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SYNTHESIS OF O-GERANYL (1-THIO)DIPHOSPHATE

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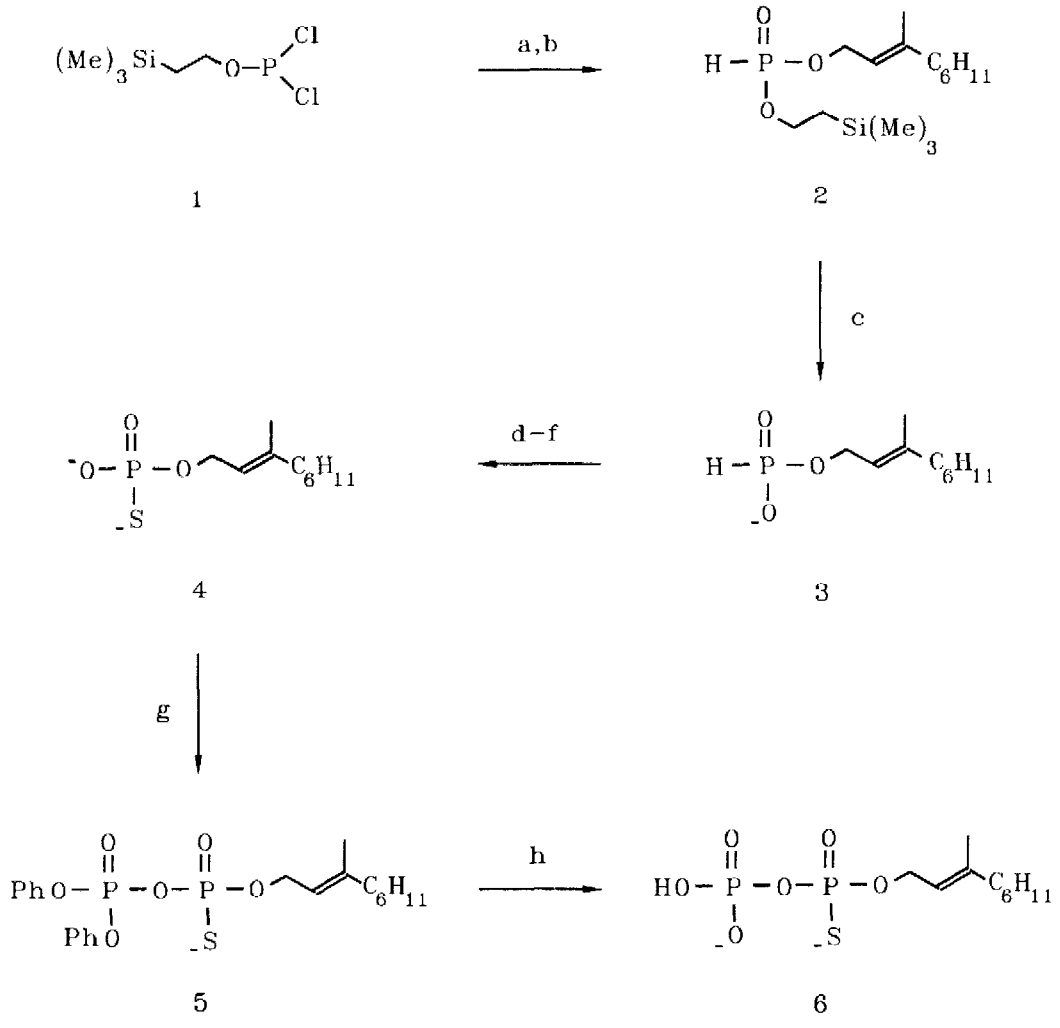
Abstract: O-Geranyl (1-thio)diphosphate was synthesized from geraniol via H-phosphonate and thiophosphate intermediates in 5 steps with an overall yield of 20%.

Mono-, di-, and trithiophosphate analogues, especially of nucleosides, have become important tools in biochemistry and molecular biology[1,2]. The structures of thiophosphate esters are not greatly perturbed from the corresponding natural phosphates, and the sulfur-containing analogues of nucleic acids are alternate substrates for many of the kinases, ligases, and restriction endonucleases used in molecular biology. (1-Thio)diphosphates of isoprenes are an unknown and potentially useful family of alternate substrates for mechanistic studies. However, the approaches commonly available to synthesize thiodiphosphate esters of nucleosides[1] are not applicable to this class of compounds because of the reactivity of the allylic and cyclopropylcarbinyl moieties usually encountered in isoprenes. Recently, Ludwig and Eckstein[3] employed phosphites to prepare nucleoside thiotriphosphates, and Shadid and coworkers[4] took advantage of the increased stability of phosphite and H-phosphonate intermediates to synthesize phosphate derivatives of allylic alcohols. As part of our effort to develop synthetic procedures for isoprene diphosphates[5-7], we had independently investigated the utility of phosphite and H-phosphonate esters to synthesize diphosphate and thiodiphosphate derivatives and now report a practical route to O-geranyl (1-thio)diphosphate.

The synthesis of O-geranyl (1-thio)diphosphate (**6**) is outlined in Scheme I. Trimethylsilylethyl dichlorophosphite (**1**) was prepared by addition of 2-(trimethylsilyl)ethanol (2.72 g, 23 mmol) in 45 mL of ether to a solution of 4.12 g (30 mmol) of phosphorus trichloride in 25 mL of ether at -40 °C[8]. After 1 h, the solution was allowed to warm to room temperature; solvent was removed; and the residue was distilled to yield 4.73 g (94%) of **1** (bp 45 °C, 1.5 mm/Hg). The dichlorophosphite can be prepared in bulk, stored at -20 °C, and used as a reagent when needed. A solution of geraniol (2.1 g, 13.7 mmol) and diisopropylethyl amine (3.9 g, 30 mmol) in 120 mL of ether was added to 3.0 g (13.7 mmol) of **1** in 60 mL of ether at -40°C. After 20 min, the mixture was allowed to warm to 0 °C, and the resulting geranyl chlorophosphite ester was immediately converted to **2** by treatment with 10 mL of water by a modification of the procedure of Reynolds et al[9]. The mixture was stirred for 10 min, and following work-up and removal of solvent, **2** was purified by flash chromatography (silica gel; 7:3 v/v hexane:ethyl acetate) in 54% yield[10]. Care must be taken to complete the chromatography rapidly to prevent decomposition of the diester on the column. Treatment of **2** (375 mg, 1.18 mmol) with tetra-n-butylammonium fluoride (372 mg, 1.18 mmol) in 5 mL of



Scheme I. a) Geraniol, Et_3N, Et_2O, -40 °C. b) H_2O, 0 °C. c) Bu_4NF, THF, rt. d) Et_3N, C_6H_6, rt. e) $(Me)_3SiCl$, 5 °C. f) S_8, rt. g) $(PhO)_2P(O)Cl$, Bu_3N, CH_2Cl_2, rt. h) $Bu_4NH_2PO_4$, pyridine, rt.

tetrahydrofuran cleanly removed the trimethylsilylethyl moiety[12] to give a quantitative recovery of **3**.

O-Geranyl H-phosphonate (**3**) was used without further purification[12]. The tetrabutylammonium salt was dried by repeated evaporation of pyridine, and the H-phosphonate monoester was converted to the corresponding *bis*-trimethylsilyl phosphite by the procedure of Hata and Sekine[13]. To a solution of **3** (968 mg, 2.0 mmol) and triethyl amine (1.20 g, 12 mmol) in 50 mL of benzene at 5 °C was added 1.28 g (12 mmol) of trimethylsilyl chloride. After 30 min, the

reaction was allowed to warm to room temperature, and 151 mg (4.7 mmol) of powdered sulfur was added. The suspension was stirred for 30 min before a 5 mL solution of 4:1 v/v methanol:triethylamine was added. The solution stirred for 30 min; solvent was removed under vacuum; and the residue was chromatographed on Dowex 50W-X8 (40 meq, ammonium form) to remove alkyl ammonium cations. Solvent was removed by lyophilization, and the residue was then chromatographed on cellulose (R_f = 0.35; Whatman CF-11, 6.1:1 v/v isopropanol:0.1 M ammonium bicarbonate, pH 7.0) to yield 265 mg (46%) of O-geranyl (1-thio)phosphate (**4**)[14].

The ammonium salt of **4** (79 mg, 0.28 mmol) was solubilized in 20 mL of methylene chloride by addition of 10 equivalents of tributylamine. To the solution was added 113 mg (0.42 mmol) of diphenyl chlorophosphate at room temperature following the general procedure of Richard and Frey[15] for conversion of O-nucleoside thiophosphates to thiodiphosphates. After 1 h, solvent was removed, and anhydride **5** was dissolved in 30 mL of dry pyridine. Anhydrous tetra-n-butylammonium phosphate (570 mg, 1.7 mmol) was added[16], and the reaction was allowed to proceed for 20 h. Following ion-exchange chromatography as previously described for **4**, a portion of the ammonium salt of **6** was chromatographed on cellulose (R_f = 0.4, Whatman CG-11; 5:2:3 v/v/v isopropanol:acetonitrile:0.1 M ammonium bicarbonate, pH 7.0). Organic solvents were removed by rotary evaporation, and the aqueous residue was lyophilized to remove water and ammonium bicarbonate to give 16 mg (80%) of the thiodiphosphate[17]. The white powder can be stored for extended periods at -70 °C. The overall yield was 20% based on geraniol.

The route shown in Scheme I for converting geraniol to **6** should, with minor modifications, be useful for synthesis of a variety of phosphate and diphosphate esters of reactive alcohols. H-Phosphonate ester **3** is readily converted to the *bis*-trimethylsilyl phosphite derivative, which is easily oxidized and hydrolyzed to give corresponding phosphate or thiophosphate. In addition, oxidation with ^{35}S can be used to provide radiolabeled material for biochemical studies. It remains to be seen if the synthesis outlined in Scheme I can be adopted to prepare optically active **6** since Richard and Frey[15] found epimerization of the thiophosphoryl center in the diphenylphosphate anhydride of AMP-αS under conditions similar to those reported in step h of Scheme I. In spite of this potential limitation, the phosphite approach offers a possible route to a wide variety of isoprene derivatives, including diphosphate esters of tertiary and cyclopropylcarbinyl systems that are currently difficult to obtain.

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REFERENCES AND NOTES

1. Eckstein, F. *Ann. Rev. Biochem.* **1985**, *54*, 367-402; and references therein.
2. Eckstein, F.; Gish, G. *TIBS* **1989**, *14*, 97-100.
3. Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631-635.
4. Shadid, B.R.; van der Plas, H.C.; deVroom, E.; van der Marcel, G.A.; van Boom, J.H. *Recl. Trav. Chim. Pays-Bas.* **1987**, *106*, 509-511.
5. Davisson, V.J.; Woodside, A.B.; Neal, T.R.; Stremler, K.E.; Muehlbacher, M.; Poulter, C.D. *J. Org. Chem.* **1986**, *51*, 4768-4779.
6. Davisson, V.J.; Woodside, A.B.; Poulter, C.D. *Meth. Enzymol.* **1985**, *110*, 130-144.
7. Davisson, V.J.; Zabriskie, T.M.; Poulter, C.D. *Bioorg. Chem.* **1986**, *14*, 46-54.
8. ^1H NMR (300 MHz, C_6D_6) δ -0.18 (9 H, s), 0.79 (2 H, t, $J_{H,H}$ = 8.4 Hz), 4.18 (2 H, dt, $J_{H,H}$ = 8.4 Hz, $J_{P,H}$ = 8.4 Hz); ^{13}C NMR (75 MHz, C_6D_6) δ -1.3 (q), 19.4 (dt), 67.8 (dt); ^{31}P NMR (121 MHz, C_6D_6) δ 173.5 ppm (br m).
9. Reynolds, M.A.; Oppenheimer, N.J.; Kenyon, G.L. *J. Am. Chem. Soc.* **1983**, *105*, 6663-6667.

10. ^{1}H NMR (300 MHz, C_6D_6) δ -0.13 (9 H, s), 0.94 (2 H, t, $J_{H,H}$ = 8.5 Hz), 1.49 (6 H, s), 1.60 (3 H, s), 1.95 (4 H, br m), 4.10 (2 H, br m), 4.52 (2 H, dd, $J_{H,H}$ = 6.8 Hz, $J_{P,H}$ = 9.5 Hz), 5.10 (1 H, br m), 5.31 (1 H, t, $J_{H,H}$ = 6.8 Hz), and 6.79 ppm (1 H, d, $J_{P,H}$ = 683 Hz); ^{13}C NMR (75 MHz, C_6D_6) δ 1.48 (q), 16.5 (q), 17.8 (q), 19.9 (dt), 25.9 (q), 26.7 (t), 39.9 (t), 62.1 (dt), 63.7 (dt), 119.7 (dd), 124.2 (d), 131.6 (s), and 142.5 ppm (s); ^{31}P NMR (121 MHz, C_6D_6) δ 4.6 ppm (dtt, $^{1}J_{P,H}$ = 683 Hz, $^{3}J_{P,H}$ = 9.5 Hz).

11. Honda, S.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 2093-2096.

12. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (12 H, s), 1.40 (8 H, six lines, $J_{H,H}$ = 7.3 Hz), 1.60 (17 H, br m), 1.95 (4 H, br m), 3.32 (8 H, br m), 4.34 (2 H, dd, $J_{H,H}$ = 6.6 Hz, $J_{P,H}$ = 7.5 Hz), 5.05 (1 H, t, $J_{H,H}$ = 6.1 Hz), 5.37 (1 H, t, $J_{H,H}$ = 6.6 Hz), and 6.92 ppm (1 H, d, $J_{P,H}$ = 584 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5 (q), 16.2 (q), 17.4 (q), 19.5 (t), 23.8 (t), 25.4 (t), 26.2 (t), 39.3 (t), 58.5 (t), 59.9 (dt), 123.1 (dd), 123.9 (d), 131.2 (s), and 136.9 ppm (s); ^{31}P NMR (121 MHz, $CDCl_3$) δ 1.2 ppm (dt, $^{1}J_{P,H}$ = 584 Hz, $^{3}J_{P,H}$ = 7.5 Hz).

13. Hata, T.; Sekine, M. *Tetrahedron Lett.* **1974**, *45*, 3943-3946.

14. ^{1}H NMR (300 MHz, D_2O) δ 1.42 (3 H, s), 1.48 (3 H, s), 1.51 (3 H, s), 1.95 (4 H, br m), 4.33 (2 H, dd, $J_{P,H}$ = 6.6 Hz, $J_{H,H}$ = 6.6 Hz), 5.01 (1 H, br m), and 5.22 ppm (1 H, t, $J_{H,H}$ = 6.6 Hz); ^{13}C NMR (75 MHz, D_2O) δ 18.4 (q), 19.8 (q), 27.7 (q), 28.5 (t), 41.7 (t), 64.2 (dt), 123.4 (dd), 126.9 (d), 136.4 (s), and 144.1 ppm (s); ^{31}P NMR (121 MHz, D_2O), δ 45.2 ppm (t, $J_{P,H}$ = 7.6 Hz).

15. Richard, J.P.; Frey, P.A. *J. Am. Chem. Soc.* **1983**, *105*, 6605-6609.

16. Attempts to use the more reactive *bis*- or *tris*-tetra-n-butyl ammonium salts resulted in lower yields.

17. ^{1}H NMR (300 MHz, D_2O) δ 1.44 (3 H, s), 1.50 (3 H, s), 1.54 (3 H, s), 1.95 (4 H, br m), 4.33 (2 H, dd, $J_{P,H}$ = 7.6 Hz, $J_{H,H}$ = 6.8 Hz), 5.00 (1 H, br m), and 5.30 ppm (1 H, t, $J_{H,H}$ = 6.8 Hz); ^{13}C NMR (75 MHz, D_2O) δ 18.4 (q), 19.7 (q), 27.5 (q), 28.2 (t), 41.5 (t), 65.6 (dt), 122.1 (d), 126.7 (d), 136.2 (s), and 145.5 ppm (s); ^{31}P NMR (121 MHz, D_2O) δ P_α 42.5 (dt, $J_{P,P}$ = 29.2 Hz, $J_{P,H}$ = 7.6 Hz), and P_β = -6.8 ppm (d, $J_{P,P}$ = 29.2 Hz).

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