with 10 mL of triethyl phosphate and a magnetic stirring bar. The stirred mixture was cooled to 0 °C and thiophosphoryl trichloride (2.50 mL, 25 mmol) added. After 30 min the reaction mixture was permitted to warm to room temperature and stirred for an additional 8 h. The flask was evacuated to 0.2 torr at 30 °C for 3 h to remove unreacted $PSCl_3$ and then cooled to 0 °C. A 25% (v/v) solution (50 mL) of aqueous triethylamine was added with constant stirring at 0 °C. Additional triethylamine was added over a period of 2 h until the pH remained above 8. The oil that appeared at the bottom of the flask was removed and stirred with 200 mL of a 25% (v/v) mixture of pyridine in water until the mixture became homogeneous (2 h). The solution was adjusted to pH 11.5 with LiOH and evaporated to dryness. The residual white solid was extracted by stirring vigorously three times with 200 mL of acetone. The solid was dissolved in water and chromatographed through a 2.5 × 60 cm column of Dowex AG-50-Li^+ eluted with 3 volumes of water. The water was removed by rotary evaporation and the solid placed in a vacuum desiccator. The yield of dilithium *O*-cyclohexyl phosphorothioates was 91%. Anal. ($C_6H_{11}O_3PSLi_2$) Calcd: C, 34.62; H, 5.29; O, 23.08; P, 14.91; S, 15.43. Found: C, 34.61; H, 5.25; O, 23.10; P, 14.88; S, 15.48. ^{31}P NMR δ 41.35 (doublet, J_{P-H} = 12.15 Hz, collapsing to a singlet with proton decoupling).

Synthesis of *n*-Propyl Phosphate. Diethyl ether (20 mL) was combined with 20 mmol of $POCl_3$ and a magnetic stirring bar inside a dry, 100-mL round-bottom flask cooled to 0 °C, and

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a 25-mL pressure-equalizing funnel was attached containing 20.0 mmol of *n*-propanol. The *n*-propanol was added dropwise over a period of 30 min with constant stirring. The cooling bath was removed and the reaction permitted to proceed for 2 h. After removing diethyl ether by rotary evaporation, the residual oil was dissolved in 200 mL of water at 0 °C. The solution was concentrated at 100 mL by rotary evaporation, filtered, and adjusted to pH 9 with NaOH, and 0.01 mol of barium chloride in 10 mL of water added. After removing barium phosphate by filtration, addition of an equal volume of ethanol induced the slow crystallization of the barium salt of *n*-propyl phosphate in 61% yield. The Ba^{2+} salt mixed as a slurry with one exchange equivalent of Dowex AG-50 in the H^+ form and was poured into a column packed with five additional exchange equivalents of Dowex AG-50 in the H^+ form. *n*-Propyl phosphate eluted from this column was adjusted to pH 11.5 by addition of 2.5 M LiOH. The solution was concentrated to dryness by rotary evaporation and the white residue dried in vacuo. The ^{31}P NMR proton-decoupled spectrum revealed a singlet at δ −4.633 and the proton-coupled spectrum a triplet, with J_{P-H} = 6.104 Hz.

Synthesis of P^1-*O*-Cyclohexyl P^2-*n*-Propyl 1-Thiodiphosphate. The Li^+ salt of *O*-cyclohexyl phosphorothioate (3 mmol) was converted to its tri-*n*-octylammonium salt and dissolved in anhydrous dioxane under dry N_2 in a glove bag. To this solution 6 mmol of tri-*n*-butylamine was added and the mixture stirred for 30 min. Diphenyl phosphorochloridate (3.3 mmol) was added and the reaction mixture stirred for 1 h. The flask was then sealed under nitrogen, removed from the glove bag, and evaporated in vacuo to a syrup. A 9:1 mixture of high boiling petroleum ether and diethyl ether was added to the syrup and stirred for 20–30 min at 0 °C. The ether layer was decanted, and the residual oil dissolved in dioxane and evaporated to a syrup. The flask was sealed and returned to the glove bag, and the syrup dissolved in a minimum amount of pyridine. The pyridinium salt of *n*-propyl phosphate (10 mmol) in a second 500-mL flask was also dissolved in a minimum of pyridine and added to the activated *O*-cyclohexyl phosphorothioate, and the mixture stirred for 6 h under nitrogen. The yellow-brown solution was quenched by addition of 100 mL of water at 0 °C and extracted with diethyl ether, and the aqueous layer adjusted to pH 8.5 by addition of triethylamine. This solution was passed into a 4 × 50 cm column of DEAE Sephadex A-25-HCO_3^-. Elution was with a 4-L linear gradient of triethylammonium bicarbonate increasing from 0.2 M to 0.6 M. The total inorganic and organic phosphates in ashed aliquots of selected column fractions were determined by colorimetric analysis.[8] Unreacted *n*-propyl phosphate emerged at 0.36 M salt and P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate at 0.5 M salt. The latter compound was contaminated with diphenyl phosphate, detected by its A_{260}. Fractions containing P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate were pooled and buffer salt removed by rotary evaporation to dryness. The residue was dissolved in 100 mL of 0.1 M triethylammonium bicarbonate, absorbed on a 3.5 × 25 cm column of Dowex AG-1 (HCO_3^-, 100–200 mesh), and eluted with 1 M triethylammonium bicarbonate. Fractions 18 mL in volume were collected and analyzed for organic phosphates as described above. P^1-*O*-Cyclohexyl P^2-*n*-propyl 1-thiophosphate emerged from the column well in advance of diphenyl phosphate. Pooled fractions of the product were evaporated to dryness and the triethylammonium salt was converted to the potassium salt by passage through a column of Dowex AG-50 in the K^+ form. The aqueous solution of the potassium salt was concentrated to 0.1 mL by rotary evaporation and then to dryness by lyophilization. Dipotassium P^1-*O*-cyclohexyl P^2-*n*-propyl 1-thiodiphosphate was obtained in 51% yield. Anal. ($C_9H_{18}O_6P_2SK_2$) Calcd: C, 32.73; O, 29.09; H, 5.46; P, 18.79. Found: C, 32.79; O, 29.20; H, 5.65; P, 18.64. Proton spin-decoupled ^{31}P NMR δ 41.04 (doublet, P^1), −11.62 (doublet, P^2, $J_{P^1P^2}$ = 28.69 Hz; ^{31}P NMR δ 41.04 (doublet of doublets, P^1), −11.62 (doublet of triplets, P^2, J_{P^1-H} = 11.00 Hz, J_{P^2-H} = 7.44 Hz).

Synthesis of P^1-*O*-Cyclohexyl 1-Thiodiphosphate. The tri-*n*-octylammonium salt of *O*-cyclohexyl phosphorothioate (0.68 mmol) was prepared and activated by reaction with diphenyl phosphorochloridate as described above. Separately, phosphoric acid (3 mmol) was dissolved in pyridine, the solvent removed by rotary evaporation, and the residue redissolved in 5 mL of dry pyridine. Tri-*n*-butyamine (3 mmol) was stirred into the solution until it became homogeneous. The solvent was again removed by rotary evaporation and the residue dried for 48 h in a vacuum desiccator over P_2O_5. Tri-*n*-butylammonium phosphate was dissolved with 10 mL of pyridine; and this solution was used to dissolve the activated *O*-cyclohexyl phosphorothioate. The reaction proceeded for 12 h at ambient temperature. After removing pyridine by rotary evaporation, the residue was dissolved in 100 mL of 0.2 M triethylammonium bicarbonate and extracted several times with diethyl ether. The aqueous layer was diluted with 400 mL of water, applied to a 2.5 × 45 cm column of DEAE Sephadex A-25 at 4 °C, and eluted with a 5-L linear gradient of triethylammonium bicarbonate increasing from 0.2 M to 0.5 M. Analysis of column fractions showed that P^1-*O*-cyclohexyl-1-thiodiphosphate appeared in fractions eluting between 0.32 M and 0.35 M salt. Unreacted *O*-cyclohexyl phosphate and inorganic phosphate appeared in fractions eluting between 0.25 M and 0.28 M salt. Product-containing fractions were pooled and solvent and volatile salt were removed by rotary evaporation. Thin-layer chromatography showed that the product was contaminated with traces of other thiophosphates, so it was rechromatographed through a 2.5 × 30 cm column of DEAE Sephadex A-25 in the HCO_3^- form. The column was washed with 200 mL of 0.2 M and then with 1200 mL of 0.3 M triethylammonium bicarbonate. P^1-*O*-Cyclohexyl 1-thiodiphosphate was obtained in 60% yield (0.4 mmol) and desalted by rotary evaporation. ^{31}P NMR δ 39.83 (doublet of doublets, P^1, −5.74 (doublet, P^2, J_{P^1-H} = 10.53 Hz, $J_{P^1P^2}$ = 28.26 Hz); proton spin-decoupled ^{31}P NMR δ 39.83 (doublet, P^1), −5.74 (doublet, P^2, $J_{P^1P^2}$ = 28.79 Hz).

Reaction of P^1-*O*-Cyclohexyl 1-Thiodiphosphate with BrCN. The triethylammonium salt of P^1-*O*-cyclohexyl 1-thiodiphosphate (20 μmol) was dissolved in 0.2 mL of 0.08 M K-borate buffer at pH 10.5, evaporated to dryness by rotary evaporation, desiccated in vacuo over P_2O_5 for 48 h, and dissolved with 0.2 M of H_2O. Cyanogen bromide (7 mg, 65 μmol) was added and the solution stirred 15 min at ambient temperature. After quenching the reaction with 7.8 mg of cysteine, it was diluted to 25 mL with 0.05 M triethylammonium bicarbonate and chromatographed through a 1.1 × 30 cm column of DEAE-Sephadex A-25-HCO_3^- eluted at 4 °C with a 1-L linear gradient of triethylammonium bicarbonate increasing from 0.1 M to 0.4 M. *O*-Cyclohexyl diphosphate appeared in the effluent at 0.25 M salt. Pooled fractions were dried by rotary evaporation. The yield by analysis of ashed samples for total phosphate was 15.7 μmol (78%). Proton spin-decoupled ^{31}P NMR of the triethylammonium salt: δ −9.73 (doublet, P^1), −5.15 (doublet, P^2, $J_{P^1P^2}$ = 20.80 Hz); ^{31}P NMR δ −9.73 (doublet of doublets, P^1), −5.13 (doublet, P^2, J_{P^1-H} = 8.05 Hz).

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Registry No. **2**, 94781-53-0; **2**·2Li, 94781-54-1; **2** triethylammonium salt, 94781-55-2; **3**·2K, 94781-66-5; **3** triethylammonium salt, 94781-65-4; $PSCl_3$, 3982-91-0; $POCl_3$, 10025-87-3; $(C_6H_5O)_2POCl$, 2524-64-3; *O*-cyclohexyl phosphorothioate, 94781-56-3; dilithium *O*-cyclohexyl phosphorothioate, 94781-57-4; *n*-propyl phosphate, 94781-58-5; barium *n*-propyl phosphate, 34296-06-5; *O*-cyclohexyl phosphorothioate tri-*n*-octylammonium salt, 94781-60-9; activated *O*-cyclohexyl phosphorothioate, 94781-62-1; *n*-propyl phosphate pyridinium salt, 94781-63-2; P^1-*O*-cyclohexyl 1-thiodiphosphate, 94781-67-6; P^1-*O*-cyclohexyl 1-thiodiphosphate triethylammonium salt, 94781-69-8; *O*-cyclohexyl diphosphate triethyl ammonium salt, 94781-71-2; benzyl alcohol, 100-51-6; cyclohexanol, 108-93-0; *n*-propanol, 71-23-8; triethylammonium bicarbonate, 15715-58-9; phosphoric acid, 7664-38-2.