1,1-Bisphosphonate Squalene Synthase Inhibitors: Interplay Between the Isoprenoid Subunit and the Diphosphate Surrogate

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Received March 16, 1995[®]

Inhibitors of squalene synthase have the potential to be superior cholesterol-lowering agents. We previously disclosed that lipophilic 1,1-bisphosphonates **I** are potent squalene synthase inhibitors and orally active cholesterol-lowering agents in animal models (Ciosek, C. P., Jr.; *et al. J. Biol. Chem.* **1993**, *268*, 24832-24837). In this paper, we describe modifications to the bisphosphonate moiety, in an attempt to reduce the number of acidic functions contained in these inhibitors. Replacing one of the acidic groups with a methyl (**II,** $R^2 = CH_3$) results in potent inhibitors when paired with a close mimic of the naturally occurring farnesyl moiety (R^1 = farnesylethyl) but not when paired with the shorter isoprene surrogates (R^1 = geranylethyl or 4-biphenylpropyl). In contrast, all three corresponding bisphosphonates **I** are potent squalene synthase inhibitors. Inhibitory potency is recovered with the shorter isoprene surrogates when R^2 is CH_2OH or CH_2OCH_3. It is proposed that these R^2 groups serve as hydrogen bond acceptors with the active site of the enzyme. The properties of these compounds as cholesterol biosynthesis inhibitors in rats are described, and synthetic routes to these and related compounds are detailed.

Squalene synthase[1] inhibition has been the focus of intensive effort in the search for new lipid-lowering agents. Current interest in squalene synthase inhibitors (SSIs) stems from two major factors: the success of the HMG-CoA reductase inhibitors[2] (HRIs) in human therapy and the realization that squalene synthase occurs, in theory, at the optimal juncture for interference with the cholesterol biosynthetic pathway.[3] The clinical results with the HRI natural products[1] demonstrate that good efficacy and safety profiles are achievable with cholesterol biosynthesis inhibitors. The location of squalene synthase at the final branch point of the isoprene biosynthesis pathway means that selective inhibition of the enzyme would not result in a decrease in the synthetic rate of the non-sterol metabolites of the pathway.[3] In this article, we describe a portion of our efforts on the discovery and evaluation of bisphosphonate squalene synthase inhibitors (structures I and II).[4,5]

HO–P(=O)(OH)–CH(R^1)–PO_3H_2 (**I**) R^2–P(=O)(OH)–CH(R^1)–PO_3H_2 (**II**)

Several classes of squalene synthase inhibitors have been described. Poulter and co-workers[1e] reported several ammonium ion mimetics of the carbocationic intermediates in the conversion of presqualene disphosphate to squalene. For significant inhibition to be observed, these analogs require either coincubation with high concentrations of inorganic diphosphate (PP_i) or covalent tethering to a diphosphate moiety. These results support the hypothesis that the squalene synthase reaction proceeds through carbocation–diphosphate ion pairs. In addition, the strict requirement for the presence of diphosphate, or a surrogate thereof, illustrates the critical role of the diphosphate in the binding of the reaction intermediates to the enzyme. In 1979, Bertolino[6] reported that substituted farnesylamines inhibit the enzyme at micromolar concentrations. The effect of added PP_i was not studied, and the inhibition by these amines was proposed to be nonspecific. Recently, Prashad[1d,7] developed the farnesylamine (and related amidine) series further, arriving at SSIs which are reported to express significant activity even in the absence of inorganic diphosphate. Several reports in the patent literature disclose lipophilic quinuclidine SSIs,[1d,8] which are likely to function as ion pairs with PP_i. In addition, three groups have published mimetics of a putative reactive intermediate in the conversion of farnesyl diphosphate (FPP) to presqualene diphosphate, which also relies on the ammonium (or sulfonium) ion analogy to carbocations.[7a,9] No significant *in vivo* actvity has been disclosed for this class of inhibitors.

A second class of SSIs relies on interactions with the enzyme via multiple acidic groups which serve as stable mimetics of the diphosphate function.[10] By incorporating a surrogate for the diphosphate as part of their covalent structures, these inhibitors do not require coincubation with PP_i, as do most of the cationic amine SSIs. Notable members of this class include the tricarboxylate fungal metabolites: the squalestatins/zaragozic acids.[11] These natural products are potent SSIs both *in vitro* and *in vivo*. Extensive structure–function studies reveal that activity is maintained with certain dicarboxylate analogs of the natural products.[11c–e] In addition, recent patent applications from Takeda and Banyu disclose interesting carboxylate SSIs.[12]

Our work has focused on rationally designed polyacidic SSIs based on the structure of FPP and putative transition states and reactive intermediates derived

® Abstract published in *Advance ACS Abstracts*, June 1, 1995.

0022-2623/95/1838-2596$09.00/0 © 1995 American Chemical Society

(a) FPP in the Donor Site

(b) Allyl Cation - Diphosphate Ion Pair

(c) Inhibitor 1c

Figure 1. Design of squalene synthase inhibitors. Bisphosphonate inhibitors (c) were designed to take advantage of interactions with proposed active site acid catalyst(s) along the reaction coordinate from substrate FPP (a) to the allyl cation–diphosphate ion pair (b).

therefrom.[3,13] We recently reported that certain lipophilic bisphosphonates are potent SSIs and effective cholesterol-lowering agents *in vivo*.[3] Farnesylethyl bisphosphonate **1c** (Figure 1c) was designed as a mimetic of the ion pair indicated in Figure 1b, which is a proposed high-energy intermediate formed from FPP (Figure 1a) in the prenyl donor site of squalene synthase.[1] Ion pair **1b** reacts with the second molecule of FPP in the prenyl acceptor site to form presqualene diphosphate. Compound **1c** was found to be a potent inhibitor of both rat and human liver microsomal squalene synthase.

We subsequently discovered that the farnesylethyl subunit of **1c** could be replaced with a wide variety of lipophilic surrogates. This structural flexibility is consistent with the recent report that certain known bisphosphonate inhibitors of bone resorption have activity against squalene synthase.[5] We report in this article a detailed study of modifications to the 1,1-bisphosphonate moiety, with an emphasis on the interplay between the diphosphate surrogate and the lipophilic subunit of the inhibitors.

Results and Discussion

***In Vitro* Activity.** We reported[3] earlier that farnesyl bisphosphonates **1a** and **1c**, as well as geranylethyl bisphosphonate **2c**, are potent inhibitors of rat liver microsomal squalene synthase (Table 1). Further studies reveal a relatively modest effect of the length of the alkylene chain connecting the isoprenyl and bisphosphonate moieties of **1** and **2** on squalene synthase inhibitory potency. In both the farnesyl (**1**) and geranyl (**2**) series, the shorter linker lengths are detrimental to activity (**1a, 2a,b**). With the exclusion of these three analogs, the potencies of the bisphosphonates in Table 1 vary by only 18-fold (IC_{50} = 1–18 nM). This is a surprising result, considering the difference in overall length between **2c** and **1f** is 8.7 Å in a fully extended conformation. Activity falls precipitously, however, with the dimethylallyl-derived bisphosphonate **3**.

While 1,1-bisphosphonates are effective squalene synthase inhibitors, they possess several attributes which render them suboptimal as cholesterol-lowering agents. These characteristics are common to the established bisphosphonate drugs such as etidronate,[5,14] which are clinically effective agents for the treatment of diseases involving bone resorption. The antiresorptive agents generally have poor oral absorption, which is attributable to the polyacidic bisphosphonate function. In addition, the highly charged nature of this pharmacophore contributes to the high affinity of bisphosphonates to calcified tissue.

Table 1. Biological Evaluation of Isoprenoid Bisphosphonates

compd	*n*	squal syn IC_{50} (nM) [95% confidence interval]	rat model ED_{50} (mg/kg) iv [SEM]	po [SEM]
1a	0	32.4 [24, 44]		
1c	2	5.7 [4.7, 6.9]	0.04 [0.01]	inactive at 30
1f	5	11.8 [9, 15]	0.13 [0.02]	26% inhibition at 15
2a	0	35.5 [22, 58]		inactive at 15
2b	1	77.7 [61, 98]		
2c	2	1.0 [0.5, 1.7]	0.05 [0.003]	0.43 [0.19]
2d	3	2.6 [1.7, 3.6]	0.11 [0.009]	0.71 [0.48]
2e	4	2.5 [1.7, 3.7]	0.04[a]	1.1 [0.20]
2f	5	17.6 [14, 22]		25% inhibition at 30
2g	6	4.2 [3.3, 5.3]	0.05 [0.01]	inactive at 30
3	2	6600 [4000, 10800]		

[a] Data based upon one determination.

In an effort to reduce the bone affinity and improve the oral absorption of bisphosphonate squalene synthase inhibitors, we examined replacing one of the acidic functions with neutral groups. In the farnesyl series **4** (Table 2), replacing one of the phosphonic acid hydroxyls with a methyl group to provide an α-phosphono phosphinate results in activity levels similar to that observed for the corresponding bisphosphonate (compare **4a** to **1a** and **4c** to **1c**, Table 2). In the geranyl series **5**, a very different relationship between inhibitory activity and alkylene linker length is observed. Although **2c** is the most potent isoprenyl bisphosphonate (IC_{50} = 1 nM), the corresponding phosphinate **5c** is 80-fold less active. Over the series of **5a–f**, the P-Me analogs are $^1/_{12}$–$^1/_{250}$ as active as the corresponding bisphosphonates **2a–f**. In contrast, geranylhexyl phosphinate **5g**, which has the same overall length as the farnesylethyl analog **4c**, has potency that is comparable to that of the latter, as well as to that of the corresponding bisphosphonates **1c** and **2g**. Thus, the potency of the P-CH_3 phosphinates **5a–g** is much more sensitive to the structure of the isoprenyl subunit than is the potency of the bisphosphonates.

The corresponding P-CH_2OH and P-CH_2OCH_3 phosphinates were prepared to examine whether the acidic function can be adequately replaced with a neutral group that could participate as a hydrogen bond acceptor and/or donor (Table 3). Both modifications in the

Table 2. Biological Evaluation of Isoprenoid *P*-Methyl α-Phosphono Phosphinates

4a, 4c, 4d

5a, 5c-5g

compd	*n*	squal syn IC_{50} (nM) [95% confidence interval]
4a	0	105.9 [88, 127]
4c	2	12.9[a] [10, 16]
4d	3	6.5[b] [5, 9]
5a	0	8600 [6000, 12000]
5c	2	81[c] [53, 124]
5d	3	605 [483, 758]
5e	4	30.7[d] [20, 47]
5f	5	374 [235, 593]
5g	6	8.7 [7.2, 9.9]

[a-d] Rat model ED_{50} values (mg/kg): (a) iv, 0.10, po, inactive at 30; (b) po, inactive at 15 (1 h); (c) iv, 1.4, po, 23% inhibition at 10 (1 h); (d) po, 48% inhibition at 10. For b and c po, drug was dosed 1 h before [^{14}C]acetate administration.

farnesylethyl series result in potent inhibitors (**8** and **11**), with comparable activity to the corresponding bisphosphonate **1c** and P-CH_3 phosphinate **4c**. In the geranylethyl series, the hydroxymethyl and methoxymethyl substitutions result in significant improvements in potency relative to the corresponding P-CH_3 derivatives. The biphenylpropyl series follows the same trend, but the effects are more dramatic. The P-CH_3 phosphinate **7** is 700-fold less potent than the corresponding bisphosphonate **6**, whereas the methoxymethyl analog **13** is only 20-fold less potent. Substituting a carboxylate for one of the phosphonates (**15**–**17**, Table 4) fails to yield good inhibitors with all three lipophilic subunit prototypes, indicating that a tetrahedral geometry is required for the acidic function. This result stands in contrast to the zaragozic acids, where an array of carboxylate residues provides strong binding to the enzyme, and suggests that the particular structural context in which the carboxylates appear is critical.

The divergent results observed for the bisphosphonates and α-phosphono phosphinates with respect to the structure of the lipophilic subunits may be due to the two inhibitor classes binding to different sites on the enzyme. Squalene synthase has two distinct FPP-binding sites,[1] one for the prenyl donor and the other for the prenyl acceptor involved in the first step of the enzymatic mechanism (FPP + FPP to presqualene diphosphate). The donor and acceptor FPP molecules can be distinguished through labeling studies[15] (the donor FPP loses the 1-S-proton). In addition, two groups[16] have reported that certain FPP analogs with modified farnesyl groups are incorporated specifically as prenyl acceptors but not as donors. Recently, Mookhtiar[17] and co-workers have utilized kinetic methods with the homogeneous, soluble yeast enzyme to distinguish between the two FPP-binding sites.

Alternatively, it is possible that the bisphosphonate is a near optimum surrogate for the diphosphate in binding to squalene synthase, whereas the α-phosphono phosphinates are suboptimal. As a near perfect surrogate, the bisphosphonate is less particular with respect to the structure of the lipophilic subunit tethered to it, as long as the latter has a lipophilicity and shape that are within an acceptible range (**3**, for example, falls out of that range). The *P*-methylphosphinate moiety of **4** and **5** is suboptimal because one of the acidic functions is deleted and the methyl group is unable to interact with the enzyme. Therefore, this diphosphate surrogate must be paired with an optimal isoprene subunit (e.g., farnesylethyl) in order to exhibit maximal potency. Subtituting the *P*-methyl group with a methoxyl provides an H-bond acceptor which partially compensates for the loss of the acidic function and thus more closely mimics diphosphate. As a result, activity is recouped by pairing the suboptimal isoprene surrogates (geranylethyl and biphenylpropyl) with the (methoxymethyl)phosphinate. Our earlier observation[13] that the activity of **18** (rat squalene synthase IC_{50} = 48 nM) is dependent on the presence of the allylic ether function is consistent with this analysis. We have proposed that the ether function of **18** participates as a hydrogen bond acceptor with a group at the active site of squalene synthase. A similar role can be proposed for the methoxyl group of **11**–**13**. This hypothesis does not require the bisphosphonates and α-phosphono phosphinates to bind to different sites on the enzyme.

Table 3. Biological Evaluation of Isoprenoid and Biphenyl Bisphosphorus Derivatives

R	rat squal syn IC_{50} (nM) [95% confidence interval]				
	X = –OH	X = –CH_3	X = –CH_2OH	X = –CH_2OCH_3	X = –H
farnesylethyl	5.7[a] [4.7, 6.9] **1c**	12.9[a] [10.3, 16.1] **4c**	4.7 [3.4, 6.5] **8**	6.5[e] [1.8, 22] **11**	
geranylethyl	1[a] [0.8, 1.2] **2c**	81[a] [53, 124] **5c**	38.7[c] [24.9, 60] **9**	13.1[f] [9.3, 18.5] **12**	4.0[g] [2.7, 5.1] **14**
biphenylpropyl	0.7[b] [0.5, 0.9] **6**	490 [379, 633] **7**	23.9[d] [13.9, 40.8] **10**	14.8 [8.9, 24.5] **13**	

[a] See Tables 1 and 2 for rat model data on **1c**, **2c**, **4c**, and **5c**. [b-g] Rat model ED_{50} values (mg/kg): (b) iv, 0.03, po, 1.0; (c) po, 8.2; (d) iv, 0.18; (e) po, >15 (1 h); (f) po, >10; (g) iv, 0.27, po, >10. For e po, drug was dosed 1 h before [^{14}C]acetate administration.

Scheme 1[a]

19a, R^1 = OEt; 19b, R^1 = CH_3 → Method A (RCH_2I, 20) → 21, R^1 = OEt; 22, R^1 = CH_3 → Method B → 1, 2, 3, 6, R^1 = OM; 4, 5, R^1 = CH_3

[a] Method A: **18** or **19**, NaH, DMF; add **20**, 0 °C to room temperature. Method B: TMSBr, 2,4,6-collidine, CH_2Cl_2, room temperature. M = H, Na, or K.

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The activity observed for the α-phosphono phosphonous acid **14** appears to be in conflict with this analysis, in that it does not bear the additional H-bond-accepting ligand, but a hydrogen instead. However, we have noted that **14** has limited stability to oxidation, and it is possible that the activity noted for **14** is due to partial conversion to **2c**.

***In Vivo* Activity.** The effect of SSIs on the incorporation of [^{14}C]acetate to [^{14}C]cholesterol in rats[3] ("rat model") was utilized as a primary assay for the effectiveness of the compounds *in vivo*. Our early studies[3] revealed that bisphosphonates which are potent squalene SSIs *in vitro* are correspondingly potent on intravenous administration in the rat model. In addition, we found[3] that bisphosphonates bearing "shorter" isoprene subunits, such as **2c** and **6**, exhibit significant activity on oral dosing to fasted rats, whereas the "longer" subunits, as found in **1c**, lead to poor oral activity. These conclusions are supported by our studies with the geranyl series **2**. When the linker length is expanded as in **2c** to **2g**, oral potency is maintained with the shorter analogs (**2c**–**e**), whereas activity is lost with additional carbon atoms in the linker (**2f,g**). Although **2c,g** have identical potencies on iv dosing, **2c** has an oral ED_{50} of 0.43 mg/kg whereas **2g** is inactive at 30 mg/kg. Note that **2g** has the same overall length as **1c**, which is also potent iv but inactive orally. The poor oral activity of the longer, more hydrophobic analogs may be due to the formation of insoluble free acids in the stomach or insoluble salts in the intestine. In our experience, salts of lipophilic bisphosphonates with divalent cations such as calcium and magnesium have poor aqueous solubility.[18] Aqueous solubility is expected to be a prerequisite for absorption of these compounds. The formation of insoluble salts may also be responsible for the lack of activity of even the short-chain analogs on oral dosing to nonfasted rats, which are expected to possess a higher divalent cation concentration in their intestine. In any event, even under fasting conditions where **2c** is expressing potent inhibition of cholesterol biosynthesis upon oral dosing, the oral absorption of **2c** in rats is less than 4%.[19]

In the P-CH_3 series (Table 2), inhibitory activity against squalene synthase is poor with the shorter chain analogs. Unfortunatey, it is the shorter chain analogs that were expected to express good oral activity in the event that squalene synthase inhibitory potency had been maintained. As an example, the most potent short-chain analog **5c** (IC_{50} = 81 nM) exhibits weak potency on iv dosing and is without significant oral activity. Not surprisingly, the longer chain analogs **4c,d**, which are good SSIs *in vitro*, are inactive on po dosing. In addition, the P-CH_2OR substituted α-phosphono phosphinates (**9, 11, 12, 14**) did not show improved oral potency commensurate with their improved activity against squalene synthase.

Table 4. Biological Evaluation of α-Phosphono Carboxylates

R = (**15**, **16**, **17**)

compd	squal syn IC_{50} (nM) [95% confidence interval]
15	880[a] [680, 1100]
16	1650 [1100, 2500]
17	2200 [1500, 3000]

[a] Rat model ED_{50} values (mg/kg): iv, 2.6, po, inactive at 100.

Chemical Synthesis. A general route to 1,1-bisphosphonates and α-phosphono phosphinates (method A) was utilized to prepare the majority of the inhibitors described herein (Scheme 1). The unsubstituted bisphosphorus ester synthons (**19a,b**[20]) were metalated with sodium hydride in DMF, THF, or mixtures thereof and alkylated[21] with a series of alkyl halides to prepare the substituted esters **21** and **22**. Dealkylation of the ester moieties was performed by treatment with excess TMSBr[22] (method B) followed by hydrolysis of the intermediate silyl esters with water or aqueous alkali to produce **1**–**6** as the free acids or salts.

Two additional general routes to the desired targets beginning with the monophosphonate **23** were exploited. Metalation of **23** with *s*-BuLi followed by quenching the anion with excess phosphoryl chloride **24a,b** provided esters **21** or **25**, respectively, in moderate yield (40–50%; method C, Scheme 2). The esters were deprotected as above to provide **2b** and **11**. Alternatively, the carbanion derived from **23** was quenched with excess diethyl chlorophosphite to provide an intermediate phosphonous diethyl ester (C-P(OEt)$_2$),[23] which was hydrolyzed *in situ* to provide **26** (method D, Scheme 3). Monoester **26** served as a precursor to **14** upon deprotection (method B), as well as to a series of alkylphosphinates, via alkylation of **26** at phosphorus[24] (**22**, method E; **25**, method G; **27**, method F).

α-Phosphono carboxylates **15**–**17** were synthesized in a manner similar to the 1,1-bisphosphonates. Unsubstituted triesters **28a,b** were alkylated according to method A to provide triesters **29a,b** (Scheme 4). The carboxyethyl ester of **29a** was hydrolyzed with alkali, and the remaining phosphonate esters were dealkylated with TMSBr[22] (method H). In the case of *tert*-butyl carboxylate ester **29b**, all three ester groups were removed in a single step with TMSI[25] (method I).

Scheme 2[a]

[a] Method C: s-BuLi, THF, −78 °C; add **24a** or **24b**. Method B: see Scheme 1. M = H or Na.

Scheme 3[a]

[a] Method D: s-BuLi, THF, −78 °C; excess $ClP(OEt)_2$; H_2O. Method E: $NaNTMS_2$, THF, −78 °C; CH_3I. Method F: $(CH_2O)_n$, i-$PrEt_2N$, EtOH, 60 °C. Method G: TMSCl, Et_3N, CH_2Cl_2; $BrCH_2OCH_3$, 0 °C. Method B: see Scheme 1. M = K or Na.

Scheme 4[a]

[a] Method A: **28a** or **28b**, NaH, DMF; add **20**, 0 °C to room temperature. Method H: NaOH, H_2O, EtOH, 55 °C; TMSBr, 2,4,6-colldine, CH_2Cl_2, room temperature. Method I: TMSI, 2,4,6-colldine, CH_2Cl_2, room temperature. M = H, Na, or K.

Conclusions

The studies described above have uncovered interesting interrelationships between the isoprene subunit and the diphosphate surrogate of bisphosphonate and α-phosphono phosphinate SSIs. While activity against squalene synthase is relatively insensitive to the overall length of the isoprenyl subunit in the bisphosphonate series, the P-CH_3 α-phosphono phosphinates are only potent when the length of the isoprenyl subunit approaches that of farnesylethyl (**4c**, **5g**). By attaching H-bond acceptors to the methyl substituent of the shorter chain analogs **5c** and **7** (e.g., **9, 10, 12, 13**), some of the lost potency is recouped. This may be due to an interaction between the additional sustituent and the active site which is compensating for the missing interaction with one of the phosphonic acid hydroxyls. In the case of oral testing in the rat model, however, only the shorter chain bisphosphonates exhibit significant potency and the α-phosphono phosphinates uniformly exhibit poor oral activity. The reason for this observation is currently unknown but may be related to the insolubility[18] of either the free acids or the calcium salts of the more lipophilic inhibitors in the digestive tract.

Experimental Section

Chemical Synthesis and Characterization. The experimental procedures utilized are described in the following examples. The inhibitors were isolated as free acids or hygroscopic sodium or potassium salts in the form of amorphous solid hydrates. The salts were purified either by reversed-phase chromatography on CHP20P (standard procedure, unless noted) or SP207SS gels (Mitsubishi Kasei, White Plains, NY), as described by Biller and Forster,[26] or by trituration of nearly pure, crude oils with acetone. Tables 5−8 display the microanalysis results, FAB (positive ion) mass spectral characterization, and synthetic methods utilized for all compounds tested. In the case of mass spectral data, the M values indicated are masses of the salt forms which were isolated. Typically, a series of positive ions are observed corresponding to M + K(Na), M + H, M + 2H − K(Na), etc. Methods which differ slightly from the standard procedures are indicated as footnotes in Tables 5−8. Unless otherwise noted, 2,4,6-collidine was utilized as an acid trap in the

Table 5. Properties and Synthesis of Bisphosphonates 1−3 and 6

compd no.	anal. (C, H, P)	MS (M)	synth methods (method, yield, %)
1a	$C_{16}H_{27}O_6P_2Na_3 + 1.25H_2O$	446	A, 62; B, 88
1c	$C_{18}H_{31}O_6P_2Na_3 + 0.85H_2O$	474	A, 84; B, 68
1f	$C_{21}H_{37}O_6P_2Na_3 + 1.75H_2O$	516	A,[a] 52; B, 63
2a	$C_{11}H_{19}O_6P_2Na_3 + 0.25H_2O$	378	A,[b] 46; B,[c] 57
2b	$C_{12}H_{21}O_6P_2Na_3 + 0.75H_2O$	392	C, 40; B,[d] 71
2c	$C_{13}H_{22}O_6P_2Na_4 + 2.25H_2O$	428	A, 80; B,[e] 98
2d	$C_{14}H_{25}O_6P_2K_3 + 1.4H_2O$	468	A, 82; B,[d] 92
2e	$C_{15}H_{26}O_6P_2Na_4 + 1.3H_2O$	456	A,[f] 56; B,[e] 69
2f	$C_{16}H_{29}O_6P_2Na_3 + 2.25H_2O$	448	A, 76; B, 78
2g	$C_{17}H_{31}O_6P_2Na_3 + 1.5H_2O$	462	A, 64; B, 77
3	$C_8H_{14}O_6P_2Na_4 + 1.3H_2O$	360	A,[f] 58; B,[e] 93
6[g]	$C_{16}H_{20}O_6P_2 + 0.2H_2O$	370	A, 56; B, 78

[a] Solvent is 2:9 THF/DMF. [b] Alkyl halide is *(E,E)*-farnesyl chloride. [c] Purification by chromatography on Whatman CF11 cellulose eluted with 2:3:1 followed by 2:1:1 2-propanol/CH_3CN/0.1 M NH_4OH followed by AG50W-X8 (Na-form) ion exchange and CHP20P gel chromatography. [d] Purification on SP207SS gel. [e] Isolated by triturating crude oil with acetone. [f] Solvent is THF. [g] Mp = 192 °C.

TMSBr/TMSI deprotections. All chiral compounds were prepared as racemic mixtures.

(*E,E*)-(6,10,14-Trimethyl-5,9,13-pentadecatrienylidene)bis(phosphonic acid), Trisodium Salt, 1c. Method A. A suspension of 132 mg (5.52 mmol) of NaH in 2 mL of dry DMF and 6 mL of dry THF at 0 °C under argon was treated with 1.58 g (5.52 mmol) of tetraethyl methylenediphosphonate (**19a**) over 10 min to give a yellow solution. The reaction mixture was allowed to warm to room temperature and stir for 0.5 h when 0.50 g (1.38 mmol) of iodide **20** (R = farnesylmethyl) was added in one portion. The reaction mixture was stirred for 18 h, and then the reaction was quenched with saturated aqueous NH_4Cl solution and the mixture diluted with ethyl acetate. The organic fraction was washed with water and brine, dried over Na_2SO_4, and evaporated to provide a crude yellow oil. Flash chromatography was performed on 100 g of silica gel eluted with 200 mL of ethyl acetate followed by 1:9 ethanol/ethyl acetate to provide 0.60 g (84%) of intermediate **21** (R = farnesylmethyl) as a pale yellow oil: TLC silica gel (1:9 ethanol/ethyl acetate) R_f = 0.53; ^{1}H NMR ($CDCl_3$) δ 5.15 (m, 3H), 4.12 (quint, 8H, *J* = 7.0 Hz), 2.30 (tt, 1H, *J* = 24.2, 6.0 Hz), 2.10−1.80 (m, 12H), 1.80 (s, 3H), 1.65 (m, 2H), 1.60 (s, 9H), 1.45 (t, 12H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 135.0, 134.4, 130.6, 124.0, 123.8, 123.3, 62.0 (d, *J* = 8.1 Hz), 61.8 (d, *J* = 8.1 Hz), 39.3 (2 C), 36.4 (t, *J* = 132.5 Hz), 28.9 (t, *J* = 6.8 Hz), 27.3, 26.3 (2 C), 25.2, 24.8 (t, *J* = 4.7 Hz), 16.0, 15.9 (d, *J* = 5.5 Hz), 15.6, 15.5.

Method B. To a stirred solution of 0.60 g (1.15 mmol) of **21** (R = farnesylmethyl) in 10 mL of dichloromethane at room temperature was added 0.71 mL (5.36 mmol) of 2,4,6-collidine followed by 0.97 mL (6.99 mmol) of bromotrimethylsilane. The reaction mixture was allowed to stir at room temperature for 6 h when the solvent was evaporated and the semisolid residue pumped under vacuum for 0.5 h. The residue was dissolved by adding 10 mL (5.0 mmol) of 0.5 M NaOH solution, diluted with 15 mL of water, and freeze-dried. The crude white residue was purified by MPLC on a column of CHP20P gel (2.5 cm × 15 cm) eluting with water (150 mL) followed by a gradient created by the gradual addition of 400 mL of acetonitrile to a reservoir of 350 mL of water. The pure fractions were combined, the acetonitrile was evaporated, and the aqueous solution was lyophilized to provide 0.37 g (68%) of **1c** as a white lyophilate: IR (KBr) 3432, 2965, 2924, 2857, 1635, 1449, 1100, 882 cm^{-1}; ^{1}H NMR (D_2O) δ 5.25 (t, 1H, *J* = 7.0 Hz), 5.15 (m, 2H), 2.15−1.90 (m, 10H), 1.70 (m, 3H), 1.63 (s, 3H), 1.58 (s, 3H), 1.53 (s, 6H), 1.50 (m, 2H); MS (FAB) *m/z* 497 (M + Na), 475 (M + H), 453 (M − Na + 2H), 431 (M − 2Na + 3H). Anal. Calcd for $C_{18}H_{31}O_6P_2Na_3$ + 0.85H_2O: C, 44.15; H, 6.73; P, 12.65. Found: C, 43.89; H, 6.64; P, 13.04.

(*E*)-(5,9-Dimethyl-4,8-decadienyl)bis(phosphonic acid), Trisodium Salt, 2b. Method C. To a stirred solution of 0.80 g (2.65 mmol) of phosphonate **23** (R = geranyl) and 10 mL of THF at −78 °C was added 2.45 mL (1.30 M, 3.18 mmol) of *sec*-butyllithium dropwise over 3 min. After 0.3 h at −78 °C, the reaction mixture was quickly added via cannula to a mixture of 0.91 g (5.3 mmol) of diethyl chlorophosphate in 5 mL of THF at −78 °C. The mixture was stirred at −78 °C for 0.5 h and warmed to −40 °C for 1 h. The reaction was quenched with NH_4Cl solution and the mixture diluted with ethyl acetate and washed with aqueous solutions of NH_4Cl, $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil was purified by flash chromatography on 50 g of silica gel eluted with 10:90 ethanol/ethyl acetate to provide 0.47 g (40%) of **21** (R = geranyl) as a colorless oil: TLC silica gel (1:9 ethanol/ethyl acetate) R_f = 0.50; IR (film) 2982, 2932, 1663, 1444, 1391, 1252, 1164, 1027, 1030, 969 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.09 (t, 2H, *J* = 6.5 Hz), 4.10 (m, 8H), 2.25 (m, 3H), 2.00 (m, 6H), 1.68 (s, 3H), 1.62 (s, 3H), 1.60 (s, 3H), 1.34 (t, 12H, *J* = 7.0 Hz); MS (CI-NH_3, + ions) *m/z* 456 (M + NH_4), 439 (M + H).

Method B. To a stirred solution of 0.44 g (1.0 mmol) of **21** (R = geranyl) in 6.0 mL of dichloromethane at 0 °C was added 2.42 g (2.0 mmol) of 2,4,6-collidine followed by 0.76 g (5.0 mmol) of bromotrimethylsilane. The reaction mixture was allowed to stir at room temperature for 13 h when the solvent was evaporated and the semisolid residue pumped under vacuum for 0.5 h. The residue was dissolved in 4.40 mL (4.40 mmol) of 1 N NaOH solution, diluted with 15 mL of water, and freeze-dried. The crude white solid was purified by MPLC on a column of SP207SS gel (2.5 cm × 20 cm) eluted with water (250 mL) followed by a gradient created by the gradual addition of 400 mL of acetonitrile to a reservoir of 350 mL of water. The pure fractions were combined, filtered, and lyophilized to provide 0.28 g (71%) of **2b** as a white lyophilate: IR (KBr) 3402, 2966, 2924, 2858, 1450, 1161, 1089, 881 cm^{-1}; ^{1}H NMR (D_2O) δ 5.20 (t, 1H, *J* = 6.4 Hz), 5.15 (t, 1H, *J* = 7.0 Hz), 2.18 (q, 2H, *J* = 7.3 Hz), 2.10 (m, 2H), 1.98 (m, 2H), 1.80 (2 m, 2H), 1.63 (s, 3H), 1.59 (s + m, 4H), 1.57 (s, 3H); MS (FAB, + ions) *m/z* 393 (M + H), 371 (M − Na + 2H), 349 (M − 2Na + 3H). Anal. Calcd for $C_{12}H_{21}O_6Na_3P_2$ + 0.75H_2O: C, 35.54; H, 5.59; P, 15.27. Found: C, 35.54; H, 5.91; P, 15.42.

Table 6. Properties and Synthesis of *P*-Methyl α-Phosphono Phosphinates 4, 5, and 7

compd no.	anal. (C, H, P)	MS (M)	synth methods (method, yield, %)
4a	$C_{17}H_{30}O_5P_2Na_2 + 0.75H_2O$	422	A, 40; B, 88
4c	$C_{19}H_{34}O_5P_2Na_2 + 0.85H_2O$	450	A, 49; B, 85
4d	$C_{20}H_{36}O_5P_2Na_2 + 0.5H_2O$	464	A, 57; B, 58
5a	$C_{12}H_{21}O_5P_2Na_3 + 0.25H_2O$	376	A,[a] 24; B,[b,c] 84
5c	$C_{14}H_{25}O_5P_2Na_3 + 1.45H_2O$	404	A, 46; B,[d] 83
5d	$C_{15}H_{28}O_5P_2K_2 + 0.8H_2O$	428	A, 50; B,[c] 87
5e	$C_{16}H_{29}O_5P_2Na_3 + 0.25H_2O$	432	A, 47; B,[d] 75
5f	$C_{17}H_{32}O_5P_2Na_2 + 1.0H_2O$	424	A, 57; B, 72
5g	$C_{18}H_{34}O_5P_2K_2 + 1.25H_2O$	470	A, 59; B, 75
7	$C_{17}H_{20}O_5P_2Na_2 + 0.4H_2O$	412	D, 60; E, 79; B,[b,c] 50

[a] solvent is THF. [b] Bis(trimethylsilyl)trifluoroacetamide used as acid trap. [c] Purification on SP207SS gel. [d] Isolated by triturating crude oil with acetone.

(*E*)-[1-(Hydroxymethylphosphinyl)-8,12-dimethyl-7,11-tridecadienyl]phosphonic Acid, Trisodium Salt, 5e. Method A. To a stirred mixture of 117 mg (4.88 mmol) of sodium hydride in 2 mL of DMF at 0 °C under argon was added dropwise, over 5 min, 1.26 g (4.88 mmol) of **19b**[20] in 2 mL of DMF. After 30 min at 0 °C, 390 mg (1.22 mmol) of iodide **20** (R = geranylpropyl) in 2 mL of DMF was added dropwise over 5 min. The mixture was maintained at 0 °C for 1 h followed by room temperature for 18 h, when it was diluted with ether and the reaction quenched with saturated NH_4Cl. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated to provide 410 mg of a crude, pale yellow oil. Flash chromatography was performed on 40 g of silica gel eluted with 49.5:49.5:1 acetone/ethyl acetate/methanol to provide 260 mg (47%) of **22** (R = geranylpropyl) as a pale yellow oil: TLC silica gel (49.5:49.5:1 acetone/ethyl acetate/methanol) R_f = 0.26; IR (CCl_4) 2978, 2928, 2857, 1445, 1385, 1244, 1028, 966 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.10 (m, 2H),

4.17 (m, 6H), 2.20 (m, 1H), 2.05 (m, 2H), 1.97 (m, 6H), 1.68 (s, 3H), 1.68 and 1.66 (2 d, 3H, J = 14.7 Hz), 1.60 (s, 3H), 1.59 (s, 3H), 1.50 (m, 2H), 1.30 (m, 4H), 1.35 (t, 6H, J = 7.0 Hz), 1.33 (t, 3H, J = 7.0 Hz); MS (CI-NH_3) m/z 468 (M + NH_4), 451 (M + H).

Method B. To a stirred solution of 260 mg (0.58 mmol) of **22** (R = geranylpropyl) in 5 mL of dichloromethane at room temperature under argon was added 0.23 mL (1.73 mmol) of 2,4,6-collidine followed by 0.38 mL (2.89 mmol) of bromotrimethylsilane. The reaction mixture was stirred at room temperature for 18 h, the solvent was evaporated, and the residue was pumped under high vacuum for 1 h. The remainder was treated with 1.91 mL (1.91 mmol) of 1 M NaOH and lyophilized. The crude lyophilate was precipitated by dissolving the sample in 5 mL of water, warming to 50 °C, treating the solution with 5 mL of acetone, and placing the cloudy mixture in an ice bath. The precipitate was filtered, and the solid was washed three times with 4:1 acetone/water and once with pure acetone. The amorphous white solid was dried under vacuum to provide 187 mg (75%) of **5e**: IR (KBr) 2926, 2852, 1446, 1377, 1292, 1149, 1091, 1026, 979, 875 cm^{-1}; ^1H NMR (D_2O) δ 5.21 (t, 1H, J = 7.0 Hz), 5.14 (t, 1H, J = 7.0 Hz), 1.95 (m, 2H), 1.85 (m, 4H), 1.65 (m, 3H), 1.63 (s, 3H), 1.57 (s, 6H), 1.35 (m, 2H), 1.30 (d, 3H, J = 13.9 Hz), 1.25 (m, 4H); MS (FAB, + ions) m/z 433 (M + H), 411 (M + 2H − Na), 389 (M + 3H − 2Na). Anal. Calcd for $C_{16}H_{29}O_5P_2Na_3$ + $0.25H_2O$: C, 43.99; H, 6.81; P, 14.18. Found: C, 44.16; H, 7.18; P, 14.05.

[4-[1,1′-Biphenyl]-4-yl-1-(hydroxymethylphosphinyl)-butyl]phosphonic Acid, Disodium Salt, 7. Method D. To a solution of 2.24 g (6.47 mmol) of **23** (R = p-biphenylethyl) in 20 mL of THF at −78 °C was added 5.93 mL (1.2 M in cyclohexane, 7.12 mmol) of *sec*-butyllithium dropwise over 5 min. The deep orange reaction mixture was stirred at −78 °C for 20 min; then 2.82 mL (19.4 mmol) of diethyl chlorophosphite was added rapidly in one portion. The orange color discharged immediately. The mixture was stirred at −78 °C for 1 h and then warmed to room temperature. Diethyl ether (50 mL) and water (25 mL) were added, and the reaction mixture was stirred vigorously for 1 h. Additional diethyl ether (100 mL) was added, and the organic layer was washed with water (10 mL) and brine (20 mL) and then dried over $MgSO_4$. Evaporation gave a colorless oil, which was purified by flash chromatography on Mallinckrodt SILICAR-CC7 buffered silica gel (200 g) eluting with a step gradient of 5:95 EtOH/EtOAc to 15:85 EtOH/EtOAc to provide 1.71 g (60%) of **26** (R = p-biphenylethyl), a colorless oil, as a 1:1 mixture of diastereomers: ^1H NMR ($CDCl_3$) δ 7.57 (d, 2H, J = 7.3 Hz), 7.51 (d, 2H, J = 8.1 Hz), 7.42 (t, 2H, J = 7.3 Hz), 7.32 (t, 1H, J = 7.5 Hz), 7.31 (d, 1H, J = 575 Hz, PH), 7.26 (d, 2H, J = 8.1 Hz), 4.15 (m, 6H), 2.70 (m, 2H), 2.40−2.20 (m, 2H), 1.97 (m, 4H), 1.32 (m, 9H, J = 7.0 Hz).

Method E. Sodium bis(trimethylsilyl)amide (2.43 mL, 1.0 M in THF, 2.43 mmol) was added dropwise over 3 min to a solution of 968 mg (2.21 mmol) of **26** (R = p-biphenylethyl) in 7 mL of THF at −78 °C. The yellow solution was stirred at −78 °C for 30 min when 0.21 mL (3.32 mmol) of methyl iodide was added dropwise. After 1 h at −78 °C, the reaction was quenched by addition of saturated NH_4Cl (5 mL). Ethyl acetate (50 mL) and water (10 mL) were added, and the organic layer was washed with brine (10 mL). The aqueous layers were combined and extracted with EtOAc (10 mL). The combined organic layers were dried over $MgSO_4$. Evaporation gave a yellow oil which was purified by flash chromatography on silica gel (100 g) eluting with 10:90 EtOH/EtOAc to give 790 mg (79%) of **22** (R = p-biphenylethyl), a yellow oil, as a 1:1 mixture of diastereomers: ^1H NMR ($CDCl_3$) δ 7.57 (d, 2H, J = 8.1 Hz), 7.50 (d, 2H, J = 7.1 Hz), 7.42 (t, 2H, J = 7.7 Hz), 7.32 (t, 1H, J = 7.2 Hz), 7.26 (d, 2H, J = 8.1 Hz), 4.13 (m, 6H), 2.70 (t, 2H, J = 7.3 Hz), 2.30−1.83 (m, 6H), 1.68 and 1.64 (2 d, 3H, J = 8.1 Hz), 1.32 (m, 9H, J = 7.0 Hz).

Method B. Bromotrimethylsilane (0.88 mL, 6.66 mmol) was added to a solution of 670 mg (1.48 mmol) of **22** (R = p-biphenylethyl) and 0.39 mL (1.48 mmol) of bis(trimethylsilyl)trifluoroacetamide in 7 mL of CH_2Cl_2 at room temperature under argon. The reaction mixture was stirred at room temperature for 19 h, concentrated *in vacuo*, and pumped under high vacuum for 1 h. The colorless oil was dissolved in 7.4 mL (7.4 mmol) of 1 N NaOH, stirred at room temperature for 2 h, and filtered through Celite with the aid of water (30 mL). The filtrate was concentrated to a volume of 3 mL and purified by MPLC on SP207SS gel (2.5 × 25 cm column), eluting with water followed by a gradient created by the gradual addition of 2-propanol to a reservoir of water. Product fractions were combined and concentrated, and the aqueous solution was lyophilized to give 302 mg (50%) of **7** as a white solid: IR (KBr) 2926, 1653, 1507, 1150, 1026, 926, 878 cm^{-1}; ^1H NMR (D_2O) δ 7.73 (d, 2H, J = 8.1 Hz,), 7.68 (d, 2H, J = 6.8 Hz), 7.56 (t, 2H, J = 7.5 Hz), 7.48 (d, 2H, J = 8.1 Hz), 7.46 (t, 1H, J = 7.3 Hz), 2.75 (m, 2H), 1.93 (m, 4H), 1.68 (m, 1H), 1.40 (d, 3H, J = 13.7 Hz); ^{13}C NMR (D_2O) δ 143.32, 140.58, 137.98, 129.47, 129.27, 127.60, 126.99, 126.91, 43.68 (dd, J = 83, 116 Hz), 35.59, 32.96 (t, J = 5.9 Hz), 26.59, 16.97 (d, J = 92 Hz); MS (FAB, + ions) m/z 391 (M + 2H − Na), 413 (M + H), 435 (M + Na), 457 (M − H + 2Na). Anal. Calcd for $C_{17}H_{20}O_5P_2Na_2$ + $0.4H_2O$: C, 48.69; H, 5.00; P, 14.77. Found: C, 48.92; H, 4.77; P, 14.50.

(E)-[1-[Hydroxy(hydroxymethyl)phosphinyl]-6,10-dimethyl-5,9-undecadienyl]phosphonic Acid, Trisodium Salt, 9. Method D. To a stirred solution of 3.00 g (9.49 mmol) of **23** (R = geranylmethyl) in 35 mL of THF at −78 °C under argon was added 8.8 mL (1.3 M in cyclohexane, 11.4 mmol) of *sec*-butyllithium dropwise over 10 min. The yellow reaction mixture was stirred at −78 °C for 75 min followed by rapid addition of 4.1 mL (28.5 mmol) of diethyl chlorophosphite, rapidly in one portion. The colorless solution was stirred at −78 °C for 1 h, allowed to warm to room temperature over 1.5 h, and stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C, whereupon Et_2O (50 mL) and water (20 mL) were added. After stirring at 0 °C for 15 min, the reaction mixture was stirred at room temperature for 15 min and partitioned between ether and water. The organic layer was washed with water (20 mL) and brine (20 mL) and then dried over $MgSO_4$. Evaporation gave a crude oil which was purified by flash chromatography on Mallinckrodt SILICAR-CC7 buffered silica gel (125 g) eluting with a gradient of 3:97−5:95 EtOH/CH_2Cl_2 to give 2.72 g (70%) of **26** (R = geranylmethyl) as a pale yellow oil, as a 2:1 mixture of diastereomers: TLC silica gel (EtOAc) R_f = 0.21; IR (CCl_4) 2980, 2932, 2336, 1715, 1551, 1443, 1391, 1236, 1165, 1028, 970, 783 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (d, 1H, J = 572 Hz, PH), 5.03 (m, 2H), 4.10 (m, 6H), 2.18 (m, 1H), 2.10−1.70 (m, 8H), 1.65−1.45 (m, 2H), 1.60 (s, 3H), 1.52 (s, 6H), 1.30 (t, 3H, J = 7.6 Hz), 1.28 (t, 6H, J = 7.3 Hz); MS (CI-NH_3, + ions) m/z 426 (M + NH_4), 409 (M + H). Anal. Calcd for $C_{19}H_{38}O_5P_2$: C, 55.87; H, 9.38; P, 15.17. Found: C, 55.83; H, 9.49; P, 14.92.

Method F. A mixture of 794 mg (1.95 mmol) of **26** (R = geranylmethyl), 234 mg (7.80 mmol) of paraformaldehyde, and 0.17 mL (0.98 mmol) of N,N-diisopropylethylamine in 5 mL of absolute EtOH was stirred at 60 °C for 25 h and concentrated *in vacuo*. The residue was dissolved in EtOAc (30 mL) and washed with saturated $NaHCO_3$ and brine (10 mL each). The aqueous washings were combined and extracted with EtOAc

Table 7. Propeties and Synthesis of Derivatives **8**−**14**

compd no.	anal. (C, H, P)	MS (M)	synth methods (method, yield, %)
8	$C_{19}H_{33}O_6P_2K_3$	536	D, 66; F, 62; B,[a] 40
9	$C_{14}H_{25}O_6P_2Na_3 + 0.2H_2O$	420	D, 70; F, 48; B,[b] 74
10	$C_{17}H_{19}O_6P_2K_3 + 1.0H_2O$	498	D, 60; F, 71; B,[c] 42
11	$C_{20}H_{36}O_6P_2Na_2 + 0.75H_2O$	480	C, 46; B, 66
12	$C_{15}H_{27}O_6P_2Na_3 + 0.5H_2O$	434	D, 70; G, 86; B,[a] 63
13	$C_{18}H_{21}O_6P_2K_3 + 0.4H_2O$	512	D, 60, G,[d] 65; B,[b,c] 18
14	$C_{13}H_{24}O_5P_2Na_2 + 0.75H_2O$	368	D, 70; B,[c,e] 73

[a] Isolated by triturating crude oil with acetone. [b] Purification on SP207SS gel. [c] Bis(trimethylsilyl)trifluoroacetamide used as acid trap. [d] $NaNTMS_2$ used as base in THF. [e] TMSBr reaction was followed by treatment with excess NaOH for 4 days to effect hydrolysis of the phosphonous ethyl ester.

(5 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to give 953 mg of a colorless oil. Flash chromatography on silica gel (50 g) eluting with a gradient of 3:97–5:95 EtOH/CH_2Cl_2 gave 406 mg (48%) of **27** (R = geranylmethyl), a 1:1 mixture of diastereomers, as a pale yellow oil. The diastereomers could be separated by flash chromatography using a gradient of 2:98–3:97 EtOH/CH_2Cl_2: TLC silica gel (5:95 EtOH/CH_2Cl_2) diastereomer A, R_f = 0.45, diastereomer B, R_f = 0.41; IR (CCl_4) 3358, 2928, 1551, 1225, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ diastereomer A, 5.12 (m, 2H), 4.19 (m, 6H), 4.04 (m, 2H), 2.30 (m, 1H), 2.20–1.70 (m, 8H), 1.68 (s, 3H), 1.60 (s + m, 8H), 1.37 (t, 3H, J = 7.0 Hz), 1.36 (t, 6H, J = 7.0 Hz), diastereomer B, 5.10 (m, 2H), 4.77 (t, 1H, J = 7.0 Hz), 4.30–4.00 (m, 7H), 3.95 (dt, 1H, J = 7.6 Hz), 2.39 (m, 1H), 2.18–1.73 (m, 8H), 1.68 (s, 3H), 1.60 (s + m, 8H), 1.36 (t, 6H, J = 7.0 Hz), 1.36 (t, 3H, J = 7.0 Hz); MS (CI-NH_3, + ions) m/z diastereomers A and B, 456 (M + NH_4), 439 (M + H). Anal. Calcd. for $C_{20}H_{40}O_6P_2$: C, 54.78; H, 9.19; P, 14.13. Found: C, 54.60; H, 9.39; P, 14.06.

Method B. Bromotrimethylsilane (0.64 mL, 4.82 mmol) was added dropwise to a mixture of 301 mg (0.69 mmol) of **27** (R = geranylmethyl) and 0.23 mL (1.72 mmol) of 2,4,6-collidine in 3 mL of CH_2Cl_2 under argon. The cloudy reaction mixture was stirred at room temperature for 27 h, concentrated *in vacuo*, and then pumped under high vacuum for 30 min. A solution of 1.1 mL (3.4 mmol) of 3.0 M pyridine in EtOH was added to the residue, and the suspension was stirred at room temperature for 30 min, concentrated *in vacuo*, and then pumped under high vacuum for 10 min. The residue was dissolved in 4.1 mL (4.1 mmol) of 1 N NaOH and stirred at room temperature for 30 min, diluted with water (10 mL), and lyophilized to give a pink solid (461 mg). The crude product was purified by column chromatography on SP207SS gel eluted with water followed by a gradient created by the gradual addition of 500 mL of 2-propanol to a reservoir of 500 mL of water. Pure fractions were combined and concentrated to a volume of approximately 20 mL. The aqueous solution was filtered through a 0.2 μm membrane filter and then lyophilized to give 213 mg (74%) of **9** as a white solid: IR (KBr) 3432, 2967, 2930, 1632, 1161, 1080, 1024 cm^{-1}; ^{1}H NMR (D_2O) δ 5.24 (t, 1H, J = 6.6 Hz) 5.15 (t, 1H, J = 6.2 Hz), 3.68 (dd, 1H, J = 4.8, 14.3 Hz), 3.53 (dd, 1H, J = 5.9, 14.3 Hz), 2.06 (m, 2H), 1.98 (m, 4H), 1.80 (m, 4H), 1.63 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H), 1.55 (m, 2H); MS (FAB) m/z 421 (M + H), 399 (M + 2H − Na), 377 (M + 3H − 2Na). Anal. Calcd for $C_{14}H_{25}O_6P_2Na_3$ + $0.2H_2O$: C, 39.67; H, 6.04; P, 14.61. Found: C, 39.73; H, 6.32; P, 14.47.

(*E*)-[1-[Hydroxy(methoxymethyl)phosphinyl]-6,10-dimethyl-5,9-undecadienyl]phosphonic Acid, Trisodium Salt, 12. Method G. Chlorotrimethylsilane (0.54 mL, 4.23 mmol) was added dropwise to a mixture of 690 mg (1.69 mmol) of **26** (R = geranylmethyl; see method D in the preparation of **9**) and 0.59 mL (4.23 mmol) of triethylamine in 10 mL of CH_2Cl_2 at 0 °C under argon. A white precipitate formed shortly after addition. The reaction mixture was stirred at 0 °C for 15 min followed by the addition of 0.41 mL (5.07 mmol) of bromomethyl methyl ether. After 1 h at 0 °C, the mixture was concentrated *in vacuo*. The residue was pumped at high vacuum for 10 min and then partitioned between EtOAc (30 mL) and water (20 mL). The organic layer was washed with saturated $NaHCO_3$ (20 mL) and brine (10 mL) and then dried over $MgSO_4$. Evaporation of the solvent gave a pale yellow oil (722 mg), which was purified by flash chromatography on silica gel eluting with 3:97 MeOH/CH_2Cl_2 to give 656 mg (86%) of **25** (R = geranylmethyl), a 3:1 mixture of diastereomers, as a colorless oil: TLC silica gel (25:25:50 acetone/EtOAc/CH_2Cl_2) diastereomer A, R_f = 0.41, diastereomer B, R_f = 0.33; IR (CCl_4) 2980, 2930, 1445, 1391, 1244, 1188, 1163, 1109, 1026, 963, 883, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.11 (m, 2H), 4.16 (m, 6H), 4.05–3.65 (m, 2H), 3.48 and 3.47 (2 s, 3H total), 2.43 (m, 1H), 2.10–1.80 (m, 8H), 1.68 (s, 3H), 1.60 (s + m, 6H), 1.34 (t, 9H, J = 7.0 Hz); MS (CI-NH_3, + ions) m/z 453 (M + H). Anal. Calcd for $C_{21}H_{42}O_6P_2$ + $0.6H_2O$: C, 54.45; H, 9.40; P, 13.37. Found: C, 54.53; H, 9.54; P, 12.99.

Table 8. Properties and Synthesis of α-Phosphono Carboxylates **15–17**

compd no.	anal. (C, H, P)	MS (M)	synth methods (method, yield, %)
15	$C_{19}H_{30}O_5PNa_3 + 1.0H_2O$	438	A, 87; H, 78
16	$C_{14}H_{23}O_5PNa_2 + 0.45H_2O$	348	A, 79; I,[a] 72
17	$C_{17}H_{16}O_5PK_3 + 2.5H_2O$	448	A, 61; H,[b] 86

[a] Isolated by triturating crude oil with acetone. b Bis(trimethylsilyl)trifluoroacetamide used as acid trap.

Method B. Bromotrimethylsilane (1.0 mL, 7.68 mmol) was added to a mixture of 577 mg (1.28 mmol) of **25** (R = geranylmethyl) and 0.34 mL (2.56 mmol) of 2,4,6-collidine in 5 mL of CH_2Cl_2 under argon. The yellow reaction mixture was stirred at room temperature for 24 h, concentrated in vacuo, and pumped under high vacuum for 30 min. The residue was dissolved in 4.5 mL (4.5 mmol) of 1 N NaOH, stirred at room temperature for 30 min, diluted with water (10 mL), and lyophilized. The light yellow solid was dissolved in water (15 mL) at 40 °C and then precipitated by addition of acetone (13 mL) in 1 mL aliquots keeping the mixture at 40 °C. The cloudy mixture was cooled to 0 °C for 20 min and then filtered and washed with 4:1 acetone/water (5 × 10 mL). The resulting solid was pumped under high vacuum overnight to give 349 mg (63%) of **12** as a white solid: IR (KBr) 3435, 2926, 1636, 1449, 1171, 1101, 972 cm^{-1}; ^{1}H NMR (D_2O) δ 5.25 (t, 1H, J = 6.8 Hz), 5.15 (dt, 1H, J = 1.1, 7.0 Hz), 3.75 (dd, 1H, J = 4.8, 12.8 Hz), 3.60 (dd, 1H, J = 6.6, 12.8 Hz), 3.36 (s, 3H), 2.05 (m, 2H), 1.98 (m, 4H), 1.80–1.40 (m, 5H), 1.63 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H); MS (FAB, + ions) m/z 457 (M + Na), 435 (M + H), 413 (M + 2H − Na), 395 (M + 2H − Na − H_2O). Anal. Calcd for $C_{15}H_{27}O_6P_2Na_3$ + $0.5H_2O$: C, 40.65; H, 6.37; P, 13.98. Found: C, 40.99; H, 6.55; P, 13.69.

Preparation of (*E*)-[1-(Hydroxyphosphinyl)-6,10-dimethyl-5,9-undecadienyl]phosphonic Acid, Disodium Salt, 14. Method B. To a solution of 1.00 g (2.45 mmol) of **26** (R = geranylmethyl; see method D in the preparation of **9**) in 10 mL of CH_2Cl_2 under argon was added 0.65 mL (2.45 mmol) of bis(trimethylsilyl)trifluoroacetamide followed by 1.00 mL (7.60 mmol) of bromotrimethylsilane. The colorless reaction mixture was stirred at room temperature for 3 h, when a solution of 2.9 mL (8.6 mmol) of 3.0 M pyridine in EtOH was added. The mixture was stirred at room temperature for 1 h and concentrated *in vacuo*. The cloudy white oil was pumped under high vacuum for 45 min, dissolved in 11 mL of 1 N NaOH, and stirred at room temperature overnight. An additional 3.7 mL of 1 N NaOH was added, and the reaction mixture was stirred at room temperature for an additional 3 days to complete the saponification of the phosphinic ester. The mixture was diluted with water (15 mL) and lyophilized to give an ivory-colored solid. The crude product was purified by column chromatography on SP207SS gel eluted with water followed by a gradient created by the gradual addition of 500 mL of 70:30 MeOH/H_2O to a reservoir of 500 mL of water. Pure fractions were combined and concentrated to a volume of approximately 30 mL, and the aqueous solution was filtered through a 0.2 μm membrane filter and then lyophilized to give 656 mg (73%) of **14** as a white solid: IR (KBr) 3414, 2967, 2926, 2856, 2315 (P-H), 1450, 1181, 1098, 893 cm^{-1}; ^{1}H NMR (D_2O) δ 7.00 (d, 1H, J = 531 Hz, PH), 5.21 (t, 1H, J = 7.0 Hz), 5.15 (t, 1H, J = 7.0 Hz), 2.10–1.90 (m, 6H), 1.85–1.40 (m, 5H), 1.63 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H); MS (FAB, + ions) m/z 391 (M + Na), 369 (M + H), 347 (M + 2H − Na), 325 (M + 3H − 2Na). Anal. Calcd for $C_{13}H_{24}O_5P_2Na_2$ + $0.75H_2O$: C, 40.90; H, 6.73; P, 16.22. Found: C, 40.75; H, 6.70; P, 16.42.

(*E,E*)-7,11,15-Trimethyl-2-phosphono-6,10,14-hexadecatrienoic Acid, Disodium Salt, 15. Method A. To a stirred solution of 400 mg (16.60 mmol) of NaH in 30 mL of THF at 0 °C under argon was added 3.10 mL (16.60 mmol) of ethyl (diethylphosphono)acetate (**28a**) over 0.5 h. The mixture was stirred for 0.5 h at 0 °C and treated with 2.0 g (5.60 mmol) of **20** (R = farnesylmethyl) over 0.2 h. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 20 h and diluted with 200 mL of Et_2O, and the reaction was quenched with 100 mL of NH_4Cl. The organic layer was washed with

water and brine and dried over $MgSO_4$. The solvent was evaporated to provide 2.36 g of a crude yellow oil. Flash chromatography was performed on 100 g of silica gel, packed, loaded, and eluted with 60:40 hexane/ethyl acetate. Pure product fractions were combined and evaporated to provide 1.69 g (68%) of **29a** (R = farnesylmethyl) as a pale yellow oil: TLC silica gel (90:10 hexane/ethyl acetate) R_f = 0.79; ^{1}H NMR ($CDCl_3$) δ 5.10 (t, 3H, J = 5.8 Hz), 4.30–4.10 (m, 6H), 2.95 (ddd, 1H, J = 22.3, 10.5, 4.1 Hz), 2.20–1.80 (m, 12H), 1.67 (s, 3H), 1.59 (s, 9H), 1.45–1.20 (m, 11H).

Method H. To a stirred solution of 1.1 g (2.40 mmol) of **29a** (R = farnesylmethyl) in 10 mL of ethanol at room temperature under argon was added 10 mL of 0.36 M NaOH. This mixture was heated to 55 °C for 20 h, cooled to room temperature, acidified with $KHSO_4$, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield 1.02 g of the carboxylic acid **29** (R = farnesylmethyl, R^2 = OH), which was used without purification in the next step. To a stirred solution of the acid in 10 mL of CH_2Cl_2 at room temperature under argon was added 0.95 mL (7.20 mmol) of 2,4,6-collidine followed by 1.42 mL (10.80 mmol) of bromotrimethylsilane. The mixture was stirred at room temperature for 20 h, the solvent was evaporated and the residue was pumped under high vacuum. The crude material was dissolved in 17.3 mL (8.65 mmol) of 0.5 M NaOH, lyophilized, and purified by MPLC on a column of CHP20P eluted with water followed by a gradient created by the gradual addition of 350 mL of CH_3CN to a reservoir of 350 mL of water. Pure product fractions were combined and evaporated to remove the CH_3CN, and the aqueous solution was lyophilized to provide 816 mg (78%) of **15** as a white lyophilate: TLC silica gel (5:4:1 *n*-propanol/concentrated NH_4OH/H_2O) R_f = 0.37; IR (KBr) 3440, 3432, 3060, 3054, 3033, 3028, 2966, 2925, 2856, 1635, 1558, 1442, 1390, 1163, 1089, 975 cm^{-1}; ^{1}H NMR (D_2O) δ 5.23 (t, 1H, J = 6.8 Hz), 5.11 (q, 2H, J = 6.6 Hz), 2.46 (ddd, 1H, J = 2.93, 13.4, 24.3 Hz), 2.05–1.96 (m, 10H), 1.72–1.67 (m, 2H), 1.63 (s, 3H), 1.57 (s, 9H), 1.29–1.20 (m, 2H); MS (FAB, + ions) *m/z* 461 (M + Na), 439 (M + H), 417 (M + 2H − Na). Anal. Calcd for $C_{19}H_{30}PO_5Na_3$ + $1.0H_2O$: C, 49.98; H, 7.07; P, 6.78. Found: C, 50.06; H, 7.21; P, 6.96.

(*E*)-7,11-Dimethyl-2-phosphono-6,10-dodecadienoic Acid, Disodium Salt, 16. Method A. To a stirred solution of 247 mg (10.27 mmol) of NaH in 14 mL of DMF at 0 °C under argon was added dropwise 2.59 g (10.27 mmol) of *tert*-butyl (diethylphosphono)acetate (**28b**) in 2 mL of DMF. The mixture was stirred for 0.5 h at 0 °C, when 1.0 g (3.42 mmol) of iodide **20** (R = geranylmethyl) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 18 h and then diluted with ether, and the reaction was quenched with saturated NH_4Cl. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated to provide 2.20 g of a pale yellow oil. Flash chromatography was performed on 200 g of silica gel, eluting with 3:97 methanol/dichloromethane. Product fractions were combined and evaporated to provide 1.64 g of a colorless oil. Volatile impurities were distilled (130 °C, ~1.0 mmHg) to leave 1.11 g (79%) of pure **29b** (R = geranylmethyl) as a colorless oil: TLC silica gel (5:95 methanol/dichloromethane) R_f = 0.70; IR (CCl_4) 2981, 2931, 1730, 1454, 1368, 1255, 1156 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.10 (m, 2H), 4.25 (m, 4H), 2.83 (ddd, 12H, J = 3.8, 10.9, 22.9 Hz), 2.00 (m, 6H), 1.83 (m, 2H), 1.68 (s, 3H), 1.59 (s, 6H), 1.48 (s, 9H), 1.40 (m, 2H), 1.33 and 1.32 (2 t, 6H, J = 7.0 Hz); MS (CI-NH_3, + ions) *m/z* 434 (M + NH_4), 417 (M + H).

Method I. To a stirred solution of 1.10 g (2.64 mmol) of **29b** (R = geranylmethyl) in 10 mL of CH_2Cl_2 at room temperature under argon was added 0.70 mL (5.28 mmol) of 2,4,6-collidine followed by 1.50 mL (10.56 mmol) of iodotrimethylsilane. The reaction was heated to 40 °C for 24 h, the solvent was evaporated, and the residue was pumped under high vacuum for 2 h. The remainder was treated with 8.70 mL (8.70 mmol) of 1 M NaOH and lyophilized. The crude lyophilate was precipitated by dissolving the sample in 5.0 mL of water, warming to 50 °C, treating the solution with 1.0 mL of acetone, and placing the mixture in an ice bath for 0.5 h. The precipitate was filtered and washed three times with 10 mL of 5:1 water/acetone followed by 20 mL of acetone. The collected solids were dried under high vacuum to provide 665 mg (70%) of **16** as a cream-colored solid: TLC silica gel (5:4:1 *n*-propanol/concentrated NH_3/H_2O) R_f = 0.37; IR (KBr) 3434, 2927, 2858, 1577, 1443, 1384, 1175, 1074 cm^{-1}; ^{1}H NMR (D_2O) δ 5.20 (t, 1H, J = 6.8 Hz), 5.12 (t, 1H, J = 6.8 Hz), 2.54 (ddd, 1H, J = 3.6, 11.5, 20.7 Hz), 2.10 and 2.02 (2 m, 6H), 1.80 (m, 2H), 1.63 (s, 3H), 1.56 (s, 6H), 1.35 (m, 2H); MS (FAB, + ions) *m/z* 393 (M + 2Na − H), 371 (M + Na), 349 (M + H), 327 (M + 2H − Na). Anal. Calcd for $C_{14}H_{23}PO_5Na_2$ + $0.45H_2O$: C, 47.19; H, 6.76; P, 8.69. Found: C, 47.19; H, 6.85; P, 8.91.

Biological Evaluation. IC_{50} values for squalene synthase in rat liver microsomes and ED_{50} values for cholesterol biosynthesis inhibition in rats ("the rat model") were determined as described in Ciosek et al.[3] In the case of oral dosing in the rat model, the drug was given by gavage to fasted rats 3 h prior to [^{14}C]acetate administration, unless otherwise noted.

Acknowledgment. We would like to thank C. Botti and J. W. Abt for technical assistance.

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JM950193E