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2-(Acyloxy)ethylphosphonate Analogues of Prenyl Pyrophosphates: Synthesis and Biological Characterization

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Abstract—2-(Acyloxy)ethylphosphonate analogues of geranyl, farnesyl, and geranylgeranyl pyrophosphate have been prepared. Horner–Wadsworth–Emmons condensation of different terpene aldehydes with an unsymmetrical bisphosphonate was the key step in syntheses of the phosphonates bearing α,β-unsaturated acyloxy groups. After preparation of the respective phosphonic acids through reaction with TMSBr, both acids and esters were tested for their effects on DNA synthesis in human-derived myeloid and lymphoid leukemia cell lines. The phosphonate esters varied substantially in their ability to impair proliferation of the different cell lines, but testing against one possible target, farnesyl protein transferase (FPTase), revealed little impact at concentrations ranging up to 10 μM. Because the corresponding 2,3-dihydro compounds showed similar biological activity, conjugate addition would not appear to be involved in the toxicity.

Introduction

Protein prenylation is an important step in the post-translational processing that facilitates protein association with membranes and their subsequent function in signal transduction.[1] Frequently these signal transduction events promote cell proliferation, and thus inhibition of protein prenylation has become a popular target for development of anti-proliferative agents.[2] For example, the farnesylation of RAS is necessary for its membrane localization and biological activity. RAS farnesylation requires reaction of a cysteine sulfhydryl group near the protein's C-terminus with farnesyl pyrophosphate (FPP, **1a**) in a process catalyzed by the enzyme farnesyl protein transferase (FPTase).[3] Inhibitors of this enzyme have attracted considerable interest as potential anti-cancer agents because mutations in RAS proteins that lead to excessive signaling for cell growth are commonly associated with a malignant phenotype.[4] In contrast, unmodified RAS proteins, wild-type or mutated, are localized to the cytoplasm and devoid of signal transduction activity.[5] Thus, diminished RAS farnesylation is an intriguing strategy to block the transforming activity of mutated *RAS* genes and activated RAS proteins.[2]

Numerous and varied inhibitors of the farnesylation process have been reported to date. Some peptide-based FPTase inhibitors homologous to the carboxy terminus of the RAS proteins have been developed and have been shown to be extremely effective competitive inhibitors when assayed in vitro, yet ineffective when added to intact cells.[6] Several natural products have been found to inhibit FPTase activity in vitro, including the chaetomellic acids,[7] oreganic acid,[8] and actinoplanic acid A.[9] Finally, both synthetic farnesol and FPP analogues have been reported as inhibitors of this enzyme or probes of its reactivity. These include bisubstrate inhibitors which mimic the lipophilic farnesyl moiety and contain a pyrophosphate isostere joined by a flexible linker,[10] as well as more basic variations on the farnesol[11] and FPP skeletons.[12]

Here we report the synthesis of a new set of terpenoid pyrophosphate analogues, centered on phosphonate **2**. When this 2-(acyloxy)ethylphosphonate is compared to FPP (**1a**), many common structural features are apparent: the common terpene tail, a similar chain length from the isoprene tail to the phosphorus,[13] and parallel hybridization of most atoms in the carbon backbone (Scheme 1). The most significant difference is the fact that the target compounds contain but one phosphorus instead of two, and thus may be more similar to farnesyl monophosphate (**1b**). However this may not be critical to interactions with enzymes such as FPTase given that compounds such as α-hydroxyfarnesylphosphonate demonstrate IC_{50}'s in the 30 nanomolar range.[12b,h] Though the structure of the central phosphonic acid **3**

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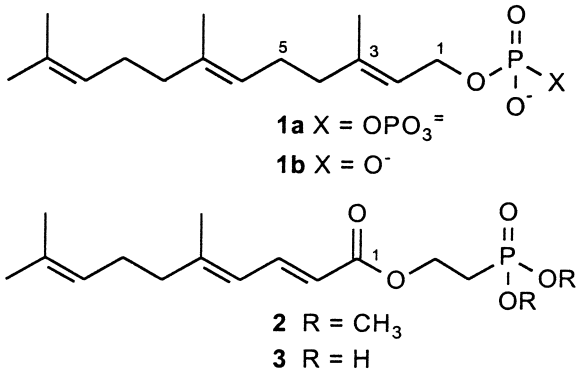


Scheme 1.

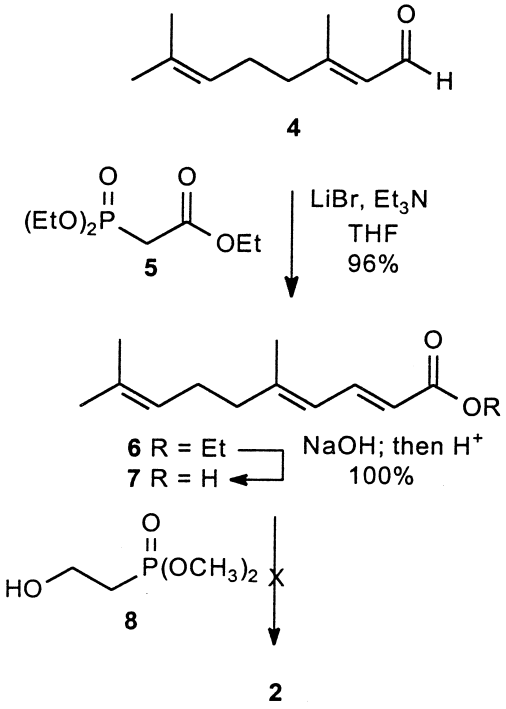
most closely resembles a farnesol derivative, other phosphonates were prepared in which the terpenoid chain length has been tailored to mimic a geranyl- and geranylgeranyl-length chain. These new phosphonates were then evaluated for their ability to impair DNA synthesis and for their ability to inhibit RAS farnesylation.

Chemical Synthesis

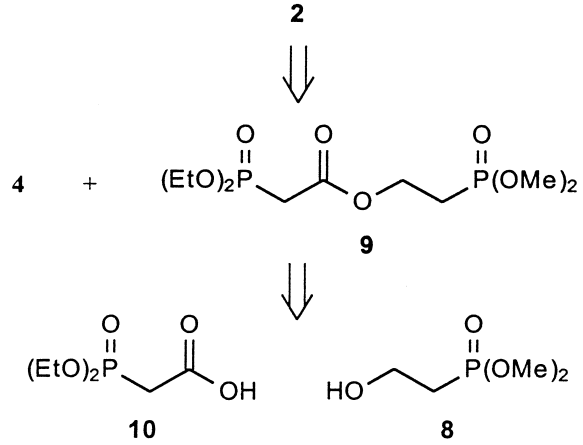
The first approach to phosphonate **3** was based on homologation of geranial[14] (**4**) through a Horner–Wadsworth–Emmons (HWE)[15] condensation with triethyl phosphonoacetate (**5**). Under conditions reported by Rathke and co-workers,[16] the ester **6**[17] was obtained in an optimized yield of 96% (Scheme 2). Saponification of ester **6** was accomplished in quantitative yield through reaction with aqueous NaOH in ethanol under reflux. Analysis of the ^{1}H NMR spectra showed that the condensation proceeded with near-complete conversion to the *trans*-olefin and that no isomerization of the conjugated olefin occurred upon hydrolysis to acid **7**. Unfortunately, Fisher esterification of acid **9** with phosphono alcohol **8** proved difficult, and attempted coupling by reaction with DCC and catalytic DMAP at room temperature[18] or by treatment with EDC[19] went unrewarded.

Problems encountered with the approach described above prompted formulation of a new strategy for synthesis of the desired ester phosphonate. By disconnection at the double bond of the α, β-unsaturated ester, geranial (**4**) could be recognized as the origin of the terpenoid tail and bisphosphonate **9** could be identified as the source of the desired phosphono group (Scheme 3). In theory, these reagents could be joined by an HWE condensation, provided that anion formation would favor the correct phosphonate and the phosphonoacetate portion would be expected to be considerably more acidic.[15] Bisphosphonate **9** could be made by coupling phosphono alcohol **8** and phosphonoacetic acid **10**, both of which are commercially available. Therefore all three components necessary for synthesis of conjugated ester phosphonate **2** would be readily accessible.

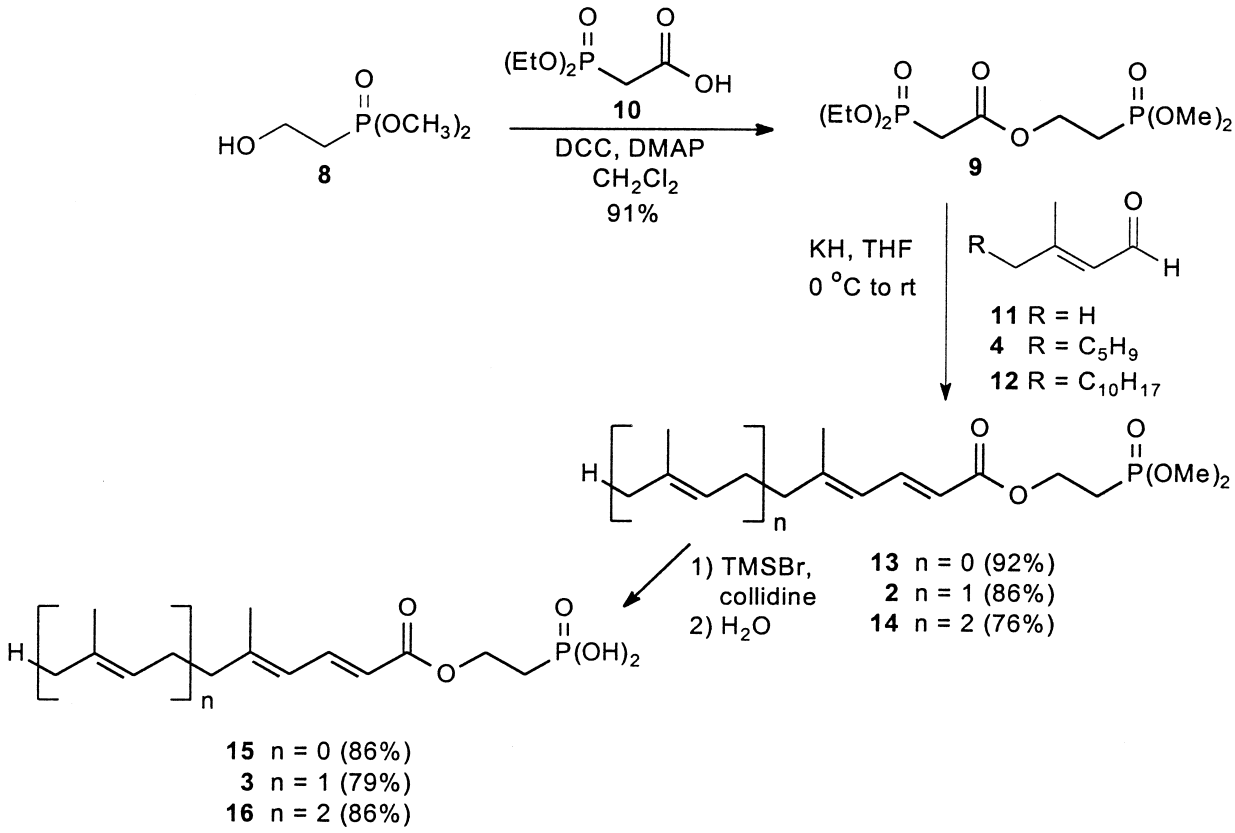
Through this approach the desired phosphonate ester **2** was readily obtained. Coupling of phosphono alcohol **8** and phosphonoacetic acid **10** was conducted under conditions similar to previous couplings but with far greater success. A mixture of acid **10** and alcohol **8** was added to DCC and catalytic DMAP (Scheme 4) and after 6–8 h the desired bisphosphonate **9** was isolated in 91% yield. The condensation of bisphosphonate **9** with isoprenoid aldehydes of different chain length (i.e., 3-methyl-2-butenal (**11**), geranial (**4**), or farnesal (**12**)[12h]) was accomplished under typical HWE conditions. For example, bisphosphonate **9** was treated with KH at 0 °C, followed by addition of the aldehyde **4** to provide the desired ester phosphonate **2** in 86% yield. After inspection of the vinylic hydrogen resonances in the ^{1}H NMR spectrum of the product, it was concluded that the HWE condensation produced phosphonate **2** as a single olefin isomer under these conditions.



Scheme 2.



Scheme 3.



Scheme 4.

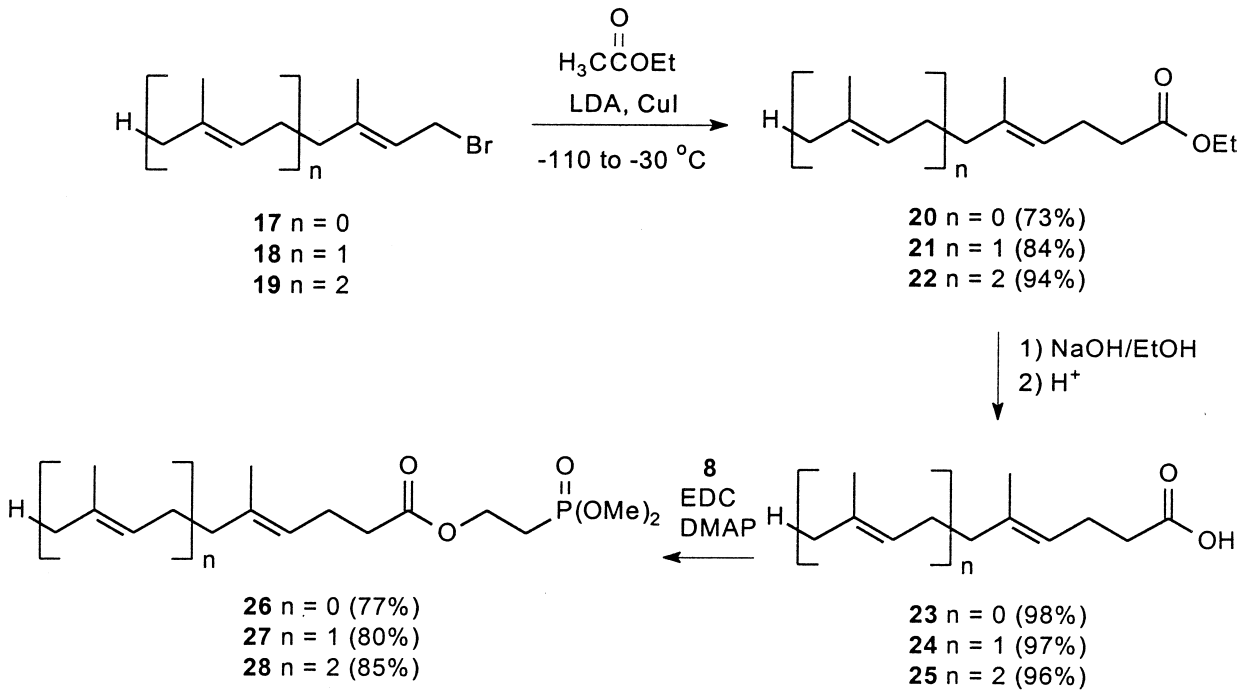
The disconnection of phosphonate ester **2** to bisphosphonate **9** also allowed facile preparation of related terpenoid phosphonates simply by changing the aldehyde employed in the HWE condensation. While phosphonic acid **3** was designed to mimic farnesyl pyrophosphate (**1a**), the corresponding geranyl and geranylgeranyl pyrophosphate analogues were readily available through HWE condensations with different terpene aldehydes. Thus condensation with the appropriate 5-carbon aldehyde (**11**) gave the phosphonate **13** while condensation with farnesal (**12**) gave the longer phosphonate **14**. In both cases, the HWE coupling proceeded with complete formation of *E* stereochemistry of the new double bond, and the desired products were isolated in high yields.

Prior to bioassays with FPTase, phosphonates **2**, **13** and **14** were converted to the corresponding phosphonic acids. Although extended times were required, in each case reaction with TMSBr/collidine and standard work-up[12b] gave the desired phosphonic acid (**3**, **15** and **16** respectively). Despite some concern for the stability of the carboxylate ester to these conditions, none of the products derived from this cleavage was observed.

In addition to these conjugated compounds (**3**, **15** and **16**) the parallel products lacking the C-2 olefin were of interest because they would more closely represent the flexibility of the natural terpenoid pyrophosphates. While it might be possible to obtain these target compounds by conjugate reduction of the phosphonates in hand, we chose instead to pursue an alternate route. Alkylation of the copper enolate derived from ethyl acetate with an allylic halide (**17**–**19**) afforded the esters **20**–**22** in high yield as long as a very low temperature (−110 °C) was maintained (Scheme 5), while at more typical temperatures (e.g., −78 °C) only much lower yields were obtained.[20] After standard saponification to the corresponding carboxylic acids (**23**–**25**), coupling with phosphono alcohol **8** in the presence of DDC and DMAP provided the desired phosphonates **26**–**28**. Surprisingly, attempted hydrolysis to the corresponding phosphonic acids by treatment with TMSBr and collidine under standard conditions resulted in cleavage of the carboxylic acid ester as the major product. Thus the phosphonic acids necessary for FPTase assays were not readily obtained. This was particularly frustrating because these dihydro compounds would better mimic the flexibility of the farnesyl chain at the C4–C5 bond. However, the dimethyl esters **26**–**28** proved useful in other bioassays, as explained further below.

Biological activity

The 2-(acyloxy)ethylphosphonate analogues of geranyl, farnesyl, and geranylgeranyl pyrophosphate were tested at 100 and 200 μM concentrations for their ability to impair cell proliferation as assessed by radiolabeled thymidine incorporation into DNA. The methods for this assay are described elsewhere[21] and in brief involve scintillation spectroscopic analysis of precipitated DNA from cells that have been incubated with the test compounds for 72 h and then pulsed with radiolabeled thymidine for 4 h. The two cells types tested were



Scheme 5.

human-derived myeloid (THP-1) and lymphoid (RPMI-8402) leukemia cell lines. The results are expressed as the ratio of thymidine incorporation with the test compound as compared to incorporation from incubations without test compound. Each assay was done in triplicate.

As shown in Table 1, the results are cell line-related. Inhibition by the unsaturated phosphonic acids (**15**, **3**, **16**) is more pronounced in the myeloid line than in lymphoid line and is correlated with alkyl chain length.

Table 1. Effects of phosphonic acid and ester chain length on ^{3}H-thymidine incorporation into DNA in THP-1 and RPMI-8402 cell lines

Compound	^{3}H-thymidine incorporation (DPM) (fraction of control)[a]			
	THP-1 cells		RPMI-8402 cells	
	100 μM	200 μM	100 μM	200 μM
15 ($n=0$)	1.14		1.14	
3 ($n=1$)	0.63		0.90	
16 ($n=2$)	0.07		0.79	
13 ($n=0$)	0.95		0.80	
2 ($n=1$)	1.14		0.12	
14 ($n=2$)	0.08		0.02	
26 ($n=0$)	0.78	0.69	0.98	0.99
27 ($n=1$)	0.61	0.79	0.60	0.00
28 ($n=2$)	ND	0.08	0.03	0.00

[a]Fraction of control (incorporation with compound/incorporation without compound). ND (no data).

While the shortest chain compound (**15**) does not impair either cell line, the farnesyl-length compound (**3**) displays more impact on the myeloid line than the lymphoid line, and the longest chain compound (**16**) has a pronounced effect on the myeloid line and much less impact on the lymphoid line. Interestingly, the dimethyl esters for these compounds (**13**, **2**, **14**) showed a different pattern than the parent acids. The shortest carbon chain compound (**13**) had minimal affect on both cell lines. The farnesyl-length compound (**2**) markedly inhibited DNA synthesis in the lymphoid, but not the myeloid, line. The geranylgeranyl-length compound (**14**) inhibited DNA synthesis in both myeloid and lymphoid lines. This general pattern is repeated with the dihydro compounds **26**–**28**. The shortest carbon chain compound (**26**) shows no effect on the lymphoid cell line and little impact on the myeloid line, while the longest carbon chain compound (**28**) completely inhibits DNA synthesis in both cell lines. At the higher concentration measured, the farnesyl-length compound (**27**) completely inhibits DNA synthesis in the lymphoid cell line but has little impact on the myeloid cell line.

Because of the increasing awareness of the importance of protein prenylation for cell proliferation, these compounds also were tested for their ability to inhibit FPTase using partially purified enzyme from bovine brain[22] in an assay modified[12h] from the method of Harwood.[23] Compounds were assayed at concentrations ranging up to 10 μM, and each assay was done in triplicate. These results, expressed as 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 2.

Compound **3**, which is most similar in structure to farnesyl pyrophosphate, showed only weak FPTase

Table 2. Effect of phosphonic acid chain length on farnesyl protein transferase

Compound	FPTase activity (fraction of control)[a]	
	1 μM	10 μM
15 (n = 0)	0.79	0.90
3 (n = 1)	0.93	0.80
16 (n = 2)	1.01	1.24
26 (n = 0)	1.04	1.11
27 (n = 1)	0.93	1.00
28 (n = 2)	0.94	1.00

[a]Fraction of control (activity with compound/activity without compound).

inhibitory activity. This effect was related to concentration with 20% inhibition observed at 10 μM, but because such weak activity is not likely to be useful higher concentrations were not tested. Compound **15** also was a weak inhibitor while compound **16** showed no inhibitory activity. In fact, compound **16** actually enhanced FPTase activity at 10 μM levels. Compounds **26**, **27** and **28** had minimal effect on FPTase. The esters **13**, **2** and **14** did not inhibit FPTase (data not shown) as might be expected on the basis of our previous results with other phosphonate esters.[12h] The basis for the activation of FPTase as observed for compounds **16** and **26** is unknown but has been observed with other compounds.[12i]

These biological findings suggest that inhibition of FPTase does not underlie the interesting ability of these compounds to differentially inhibit DNA synthesis in human-derived myeloid and lymphoid cell lines. While the basis of this selectivity is not yet clear, it may be useful because this differential activity suggests potential immunosuppressive effects (lymphoid inhibition) without suppression of bone marrow function (myeloid inhibition). Further studies will be needed to delineate the significance and eventual application of this finding.

Experimental

(*E,E*)-Farnesol and (*E*)-geraniol were purchased from Aldrich and Fluka, respectively, and were used directly. Tetrahydrofuran was freshly distilled from sodium/benzophenone, while toluene, dichloromethane, triethylamine, and diisopropylamine were freshly distilled from calcium hydride. All reactions in non-hydroxylic solvents were conducted in oven-dried glassware, under a positive pressure of nitrogen. Oil-free KH was prepared by washing mineral oil dispersions five times with an equal volume of pentane. Flash column chromatography was carried out on Baker silica gel with 40 μm average particle diameter. Melting points are uncorrected. NMR spectra (^{1}H at 300 MHz and ^{13}C at 75 MHz) were recorded with $CDCl_3$ as solvent using $(CH_3)_4Si$ (^{1}H, 0.0 ppm) or $CDCl_3$ (^{13}C, 77.0 ppm) as internal standards; ^{31}P chemical shifts are reported in ppm relative to 85% H_3PO_4 (external standard). Low-resolution electron impact (EI) mass spectra were recorded at 70 eV. High-resolution mass spectra were obtained on either a ZAB-HF reversed geometry mass spectrometer or an Autospec reverse geometry mass spectrometer at The University of Iowa Mass Spectrometry Facility. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA) or Supersun Technology (Stoney Brook, NY).

Ethyl (*E,E*)-5,9-dimethyl-2,4,8-decatrienoate (6).[17] To a mixture of LiBr (4.11 g, 47.4 mmol) in THF (60 mL) at room temperature was added triethyl phosphonoacetate (**5**, 7.2 mL, 36.3 mmol) followed by dropwise addition of triethylamine (6.6 mL, 47.4 mmol). After the mixture was stirred for 10 min, geranial (**4**, 5.53 g, 36.3 mmol) was added via cannula, and the cannula was rinsed into the reaction mixture with THF (15 mL). After the reaction mixture was stirred at room temperature for 50 h, a solution of 1% HCl (100 mL) was added and the mixture was extracted with ether (3×75 mL). The organic layers were combined and extracted with 1 M NaOH (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash column chromatography on silica gel (gradient of 95:5 hexane:ethyl acetate to 92:8 hexane:ethyl acetate) gave ester **6** (7.77 g, 96%) as a yellow oil: ^{1}H NMR δ 7.58 (dd, J = 15.0, 11.5 Hz, 1H), 5.99 (d, J = 11.5 Hz, 1H), 5.78 (d, J = 15.0 Hz, 1H), 5.03–5.15 (m, 1H), 4.19 (q, J = 7.1 Hz, 2H), 2.10–2.18 (m, 4H), 1.89 (d, J = 1.1 Hz, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C NMR δ 167.4, 149.4, 140.7, 131.9, 123.2, 123.1, 118.8, 59.8, 40.1, 26.1, 25.4, 17.5, 17.1, 14.2; EIMS m/z (relative intensity) 222 (M^+, 6), 154 (25), 139 (17), 81 (35), 69 (100).

(*E,E*)-5,9-Dimethyl-2,4,8-decatrienoic acid (7).[24] To a solution of ester **6** (402 mg, 1.81 mmol) in ethanol (7 mL) was added 2 M NaOH (1.4 mL, 2.80 mmol) and the reaction mixture was heated to reflux. After 18 h at reflux, the reaction mixture was allowed to cool to rt. The resulting solution was acidified to pH 2 by addition of 1 M HCl, extracted with ethyl acetate (3×50 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated to give acid **7** as a white crystalline solid (351 mg, 100%):[24] mp 63–65 °C; lit. Mp 62.5–64.5 °C; ^{1}H NMR δ 12.43–12.54 (br s, 1H), 7.68 (dd, J = 15.1, 11.5 Hz, 1H), 6.03 (d, J = 11.5 Hz, 1H), 5.78 (d, J = 15.1 Hz, 1H), 5.02–5.16 (m, 1H), 2.10–2.30 (m, 4H), 1.91 (s, 3H), 1.69 (s, 3H), 1.61 (s, 3H); ^{13}C NMR δ 173.3, 151.2, 143.1, 132.1, 123.1 (2C), 118.0, 40.2, 26.1, 25.5, 17.5, 17.3; EIMS m/z (relative intensity) 194 (M^+, 1), 126 (15), 111 (10), 81 (16), 69 (100).

2-(Dimethoxyphosphinyl)ethyl (diethoxyphosphinyl)acetate (9). A solution of diethyl phosphonoacetic acid **10** (2.60 g, 13.3 mmol), dimethyl 2-hydroxyethylphosphonate **8** (1.5 mL, 12.0 mmol) and DCC (3.27 g, 15.9 mmol) in dichloromethane (70 mL) was stirred at rt. After 24 h, precipitated dicyclohexylurea was removed by filtration through Celite, and the urea cake was washed with dichloromethane (20 mL). The solvent was removed in vacuo to afford a clear oil which was contaminated with

dicyclohexylurea. Final purification of the oil by flash column chromatography (85:15 ethyl acetate:methanol) gave bisphosphonate **9** as a clear oil (3.62 g, 91%): ^{1}H NMR δ 4.37 (dt, J = 11.2, 7.6 Hz, 2H), 4.18 (dq, J = 8.4, 7.1 Hz, 4H), 3.77 (d, J = 11.1 Hz, 6H), 2.99 (d, J = 21.6 Hz, 2H), 2.21 (dt, J = 19.1, 7.7 Hz, 2H), 1.35 (dt, J = 7.1, 0.5 Hz, 6H); ^{13}C NMR δ 165.0 (d, J_{CP} = 6.1 Hz), 62.3 (d, J_{CP} = 6.1 Hz), 59.0, 52.1 (d, J_{CP} = 6.4 Hz), 33.7 (d, J_{CP} = 134.5 Hz), 24.4 (d, J_{CP} = 140.4 Hz), 15.9 (d, J_{CP} = 6.1 Hz); ^{31}P NMR δ 29.6, 19.9. Anal. calcd for $C_{10}H_{22}O_8P_2$: C, 36.14; H, 6.68. Found: C, 36.36; H, 6.73.

2-(Dimethoxyphosphinyl)ethyl (*E,E*)-5,9-dimethyl-2,4,8-decatrienoate (2). To a suspension of KH (42 mg, 1.05 mmol) in THF (2 mL), at 0 °C was added via cannula bisphosphonate **9** (352 mg, 1.06 mmol) in THF (2 mL). After the solution was stirred for 20 min, geranial (**4**, 134 mg, 0.880 mmol) in THF (1.5 mL) was added via cannula. The reaction mixture was stirred at 0 °C for an additional 10 min, allowed to warm to rt and stirred for 12 h. The reaction was quenched by addition of 1 M acetic acid in ether (1.1 mL) and concentrated in vacuo to afford a yellow oil. Purification by flash column chromatography (ethyl acetate) gave compound **2** as a clear oil (250 mg, 86%): ^{1}H NMR δ 7.61 (dd, J = 15.1, 11.6 Hz, 1H), 6.00 (d, J = 11.6 Hz, 1H), 5.77 (d, J = 15.1 Hz, 1H), 5.11–5.02 (m, 1H), 4.38 (dt, J = 13.1, 7.5 Hz, 2H), 3.77 (d, J_{HP} = 11.0 Hz, 6H), 2.22 (dt, J = 18.8, 7.5 Hz, 2H), 2.12–2.18 (m, 4H), 1.90 (d, J = 1.0 Hz, 3H), 1.68 (s, 3H), 1.60 (s, 3H); ^{13}C NMR δ 167.0, 150.4, 141.6, 132.1, 123.1, 123.0, 117.9, 57.9 (d, J_{CP} = 1.2 Hz), 52.3 (d, J_{CP} = 6.4 Hz), 40.1, 26.1, 25.5, 25.0 (d, J_{CP} = 140.4 Hz), 17.5, 17.2; ^{31}P NMR δ 30.5; HRMS calcd for $C_{16}H_{28}O_5P$ $(M+H)^+$ 331.1674, found 331.1698.

2-(Dimethoxyphosphinyl)ethyl (*E*)-5-methyl-2,4-hexadienoate (13). 3-Methyl-2-butenal (114 mg, 1.36 mmol) was treated with bisphosphonate **9** (519 mg, 1.56 mmol) and KH (63 mg, 1.57 mmol) in THF as described for compound **2**. After 12 h, standard work up and purification by flash column chromatography (95:5 ethyl acetate:methanol) gave compound **13** as a clear oil (328 mg, 92%): ^{1}H NMR δ 7.59 (dd, J = 15.2, 11.7 Hz, 1H), 6.00 (dd, J = 11.7, 0.8 Hz, 1H), 5.75 (d, J = 15.2, 1H), 4.38 (dt, J = 13.0, 7.3 Hz, 2H), 3.77 (d, J = 11.0 Hz, 6H), 2.22 (dt, J = 18.7, 7.5 Hz, 2H), 1.90 (s, 3H), 1.89 (s, 3H); ^{13}C NMR δ 166.9, 146.8, 141.5, 123.4, 117.5, 57.9 (d, J_{CP} = 0.9 Hz), 52.2 (d, J_{CP} = 6.4 Hz), 26.3, 24.9 (d, J_{CP} = 140.4 Hz), 18.7; ^{31}P NMR δ 30.5; HRMS calcd for $C_{11}H_{19}O_5PNa$ $(M+Na)^+$ 285.0868, found 285.0866.

2-(Dimethoxyphosphinyl)ethyl (*E,E,E*)-5,9,13-trimethyl-2,4,8,12-tetradecatetraenoate (14). Farnesal (**12**, 187 mg, 0.848 mmol) was treated with bisphosphonate **9** (338 mg, 1.02 mmol) and KH (41 mg, 1.02 mmol) as described above for compound **2**. After 6 h, standard work up and purification by flash column chromatography (98:2 ethyl acetate:methanol) gave compound **14** as a clear oil (257 mg, 76%): ^{1}H NMR δ 7.61 (dd, J = 15.1, 11.6 Hz, 1H), 6.00 (d, J = 11.6 Hz, 1H), 5.77 (d, J = 15.1 Hz, 1H), 5.03–5.13 (m, 2H), 4.38 (dt, J = 13.0, 7.3 Hz, 2H), 3.77 (d, J_{HP} = 11.0 Hz, 6H), 2.22 (dt, J = 18.8, 7.5 Hz, 2H), 2.13–2.19 (m, 4H), 1.94–2.10 (m, 4H) 1.90 (s, 3H), 1.67 (s, 3H), 1.60 (s, 6H); ^{13}C NMR δ 166.9, 150.3, 141.6, 135.7, 131.1, 124.0, 123.0, 122.9, 117.8, 57.9, 52.2 (d, J_{CP} = 6.4 Hz), 40.1, 39.5, 26.5, 26.0, 25.5, 24.9 (d, J_{CP} = 140.3 Hz), 17.5, 17.2, 15.8; ^{31}P NMR δ 30.6; HRMS calculated for $C_{21}H_{36}O_5P$ $(M+H)^+$ 399.2300, found 399.2325.

(*E,E*)-2-[(5,9-Dimethyl-1-oxo-2,4,8-decatrienyl)ethoxy]-phosphonic acid (3). Trimethylsilyl bromide (98 μL, 0.74 mmol) was added to a stirred solution of phosphonate **2** (85 mg, 0.26 mmol) and 2,4,6-collidine (98 μL, 0.74 mmol) in dichloromethane (4 mL) at 0 °C and the resulting solution was stirred for 30 min and then at room temperature for 6 h. The solution was diluted with toluene (5 mL) followed by removal of solvents in vacuo. The white residue was dissolved in water (3 mL) and allowed to stir at room temperature. After this mixture was stirred for 8 days, the solution was adjusted to pH 1 through addition of 1 M HCl, and extracted with ethyl acetate (3×20 mL). All organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo to yield phosphonic acid **3** as a yellow oil (66 mg, 86%): ^{1}H NMR δ 9.60–10.40 (br s, 2H), 7.59 (dd, J = 15.0, 11.7 Hz, 1H), 5.98 (d, J = 11.7 Hz, 1H), 5.77 (d, J = 15.0 Hz, 1H), 5.02–5.13 (m, 1H), 4.30–4.49 (m, 2H), 2.10–2.30 (m, 6H), 1.88 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H); ^{31}P NMR δ 29.6; HRMS calcd for $C_{14}H_{22}O_5P$ $(M-H)^-$ 301.1205, found 301.1202.

(*E*)-2-[(5-Methyl-1-oxo-2,4-hexadienyl)ethoxy]phosphonic acid (15). Phosphonate **13** (72 mg, 0.28 mmol) was treated with TMSBr (80 μL, 0.61 mmol) and 2,4,6-collidine (80 μL, 0.61 mmol) as described for compound **3**. After the resulting solid was dissolved in water, the mixture was stirred at rt for 72 h, the solution was adjusted to pH 1 through addition of 1 M HCl, and extracted with ethyl acetate (3×15 mL). All organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo to yield phosphonic acid **15** as a yellow oil (51 mg, 79%): ^{1}H NMR δ 9.00–10.00 (br s, 2H), 7.55 (dd, J = 15.1, 11.9 Hz, 1H), 5.96 (d, J = 11.9 Hz, 1H), 5.73 (d, J = 15.1 Hz, 1H), 4.30–4.50 (br s, 2H), 2.10–2.35 (br s, 2H), 1.87 (s, 3H), 1.86 (s, 3H); ^{31}P NMR δ 28.7; HRMS calcd for $C_9H_{14}O_5P$ $(M-H)^-$ 233.0579, found 233.0560.

(*E,E,E*)-2-[(5,9,13-Trimethyl-1-oxo-2,4,8,12-tetradecatetraenyl)ethoxy]phosphonic acid (16). Phosphonate **14** (95 mg, 0.24 mmol) was treated with TMSBr (70 μL, 0.53 mmol) and 2,4,6-collidine (70 μL, 0.53 mmol) as described for compound **3**. After the resulting solid was dissolved in water, this mixture was stirred for 9 days, the solution was adjusted to pH 1 through addition of 1 M HCl, and extracted with ethyl acetate (3×20 mL). All organic layers were combined, dried over $MgSO_4$, filtered, and concentrated in vacuo to yield phosphonic acid **16** as a yellow oil (76 mg, 86%): ^{1}H NMR δ 9.40–11.40 (br s, 2H), 7.59 (dd, J = 15.0, 11.0 Hz, 1H), 5.98 (d J = 11.0 Hz, 1H), 5.77 (d, J = 15.0 Hz, 1H), 5.03–5.14 (m, 2H), 4.28–4.52 (m, 2H), 1.85–2.30 (m, 13H), 1.67 (s, 3H), 1.59 (s, 6H); ^{31}P NMR δ 29.3; HRMS calcd for $C_{19}H_{30}O_5P$ $(M-H)^-$ 369.1831, found 369.1847.

Ethyl 5-methyl-4-hexenoate (20).[25] To a solution of ethyl acetate (1.6 mL, 16.4 mmol) and copper(I) iodide (6.10 g, 32.0 mmol) in THF (60 mL) at −110 °C (bath temperature) was added a solution of LDA (made by addition of *n*-butyllithium (12.6 mL, 1.3 M in hexane, 16.4 mmol) to a solution of diisopropylamine (2.3 mL, 16.4 mmol) in THF (20 mL) at 0 °C and stirred for 30 min) via cannula. The reaction mixture was allowed to warm to −30 °C over 2 h, followed by addition of 4-bromo-2-methyl-2-butene (1.19 g, 8.0 mmol) in THF (20 mL) via cannula. After the reaction was stirred at −30 °C for 1 h, it was quenched by addition of satd ammonium chloride (40 mL) and allowed to warm to rt. The mixture was extracted with hexane (3×60 mL), the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford an orange oil. Purification by flash column chromatography (97:3 hexane:ethyl acetate) gave ester **20** as a clear oil (0.92 g, 73%):[25] ^{1}H NMR δ 5.04–5.14 (m, 1H), 4.12 (q, J= 7.1 Hz, 2H), 2.27–2.33 (m, 4H), 1.68 (d, J=0.6 Hz, 3H), 1.62 (d, J=0.7 Hz, 3H), 1.25 (t, J=7.1 Hz, 3H); ^{13}C NMR δ 173.2, 132.7, 122.4, 60.0, 34.4, 25.5, 23.5, 17.4, 14.1; EIMS m/z (relative intensity) 156 (M^+, 57), 85 (52), 82 (100), 69 (100), 67 (38), 55 (34).

Ethyl (*E*)-5,9-dimethyl-4,8-decadienoate (21).[20,25a] A solution of ethyl acetate (1.6 mL, 16.4 mmol) and copper(I) iodide (6.10 g, 32.0 mmol) in THF (60 mL) was treated with LDA and geranyl bromide (**18**, 1.74 g, 8.0 mmol) as described for compound **20**. A parallel work up and final purification by flash column chromatography (97:3 hexane:ethyl acetate) gave ester **21** as a clear oil (1.51 g, 84%):[25a] ^{1}H NMR δ 5.03–5.14 (m, 2H), 4.12 (q, J= 7.2 Hz, 2H), 2.28–2.35 (m, 4H), 1.93–2.11 (m, 4H), 1.67 (d, J=1.0 Hz, 3H), 1.62 (d, J=1.1 Hz, 3H), 1.59 (d, J= 0.3 Hz, 3H), 1.25 (t, J=7.2 Hz, 3H); ^{13}C NMR δ 173.2, 136.4, 131.2, 124.1, 122.3, 60.0, 39.5, 34.4, 26.5, 25.5, 23.5, 17.5, 15.8, 14.1; EIMS m/z (relative intensity) 224 (M^+, 2), 181 (54), 109 (43), 81 (43), 69 (100).

Ethyl (*E,E*)-5,9,13-trimethyl-4,8,12-tetradecatrienoate (22).[25a,26] A solution of ethyl acetate (1.6 mL, 16.4 mmol) and copper(I) iodide (6.10 g, 32.0 mmol) in THF (60 mL) was treated with LDA and farnesyl bromide (2.28 g, 8.00 mmol) as described for compound **20**. Parallel work up and final purification by flash column chromatography (97:3 hexane:ethyl acetate) gave ester **22** as a clear oil (2.19 g, 94%):[25a] ^{1}H NMR δ 5.05–5.15 (m, 3H), 4.12 (q, J=7.2 Hz, 2H), 2.28–2.34 (m, 4H), 1.93–2.12 (m, 8H), 1.67 (d, J=0.9 Hz, 3H), 1.62 (d, J=0.9 Hz, 3H), 1.59 (s, 6H), 1.25 (t, J=7.2 Hz, 3H); ^{13}C NMR δ 173.2, 136.4, 134.8, 131.0, 124.3, 124.0, 122.3, 60.0, 39.6, 39.5, 34.4, 26.6, 26.4, 25.5, 23.5, 17.5, 15.8, 15.8, 14.1; EIMS m/z (relative intensity) 292 (M^+, 2), 136 (27), 135 (24), 81 (43), 69 (100).

5-Methyl-4-hexenoic acid (23).[27] To ester **20** (837 mg, 5.36 mmol) in ethanol (40 mL) was added 2 M NaOH (4.3 mL, 8.57 mmol), and the mixture was heated to reflux. After heating for 6 h, the mixture was allowed to cool to rt and all volatiles were removed in vacuo. After the addition of water (50 mL), the solution was acidified to pH 1 with 1 M HCl and extracted with ether (3×25 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo to afford acid **23** as a clear oil (673 mg, 98%):[27] ^{1}H NMR ($CDCl_3$) δ 11.05–11.40 (br s, 1H), 5.06–5.16 (m, 1H), 2.26–2.41 (m, 4H), 1.69 (d, J=0.9 Hz, 3H), 1.62 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 180.0, 133.2, 122.1, 34.2, 25.5, 23.2, 17.4; EIMS m/z (relative intensity) 128 (M^+, 42), 82 (18), 69 (100), 67 (30), 55 (19).

(*E*)-5,9-Dimethyl-4,8-decadienoic acid (24).[28] To ester **21** (1.30 g, 5.79 mmol) in ethanol (35 mL) was added 2 M NaOH (4.6 mL, 9.27 mmol), and the mixture was heated to reflux. After 8 h, the mixture was allowed to cool to room temperature and all volatiles were removed in vacuo. After addition of water (50 mL), the mixture was acidified to pH 1 with 1 M HCl and extracted with ethyl acetate (3×50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford acid **24** as a clear, colorless oil (1.11 g, 97%): ^{1}H NMR δ 11.25–11.64 (br s, 1H), 5.03–5.17 (m, 2H), 2.27–2.43 (m, 4H), 1.94–2.11 (m, 4H), 1.67 (d, J= 0.9 Hz, 3H), 1.62 (d, J=1.2 Hz, 3H), 1.59 (d, J=0.3 Hz, 3H); ^{13}C NMR δ 180.0, 136.8, 131.2, 124.1, 122.0, 39.5, 34.2, 26.5, 25.5, 23.2, 17.5, 15.8; EIMS m/z (relative intensity) 196 (M^+, 1), 153 (32), 85 (19), 69 (100).

(*E,E*)-5,9,13-Trimethyl-4,8,12-tetradecatrienoic acid (25).[28a] To ester **22** (2.15 g, 7.33 mmol) in ethanol (45 mL) was added 2 M NaOH (5.9 mL, 11.8 mmol), and the mixture was heated to reflux. After 6 h, the mixture was allowed to cool to room temperature and all volatiles were removed in vacuo. After addition of water (50 mL), the mixture was acidified to pH 1 with 1 M HCl and extracted with ethyl acetate (3×25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford acid **25** as a clear oil (1.85 g, 96%): ^{1}H NMR δ 11.55–11.82 (br s, 1H), 5.05–5.18 (m, 3H), 2.27–2.41 (m, 4H), 1.93–2.13 (m, 8H), 1.67 (d, J=0.9 Hz, 3H), 1.62 (d, J=0.9 Hz, 3H), 1.59 (s, 6H); ^{13}C NMR δ 180.0, 136.9, 134.9, 131.1, 124.3, 124.0, 121.9, 39.6, 39.5, 34.3, 26.7, 26.5, 25.6, 23.2, 17.5, 15.9, 15.9; EIMS m/z (relative intensity) 264 (M^+, 0.4), 136 (19), 81 (38), 69 (100), 67 (15).

2-(Dimethoxyphosphinyl)ethyl 5-methyl-4-hexenoate (26). A catalytic amount of DMAP (32 mg, 0.26 mmol) was added to a solution of acid **23** (300 mg, 2.34 mmol), phosphonate **8** (320 μL, 2.57 mmol), and DCC (537 mg, 2.60 mmol) in dichloromethane (24 mL). After 6 h, precipitated dicyclohexylurea was removed by filtration through Celite, and the urea cake was washed with hexane. The filtrate was washed successively with 1 M HCl (50 mL), satd $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a clear oil. Purification by flash column chromatography (97:3 ethyl acetate: methanol) gave compound **26** as a clear oil (474 mg, 77%): ^{1}H NMR δ 5.04–5.12 (m, 1H), 4.30 (dt, J=13.0, 7.4 Hz, 2H), 3.76 (d, J=11.0 Hz, 6H), 2.27–2.38 (m, 4H), 2.17 (dt, J=18.9, 7.4 Hz, 2H), 1.68 (s, 3H), 1.62 (s, 3H); ^{13}C NMR δ 172.5, 132.7, 121.9, 57.9, 52.0 (d, J_{CP}=6.1 Hz), 33.9, 25.2, 24.7 (d, J_{CP}=140.4 Hz), 23.1, 17.2; ^{31}P NMR δ 30.3. Anal. calcd for $C_{11}H_{21}O_5P$: C, 49.98; H, 8.01. Found C, 49.78; H, 7.92.

2-(Dimethoxyphosphinyl)ethyl *(E)*-5,9-dimethyl-4,8-decadienoate (27). A solution of acid **24** (165 mg, 0.84 mmol), phosphonate **8** (116 μL, 0.931 mmol), DCC (191 mg, 0.926 mmol) and DMAP (11 mg, 0.09 mmol) in dichloromethane (8.4 mL) was allowed to react as described for compound **26**. After 4 h, standard work up and final purification by flash column chromatography (ethyl acetate) gave compound **27** as a clear oil (224 mg, 80%): ^{1}H NMR δ 5.03–5.14 (m, 2H), 4.30 (dt, J = 12.8, 7.4 Hz, 2H), 3.76 (d, J = 10.9 Hz, 6H), 2.29–2.37 (m, 4H), 2.17 (dt, J = 18.8, 7.4 Hz, 2H), 1.93–2.10 (m, 4H), 1.67 (d, J = 0.9 Hz, 3H), 1.62 (d, J = 0.9 Hz, 3H), 1.60 (s, 3H); ^{13}C NMR δ 172.7, 136.5, 131.1, 123.9, 121.9, 58.0, 52.2 (d, J_{CP} = 6.7 Hz), 39.4, 34.1, 26.4, 25.4, 24.8 (d, J_{CP} = 141.0 Hz), 23.2, 17.4, 15.7; ^{31}P NMR δ 30.3. Anal. calcd for $C_{16}H_{29}O_5P$: C, 57.80; H, 8.80. Found C, 57.80; H, 8.84.

2-(Dimethoxyphosphinyl)ethyl (*E,E*)-5,9,13-trimethyl-4,8,12-tetradecatrienoate (28). A solution of acid **25** (500 mg, 1.89 mmol), phosphonate **8** (270 μL, 2.17 mmol), and DCC (429 mg, 2.08 mmol) and DMAP (25 mg, 0.20 mmol) in dichloromethane (8.4 mL) was allowed to react as described for compound **26**. After 4 h, standard work up and final purification by flash column chromatography (ethyl acetate) gave compound **28** as a clear oil (647 mg, 85%): ^{1}H NMR δ 5.05–5.13 (m, 3H), 4.30 (dt, J = 12.8, 7.4 Hz, 2H), 3.76 (d, J = 11.0 Hz, 6H), 2.29–2.37 (m, 4H), 2.16 (dt, J = 18.9, 7.4 Hz, 2H), 1.93–2.10 (m, 8H), 1.67 (d, J = 0.9 Hz, 3H), 1.62 (d, J = 1.0 Hz, 3H), 1.59 (s, 6H); ^{13}C NMR δ 172.5, 136.4, 134.6, 130.7, 124.0, 123.7, 121.8, 57.8, 52.0 (d, J_{CP} = 6.7 Hz), 39.3, 39.2, 33.9, 26.4, 26.1, 25.3, 24.7 (d, J_{CP} = 140.4 Hz), 23.0, 17.3, 15.6, 15.6; ^{31}P NMR δ 30.2. Anal. calcd for $C_{21}H_{37}O_5P$: C, 62.96; H, 9.32. Found C, 62.71; H, 9.35.

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