Synthesis of (*S*)-Isoprenoid Thiodiphosphates as Substrates and Inhibitors

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Thiolo thiophosphate analogues of isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP) were synthesized. Inorganic thiopyrophosphate (SPP_i) was prepared from trimethyl phosphate in four steps. The tris(tetra-*n*-butylammonium) salt was then used to convert isopentenyl tosylate to (*S*)-isopentenyl thiodiphosphate (ISPP). (*S*)-Dimethylallyl (DMASPP), (*S*)-geranyl (GSPP), (*S*)-farnesyl (FSPP), and (*S*)-geranylgeranyl thiodiphosphate (GGSPP) were prepared from the corresponding bromides in a similar manner. ISPP and GSPP were substrates for avian farnesyl diphosphate synthase (FPPase). Incubation of the enzyme with ISPP and GPP gave FSPP, whereas incubation with IPP and GSPP gave FPP. GSPP was a substantially less reactive than GPP in the chain elongation reaction and was an excellent competitive inhibitor, $K_I^{GSPP} = 24.8\ \mu M$, of the enzyme. Thus, when ISPP and DMAPP were incubated with FPPase, GSPP accumulated and was only slowly converted to FSPP.

Introduction

Isoprenoid molecules are constructed from simple five-carbon building blocks through a series of fundamental "building reactions" in the central part of the pathway mediated by prenyltransferases. These enzymes catalyze the alkylation of electron-rich functional groups by allylic isoprenoid diphosphates. Some common prenyl transfer reactions include the chain elongation of dimethylallyl diphosphate (DMAPP) to farnesyl diphosphate (FPP)[1] by sequential addition of two molecules of isopentenyl diphosphate (IPP),[2] the joining of two molecules of farnesyl diphosphate (FPP) to give squalene,[3] the post-translational modification of proteins by attachment of farnesyl and geranylgeranyl groups,[4] and the modification of adenosine residues in tRNA with a dimethylallyl moiety.[5] Isoprenoid cyclases catalyze intramolecular versions of prenyl-transfer reactions by electrophilic alkylation of distal double bonds to create a variety of cyclic isoprenoids.[6] The intermediates produced by prenyl transfer and cyclization reactions are ultimately transformed into over 30 000 different metabolites[7] that perform a variety of functions in their respective hosts.

Prenyl transfers are nucleophilic substitution reactions. Mechanistic studies indicate that the reactions are associative for strong nucleophiles, such as the zinc thiolate in protein prenylation, but proceed through a "late" transition state with substantial development of positive charge in the allylic moiety.[8] For weak nucleophiles, such as a carbon−carbon double bond, the reaction appears to occur by a dissociative mechanism.[9] We sought to obtain direct evidence for the dissociative reaction by detecting recombination of the allylic cation and PP_i through positional isotope exchange of oxygen atoms in the pyrophosphate leaving group[10] and by an enzyme-catalyzed thiono → thiolo rearrangement of thiodiphosphate analogues.[11] Thiono → thiolo isomerization was a particularly attractive technique for detecting ion-pair recombination. Model studies showed that (*O*)-dimethylallyl-(*O*,*O*)-dimethyl thiophosphate readily underwent ion-pair return accompanied by both allylic and thiono → thiolo rearrangement during solvolysis[12] and that the allylic thiolo derivatives were ∼10^6-fold less reactive than their thiono counterparts. However, neither positional isotope exchange nor thiono to thiolo rearrangements were detected in these experiments. Recently published X-ray structures for binary complexes of FPPase and allylic diphosphate substrates show that the negatively charged nonbridging oxygen atoms are coordinated to

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(1) Abbreviations used: BHDA, bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid; BME, 2-mercaptoethanol; BSA, bovine serum albumin; DMAPP, dimethylallyl diphosphate; DMASPP, (*S*)-dimethylallyl thiodiphosphate; FPP, farnesyl diphosphate; FPPase, farnesyl diphosphate synthase; FSPP, (*S*)-farnesyl thiodiphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; GGSPP, (*S*)-geranylgeranyl thiodiphosphate; GSPP, (*S*)-geranyl thiodiphosphate; IPP, isopentenyl diphosphate; ISPP, (*S*)-isopentenyl thiodiphosphate; PP_i, inorganic pyrophosphate salt; SPP_i, inorganic thiopyrophosphate salt.

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positively charged groups in the active site.[13] Presumably, these interactions are sufficiently strong to prevent bond rotations in the enzyme-bound pyrophosphate group in the allylic cation−PP_i complex.

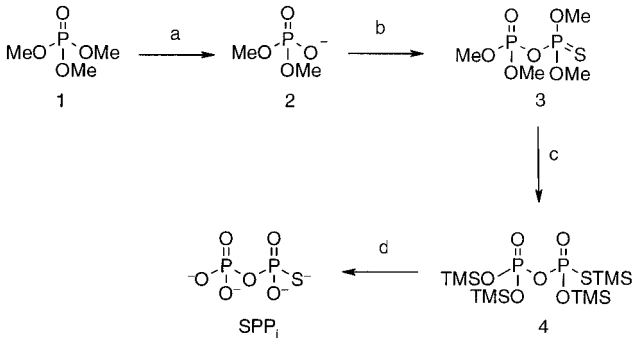
Several groups have synthesized prenyltransferase inhibitors by replacing the diphosphate leaving group with less reactive or unreactive "diphosphate" mimics. These include phosphonophosphates,[14] phosphonophosphinates,[15] and bisphosphonates.[16] Typically, these compounds are modest to poor prenyltransferase inhibitors. Although the structures of the analogues do not precisely duplicate those of the normal substrates, these differences do not appear to adequately account for their typically poor binding properties. Diphosphate esters are trianions at physiological pH. One possible reason for the loss in binding affinity is the increase in the pK_a's of the analogues as the bridging oxygen atoms in the diphosphate group are replaced by less electron-withdrawing carbons.[17] These considerations led us to design a new class of prenyltransferase inhibitors with structures and ionization properties very similar to their normal substrates by replacing the diphosphate oxygen atom attached to the isoprenoid moiety with sulfur.[18]

Results and Discussion

Synthesis of Tris(tetra-*n*-butylammonium) Thiopyrophosphate. We based our syntheses of (*S*)-isopentenyl thiodiphosphate (ISPP), (*S*)-dimethylallyl (DMASPP), (*S*)-geranyl (GSPP), (*S*)-farnesyl (FSPP), and (*S*)-geranylgeranyl thiodiphosphate (GGSPP) on the general procedure for isoprenoid diphosphates developed by Davisson et al.[19] This method uses the tris(tetra-*n*-butylammonium) salt of inorganic pyrophosphate to convert activated isoprenoid compounds to the corresponding diphosphate esters by nucleophilic displacement. We reasoned that the sulfur atom in inorganic thiodiphosphate would be sufficiently more nucleophilic than the oxygen atoms to produce the (*S*)-alkyl thiodiphosphates regioselectivity.

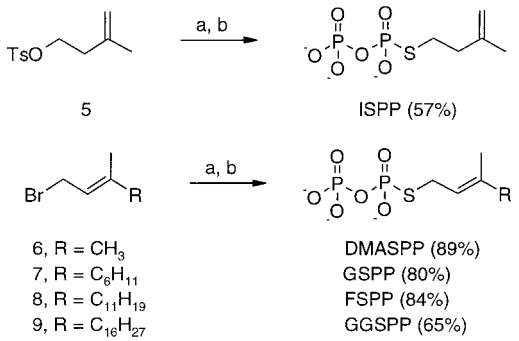
The synthesis of tris(tetra-*n*-butylammonium) thiopyrophosphate (SPP_i) is shown in Scheme 1. Trimethyl phosphate (**1**) was selectively monodemethylated with tetrabutylammonium hydroxide. Monodemethylation was also seen for sodium hydroxide, ammonium hydroxide, and trimethylamine; however, the sodium, ammonium, and tetramethylammonium SPP_i salts were not sufficiently soluble in organic solvents and had to be converted to the tetra-*n*-butylammonium form by ion-exchange chromatography before proceeding. The synthesis of anhydride **3** from methyl phosphate (**2**) and

Scheme 1. Synthesis of Tris(tetra-*n*-butylammonium) Thiopyrophosphate



[a] (a) nBu$_4$NOH; (b) $(MeO)_2P(S)Cl$; (c) TMSI; (d) nBu$_4$NOH/H_2O.

Scheme 2. Synthesis of (*S*)-Alkyl Thiodiphosphates



[a] (a) Tris-(tetra-*n*-butylammonium)thiopyrophosphate, CH_3CN. (b) Dowex AG 50W-X8 (NH_4^+ form).

dimethyl thiophosphorochloridate was touchy. The best yields were achieved when the progress of the reaction was carefully monitored at −35 °C. Tetramethylthiophosphate **3** was unstable upon extended exposure to **2** or dimethyl thiophosphorochloridate. Thus, the cold reaction mixture was passed through a silica column to separate **3** from the other components in the reaction mixture. The anhydride purified in this manner can be stored at −20 °C.

The methyl groups were removed from anhydride **3** by treatment with trimethylsilyl iodide (TMSI) at −35 °C. The mechanism for dealkylation, displacement of iodide from TMSI by the phosphoryl oxygen or thiophosphoryl sulfur followed by a second displacement at the methyl groups by iodide,[20] predicts formation of thiolo-TMS diphosphate **4**. The TMS derivative obtained from the reaction could not be chromatographed and was treated with tetra-*n*-butylammonium hydroxide without purification to give $[(n\text{-Bu})_4N]_3(SPP_i)$. The salt is stable for up to a year when stored as a lyophilized powder at −80 °C. Attempts to remove the methyl groups with trimethylsilyl bromide (TMSBr) or TMSI generated in situ by treatment TMSBr with NaI were not successful.

Synthesis of (*S*)-Alkyl Thiodiphosphates. (*S*)-Alkyl isopentenyl and allylic thiodiphosphates were obtained by the procedure of Davisson et al.[19a] with minor modifications. As illustrated in Scheme 2, ISPP was prepared from isopentenyl tosylate (**5**), while DMASPP, GSPP,

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FSPP, and GGSPP were prepared from the corresponding allylic bromides. Previously, attempts to synthesize IPP from isopentenyl chloride or bromide gave poor yields because of a competing elimination that was not seen when the displacement reaction was conducted with tosylate **5**.[19b] DMASPP, GSPP, and FSPP were synthesized from the corresponding commercially available bromides. GGSPP was prepared from geranylgeranyl bromide[19b] obtained from geranylgeraniol.

Typically, the activated isoprenoid derivatives were slowly added to acetonitrile solutions containing 2−3 equiv of SPP_i cooled to −35 °C. After 30−45 min, the reaction was complete, and the solvent was removed at reduced pressure. Although sulfur is substantially more nucleophilic than oxygen, excess SPP_i was used, and the reaction was run at −30 °C to ensure high regioselectivity for displacement by sulfur. When the order of addition of the reactants was reversed, small amounts diphosphate esters were formed from displacement by oxygen. The residue was dissolved in water and passed through an ion-exchange column replace the tetra-*n*-butylammonium cations with ammonium. This step was taken because the tetrabutylammonium salts streaked when chromatographed on cellulose and exchange of tetrabutylammonium for ammonium facilitated the final purification step by chromatography on cellulose.

The ammonium salts of allylic isoprenoid diphosphates decompose upon prolonged lyophilization because the tris-ammonium forms are converted to less stable di- or mono-ammonium species due to removal of NH_3 under vacuum. Decomposition is less of a problem for the ammonium salts of the corresponding allylic thiolodiphosphates because of the increased kinetic stability of the thiolo linkage. However, we always store all of these materials as lyophilized powders at −80 °C.

The thiolo structures for the thiodiphosphates were confirmed from their NMR spectra. The ^{1}H-decoupled ^{31}P spectra have characteristic AB patterns with doublets ($J \sim 29$ Hz) at approximately 8 and −8 ppm for P_α and P_β, respectively. In ^{1}H-coupled ^{31}P spectra, the doublet at 8 ppm was further split into triplets ($J \sim 8$ Hz) by the C(1) methylene protons. The ^{1}H NMR spectra were also consistent with the thiolo structure with the bridging sulfur attached to C(1) of the isoprenoid moiety. The C(1) protons in allylic thiodiphosphates DMASPP, GSPP, FSPP, and GGSPP appear as triplets at ∼3.5 ppm as a result of similar coupling constants to the proton at C(2) and P_α and as a doublet of triplets at 3.0 ppm in ISPP. The C(1) methylene protons appear at ∼4.5 ppm in the normal allylic diphosphate esters and at ∼4.0 ppm in IPP.

Studies with Farnesyl Diphosphate Synthase. We conducted a series of experiments with recombinant avian FPPase to study the effects of replacing the bridging oxygen atom in the normal substrates for chain elongation. Avian FPPase was incubated with GSPP in 2-fold molar excess over [^{14}C]IPP for 20 h at 37 °C. A control reaction was conducted at the same time where GSPP was replaced by GPP in a 10-min incubation. A 10 μL portion of each sample was spotted on a silica gel TLC plate, the plate was developed with $CHCl_3$/methanol/water/acetic acid, and the radioactive materials were visualized by phosphorimaging. The incubation of GSPP and [^{14}C]IPP gave single new spot whose mobility was similar to FPP formed in the control reaction. All of the [^{14}C]IPP was consumed in the 10-minute incubation with GPP, while only 4% of the radioactivity comigrated with FPP when GSPP was the allylic substrate (see Figure 1).

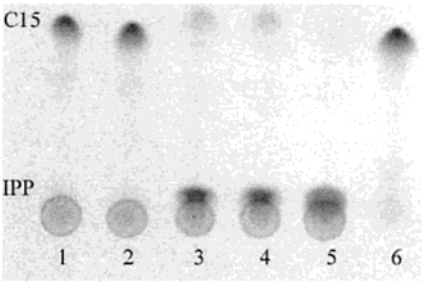

Figure 1. Products from incubation of avian FPPase and [^{14}C]-IPP with GSPP or GPP: lanes 1 and 2, incubations with GPP; lanes 3 and 4, incubations with GSPP; lane 5, [^{14}C]IPP; lane 6, [^{14}C]FPP.

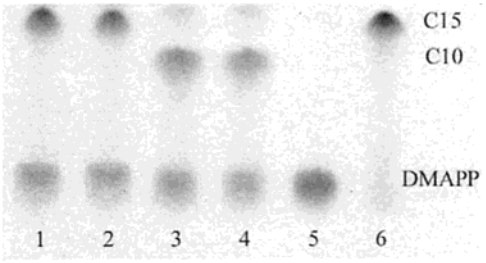

Figure 2. Products from incubation of avian FPPase and [^{14}C]-DMAPP with IPP or ISPP: lanes 1 and 2, incubations with IPP; lanes 3 and 4, incubations with ISPP; lane 5, [^{14}C]DMPP; lane 6, [^{14}C]FPP.

In the normal chain elongation reaction catalyzed by FPPase, DMAPP condenses with IPP to form GPP, and the newly formed allylic product is then the substrate for a second condensation with IPP to give FPP. The enzyme-catalyzed condensation is an electrophilic alkylation of the double bond in IPP by the allylic carbocation generated from the allylic substrate.[9] Thus, one would anticipate that ISPP would be a more efficient substrate for FPPase than GSPP. This assumption was verified by the results shown in Figure 2, where FPPase was incubated with [^{14}C] DMAPP and a 6-fold excess of ISPP at 37 °C for 20 h. A control experiment was run in parallel under similar conditions except that IPP replaced ISPP and the incubation time was 10 min. As described above for GSPP, a portion of each sample was co-spotted with cold GPP and analyzed by TLC. When ISPP was the homoallylic substrate, new spots were seen at R_f = 0.23 and 0.16, corresponding to FSPP (8%) and GSPP (92%), respectively, along with a residual spot at the origin that is presumably an impurity in the [^{14}C]-DMAPP. As anticipated, in the incubation with IPP, [^{14}C]-DMAPP was converted to FPP within 10 min.

The identity of the products was verified by HPLC−MS. Negative-ion HPLC of a reaction mixture from an incubation of FPPase with ISPP and DMAPP on a Nucleodex β-OH column gave peaks with retention times of 8.26 (*m*/*z* 245, M − H^+ for DMAPP), 9.53 (*m*/*z* 261, M − H^+ for ISPP), and 11.56 min (*m*/*z* 329, M − H^+ for GSPP). Analysis of the products from a similar incubation with IPP and DMAPP gave a small peak at 11.39 (*m*/*z* 313, M − H^+) for GPP and a large peak at 14.13 min (*m*/*z* 381, M − H^+) for FPP. These results confirm that ISPP condenses with DMAPP in the first round of chain

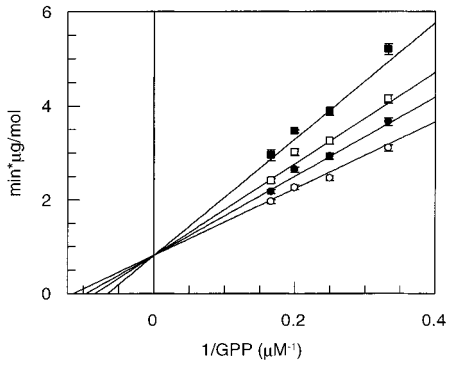

Figure 3. Double-reciprocal plot of v^{-1} versus $[GPP]^{-1}$ at different fixed concentrations of GSPP: [GSPP] = 0 (○), [GSPP] = 5 μM (●), [GSPP] = 10 μM (□), and [GSPP] = 20 μM (■).

elongation, but the product of the reaction, GSPP, is a poor substrate for the second round.

Kinetic Studies with GSPP. In a preliminary communication of this work, we reported that GSPP reacts much more slowly with IPP than does GPP.[18] This experiment was performed by the incubation of [^{14}C]IPP and GSPP or GPP with recombinant FPPase, and the initial rate of the reactions were determined by the standard acid lability assay.[21] Under conditions where the condensation of [^{14}C]IPP and GPP gave a predictable amount of product, no detectable formation of product was seen for [^{14}C]IPP and GSPP. When the concentration of FPPase was increased 100-fold and the time of the incubation was lengthened 10-fold, only slight amount of product was observed. When compared to the normal assay conditions for this enzyme, the reaction of IPP with GSPP is estimated to be 10^5 times slower than for GPP.

Additional kinetic measurements revealed that GSPP was an efficient competitive inhibitor of FPPase with $K_I^{GSPP} = 24.8$ μM, as illustrated by the double-reciprocal plot of initial velocity versus $[GPP]^{-1}$ at different fixed concentrations of GSPP (see Figure 3), in comparison, $K_{Mapp}^{GPP} = 4.5$ μM under similar conditions.[22] The steady-state kinetic measurements show that GSPP binds to the allylic site in FPPase with excellent selectivity and affinity.

Conclusion

In summary, the thiolo isomers of isoprenoid thiodiphosphate esters were synthesized by treating allylic bromides and homoallylic tosylates with tris(tetra-*n*-butylammonium)thiopyrophosphate. The thiolo analogue of geranyl diphosphate was substrate for condensation with IPP catalyzed by FPPase; however, GSPP was ~10^5-fold less reactive than GPP itself. ISPP was also a substrate for the chain elongation reaction. Condensation with DMAPP produced GSPP, which accumulated because of its poor reactivity. The direct displacement protocol using the tris(tetra-*n*-butylammonium) salt of SPP_i should be generally applicable to a variety of thiodiphosphate analogues where the center undergoing substitution is not sterically hindered.

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Experimental Section

General Methods. All experiments were performed under an inert atmosphere unless otherwise indicated. NMR spectra were collected at 23 °C. ^{1}H chemical shifts were referenced to internal $CDCl_3$ at 7.26 or D_2O at 4.8. Coupling constants are reported in hertz. ^{13}C NMR chemical shift was referenced to internal $CDCl_3$ at 77.23. ^{13}C NMR spectra for $[(n\text{-}Bu)_4N]_3SPP_i$ and the isoprenoid thiodiphosphates were referenced to external methanol (10%) at 49.15. ^{31}P NMR spectra were referenced to external phosphoric acid (5%) in D_2O. A DB-5 capillary column was used for GC−MS. FAB, CI, or EI mass spectroscopy were used to obtain high-resolution mass spectra. Analytical HPLC separations were performed with a 4 × 200 mm Nucleodex β-OH column from Machery-Nagel Inc.[23] Radioactivity was measured in Cytoscint scintillation cocktail.

Materials. THF was freshly distilled over Na and benzophenone. Acetonitrile and CH_2Cl_2 were freshly distilled over CaH_2. All other solvents were HPLC grade and used without further purification. [C^{14}-IPP] was purchased from Amersham. [C^{14}-DMAPP] and [C^{14}-FPP] were from American Radiolabeled Chemicals, Inc. BME was from Fisher. All other chemical reagents were purchased from Aldrich. Tris(tetra-*n*-butylammonium) salts of PP_i, isopentenyl tosylate, farnesyl bromide, IPP, DMAPP, and GPP were prepared as described by Davisson et al.[19] TsCl was recrystallized from $CHCl_3$/hexanes and dried under vacuum for 24 h. Isopentenyl alcohol was distilled under nitrogen prior to use. BHDA was prepared from the corresponding anhydride (Fluka).[24] Silica flash chromatography was performed with Merck silica gel (200−245 mesh). Thin-layer chromatography was on Merck silica gel 60 Å F_{254} TLC plates. Silica gel was visualized with phosphomolybdic acid. Cellulose for flash chromatography was purchased from Whatman and used according to method of Woodside.[25] Cellulose TLC plates were developed with a sulfosalicylic acid−ferric chloride spray.[19a]

Tetra-*n*-butylammonium Dimethyl Phosphate (2). To 6 mL of neat trimethyl phosphate (51.3 mmol) was added 33.6 mL (51.3 mmol) of 40% (w/w) tetra-*n*-butylammonium hydroxide. The mixture was heated at reflux at 100 °C for 24 h. Methanol was removed by rotary evaporation, and the residue was lyophilized for 24 h. Traces of water were removed by the addition of benzene, followed by its removal at reduced pressure, to give 18 g (95%) of a white solid: ^{1}H NMR (D_2O) 1.02 (12H, t, $J_{H,H} = 7.6$), 1.44 (8H, m), 1.74 (8H, m), 3.25 (8H, t, $J_{H,H} = 8.6$), 3.54 (6H, d, $J_{H,P} = 10.8$); ^{13}C NMR (D_2O) 12.73 (s), 18.93 (s), 22.88 (s), 52 (d, $J_{C,P} = 6$), 57.78 (s); ^{31}P NMR (D_2O) 2.83 (s); HRMS (FAB^-) $[M - 1]^-$ calcd for $C_2H_7PO_4$ 125.00090, found 125.00037.

Tetramethyl Thiodiphosphate (3). Tetra-*n*-butylammonium dimethyl phosphate (**2**) (9.8 g, 26.7 mmol) was dissolved in 7 mL of acetonitrile. The mixture was cooled to −35 °C before dropwise addition of 3 mL of dimethyl chlorothiophosphate (24.7 mmol). The mixture was allowed to stir at −35 °C for 30 min, immediately loaded onto a silica gel column (250 mL), and eluted with CH_2Cl_2. Solvent was removed in vacuo to give 1.85 g (30%) of a yellow oil: ^{1}H NMR ($CDCl_3$) 3.86 (d, 6 H, $J_{H,P} = 0.73$), 3.9 (d, 6H, $J_{H,P} = 0.9$); ^{13}C NMR ($CDCl_3$) δ 55.1 (d, $J_{C,P} = 6$), δ 55.4 (d, $J_{C,P} = 5.5$); ^{31}P NMR ($CDCl_3$) δ −13.8 (d, $J_{P,P} = 20$, P(O)), δ 55.6 (d, $J_{P,P} = 20$, P(S)); HRMS (EI) $[M]^+$ calcd for $C_{10}H_{12}SP_2O_6$ 249.9830, found 249.9841.

Tris(tetra-*n*-butylammonium)thiodiphosphate ([(*n*-Bu)$_4$N]$_3$SPP$_i$). Iodotrimethylsilane (3.41 g, 33.84 mmol) was added dropwise to 1.5 g of neat tetramethyl thiodiphosphate (6 mmol) at −35 °C, and the mixture was slowly allowed to warm to room temperature over 6 h. The solvent was removed in vacuo to give tris(trimethylsilane) thiodiphosphate (**4**) as a brown semisolid: ^{31}P NMR (CD_3CN in 1 M EDTA) −32.64 (d,

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$J_{P,P}$ = 16.5, P(O)), 29.94 (d, $J_{P,P}$ = 16.5, P(S)). TMS derivative **4** was dissolved in 20 mL of acetonitrile. The solution was titrated with 40% (w/w) tetra-*n*-butylammonium hydroxide to pH 7.2 and acetonitrile was then removed at reduce pressure. Benzene (10 mL) was added to give a heterogeneous mixture composed of three layers. The bottom layer was separated, concentrated in vacuo, and lyophilized to give 5.36 g (96%) of a yellow gelatinous residue, which was used directly in next step: ^{31}P NMR ($CDCl_3$), −9.2 (d, $J_{P,P}$ = 30, P(O)), 36.8 (d, $J_{P,P}$ = 30, P(S)); ^{1}H NMR ($CDCl_3$), 1.02 (12H, t, $J_{H,H}$ = 7.1), 1.44 (8H, m), 1.74 (8H, m), 3.25 (8H, t, $J_{H,H}$ = 8.2); HRMS (FAB^-) $[M - 1]^-$ calcd for $H_4SP_2O_6$ 192.91256, found 192.91181.

General Procedure for Preparation of Thiodiphosphates. Two equivalents of tris(tetra-*n*-butylamonium)thiodiphosphate in acetonitrile was placed in a dried flask, and the solution was cooled to −35 °C. To the cold, well-stirred solution was slowly added 1 equiv of allylic bromide or homoallylic tosylate. The mixture was allowed to stir for 30−45 min at −35 °C. The solvent was then removed at reduced pressure to give an opaque semisolid residue. This residue was dissolved in a minimum amount of water and passed through a column of 40−60 equiv of DOWEX AG 50W-X8 (100−200 mesh) cation-exchange resin (NH_4^+ form). The column was eluted with two volumes of ion-exchange buffer (25 mM NH_4HCO_3 and 2% (v/v) of 2-propanol in water), and the eluent was lyophilized to give a light yellow powder. The resulting powder was dissolved in a limited amount of water and purified by chromatography on cellulose with elution by 50 mM ammonium bicarbonate in 1:2:1 (v/v/v) acetonitrile/2-propanol/water. The desired fractions were pooled, concentrated by rotatory evaporation, and lyophilized to give a yellowish powder that was then stored at −80 °C.

(*S*)-3-Methyl-3-butene-1yl Thiodiphosphate (ISPP). Isopentenyl tosylate (0.11 g, 0.45 mmol) was treated with tris-(tetra-*n*-butylamonium) thiodiphosphate (0.97 g, 1 mmol) in acetonitrile (5 mL) for 45 min. The resulting tetrabutylamonium salt was converted to ammonium form with 50 equiv of resin. After the lyophilization, the resulting powder was dissolved in 1 mL of water. The mixture was passing through the flash cellulose column to yield 0.126 g (57%) of white-yellowish solid: R_f 0.33; ^{1}H NMR (D_2O) 4.85 (d, 2H, $J_{H,H}$ = 5 Hz), 3 (m,2H), 2.46 (dt, 2H, $J_{H,H}$ = 7 Hz), 1.77 (s, 3H); ^{13}C NMR (D_2O) 21.6 (s), 28.4 (d), 38.3 (d), 111.3 (s), 145.8 (s); ^{31}P NMR (D_2O) 7 (d, PS, $J_{P,P}$ = 29 Hz), −7.9 (d, $J_{P,P}$ = 29 Hz); HRMS (FAB^-) $[M - 1]^-$ calcd for $C_5H_{11}SP_2O_6$ 260.97516, found 260.97402.

(*S*)-3-Methyl-2-buten-1-yl Thiodiphosphate (DMASPP). Dimethylallyl bromide (0.3 g, 2 mmol) was added dropwise to a cold solution of tris(tetra-*n*-butylamonium) thiodiphosphate (3.64 g, 3.91 mmol) in 15 mL of acetonitrile,. The mixture was allowed to stir for 30 min. After the workup, the resulting tetrabutylamonium salt was converted to the ammonium form with 40 equiv of resin and lyophilized. The resulting powder was dissolved in a limited amount of elution buffer and chromatographed on cellulose with a flow rate 2 mL/min to yield 0.5 g (89%) of white-yellowish solid: R_f 0.3; ^{1}H NMR (D_2O) 5.3 (t, 1H, $J_{H,H}$ = 7.8 Hz), 3.5 (t, 2H, $J_{H,H}$ = 8.8 Hz), 1.8 (d, 6H, $J_{H,H}$ = 8 Hz); ^{13}C NMR (D_2O) 17.3 (s), 25.1 (s), 28.3 (s), 120.3 (d), 137.8 (s); ^{31}P NMR (D_2O) 8.6 (d, PS, $J_{P,P}$= 28 Hz), −9.0 (d, $J_{P,P}$ = 28 Hz); HRMS (FAB^-) $[M - 1]^-$ $C_5H_{11}SP_2O_6$ 260.9755, found 260.9740.

(*S*)-(*E*)-3,7-Dimethyl-2,6-octadien-1-yl Thiodiphosphate (GSPP). Geranyl bromide (0.438 g, 2 mmol) was added dropwise to a cold solution of tris(tetra-*n*-butylamonium) thiodiphosphate (4.30 g, 4.62 mmol) in 20 mL of acetonitrile. The mixture was allowed to stir for 30 min. After the workup, the resulting tetrabutylamonium salt was converted to ammonium form with 40 equiv of resin and lyophilized. The residual powder was dissolved in a limited amount of elution buffer and chromatographed on cellulose with a flow rate 2 mL/min to yield 0.61 g (80%) of white-yellowish solid: R_f 0.38; ^{1}H NMR (D_2O) 5.4 (t, 1H, $J_{H,H}$ = 9 Hz), 5.15 (b, 1H), 3.5 (t, 2H, $J_{H,P}$ = 8.79 Hz), 2.1 (m, 4H), 1.7 (d, 6H, $J_{H,H}$ = 5.13 Hz), 1.6 (s, 3H); ^{13}C NMR (D_2O) 15.7 (s), 17.5 (s), 25.3 (s), 26.1(s), 28.3 (s), 39.2 (s), 120.3 (d), 124.5 (s), 133.6 (s), 141.0 (s); ^{31}P NMR (D_2O) 8.0 (d, P(S), $J_{P,P}$ = 29 Hz), −10.5 (d, P(O), $J_{P,P}$ = 29 Hz); HRMS (FAB^-) $[M - 1]^-$ calcd for $C_5H_{11}SP_2O_6$ 329.03776, found 329.03612.

(*S*)-(*E*,*E*)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-yl Thiodiphosphate (FSPP). Farnesyl bromide (0.347 g, 1.22 mmol) was added dropwise to a cold solution of tris(tetra-*n*-butylamonium) thiodiphosphate (2.5 g, 2.68 mmol) in 15 mL of acetonitrile. The reaction mixture was allowed to stir for 30 min. After the workup, the resulting tetrabutylamonium salt was converted to ammonium form with 55 equiv of resin and lyophilized. The resulting powder was dissolved in a limited amount of elution buffer and chromatographed on cellulose with a flow rate 2 mL/min to yield 0.46 g (84%) white-yellowish solid: R_f 0.4; ^{1}H NMR (D_2O) 5.4 (t, 1H, $J_{H,H}$ = 7.69 Hz), 5.2 (b, 2H), 3.5 (t,2H, $J_{H,P}$ = 8.3 Hz), 2.1 (m, 8H), δ1.7 (d, 6H, $J_{H,H}$ = 6.95 Hz), 1.6 (s, 6H); ^{13}C NMR (D_2O) 16.0 (s), 17.7 (s), 25.7 (s), 26.6 (s), 26.8 (s), 28.3 (s), 39.8 (d), 120.2 (d), 124.5 (s), 124.9 (s), 132.0 (s), 135.9 (s), 140.9 (s); ^{31}P NMR (D_2O) 7.6 (d, $J_{P,P}$ = 29.31 Hz); −7.4 (d, $J_{P,P}$ = 29.31 Hz); HRMS (FAB^-) $[M - 1]^-$ calcd for $C_{15}H_{27}SP_2O_6$ 397.10036, found 397.10170.

(*S*)-(*E*,*E*,*E*)-3,7,11,15-Tetramethylhexadeca 2,6,10,14,-tetraen-1-yl Thiodiphosphate (GGSPP). Geranylgeranyl bromide (0.34 g, 0.91 mmol) was added dropwise to a cold solution of tris(tetra-*n*-butylamonium)thiodiphosphate (1.56 g, 1.71 mmol) in 5 mL of acetonitrile. The mixture was allowed to stir for 30 min. After the workup, the resulting tetrabutylamonium salt was converted to ammonium form with 60 equiv of resin and lyophilized. The resulting powder was dissolved in a limited amount of elution buffer and chromatographed on cellulose with a flow rate 2 mL/min to yield 0.32 g (65%) of white-yellowish solid: R_f 0.47; ^{1}H NMR (D_2O) 5.4 (t, 1H, $J_{H,H}$ = 8.3 Hz), 5.2 (b, 3H), 3.5 (t,2H, $J_{H,P}$ = 8.79 Hz), 2.1 (m, 12H), 1.7 (d, 6H, $J_{H,H}$ = 12.7 Hz), 1.6 (d, 9H, $J_{H,H}$ = 5.3 Hz); ^{13}C NMR (D_2O) 15.8 (s), 17.5 (s), 23.5 (s), 25.5 (s), 26.7 (s), 27.9 (s), 31.6 (s), 39.7 (s), 124.4 (d), 124.6 (s), 125.3 (s), 131.0 (s), 133. 8 (s), 134.8 (s), 135.7 (s), 139.7 (s); ^{31}P NMR (D_2O) 7.6 (d, PS, $J_{P,P}$ = 27.5 Hz), −7.1 (d, $J_{P,P}$ = 28.8 Hz); high resolution (FAB^-) mass spectrum $[M - 1]^-$ calcd for $C_{20}H_{35}SP_2O_6$ 465.16296, found 465.16190.

Kinetic Studies with FPPase. The acid lability assay was used to measure initial velocities of coupling reaction of [^{14}C]-IPP with GPP or GSPP.[21] Each standard assay contained 70 ng of purified enzyme, 20 μM [^{14}C]IPP (10 μCi/μmol), 50 μM GPP or GSPP, with a buffer solution containing 40 mM BHDA at pH 7.3, 20 mM BME, 2 mM $MgCl_2$, 2 mg/mL BSA in a total volume of 200 μL. The assay mixture was incubated at 37 °C for 10 min. The assays were quenched with 200 μL of methanol/HCl (4:1, v/v), incubated for an addition of 10 min, and extracted with 1 mL of ligroine. The radioactivity in a 0.5 mL sample of the organic layer was measured by liquid scintillation spectrometry. The IC_{50}^{GSPP} was measured with 4.5 μM GPP, 10 μM [^{14}C]IPP (10 μCi/μmol), and varied concentrations of the thiodiphosphate (0.5, 1, 5, 10, 50, 100 μM). K_I^{GSPP} was measured with 10 μM [^{14}C]IPP (10 μCi/μmol) and varied concentrations of GPP (3, 4, 5, 6 μM) and GSPP (0, 5, 10, 20 μM).

Product Studies. Incubations with GSPP were in a total volume of 50 μL, which included 25 μL of buffer solution (containing 40 mM BHDA at pH 7.3, 20 mM BME, 2 mM $MgCl_2$, 2 mg/mL BSA), 3 μL of 1mM GSPP (50 μM) or 3 μL of 1mM GPP (50 μM), 1 μL of 1mM [^{14}C]IPP (20 μM, 55 μCi/μmol), 14.5 μL of water and 7 μL of FPPase (6.87 mg/mL, 20 μM). Similar conditions were used for ISPP and included 25 μL of buffer, 6 μL of 1mM ISPP (120 μM) or 6 μL of 1mM IPP (120 μM), 1 μL of 1mM [^{14}C]DMAPP (20 μM, 55 μCi/μmol), 11.5 μL of water and 7 μL of FPPase (6.87 mg/mL, 20 μM). Samples containing GSPP and ISPP were incubated at 37 °C for 20 h, while those containing GPP and IPP were incubated at 37 °C for 10 min. A 10 μL portion of each sample was spotted on a silica gel 60 F_{254} TLC plate and developed with 125:75:20:10 (v/v/v/v) $CHCl_3$/methanol/water/acetic acid. The plates were wrapped with plastic wrap and imaged for 20 h.

HPLC−MS for Products from ISPP. A mixture of 32 μL of 500 mM BHDA buffer, 5.5 μL of 72 mM $MgCl_2$, 40 μL of 10 mg/mL BSA, 2.8 μL of 1.4 M BME, 56 μL water, 6.5 μL of

174.89 mM ISPP (2.98 mmol), 17 μL of 153 mM DMAPP (0.99 mmol), and 40 μL of FPPase (24.9 mg/mL, 30 nmol) was placed in a 1.5 mL Eppendorf tube. A control reaction with IPP was run in parallel. The two mixtures were incubated at 37 °C for 20 h. The solutions were placed in the 10 μm centrifuge filters, and centrifuged at 10 000 rpm for 20 min. The filters were rinsed with 100 μL of 25 mM ammonium bicarbonate solution, and the combined filtrates were lyophilized. The residues were dissolved with 200 μL of 1:1 (v/v) water/acetonitrile, and a 5 μL portion was analyzed by HPLC−MS.

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Supporting Information Available: ^{1}H and ^{31}P NMR spectra for obtained compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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