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Phosphonate and Bisphosphonate Analogues of Farnesyl Pyrophosphate as Potential Inhibitors of Farnesyl Protein Transferase

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Abstract—Several phosphonate and bisphosphonate analogues of farnesyl pyrophosphate have been prepared for an examination of their ability to inhibit farnesyl protein transferase (FPTase). A Horner–Wadsworth–Emmons condensation of farnesal or geranial with tetraethyl methylenediphosphonate gave the desired vinyl phosphonates, while alkylation of the dimethyl methylphosphonate anion with a terpenoid bromide gave the corresponding saturated phosphonates. Alkylation of tetraethyl methylenediphosphonate with farnesyl bromide gave the expected alkyl bisphosphonate, which was converted to its α,β-unsaturated derivative by preparation of the phenyl selenide, oxidation to the selenoxide, and elimination. In a similar fashion, triethyl phosphonoacetate was converted to a farnesyl pyrophosphate analogue by reaction with farnesyl bromide. After preparation of the respective acids, each compound was tested for inhibition of FPTase at concentrations ranging up to 10 μM. The effect of these compounds on FPTase activity varied substantially, ranging from depressed to surprisingly enhanced enzymatic activity.

Introduction

The Ras proteins are relatively small guanine nucleotide binding proteins, that play an important role in a signaling pathway that controls cell proliferation.[1] Ras proteins bound to GTP convey a signal that results in growth promotion, while those that are GDP-bound reflect an inactive state.[2] Normal, wild-type Ras is capable of a GTPase activity that converts bound GTP to GDP and thereby terminates the signal for cell growth. However, mutated forms of Ras that lack GTPase activity signal for continuous cell proliferation. In fact, point mutations in the *Ras* genes can result in the loss of catalytic activity in the Ras proteins, and are among the most frequently identified mutations in human cancers.[3]

To function in this signal transduction pathway Ras proteins must become membrane bound, and binding to the cell membrane is facilitated by a sequence of post translational reactions.[1,4] The paramount step in this sequence is the alkylation of a cysteine residue near the Ras C-terminal through reaction of the sulfhydryl group with farnesyl pyrophosphate catalyzed by farnesyl protein transferase (FPTase). A number of research efforts have recognized that inhibition of this enzyme would impair membrane localization of Ras proteins, and might lead to new types of antiproliferative agents. Most of these efforts have focused on preparation of peptides or peptidomimetics that imitate the C-terminal RAS sequence,[5] bisubstrates that resemble both the peptide and farnesyl pyrophosphate,[6] or natural products isolated by screening.[7] Relatively few studies have considered farnesyl pyrophosphate analogues as potential inhibitors of this enzyme.[8]

Our interest in phosphonate chemistry[9] led us to consider the possibility that unexplored phosphonates

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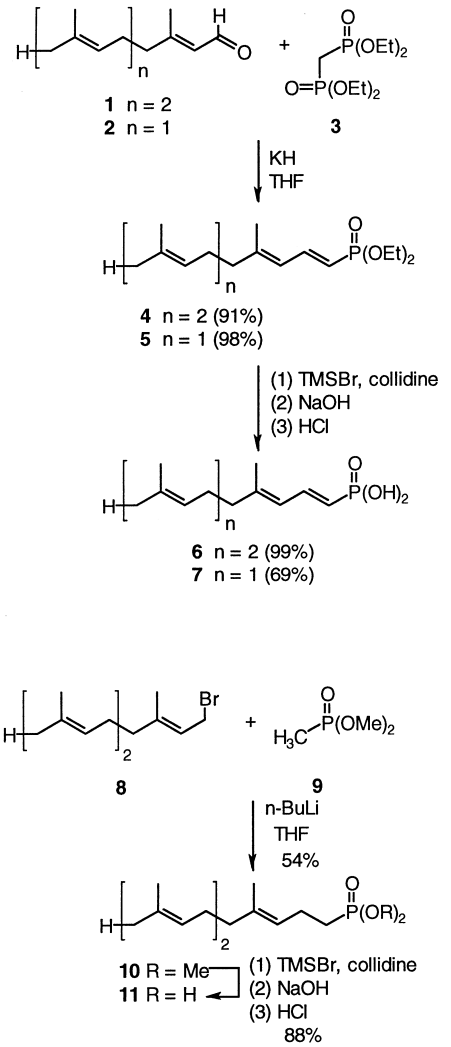
and bisphosphonates analogous to farnesyl pyrophosphate might inhibit this enzyme. To allow a systematic examination of this hypothesis, we have prepared several terpenoid phosphonates of different chain lengths, degrees of unsaturation, and bearing one or two phosphonate moieties. In this report, we present the synthesis of these compounds, along with our assays of their impact on the biological activity of FPTase.

Chemical Synthesis

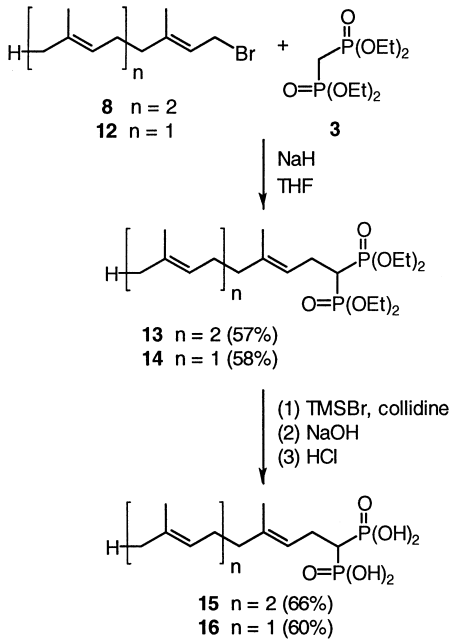
The first compounds targeted, vinyl phosphonates derived from farnesal (**1**) and geranial (**2**), were readily prepared[10] by condensation of the respective aldehydes with the commercial bisphosphonate **3**. Treatment of farnesal with compound **3** and KH in THF gave the expected vinyl phosphonate **4**[11] in virtually quantitative yield, and geranial reacted in a similar fashion to afford the vinyl phosphonate **5**. The resulting phosphonate esters were converted to the corresponding phosphonic acids (**6** and **7**) by standard treatment with TMSBr and subsequent aqueous hydrolysis.[12] To allow evaluation of the importance of the C1–C2 double bond on the biological activity of these compounds, the saturated analogues were also prepared. This was readily accomplished by reaction of farnesyl bromide (**8**) with the dimethyl methylphosphonate (**9**) anion to obtain the ester **10**,[13] and subsequent hydrolysis under standard conditions to obtain the phosphonic acid **11** (Scheme 1).



Scheme 1.

Both the vinyl phosphonic acids **6** and **7** incorporate a single phosphorus atom and would exist at most as dianions at physiological pH, while farnesyl pyrophosphate contains two phosphorus atoms and can hold a higher negative charge. To obtain an analogue more strongly parallel to farnesyl pyrophosphate, addition of a second phosphorus to the C-1 position of vinyl phosphonate **6** was desirable. Vinyl bisphosphonates have been prepared by a Knoevenagel-like condensation with methylene bisphosphonates[14] and condensation of triethyl phosphonoacetate with citronellal has been reported.[15] However, attempted Knoevenagel condensation of geranial with bisphosphonate **3** under parallel reaction conditions gave only the elimination product **5**, necessitating a longer synthetic sequence for preparation of the vinyl bisphosphonates. To initiate that sequence, the corresponding α,β-saturated analogues were prepared by alkylation of bisphosphonate **3** with either farnesyl or geranyl bromide. For example, reaction of compound **3** with NaH and farnesylbromide (**8**) according to a literature procedure gave the mono-alkylated product **13**[16] in 57% yield, accompanied by lesser amounts of unalkylated and dialkylated material. Comparable results were observed with geranyl bromide (**12**), yielding the bisphosphonate **14**. For our studies on their biological activity, each of the bisphosphonates **13** and **14** was converted to the corresponding phosphonic acid (**15**[16] and **16**) under standard conditions (Scheme 2).

To convert the saturated bisphosphonate **13** into its α,β-unsaturated analogue, an extension of methodology[10] used to prepare vinyl phosphonates was explored. After compound **10** was treated with NaH, the resulting anion reacted cleanly with phenyl selenyl bromide. The intermediate phenyl selenide was not isolated, but instead treated immediately with H_2O_2 to bring about oxidation to the selenoxide. This compound undergoes rapid elimination to afford the desired product **17** in an overall yield of 50% following purification by column chromatography. Use of larger amounts of peroxide resulted in more complex reaction mixtures presumably resulting from competing olefin oxidations. Our efforts to

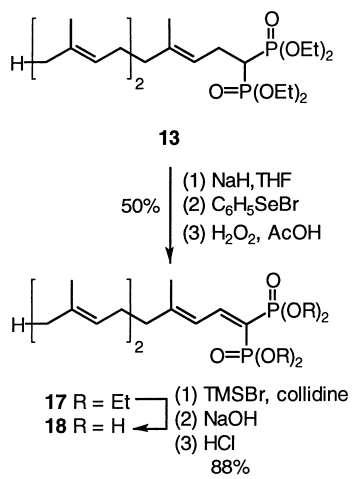


Scheme 2.

apply a parallel reaction sequence to preparation of the geranyl derivative also were frustrated by competing olefin oxidations, which may not be surprising given that oxidation of benzenseleninic acid (formed upon elimination) to the known epoxidation reagent benzeneperoxyseleninic acid has been proposed.[17] Conversion of compound **17** to the bisphosphonic acid **18** was straightforward under standard reaction conditions (Scheme 3).

The final target compounds, the ester-phosphono ester **20** and its various hydrolysis products, were designed to allow variation of the negative charge at the terpenoid head. The parent triester was prepared by alkylation of triethyl phosphonoacetate (**19**) with farnesyl bromide. Selective cleavage of the carboxylate ester could be accomplished by reaction of triester **20** with ethanolic NaOH, followed by acidification of the reaction mixture. The resulting carboxylic acid phosphonate (**21**) undergoes cleavage to phosphonic acid (**22**) upon reaction with TMSBr. The final member of this family, compound **23**, was obtained as its disodium salt by selective hydrolysis of the phosphonate esters with TMSBr in the presence of the carboxylate ester (Schemes 4 and 5).

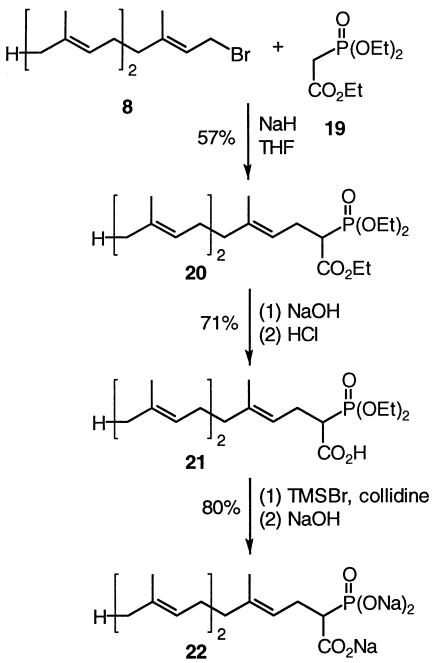
In summary, the desired phosphonic acids and bisphosphonates are readily available via reasonably short reaction sequences. Extension of the selenoxide elimination to preparation of vinyl bisphosphonate **17**, a substrate containing other reactive olefins, suggests that parallel reaction sequences may be viable routes for preparation of still higher terpenoids or other polyenes.



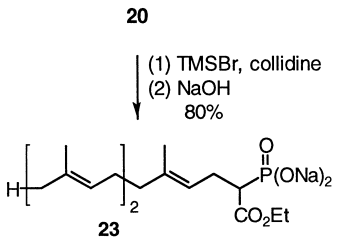
Scheme 3.

Farnesyl Protein Transferase Inhibition

The phosphonic acids and bisphosphonates described above were tested for their ability to inhibit FPTase using partially purified enzyme from bovine brain[18,19] in an assay modified[20] from the method of Harwood.[21] Compounds were assayed at concentrations ranging up



Scheme 4.



Scheme 5.

to 10 μM. These results, expressed as the FPTase activity in the presence of the compound divided by the FPTase activity in the absence of the compound (i.e., fraction of control), are shown in Table 1.

Bisphosphonate **15** is the most active FPTase inhibitor with slightly less activity displayed by vinyl bisphosphonate **18** and phosphonate **6**. The phosphonate analogue **11** of compound **15** is a weak inhibitor. Therefore the most active inhibitor in this group of compounds is the farnesyl bisphosphonate that lacks the C-1 double bond. The geranyl bisphosphonate **16** slightly enhances FPTase activity, suggesting that longer chain length is important for FPTase inhibition in this series. However, the geranyl-length vinyl phosphonate **7** is a weak inhibitor of FPTase suggesting that the structure–activity relationships differ for the C-10 and C-15 compounds. Finally, it is of interest that the most active inhibitor of this collection is compound **15** (IC_{50} of 0.85 μM) which has been described as a potent inhibitor of squalene synthase.[8c]

An alternate classification of the phosphonate analogues is related to their potential for anion formation. The most active inhibitor **15** has the potential for four negative charges if all phosphonic acid positions were fully ionized at physiological pH, whereas a less active inhibitor, compound **22**, has at most three negative charges. Compound **23** has the potential for two negative charges and is a weak inhibitor. Compound **21** is the least acidic of these compounds, with the potential for only one negative charge. Nevertheless, the finding that compound **21** substantially enhances FPTase activity is unexpected. The mechanism for enhancement of FPTase activity by compounds **16** and **21** is as yet unknown, but they are the only compounds thus far described that possess this property.

Table 1. Effect of terpenoid phosphonates on farnesyl protein transferase

Compound	FPTase activity (fraction of control)[a] 1 μM	10 μM
Farnesyl length compounds		
6	0.56	ND[b]
11	0.87	ND
15	0.46	0.18
18	0.53	0.20
21	2.0	1.8
22	0.65	ND
23	0.75	0.85
Geranyl length compounds		
7	0.87	0.59
16	1.2	1.4

[a]Fraction of control = (activity with compound/activity without compound).
[b]ND = no assay at this concentration.

In summary, the phosphonic acids and bisphosphonic acids described here show varied effects on FPTase, ranging from the inhibition observed with compound **15** to the increased enzymatic activity observed with compound **21**. A detailed molecular rational for this variation in activity is not yet possible. However a diffraction analysis of FPTase appeared recently,[22] and in time it may be possible to gain understanding of the interactions of these different phosphonates with FPTase through molecular modeling. In the meantime, the synthesis and bioassay of varied terpenoid phosphonates provides necessary reference points for that modeling, and may uncover new analogues with more potent activities.

Experimental

Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone while CH_2Cl_2 was freshly distilled from calcium hydride. All nonaqueous reactions were conducted in oven-dried glassware, under an atmosphere of nitrogen, and with magnetic stirring. Oil-free KH and NaH were prepared by washing mineral oil dispersions with pentane. Flash column chromatography was carried out on Baker silica gel with an average particle diameter of 40 μm. Reverse phase column chromatography was carried out on Diaion HP-20 adsorbent. NMR spectra (^1H at 300 MHz and ^{13}C at 75 MHz) were recorded on a Bruker AC-300 instrument using $CDCl_3$ as the solvent (unless noted otherwise) and $(CH_3)_4Si$ or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards. ^{31}P chemical shifts are reported in ppm relative to an external standard of 85% H_3PO_4. High-resolution and FAB mass spectra were obtained from The University of Iowa Mass Spectrometry Facility on a ZAB-HF reversed geometry mass spectrometer; only selected ions are reported here.

Diethyl [4,8,12-trimethyl-(*E*,*E*,*E*)-1,3,7,11-tridecatetraenyl] phosphonate (4).[11] To a mixture of KH (91 mg, 2.27 mmol) in THF (2.0 mL) at 0 °C was added tetraethyl

methylenediphosphonate (**3**, 654 mg, 2.27 mmol) in THF (2.5 mL) via cannula. After 1 h at 0 °C, *trans*, *trans*-farnesal (**1**, 400 mg, 1.82 mmol) in THF (2.5 mL) was added via cannula. The mixture was stirred at 0 °C for 10 min, and then allowed to warm to rt. After stirring for 6 h, the reaction was quenched by addition of 1 M acetic acid in ether (2.3 mL) and the solvents were removed in vacuo to provide a yellow oil. Final purification by flash column chromatography (1:1 ethyl acetate:hexanes) provided vinyl phosphonate **4** as a clear oil (584 mg, 91%): ^{1}H NMR δ 7.38 (ddd, J = 21.0, 16.7, 11.3 Hz, 1H), 5.96 (d, J = 11.2 Hz, 1H), 5.55 (dd, J = 20.1, 16.7 Hz, 1H), 5.13–5.04 (m, 2H), 4.08 (m, 4H), 2.20–1.93 (m, 8H), 1.88 (s, 3H), 1.68 (d, J = 0.9 Hz, 3H), 1.60 (s, 6H), 1.33 (dt, J = 7.0, 0.3 Hz, 6H); ^{13}C NMR δ 148.6, 145.2 (d, J_{CP} = 6.7 Hz), 135.7, 131.2, 124.4, 124.1 (d, J_{CP} = 3.3 Hz), 123.1, 113.9 (d, J_{CP} = 191.9 Hz), 61.4 (d, J_{CP} = 5.5 Hz, 2C), 40.0 (d, J_{CP} = 1.5 Hz), 39.6, 26.6, 26.0 (d, J_{CP} = 0.6 Hz), 25.6, 17.5, 17.2, 16.3 (d, J_{CP} = 6.7 Hz, 2C), 15.9; ^{31}P NMR δ 21.3; HRMS calculated for $C_{20}H_{36}O_3P$ $(M+H)^+$ 355.2402, found 355.2380.

4,8,12-Trimethyl-(*E*,*E*,*E*)-1,3,7,11-tridecatetraenylphosphonic acid (6). To a mixture of vinyl phosphonate **4** (92 mg, 0.26 mmol) and collidine (153 μL, 1.16 mmol) in CH_2Cl_2 (4.5 mL) at 0 °C was added trimethylsilyl bromide (153 μL, 1.16 mmol). After 30 min at 0 °C, the reaction mixture was allowed to warm to rt and was stirred for 18 h. Toluene (5 mL) was added and all volatiles were removed in vacuo. A solution of 0.5 M NaOH (4.3 mL) was added and the resulting clear solution was stirred for 10 d. The solution was acidified with 1 M HCl until pH 1, extracted with ethyl acetate (3×15 mL), and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo to provide phosphonic acid **6** as an oil (77 mg, 99%); ^{1}H NMR δ 11.5–10.5 (br s, 2H), 7.4–7.1 (m, 1H), 5.89 (d, J = 8.1 Hz, 1H), 5.75–5.55 (m, 1H), 5.20–5.05 (m, 2H), 2.15–1.90 (m, 8H), 1.82 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H); ^{31}P NMR δ 21.9; HRMS calculated for $C_{16}H_{26}O_3P$ $(M-H)^-$ 297.1620, found 297.1610.

Diethyl [4,8-dimethyl-(*E*,*E*)-1,3,7-nonatrienyl] phosphonate (5). Tetraethyl methylenediphosphonate (**3**, 530 mg, 1.84 mmol) in THF (2 mL) was added via cannula to a mixture of KH (73 mg, 1.83 mmol) in THF (2.0 mL) at 0 °C. After 30 min at 0 °C, geranial (**2**, 220 mg, 1.45 mmol) in THF (2 mL) was added via cannula. The mixture was stirred at 0 °C for 15 min and then was allowed to warm to rt. After stirring overnight, the reaction was quenched by addition of 1 M acetic acid in ether (1.9 mL) and the solvents were removed in vacuo. Purification by flash chromatography (1:1, ethyl acetate:hexanes) provided the vinyl phosphonate **5** as a clear, colorless oil (408 mg, 98%): ^{1}H NMR δ 7.38 (ddd, J = 20.9, 16.5, 11.1 Hz, 1H), 5.96 (d, J = 11.1 Hz, 1H), 5.55 (dd, J = 20.3, 16.7 Hz, 1H), 5.07 (m, 1H), 4.08 (m, 4 H), 2.13 (m, 4H), 1.87 (s, 3H), 1.69 (s, 3H), 1.61 (s, 3H), 1.33 (t, J = 7.1 Hz, 6H); ^{13}C NMR δ 148.7, 145.2 (d, J_{CP} = 6.7 Hz), 132.1, 124.2 (d, J_{CP} = 26.6 Hz), 123.2, 114.0 (d, J_{CP} = 192.3 Hz), 61.4 (d, J_{CP} = 6.4 Hz, 2C), 40.0, 26.2, 25.6, 17.6, 17.2, 16.3 (d, J_{CP} = 6.4 Hz, 2C); ^{31}P NMR δ 21.4; HRMS calculated for $C_{15}H_{28}O_3P$ $(M+H)^+$ 287.1776, found 287.1770.

4,8-Dimethyl-(*E*,*E*)-1,3,7-nonatrienylphosphonic acid (7). According to the general procedure described for preparation of compound **6**, phosphonate **5** (109 mg, 0.38 mmol) was treated with TMSBr (230 μL, 1.74 mmol) and collidine (226 μL) in CH_2Cl_2 (7.0 mL) for 4 d, and then with 0.5 M NaOH (6.1 mL). After 15 d when the reaction was complete as indicated by analysis of the ^{31}P spectrum of the reaction mixture, standard workup gave the vinyl phosphonic acid **7** (61 mg, 69%) as a white film: ^{1}H NMR δ 11.4–9.4 (br s, 2H), 7.5–7.2 (m, 1H), 6.05–5.85 (m, 1H), 5.8–5.5 (m, 1H), 5.06 (m, 1H), 2.12 (m, 4H), 1.84 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H); ^{31}P NMR δ 24.0; HRMS calculated for $C_{11}H_{18}O_3P$ $(M-H)^-$ 229.0994, found 229.0990.

Dimethyl [4,8,12-trimethyl-(*E*,*E*)-3,7,11-tridecatrienyl] phosphonate (10).[13] To a mixture of dimethyl methylphosphonate (**9**, 275 mg, 2.22 mmol) in THF (5 mL) at −78 °C was added *n*-butyllithium (1.90 mL, 1.15 M in hexanes, 2.19 mmol). After 30 min at −78 °C, the solution was added dropwise via cannula to a solution of farnesyl bromide (**8**, 500 mg, 1.75 mmol) in THF (2 mL) at −78 °C over a period of 1 h. The solution was allowed to stir at −78 °C for 1.5 h and then the mixture was quenched by the addition of water (25 mL). The mixture was extracted with ethyl acetate (3×20 mL), the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give an orange oil. Purification by flash column chromatography (gradient of 8:2–9:1 ethyl acetate:hexanes) afforded phosphonate **10**[13] as a clear oil (311 mg, 54%): ^{1}H NMR δ 5.17–5.05 (m, 3H), 3.73 (d, J = 10.7 Hz, 6H), 2.36–2.22 (m, 2H), 2.13–1.93 (m, 8H), 1.84–1.70 (m, 2H), 1.67 (d, J = 0.6 Hz, 3H), 1.62 (s, 3H), 1.59 (s, 6H); ^{13}C NMR δ 136.5 (d, J_{CP} = 1.8 Hz), 134.9, 131.1, 124.2, 123.8, 122.6 (d, J_{CP} = 17.1 Hz), 52.2 (d, J_{CP} = 6.8 Hz, 2C), 39.5 (d, J_{CP} = 11.0 Hz), 26.5 (d, J_{CP} = 20.8 Hz), 26.3, 25.5, 24.7 (d, J_{CP} = 139.2 Hz), 20.7, 20.6, 17.5, 15.8 (2C); ^{31}P NMR δ 35.5; HRMS calculated for $C_{18}H_{34}O_3P$ $(M+H)^+$ 329.2246, found 329.2250.

4,8,12-Trimethyl-(*E*,*E*)-3,7,11-tridecatrienylphosphonic acid (11). According to the general procedure described for preparation of compound **6**, phosphonate **10** (100 mg, 0.30 mmol) was treated with TMSBr (180 μL, 1.36 mmol) and collidine (180 μL) in CH_2Cl_2 (5 mL)

for 30 h, and then with 0.5 M NaOH (5 mL) for 5 d. Standard workup gave phosphonic acid **11** as an oil (80 mg, 88%): ^{1}H NMR δ 10.70–9.70 (br s, 2H), 5.20–5.05 (m, 3H), 2.40–2.20 (m, 2H), 2.10–1.90 (m, 8H), 1.90–1.70 (m, 2H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 6H); ^{31}P NMR δ 36.5; HRMS calculated for $C_{16}H_{28}O_3P$ $(M\text{-}H)^-$ 299.1776, found 299.1781.

Tetraethyl [4,8,12-trimethyl-(*E*,*E*,*E*)-3,7,11-tridecatrienyl]-1,1-bisphosphonate (13).[16] Bisphosphonate **3** (1.646 g, 5.71 mmol) in THF (20 mL) was added via cannula to a mixture of NaH (137 mg, 5.30 mmol) in THF (20 mL). After 30 min, the reaction mixture was cooled to 0 °C and added via cannula over a period of 1 h to a mixture of bromide **8** (1.511 mg, 5.30 mmol) in THF (20 mL) at 0 °C. The reaction mixture was allowed to warm slowly to rt and, after 1 day, it was quenched by addition of H_2O (25 mL) and extracted with ethyl acetate (3×25 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo to give an orange oil. Purification by flash chromatography (95:5, ethyl acetate: methanol) afforded bisphosphonate **13** (1.491 g, 57%) as a clear oil: ^{1}H NMR δ 5.32 (t, J = 6.7 Hz, 1H), 5.10 (m, 2H), 4.17 (m, 8H), 2.64 (m, 2H), 2.31 (tt, J = 23.7, 6.1 Hz, 1H), 2.13–1.93 (m, 8H), 1.68 (s, 3H), 1.65 (s, 3H), 1.60 (s, 6H), 1.34 (dt, J = 7.0, 0.6 Hz, 12H); ^{31}P NMR data matched reported data.[16]

4,8,12-Trimethyl-(*E*,*E*,*E*)-3,7,11-tridecatrienyl-1,1-bisphosphonic acid (15).[16] According to the general procedure described for preparation of compound **6**, phosphonate **13** (213 mg, 0.43 mmol) was treated with TMSBr (540 μL, 4.09 mmol) and collidine (540 μL) in CH_2Cl_2 (8.0 mL) for 1 day, and then with 0.5 M NaOH (6.1 mL) for 16 days. Standard workup gave phosphonic acid **15** (109 mg, 66%). Both ^{1}H and ^{31}P NMR spectra were in agreement with literature data.[16]

Tetraethyl [4,8-dimethyl-(*E*,*E*)-3,7-nonadienyl]-1,1-bisphosphonate (14). According to the procedure described for preparation of compound **13**, bisphosphonate **3** (727 mg, 2.53 mmol) in THF (10 mL) was treated with NaH (60 mg, 2.49 mmol) and geranyl bromide (**12**, 504 mg, 2.32 mmol in 8 mL THF). After 3 days, a parallel workup and final purification by flash chromatography (95:5 ethyl acetate: methanol) afforded bisphosphonate **14** (569 mg, 58%) as a clear oil: ^{1}H NMR δ 5.32 (t, J = 7.6 Hz, 1H), 5.09 (m, 1H), 4.18 (m, 8H), 2.64 (m, 2H), 2.31 (tt, J = 23.9, 6.2 Hz, 1H), 2.12–1.94 (m, 4H), 1.68 (s, 3H), 1.64 (s, 3H), 1.60 (s, 3H), 1.34 (dt, J = 7.1, 0.7 Hz, 12H); ^{13}C NMR δ 136.1, 130.7, 123.7, 121.5, 61.9 (t, J_{CP} = 7.3 Hz, 4C), 39.2, 37.1 (t, J_{CP} = 133 Hz), 26.1, 23.6, 25.1, 17.1, 15.9 (4C), 15.6; ^{31}P NMR δ 24.2; HRMS calculated for $C_{19}H_{39}O_6P_2$ $(M+H)^+$ 425.2222, found 425.2244.

4,8-Dimethyl-(*E*,*E*)-3,7-nonadienyl-1,1-bisphosphonic acid (16). According to the general procedure described for preparation of compound **6**, phosphonate **14** (157 mg, 0.37 mmol) was treated with TMSBr (450 μL. 3.41 mmol) and collidine (460 μL) in CH_2Cl_2 (6 mL) for 3 d, and then with 0.5 M NaOH for 15 days. Standard workup gave the bisphosphonic acid **16** (69 mg, 60%). ^{1}H NMR (CD_3OD) δ 5.4 (m, 1H), 5.1 (m, 1H), 2.7–2.5 (m, 2H), 2.4–1.9 (m, 5H), 1.65 (s, 6H), 1.58 (s, 3H); ^{31}P NMR δ 23.2; HRMS calculated for $C_{11}H_{21}O_6P_2$ $(M\text{-}H)^-$ 311.0813, found 311.0811.

Tetraethyl [4,8,12-trimethyl-(*E*,*E*,*E*)-1,3,7,11-tridecatetraenyl]-1,1-bisphosphonate (17). The bisphosphonate (**13**, 306 mg, 0.62 mmol) in THF (3.8 mL) was added via cannula to a solution of NaH (21 mg, 0.85 mmol) in THF (3.4 mL). After 80 min at rt, the reaction mixture was cooled to 0 °C. Phenyl selenyl bromide (211 mg, 0.89 mmol) in THF (3.8 mL) was added via cannula and the mixture was stirred for 3 h. The mixture was then poured into a solution of sat. $NaHCO_3$ (20 mL) and was extracted with ether (3×20 mL). The combined organic extracts were washed with H_2O (20 mL), dried over $MgSO_4$, filtered, and then concentrated in vacuo. THF (8 mL), H_2O (1 mL), and acetic acid (80 μL) were added and the reaction mixture was cooled to 0 °C. A solution of 30% hydrogen peroxide (205 μL) was added and the mixture was stirred for 90 min. Additional hydrogen peroxide (10 μL) was then added and, after a period of 20 min, a third portion was added (10 μL). After 20 min, the mixture was poured into H_2O (20 mL) and extracted with ether (4×15 mL). The combined extracts were washed with H_2O (20 mL), dried over $MgSO_4$, filtered, and then concentrated in vacuo. Purification by flash chromatography (4:1 chloroform:acetone) provided the bisphosphonate **17** as a clear, colorless oil (152 mg, 50%): ^{1}H NMR δ 8.12 (ddd, J = 46.6, 26.6, 12.1 Hz, 1H), 6.98 (d, J = 12.2 Hz, 1H), 5.08 (m, 2H), 4.20–4.06 (m, 8H), 2.30–2.00 (m, 8H), 1.98 (s, 3H), 1.68 (s, 3H) 1.60 (s, 6H), 1.34 (dt, J = 7.0, 3.1 Hz, 12H); ^{13}C NMR δ 157.7, 156.7, 136.0, 131.3, 124.1, 122.9, 122.4 (dd, J_{CP} = 21.3, 8.5 Hz), 114.2 (dd, J_{CP} = 167.3, 172.2 Hz), 62.2 (d, J_{CP} = 5.5 Hz, 2C), 61.9 (d, J_{CP} = 5.5 Hz, 2C), 41.0, 39.6, 26.6, 26.3, 25.6, 17.6, 17.5, 16.3 (2C), 16.2 (2C), 15.9; ^{31}P NMR δ 16.5 (d, J = 51.1 Hz), 12.0 (d, J = 51.2 Hz); HRMS calculated for $C_{24}H_{45}O_6P_2$ $(M+H)^+$ 491.2691, found 491.2694.

4,8,12-Trimethyl-(*E*,*E*,*E*)-1,3,7,11-tridecatetraenyl-1,1-bisphosphonic acid (18). According to the general procedure described for preparation of compound **6**, phosphonate **17** (72 mg, 0.15 mmol) was treated with TMSBr (180 μL, 1.36 mmol) and collidine (180 μL) in CH_2Cl_2 (3 mL) for 1 day, and then with 0.5 M NaOH for 7 d. Standard workup gave phosphonic acid **18** (49 mg, 88%) as a yellow film: ^{1}H NMR (CD_3OD) δ 8.0–7.6 (br

s, 1H), 6.9–6.7 (m, 1H), 5.17–5.05 (m, 2H), 2.25–1.95 (m, 8H), 1.92 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H); ^{31}P NMR δ 18, 13; HRMS calculated for $C_{16}H_{27}O_6P_2$ (M-H)$^-$ 377.1282, found 377.1258

Ethyl 2-(diethoxyphosphinyl)-5,9,13-trimethyl-(*E*,*E*,*E*)-4,8,12-tetradecatrienoate (20). To a mixture of NaH (39 mg, 1.64 mmol) in THF (10 mL) was added triethyl phosphonoacetate (**19**, 375 mg, 1.67 mmol) in THF (6 mL) via cannula. After 20 min, the mixture was cooled to 0 °C and transferred via cannula over a period of 35 min to a mixture of farnesyl bromide (**8**, 427 mg, 1.50 mmol) in THF (10 mL), also at 0 °C. The resulting mixture was allowed to slowly warm to rt, and after 1 day, the reaction was quenched by addition of H_2O (25 mL). The mixture was extracted with ethyl acetate (4×20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo to give an orange oil. Purification by flash chromatography (1:1, ethyl acetate:hexanes) afforded phosphonate **20** (367 mg, 57%) as a clear oil: ^{1}H NMR δ 5.08 (m, 3H), 4.23–4.10 (m, 6H), 2.93 (ddd, J = 21.7, 11.1, 4.0 Hz, 1H), 2.78–2.61 (m, 1 H), 2.59–2.45 (m, 1 H), 2.11–1.93 (m, 8 H), 1.68 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.34 (dt, J = 7.0, 0.5 Hz, 3H), 1.34 (dt, J = 7.0, 0.5 Hz, 3H), 1.27 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 168.6 (d, J_{CP} = 4.2 Hz), 137.9, 134.7, 130.8, 124.0, 123.6, 120.0 (d, J_{CP} = 16.1 Hz), 62.3 (d, J_{CP} = 6.4 Hz), 62.2 (d, J_{CP} = 6.7 Hz), 60.9, 45.8 (d, J_{CP} = 129.7 Hz), 39.4, 39.3, 26.4, 26.2, 25.4 (d, J_{CP} = 4.3 Hz), 25.3, 17.3, 16.0 (d, J_{CP} = 16.1 Hz, 2C), 15.8, 15.6, 13.8; ^{31}P NMR δ 23.0; HRMS calculated for $C_{23}H_{42}O_5P$ (M + H)$^+$ 429.2770, found 429.2756.

2-(Diethoxyphosphinyl)-5,9,13-trimethyl-(*E*,*E*,*E*)-4,8,12-tetradecatrienoic acid (21). A solution of phosphonate **20** (251 mg, 0.59 mmol) and 2 M NaOH (1.17 mL, 2.35 mmol) in EtOH (16 mL) was heated at reflux for 4 h. After the mixture had cooled to rt, it was acidified to pH 1 by addition of 1 M HCl. Water was added (20 mL) and the reaction mixture was extracted with ethyl acetate (3×20 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo to yield the product as a yellow oil. Final purification by flash chromatography (1 mL acetic acid per 250 mL ethyl acetate) yielded carboxylic acid **21** as a clear oil (168 mg, 71%): ^{1}H NMR δ 10.1–9.8 (br s, 1H), 5.09 (m, 3H), 4.19 (m, 4H), 2.96 (ddd, J = 22.2, 10.5, 4.2 Hz, 1H), 2.75–2.45 (m, 2H), 2.10–1.92 (m, 8H), 1.68 (s, 3H), 1.63 (s, 3H), 1.60 (s, 3H), 1.58 (s, 3H), 1.34 (dt, J = 7.0, 2.3 Hz, 6H); ^{13}C NMR δ 171.0, 138.2, 135.0, 131.1, 124.3, 123.9, 120.2 (d, J_{CP} = 14.7 Hz), 63.4 (d, J_{CP} = 6.1 Hz), 62.8 (d, J_{CP} = 6.7 Hz), 45.9 (d, J_{CP} = 130.6 Hz), 39.6 (2C), 26.7, 26.5, 25.6 (2C), 17.6, 16.2 (d, J_{CP} = 5.5 Hz, 2C), 16.0, 15.9; ^{31}P NMR δ 23.9.

2-Phosphono-5,9,13-trimethyl-(*E*,*E*,*E*)-4,8,12-tetradecatrienoic acid, trisodium salt (22). According to the procedure described for compound **6**, compound **21** (93 mg, 0.23 mmol) was treated with TMSBr (140 μL, 1.06 mmol) and collidine (140 μL) in CH_2Cl_2 overnight, and then with 0.5 M NaOH for 17 days. The volatiles were removed in vacuo, and the initial product was placed on an HP-20 column. Elution with water, 10% acetonitrile, and 20% acetonitrile afforded the sodium salt of the phosphonic acid **22** as a white solid (76 mg, 80%): ^{1}H NMR (D_2O) δ 5.2–5.1 (m, 3H), 2.5–2.3 (m, 3H), 2.1–1.9 (m, 8H), 1.65 (s, 3 H), 1.61 (s, 3H), 1.58 (s, 6H); ^{31}P NMR δ 20.5. HRMS calculated for $C_{17}H_{28}O_5PNa_3$ (M-3Na + 2H)$^-$ 343.1674, found 343.1678.

Ethyl 2-phosphono-5,9,13-trimethyl-(*E*,*E*,*E*)-4,8,12-tetradecatrienoate, disodium salt (23). According to the procedure described for compound **6**, phosphonate **20** (166 mg, 0.39 mmol) was treated with TMSBr (230 μL, 1.4 mmol) and collidine (230 μL) in CH_2Cl_2 overnight, and then with 0.5 M NaOH for 14 days. The volatiles were removed in vacuo, and the initial product was placed on an HP-20 column. Elution with water afforded the sodium salt of phosphonic acid **23** as a white solid (115 mg, 80%): ^{1}H NMR (D_2O) δ 5.2–5.1 (m, 3H), 4.09 (q, J = 7 Hz, 2H), 2.7–2.6 (m, 2H), 2.4–2.3 (m, 1H), 2.2–1.9 (m, 8H), 1.66 (s, 3H), 1.63 (s, 3H), 1.58 (s, 6H), 1.22 (t, J = 7.1 Hz, 3H); ^{31}P NMR δ 13.9; HRMS calculated for $C_{19}H_{32}O_5PNa_2$ (M-2Na + H)$^-$ 371.1987, found 371.2005.

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