Farnesyl Diphosphate-Based Inhibitors of Ras Farnesyl Protein Transferase

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The rational design, synthesis, and biological activity of farnesyl diphosphate (FPP)-based inhibitors of the enzyme Ras farnesyl protein transferase (FPT) is described. Compound **3**, wherein a β-carboxylic phosphonic acid type pyrophosphate (PP) surrogate is connected to the hydrophobic farnesyl group by an amide linker, was found to be a potent (I_{50}(FPT) = 75 nM) and selective inhibitor of FPT, as evidenced by its inferior activity against squalene synthetase (I_{50}(SS) = 516 μM) and mevalonate kinase (I_{50}(MK) = >200 μM). A systematic structure–activity relationship study involving modifications of the farnesyl group, the amide linker, and the PP surrogate of **3** was undertaken. Both the carboxylic and phosphonic acid groups of the β-carboxylic phosphonic acid PP surrogate are essential for activity, since deletion of either group results in 50–2600-fold loss in activity (**6–9**, I_{50} = 4.6–220 μM). The farnesyl group also displays very stringent requirements and does not tolerate one carbon homologation (**12**, I_{50} = 17.7 μM), substitution by a dodecyl fragment (**14**, I_{50} = 9 μM), or introduction of an extra methyl group at the allylic position (**18**, I_{50} = 55 μM). Modifications around the amide linker group of **3** were more forgiving, as evidenced by the activity of *N*-methyl analog (**21**, I_{50} = 0.53 μM), the one carbon atom shorter farnesoic acid-derived retroamide analog (**32**, I_{50} = 250 nM), and the exact retroamide analog (**49**, I_{50} = 50 nM). FPP analogs such as **3**, **32**, and **49** are novel, potent, selective, small-sized, nonpeptidic inhibitors of FPT that may find utility as antitumor agents.

Introduction

The frequent detection of *ras* oncogenes in a wide variety of human malignancies highlights their fundamental role in cellular proliferation and differentiation.[1] For example, mutated *ras* genes are found in 50% of lung and colorectal carcinomas and in up to 95% of pancreatic carcinomas.[2] Ras proteins are members of the low molecular weight superfamily of GTP-binding proteins that bind to guanine nucleotides GTP and GDP and possess intrinsic GTPase activity.[3] Unlike normal ras proteins, mutated ras proteins are unable to hydrolyze GTP and are locked in the biologically active GTP-bound state, thereby triggering a continous growth signal resulting in malignant transformation.[4] Agents that would obstruct one or more of the critical events in ras-mediated signal transduction pathway may therefore be expected to function as antitumor agents. This hypothesis has formed the basis for rational design and discovery of novel anticancer agents in recent years.[5]

Critical for the transforming activity of ras proteins is their migration from cytoplasm to the plasma membrane, an event which is facilitated by a series of well-defined posttranslational modifications.[6] The first essential step in this process is the farnesylation of a cysteine residue located at the fourth position from the carboxyl terminus of the ras protein.[7] A "CAAX" motif at the C-terminus is a characteristic consensus sequence for farnesylation, where C is cysteine, A is an aliphatic amino acid, and X is serine or methionine.[8] Farnesyl pyrophosphate (FPP), an intermediate formed in the cholesterol biosynthetic pathway, serves as the farnesyl group donor in this reaction.[9] Next, proteolysis occurs to remove the C-terminal tripeptide fragement "AAX",[10] followed by carboxymethylation[11] of the newly exposed free carboxyl group of the S-farnesylated cysteine residue. This completes the conversion of pro-p21^{ras} to the hydrophobic c-p21^{ras}. The protein can now become anchored to the membrane, interact with it, and function as a transforming, mature, m-p21^{ras}.[12] At the membrane, palmitoylation of one of the upstream cysteines occurs in some but not all ras proteins.[13] The first and mandatory farnesylation step in this cascade of posttranslational events is catalyzed by the enzyme farnesyl protein transferase (FPT).[14] Thus, the search for inhibitors of FPT as antitumor agents has expectedly witnessed intense activity recently from academic and industrial groups.[15]

Inhibitor Design Strategy

Since FPT catalyzes a bisubstrate reaction (Figure 1), inhibitor design based on either one or both of the two reacting substrates can be pursued. We have recently reported on the first examples of bisubstrate inhibitors of FPT.[16] Inhibitors based on the CAAX motif of ras have been studied most extensively by several groups.[17] In this paper, we describe our approach toward designing inhibitors of FPT based on the other substrate FPP.[18] On the basis of prior information about the structure of the enzyme and its mechanism of action,[19] multiple binding interactions at its active site stabilizing the two reactants and facilitating the farnesylation event can be hypothesized. Such a model can form the basis for rational design of inhibitors of FPT.[16] The substrate FPP can be viewed as being composed of two structural units, namely the hydrophobic farnesyl group and the highly charged pyrophosphate moiety. Whereas a hydrophobic pocket may accommodate the lipophilic farnesyl moiety, the PP group is probably stabilized by

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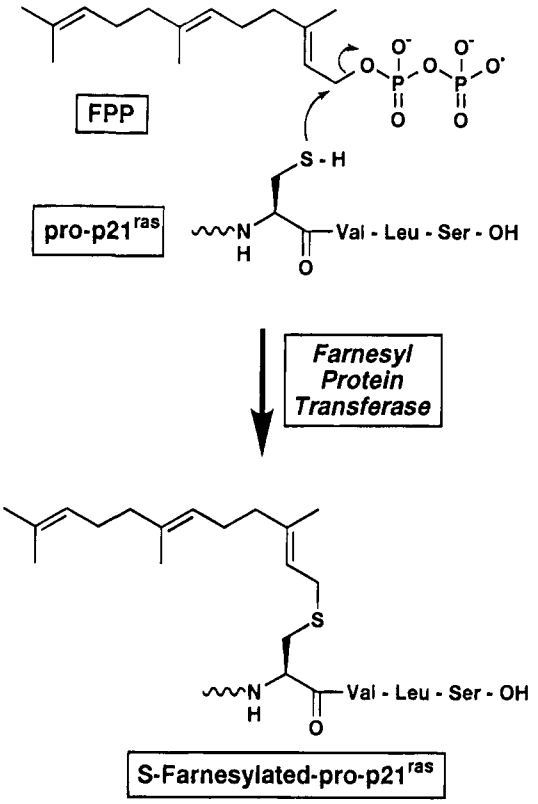


Figure 1. FPT-catalyzed farnesylation of p21[ras].

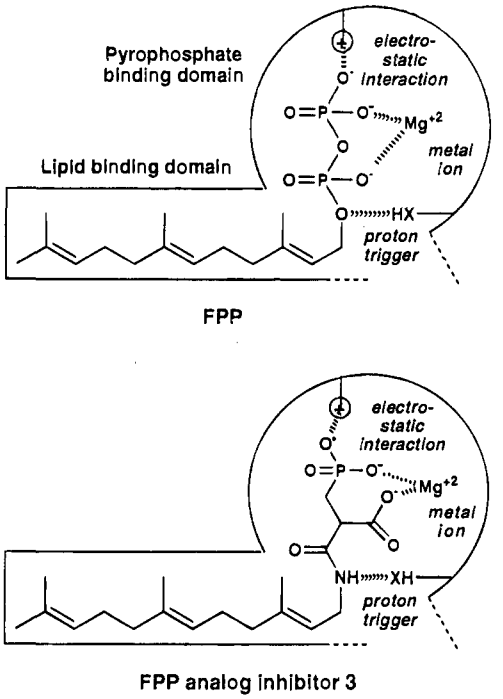


Figure 2. Hypothesized binding interactions of FPP vs FPP analog inhibitor **3** at the active site of FPT.

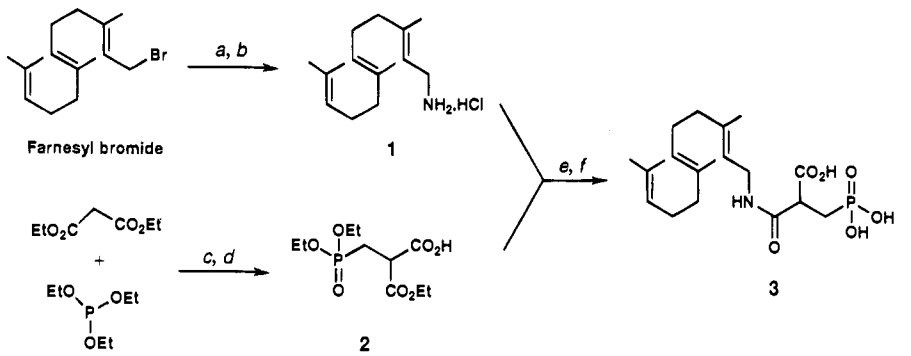
complexation with a magnesium ion and by electrostatic interaction with a positively charged enzyme residue. An H-bond donor may serve to stabilize the growing negative charge on the allylic oxygen atom being displaced, thereby facilitating the departure of the leaving PP group.[20a] Thus, the basic structural elements in our FPP-based inhibitors were a farnesyl group, a pyrophosphate isostere, and a flexible linker connecting these two subunits. The farnesyl group can be retained or substituted with appropriate hydrophobic residues to capitalize on potential hydrophobic interactions. The length and nature of the linker can be varied so as to mimic the electronic and structural features of an early or late transition state geometry. The replacement of the highly charged and biologically labile PP group of FPP with a more stable bioisosteric moiety is probably the most critical aspect of FPP-based inhibitors of FPT.[20] In the early stages of inhibitor design, the farnesyl group was retained as such without any changes, an amide group was used as a linker, and various combinations of phosphonic and/or carboxylic acid groups were used to approximate the arrangement of charges of the PP moiety. This led to the design of inhibitor **3**, wherein the β-carboxylphosphonic acid group was introduced as a substitution for the pyrophosphate head group of FPP (Figure 2). The good potency and selectivity displayed by **3** (I_{50} = 75 nM) as an inhibitor of FPT warranted further SAR study centered around the nucleus of **3** and form the subject matter of this paper.

Chemistry

Farnesylamine **1** was prepared by N-alkylation of farnesyl bromide with potassium phthalimide, followed by treatment with methyl hydrazine.[21] Thermal condensation of diethyl malonate and triethyl phosphite with paraformaldehyde afforded the tetraethyl ester adduct,[22] whose carboxyethyl group was selectively monohydrolyzed by careful treatment with 1.0 equiv of 2 N KOH to acid **2**. Carbonyl diimidazole (CDI)-mediated coupling of amine **1** with **2** gave the triester amide intermediate, whose phosphonate and carboxyl ester moities were sequentially deprotected by treatment with 2,4,6-collidine and bromotrimethylsilane (TMSBr) followed by aqueous 1 N NaOH to provide triacid **3**, the lead FPP anlog-based inhibitor of FPT (Scheme 1).

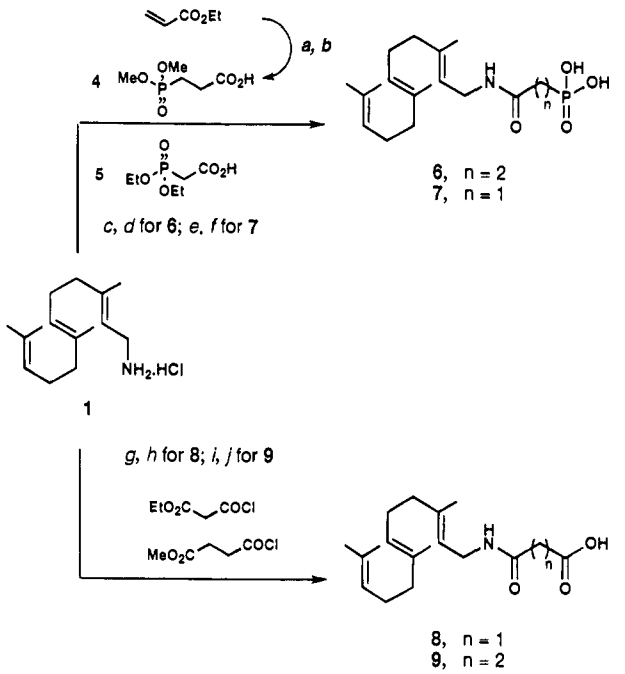
Preparation of analog **6**, the descarboxyl analog of **3**, is summarized in Scheme 2. Michael addition of dimethyl trimethylsilyl phosphite to ethyl acrylate followed by careful hydrolysis of the resulting silyl ester with 1 equiv of NaOH yielded acid **4** in 36% overall yield.[23] CDI-mediated coupling of **4** with farnesylamine **3** proceeded very efficiently. Hydrolysis of the dimethyl phosphonate group of the penultimate precursor of **6** was best accomplished by treatment with TMSBr buffered with excess bis(trimethylsilyl)trifluoroacetamide (BSTFA, which scavenges off the liberated HBr), followed by treatment with NaOH to convert the resulting silyl ester to the sodium salt. This final hydrolysis step in the preparation of **6** proceeded in 43% overall yield. In order to prepare **7**, the one carbon lower homolog of **6**, farnesylamine **1** was coupled with acid **5** using CDI and the resulting diethyl phosphonate ester intermediate (58%) was hydrolyzed by treatment with collidine/TMSBr (63%). Intermediate **5** required for this coupling was synthesized by selective monohydrolysis of triethyl phosphonoacetate (Aldrich) with 1.0 equiv of 1 N NaOH in ethanol (95%). Acylation of farnesyl amine **1** with the ethyl ester of malonyl chloride followed by base hydrolysis yielded acid **8**, an analog involving deletion of the $CH_2PO(OH)_2$ moiety from **3**. Acid **9** is the one-carbon homolog of **8** and was prepared in a similar fashion by employing carbomethoxypropionyl chloride as the acylating reagent.

Scheme 1[a]



[a] Reagents: (a) potassium phthalimide, 81%; (b) $MeNHNH_2$, reflux, 89%; (c) $(CH_2O)_n$, 170 °C, 48%; (d) NaOH, H_2O, EtOH, H_2SO_4, 70%; (e) CDI, iPr_2NEt, 78%; (f) BSTFA, TMSBr, NaOH, 80%.

Scheme 2[a]



[a] Reagents: (a) $P(OMe)_2OSiMe_3$, 40%; (b) NaOH, MeOH, H_2SO_4, 58%; (c) CDI, iPr_2NEt, **4**, 85%; (d) BSTFA, TMSBr; then NaOH, 90%; (e) CDI, iPr_2NEt, **5**, 58%; (f) BSTFA, TMSBr; then NaOH, 63%; (g) EtO_2CCH_2COCl, iPr_2NEt 59%; (h) NaOH, MeOH, 71%; (i) $EtO_2C(CH_2)_2COCl$, iPr_2NEt, 98%; (j) NaOH, MeOH, 91%.

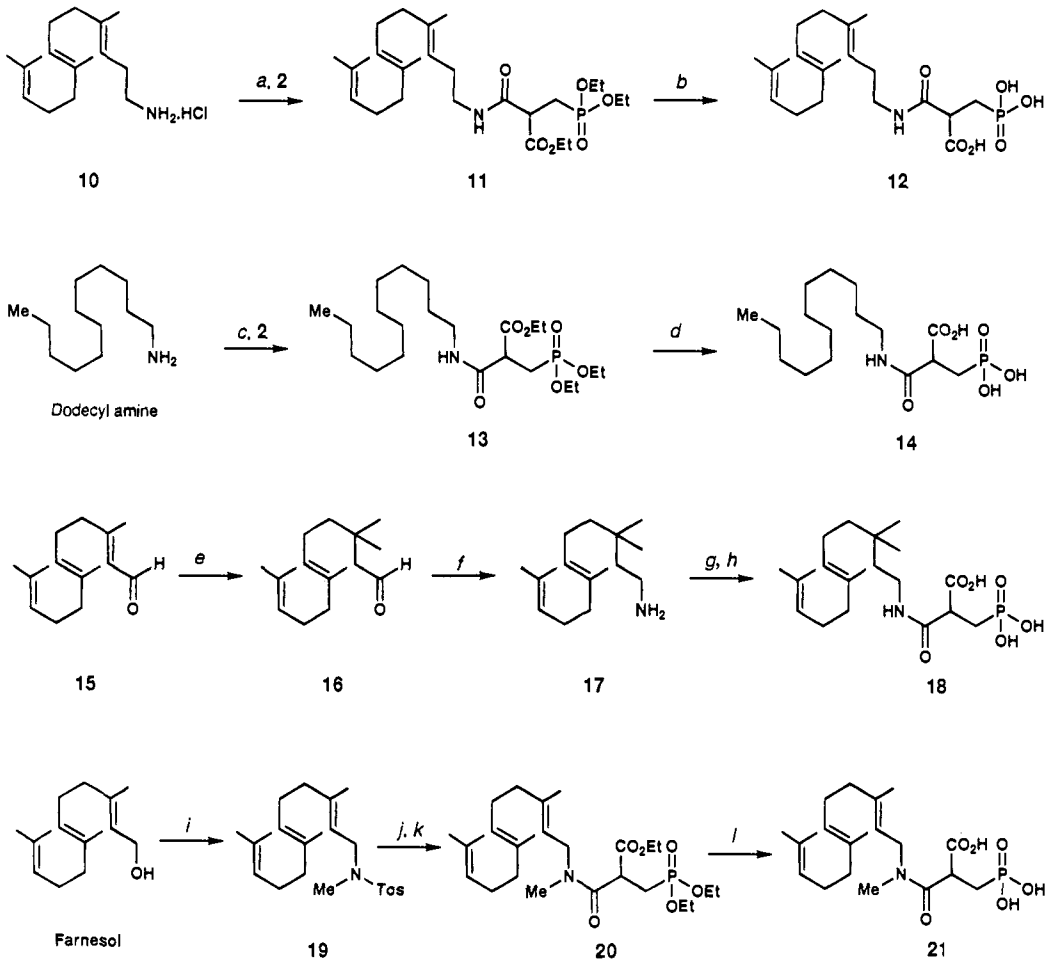
Coupling of homofarnesylamine[24] with acid **2** provided the triester **11** which upon sequential hydrolysis with BSTFA/TMSBr and NaOH gave **12** (Scheme 3). Similarly, coupling of **2** with dodecylamine followed by sequential hydrolysis of intermediate **13** with BSTFA/TMSBr and NaOH gave **14**. It should be noted that while hydrolysis of phosphonate group of **13** by treatment with TMSBr/BSTFA was facile, conversion of intermediate diacid to the final triacid was very sluggish and necessitated the use of a large excess of NaOH (8 equiv).

We next undertook the preparation of dimethyl analog **18** which involved the introduction of an extra methyl group to the double bond that was allylic to the pyrophosphate surrogate. Such an alteration eliminated the reactive and chemically susceptible allylic olefin functionality. Michael addition of dimethylcopper lithium to farnesaldehyde **15**[25] turned out be fairly challenging, the main competing reaction being direct 1,2-addition to form the secondary alcohol. Superior yields of the desired adduct **16** were finally realized when an excess of TMSCl (5.0 equiv) was employed, thereby highlighting its role in enhancing the rate and selectivity of 1,4-addition reactions.[26] Aldehyde **16** was fairly sensitive and had to be purified over neutral silica gel. While the purified material could be stored at −40 °C under argon for a few weeks with minimal decomposition, it was found best to use it as soon as possible. Thus, **16** was reductively aminated under the standard conditions employing $NaBH_3CN/NH_4OAc$[27a] but gave only 27% yield of the desired amine **17** along with the corresponding dialkylated amine (45%) as the major product.[27b] Coupling of **17** with acid **2** afforded moderate amounts of the coupled product (34%) which was hydrolyzed in the standard fashion to provide **18** (87%).

The recently reported procedure[28] for direct conversion of alcohols to *N*-methylamines via a Mitsunobu type of coupling reaction was employed for preparing **21**, the *N*-methyl analog of **3**. Although *N*-methyltriflate is the recommended reagent, reaction with the analogous tosylate was attempted since it is commercially available (Aldrich). Indeed, upon treatment of farnesol with *N*-methyl-*p*-toluenesulfonamide under Mitsunobu conditions, the desired intermediate **19** was obtained in very good yield (91%) and was accompanied with minor amounts of easily separable DEAD N-alkylation side product. The tosyl group was cleanly cleaved with sodium naphthalide and the resulting secondary amine treated directly with acid **2** and CDI to provide the coupled product **20** in 42% overall yield. Hydrolysis under standard conditions gave the *N*-methyl analog **21**.

Preparation of the retro-amide analogs of **3** shorter by one atom is summarized in Scheme 4. Starting with Boc-L-serine, the dimethyl phosphonate **25** was prepared by treatment of the Vederas lactone **23**[29] with trimethyl phosphite according to literature procedure.[30] Coupling of amine **27** with farnesoic acid **22** could not be accomplished with CDI. Inferior yields of the desired product **29** and extensive *E*/*Z* isomerization problems were encountered when coupling of **22** was attempted via its acid chloride. After some experimentation, the BOP[31] reagent was found to be most effective and afforded the *E* isomer **41** in moderate yields. In general, for deactivated unsaturated acids, CDI[31] and EDC[31] have been unsuccessful and even coupling via acid chloride has been less satisfactory. BOP is clearly the reagent of choice under these circumstances. Hydrolysis of **29** utilizing the previously optimized protocol afforded the final triester **31**. Similarly, starting from Boc-D-

Scheme 3[a]



[a] Reagents: (a) CDI, iPr_2NEt, 90%; (b) BSTFA, TMSBr, NaOH, 71%; (c) CDI, iPr_2NEt, 61%; (d) BSTFA, TMSBr, NaOH, 36 °C; (e) MeLi, CuI, TMEDA, TMSCl, 4:2, 5:5, 66%; (f) NH_4OAc, $NaBH_3CN$, 27%; (g) BOP, iPr_2NEt, **2**, 34%; (h) BSTFA, TMSBr NaOH, 87%; (i) *N*-methyl-*p*-toluenesulfonamide, DEAD, PPh_3, 91%; (j) naphthalene, Na, DME, 99%; (k) **2**, CDI, 42%; (l) BSTFA, TMSBr, NaOH, 67%.

serine, the D isomer **32** was prepared in an analogous manner.

In an attempt to reduce the overall charge in the retro series, preparation of the D and L isomers of *N*-farnesoylated glutamic (**37, 38**) and aspartic acid (**43, 44**) analogs was undertaken (Scheme 4). L-Aspartic, D-aspartic, and L-glutamic dialkyl esters **39, 40,** and **35,** respectively, are commercially available, and D-glutamic diethyl ester **36** was conveniently prepared by treatment of glutamic acid with acid and ethanol.[32] Coupling of these amines with (*E*)-farnesoic acid **22** utilizing BOP was uneventful, and isomerization products (*Z* vs *E*) were not observed. Base hydrolysis of the protected diesters **35, 36, 41,** and **42** gave the final diacids **37, 38, 43,** and **44,** respectively, in a straightforward manner.

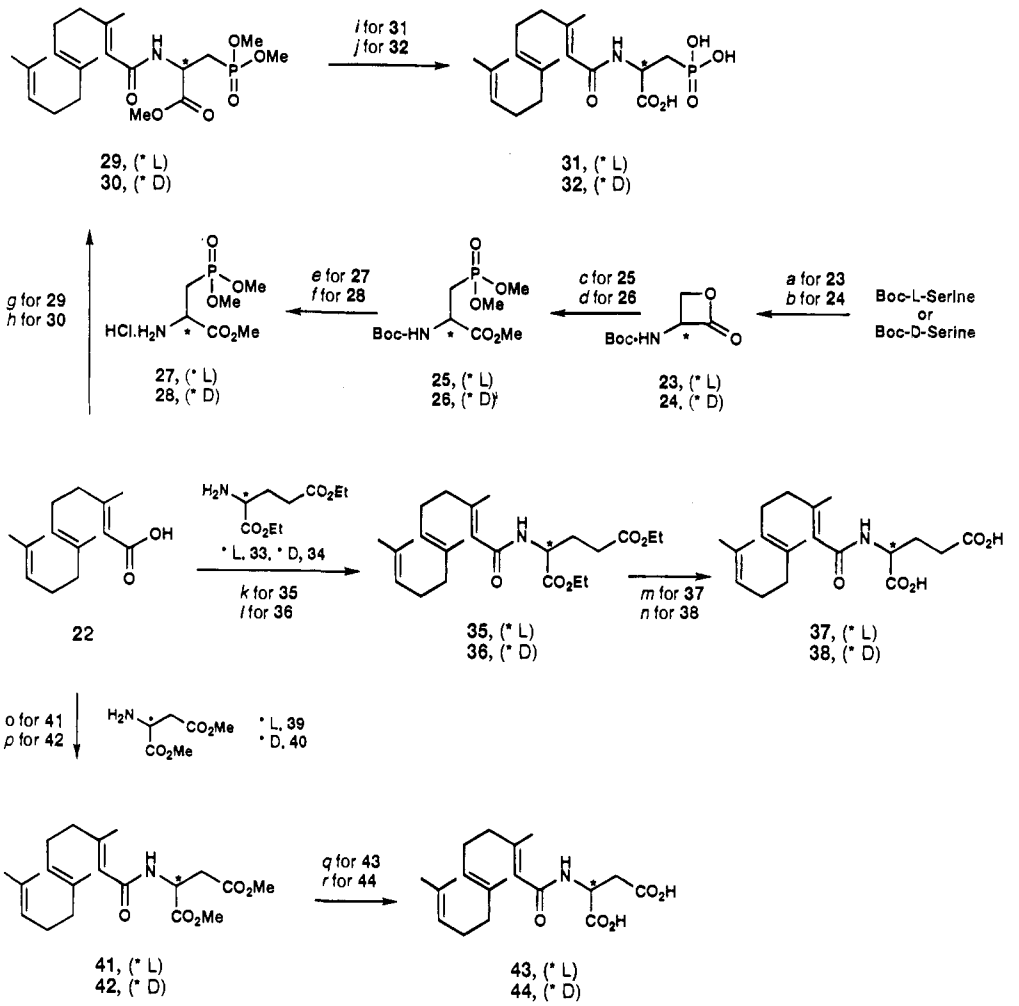
For preparation of **49** and **50,** the exact retro-amide analogs of **3,** a good preparation of stereochemically pure (*E*)-homofarnesoic acid **46** was needed (Scheme 5). Not surprisingly, alkaline hydrolysis of (*E*)-farnesyl nitrile **45**[24] yielded a nonseparable mixture of isomeric acids **46**.[33] Coupling with chiral amino esters **39** and **40** derived from D- and L-serine followed by hydrolysis gave the retro analogs **49** and **50** respectively as a 2:1 *E*/*Z* mixture in each instance.

Biology and Discussion

Since FPT catalyzes a bisubstrate reaction, it offers several different choices ranging from individual substrate to bisubstrate analog-based design of inhibitors.[5,15-18] The study of FPP-based inhibitors described here offers the advantage of starting out with small-sized, nonpeptidic molecules; features that are highly desirable in the area of drug discovery. However, FPP is fairly ubiquitous in nature and is involved in several biochemical pathways incuding cholesterol biosynthesis, raising concerns regarding selectivity of FPP based inhibitors.[9,35] Second, the PP group is highly charged and labile, necessitating its replacement with suitable isosteres to achieve the desired level of metabolic stability and cell permeability.[20]

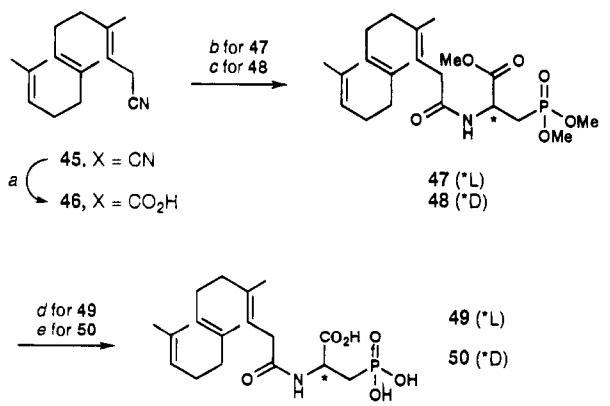
The first set of FPP-based inhibitors consisted of a farnesyl group, an amide linker, and a β-carboxylic phosphonic acid group as a PP surrogate. This led to inhibitor **3,** which was found to be a potent (I_{50}(FPT) = 0.075 μM, Table 1) and selective inhibitor of FPT, as demonstrated by its relatively inferior activity against squalene synthetase (I_{50}(SS) = 516 μM), an enzyme in the cholesterol biosynthetic pathway that also utilizes FPP as a substrate.[35] It was also inactive against mevalonate kinase (I_{50}(MK) = > 200 μM), an enzyme against which FPP has moderate activity (I_{50}(MK) = 5 μM).[38] As shown in Figure 2, this novel inhibitor can possibly benefit from similar binding interactions as FPP at the active site of the enzyme. Thus, the amide linker group of **3** may be substituting for the allylic oxygen of FPP and the carboxylic and phosphonic acid groups of **3** may be working in concert to benefit from

Scheme 4[a]



[a] Reagents: (a) Boc-L-serine, DEAD, PPh_3; (b) Boc-D-serine, DEAD, PPh_3, 54%; (c) $P(OMe)_3$, 89%; (d) $P(OMe)_3$, 86%; (e) 4 M HCl/dioxane, 90%; (f) 4 M HCl/dioxane, 99%; (g) BOP, iPr_2NEt, **27**, 52%; (h) BOP, iPr_2NEt, **28**, 42%; (i) BSTFA, TMSBr, NaOH, 46%; (j) BSTFA, TMSBr, NaOH, 69%; (k) BOP, iPr_2NEt, **33**, 88%; (l) BOP, iPr_2NEt, **34**, 40%; (m) 1N NaOH, 67%; (n) 1 N NaOH, 97%; (o) BOP, iPr_2NEt, **39**, 86%; (p) BOP, iPr_2NEt, **40**, 78%; (q) 1 N NaOH, 79%; (r) 1 N NaOH, 95%.

Scheme 5[a]

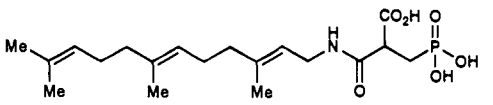
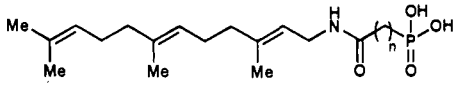
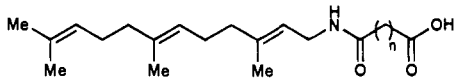
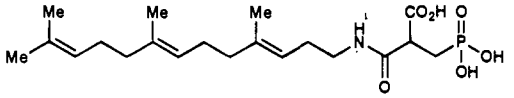
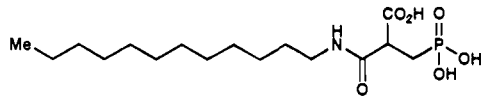
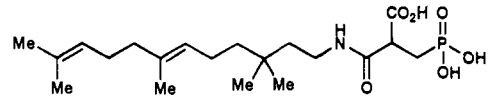
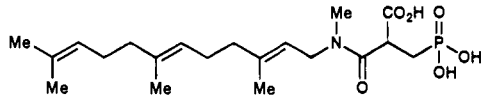
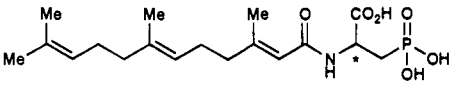
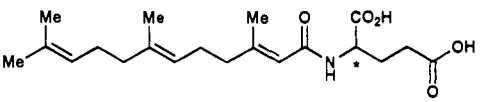
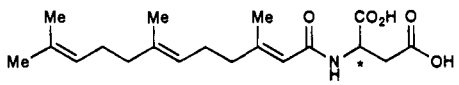
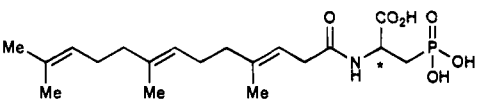


[a] Reagents: (a) KOH, EtOH, 78%; (b) BOP, iPr_2NEt, **39**, 46%; (c) BOP, iPr_2NEt, **40**, 56%; (d) BSTFA, TMSBr, NaOH, **47**, 53%; (e) BSTFA, TMSBr, NaOH, **47**, 10%.

various metal chelation and ionic interactions normally available for stabilization of the PP group of the substrate FPP.[18c]

Systematic modifications of the three structural subunits (farnesyl group, amide linker, and the carboxylic–phosphonic PP surrogate) of **3** were examined with the objective of determining the minimal structural features essential for FPT inhibition. As a starting point, we decided to remove the acidic groups individually from the densely functionalized β-carboxylic phosphonic acid diphosphate surrogate of **3** in order to gain an understanding about their relative contribution toward the overall activity of this compound. If any of these deletions are tolerable, it would lead us toward a molecule with less overall charge, a factor of great importance with respect to cell penetration ability of these type of compounds. Several analogs were prepared in which only one of the acidic groups of **3** was retained at a time. Compound **6** is the descarboxyl analog of **3** and suffered a 500-fold loss in activity in comparison to the parent compound (**6**, I_{50} = 41.5 μM). Shortening the linker in **6** to facilitate better alignment of inhibitor with the substrate molecule FPP led to a 10-fold improvement in potency (**7**, I_{50} = 4.6 μM). Compound **7** was however still 50-fold less active compared to the parent molecule **3**. The β- and γ-carboxyl amides **8** and **9** are the complementary set of analogs involving deletion of the phosphonic acid moiety from **3** and were found to sustain even greater loss in activity (**8**, I_{50} = 115 μM; **9**, I_{50} = 220 μM). These studies demonstrate that both the carboxylic and phosphonic acid groups are critical structural components of **3** since deletion of either of them is detrimental to its *in vitro* potency. Overall, these results are not surprising and

Table 1

Compound		I_{50} (FPT)
	**3**	0.075 ± 0.01 uM 516 uM (SS) >200 uM (MK)
	**6** (n = 1) **7** (n = 2)	41.5 ± 6.5 uM 4.6 ± 3.5 uM
	**8** (n = 1) **9** (n = 2)	115 ± 30 uM 220 ± 80 uM
	**12**	17.75 ± 5.3 uM
	**14**	9.0 ± 2.9 uM
	**18**	55 ± 19 uM
	**21**	0.53 ± 0.03 uM
	**31** (*L) **32** (*D)	0.50 uM 0.25 uM
	**37** (*L) **38** (*D)	30 uM 115 ± 7.5 uM
	**43** (*L) **44** (*D)	25 ± 5 uM 12.6 ± 4.4 uM
	**49** (*L) **50** (*D)	0.05 ± 0 uM 1.7 ± 0.2 uM

indicate that the β-carboxylic phosphonic acid group is collectively serving as a surrogate for the triacidic PP moiety of FPP.

Next, modifications around the farnesyl region of **3** were examined. Analog **12** is the one carbon higher homolog of **3** and is >200-fold less active (I_{50} = 17.7 μM). Since **3** itself is out of register by one carbon atom with respect to the overall length of FPP, compound **12** represents even further deviation from FPP and may provide an explanation for this loss in activity. Substitution of farnesyl group of **3** by a dodecyl group, where the overall length of a farnesyl chain is maintained but the double bonds and side chain methyl groups have been removed, was also found to have a detrimental effect on activity (**14**, I_{50} = 9.0 μM). This highlights the important conformational and/or electronic effect that the olefin and side chain alkyl groups may be exerting on the farnesyl moiety of **3**. The dimethyl analog **18** that involves the removal of a single olefin group and the introduction of an extra methyl group at the same position is a less drastic modification compared to the dodecyl analog **14**. It, too, however, also suffered a >600-fold loss in activity (**18**, I_{50} = 55 μM). The results of analogs **12**, **14**, and **18** collectively suggest that requirements around the farnesyl group of **3** are very stringent.

The *N*-methyl analog **21** was prepared in order to derive some understanding regarding the importance of H-bond-donating ability of the amide proton in **3**. A 6-fold loss in activity of **21** was observed (I_{50} = 0.53 μM). While this result does highlight the importance of the H-bond-donating ability of the amide functionality in **3**, it needs to be interpreted with caution since N-methylation of an amide group can also enhance its overall *cis* population, which can in turn affect the relative orientation of the farnesyl and PP surrogate moieties.

While investigating the criticality of the amide group

of **3**, it occurred to us that unlike **3** which is a racemic mixture, its retroamide analogs **49** and **50** could be prepared as individual diastereomers by utilizing chiral amino acids as starting materials. Additionally, besides synthesizing the exact retro-amide analog, we could now also prepare analogs **31** and **32** which are shorter by one carbon atom and which overlay more accurately with the natural substrate FPP. Such analogs may end up displaying better overall activity compared to **3**, since the latter is longer by one atom than FPP.

Compounds **31** and **32** (Scheme 4) are the L- and D-enantiomers respectively of retro analogs of **3**, wherein the alignment of acidic groups is in line with that of the natural substrate FPP. Inhibitor **32** (I_{50} = 0.25 μM) that was derived from D-aspartic acid was only 3-fold less active than the parent compound **3** and represented the first acceptable structural modification of the lead compound. Additionally, there was only a 2-fold difference in activity between the D- and L-enantiomers (**31**, I_{50} = 0.50 μM), suggesting that absolute chirality is not very crucial in this retro series. Olefin isomerization problems were encountered in the preparation of individual L- and D-enantiomers **49** and **50** (Scheme 5) of the exact retro analogs of **3** and were submitted as a mixture of isomers (*E*:*Z* = 2:1). This modification was well-tolerated, as evident from the equipotency of retro analog **49** (I_{50} = 0.05 μM). It is important to note that since **49** is a 2:1 *E*/*Z* mixture, the observed potency might be a slight underestimation of its true activity. The good potency of retroamide analog **49** (I_{50} = 0.05 μM) versus the parent compound **3** (I_{50} = 0.075 μM) may be suggestive of the hypothetical H-bond donor group at the active site at such a position so that it can form good interactions with either a normal or a retro amide bond. In contrast to the observations made with one carbon shorter retro analogs **31** and **32**, the L- and D-enantiomers of these exact retro analogs registered a substantial 34-fold difference in activity among themselves (**49**, I_{50} = 0.05 μM; **50**, I_{50} = 1.7 μM).

The D and L isomers **37** and **38** and **43** and **44** of N-farnesoylated glutamic and aspartic acid analogs, respectively, were prepared in an attempt to reduce the overall charge in the retro series. The *in vitro* activity of both the aspartic (**37**, I_{50} = 30 μM; **38**, I_{50} = 115 μM) and glutamic acid (**43**, I_{50} = 25 μM; **44**, I_{50} = 12.6 μM)-derived analogs reiterates the fact that a diacidic phosphonic acid group cannot be substituted by a monoacidic carboxylic acid group since both charges of the former are required for good binding of this type of FPP-based inhibitors of FPT. The D isomer **44** was slightly more active than the L isomer **43** in the aspartyl series whereas the L isomer **37** displayed 4-fold better potency amongst the two glutamic acid analogs **37** and **38**.

Summary

An approach toward the preparation of novel FPP-based inhibitors of the enzyme FPT was undertaken. This led to the design and synthesis of **3**, wherein an amide linker connected the farnesyl group and a β-carboxylic phosphonic acid type pyrophosphate surrogate. Compound **3** was found to be a potent inhibitor of FPT (I_{50}(FPT) = 75 nM). It is also highly selective, as witnessed by its inferior activity against other FPP utilizing enzymes such as squalene synthetase (I_{50}(SS) = 516 μM) and mevalonate kinase (I_{50}(MK) = >200 μM). A systematic SAR study of **3** was undertaken. The molecule was divided into three subunits—the farnesyl group, the amide linker, and the PP surrogate—and modifications of each component were carried out to probe the critical structural requirements of **3** for good binding. Both the carboxylic and phosphonic acid groups of the PP surrogate of **3** are essential for activity, since deletion of either group results in 50–2600-fold fold loss in activity (compounds **6**–**9**). The farnesyl group has very stringent requirements and does not tolerate one-carbon homologation, substitution by a dodecyl fragment, or introduction of an extra methyl group at the allylic position (Inhibitors **12**, **14**, and **18**; 100–500-fold less active compared to **3**). The amide linker group in **3**, besides exerting the expected electronic and conformational effect (*N*-methyl analog **21**; 6-fold less active), also appears to provide the right degree of separation between the farnesyl moiety and the PP surrogate. Thus, studies involving reversal of orientation of the amide group reveal that the one carbon atom shorter farnesoic acid-derived retroamide analog **32** (I_{50} = 250 nM) is 4-fold less active, whereas the exact retroamide analog **49** (I_{50} = 50 nM) is equiactive to **3** (I_{50} = 75 nM).

In summary, compounds such as **3**, **32**, and **49** are small sized, nonpeptidic, novel, potent, and selective inhibitors of FPT that may find utility as antitumor agents. These compounds are the outcome of a rational design approach based on the structure and binding interactions of substrate FPP and are thus structurally different from the previously reported CAAX-based[17] or bisubstrate type[16] FPT inhibitors.

Experimental Section

General. All reactions were carried out under a positive pressure of dry argon, unless otherwise specified. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium or potassium benzophenone ketyl prior to use. Acetonitrile, benzene, dichloromethane, diisopropylamine, hexane, methanol, pyridine, and toluene were distilled from calcium hydride prior to use.

TLC was performed using EM Science 5 × 10 cm plates precoated with silica gel 60 F_{254} (0.25 mm thickness), and the spots were visualized by any of the following: UV, iodine, phosphomolybdic acid (PMA), ceric ammonium sulfate, anisaldehyde, vanillin, or Rydons stain. EM Science silica gel 60 (230–400 mesh ASTM) was used for flash chromatography. A ratio of 25–100:1 silica gel/crude product by weight and a nitrogen pressure of 5–25 psi was normally employed for flash columns. Reverse phase chromatographic purification of final compounds was carried out using CHP20P gel, a 75–150 μm polystyrene–divinyl benzene copolymer purchased from Mitsubishi Chemical Industries. Analytical HPLC was performed using two Shimadzu LC-6A pumps with an SCL-6B system controller, a C-R4AX Chromatopac integrator, and an SPD-6AV UV-VIS spectrophotometric detector. HPLC columns were from YMC Corporation (YMC S3 120A ODS, 6.0 × 150 mm) and were eluted with gradients of methanol/water containing 0.2% phosphoric acid.

Melting points were determined on an electrothermal Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on one of the following instruments: JEOL GX-400 operating at 400 (^{1}H) or 100 MHz (^{13}C), JEOL FX-270 operating at 270 (^{1}H) or 67.8 (^{13}C) MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (*J*) are in hertz (Hz). IR spectra were recorded on a Mattson Sirius 100 FT-IR spectrophotometer, and the absorption maxima are reported in cm^{-1}. Mass spectra were

recorded on a Finnigan MAT TSQ-4600 mass spectrometer (chemical ionization, CI) or a VG-ZAB-2F mass spectrometer (fast atom bombardment, FAB). High-resolution mass spectra (HRMS) were determined using peak-matching techniques versus PEG standards on a VG-ZAB-2F spectrometer. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter and a 10 cm path length optical cell. All compounds were homogeneous by TLC and HPLC. Microanalysis results were adjusted to obtain the best fit assuming nonstoichiometric hydration.

Farnesyl Protein Transferase (FPT) Inhibition Assay. Farnesyl protein transferase was isolated from pig brain as described by Manne et al.[14a] and further purified by a 30–55% ammonium sulfate precipitation and subsequent FPLC using a DE52 column with a linear 0–400 mM NaCl gradient, hydoxylapatite column with a 10–110 mM potassium phosphate gradient, and Mono Q column with a 0–1000 mM NaCl gradient. Fractions containing farnesyl protein transferase were identified on the basis of enzyme activity using the assay described below. Active fractions were combined and dialyzed overnight into 20 mM Tris-HCl, pH 7.4 (for DE52 and Mono Q), or 10 mM potassium phosphate, pH 7.6 containing 100 mM NaCl (for hydroxlapatite). All dialysis buffers contained 1 mM DTT, 5% glycerol, 0.1–0.25 mM EDTA, 0.1–0.25 mM EGTA, 1 mM benzamidine, 10 μg/mL soybean trypsin inhibitor and for the DE52 and Mono Q dialysis buffers 1 μg/mL leupeptin and 0.25–0.5 mM PMSF. Enzyme activity was purified approximately 2000-fold relative to the initial crude pig brain cytosol. The PXCR expression vector containing H-ras was kindly provided by Dr. Larry Feig (Tufts University, Medford, MA). Recombinant p21 H-ras was expressed in the *E. coli* strain PR13Q and processed as described by Farnsworth et al.[36] Following processing and ammonium sulfate precipitation the pellet was resuspended in 50 mM Tris-HCl, pH 7.5 20 mM NaCl, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, 10 μg/mL soybean trypsin inhibitor, 10 μM E64, and 1 μM pepstatin and dialyzed overnight. The recombinant p21 H-ras was then partially purified by FPLC using a DE52 column and a linear NaCl gradient from 20 to 320 mM. Fractions containing the p21 H-ras were visualized by Coomassie blue-stained SDS–polyacrylamide gels and assayed for substrate capacity using the farnesylation assay described below. p21 H-ras with a purity of >60% was obtained with this single column purification. Additional processing often led to a more pure protein with a reduced capacity to be farnesylated (assumed to be a consequence of the carboxyl terminus cleavage described in Farnsworth et al.[36]).

Farnesyl protein transferase assays were run in 96-well dishes in a reaction volume of 20 μL. The final reaction mixture contained 1 μM [^{3}H]FPP (NEN Dupont), 7 μM p21 H-ras, 25 mM $MgCl_2$, 10 mM DTT, 100 mM HEPES 7.4, and serial dilutions of inhibitor usually ranging from 360 to 0.02 μM. Reactions were started by adding sufficient enzyme to produce approximately 2 pmol of [^{3}H]FPP incorporation in 1 h in the control wells. Following incubation at 37 °C for 1 h, the reactions were stopped by adding 90 μL of 4% sodium dodecyl sulfate (SDS) followed by 90 μL of 30% TCA. Plates were incubated overnight at 4 °C and then the precipitates were transferred to Millipore multiscreen filtration 96-well plates with 0.65 PVDF membranes (Millipore Corp. Bedford, MA). Following filtration using the multiscreen vacuum manifold, the wells were washed once with 200 μL of 4% SDS/6%TCA and five times with 200 μL of 6% TCA. Following removal of the bottom seal, excess washing fluid was blotted and the plates were allowed to dry before the filters were punched into 4 mL vials using the multiscreen punch. After incubation at 60–70 °C with 300 μL of Solvable (NEN Dupont), 3 mL of Formula 989 (Dupont) scintillation fluid was added and radioactivity determined by scintillation counting. Dose-response curves for inhibitors used triplicate estimates at each drug concentration, and the IC_{50} estimation were made from percent control versus log drug concentration plots. Each compound was tested at least twice.

Squalene Synthetase (SS) and Mevalonate Kinase (MK) Inhibition Assay. Squalene synthetase was prepared from rat liver microsomes and used for I_{50} determinations as described before.[37] Mevalonte kinase was purified to homogenity from rat liver and used for inhibition as described before.[38]

Preparation of (*E,E*)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-amine (1). A solution of 2.47 g of farnesyl bromide in 20 mL of dry DMF at room temperature under argon was treated with 1.83 g (9.9 mmol, 1.1 equiv) of potassium phthalimide and stirred for 3 h at room temperature. The solvent was removed under reduced pressure, the residue was titurated with 150 mL of Et_2O, and the precipitate was filtered off. The ethereal solution was washed with 50 mL of H_2O and 50 mL of brine, dried over $MgSO_4$, and evaporated to yield 2.96 g of crude product as a milky oil. Purification by flash chromatography on 300 g of Merck 9385 silica, eluting with 7:93 EtOAc/petroleum ether afforded 2.56 g (81%) of the phthalimide intermediate as a colorless oil: TLC silica gel (2:8 EtOAc/hexane) R_f = 0.37; MS (CI-CH_4/N_2O, + ions) *m/e* 392 (M + C_3H_5), 380 (M + C_2H_5), 352 (M + H), 296, 284, 270, 228, 216; IR (neat) 2967, 2930, 2856, 1772, 1716, 1468, 1432, 1394, 1366, 1325, 1112, 1088, 1073, 947, 721, 531 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) 1.56 (s, 6H), 1.66 (s, 3H), 1.83 (s, 3H), 1.9–2.1 (m, 8H), 4.27 (d, 2H, J = 7.0 Hz), 4.27 (d, 2H, J = 7.0 Hz), 5.05 (d, 2H, J = 7.0 Hz), 5.27 (t, 1H, J = 7.0 Hz), 7.68 (dd, 2H, J = 3.0, 5.5 Hz), 7.82 (dd, 2H, J = 3.0, 5.5 Hz).

A solution of 2.50 g (7.1 mmol) of the above intermediate in 15 mL of absolute EtOH at room temperature under argon was treated with 1.9 mL (35.57 mmol, 5.0 equiv) of methylhydrazine and stirred for 2 h at room temperature and 4 h at reflux. After cooling and the addition of 7.1 mL (7.1 mmol, 1.0 equiv) of 1 M NaOH, the ethanol was removed under reduced pressure. The residue was extracted with 350 mL of Et_2O, and the ether layer was washed with 100 mL of 1 M NaOH, 50 mL of H_2O, and 50 mL of brine, dried ($MgSO_4$), and evaporated to obtain 1.45 g of crude product. Purification by Kugelrohr distillation at 120 °C/0.005 mm provided 1.405 g (89%) of 1 as a colorless oil: TLC silica gel (8:1:1 1-propanol/concentrated NH_3/H_2O) R_f = 0.64; MS (CI-CH_4/N_2O, + ions) *m/e* 443 (2M + H), 222 (M + H), 205, 137; IR (neat) 3291, 2967, 2923, 2856, 1574, 1483, 1453, 1383, 1378, 1347, 1288, 819, 777 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) 1.20 (br s, 2H, NH_2), 1.60 (s, 6H, H_{13}, H_{14}), 1.63 (s, 3H, H_{15}), 1.68 (s, 3H, H_{12}), 1.9–2.1 (m, 8H, H_4, H_5, H_8, H_9), 3.27 (d, 2H, J = 7.0 Hz, H_1), 5.10 (br, 2H, H_6, H_{10}), 5.26 (t, 1H, J = 7.0 Hz, H_2).

Preparation of [(Diethoxyphosphinyl)methyl]propanedioic Acid, Monoethyl Ester (2). A solution of 15.16 mL (100 mmol, Aldrich) of diethyl malonate, 3.0 g (100 mmol) of paraformaldehyde, and 24.9 g (150 mmol, Aldrich) of triethyl phosphite was heated to 180 °C and stirred for 16 h. The reaction was cooled and placed under vacuum to remove volatiles. Purification was achieved after two fractional distillations (bp 142 °C, 0.046 mmHg) and flash chromatography on Merck silica gel, eluting with EtOAc to afford 14.76 g (48%) of penultimate tetraester precursor of **2** as a colorless oil: TLC R_f (silica gel, EtOAc) = 0.36; IR ($CHCl_3$) 3025, 2992, 2939, 2908, 1732, 1370, 1253, 1053, 1028, 974 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) 1.28 (t, 6H, J = 7 Hz), 1.31 (t, 6H, J = 7 Hz), 2.41 (dd, 2H, J = 7, 17 Hz), 3.67 (dt, 1H, J = 7, 12 Hz) 4.09 (m, 4H), 4.22 (m, 4H).

To a stirred solution of 5.00 g (16.1 mmol) of the above intermediate in 16 mL of 70% aqueous EtOH was added an 8 mL (16 mmol) solution of 2 M KOH in 70% aqueous EtOH over 55 min. The reaction was stirred 18 h, the solvent was concentrated *in vacuo* at 30 °C, and the mixture was partitioned between Et_2O and H_2O. The aqueous layer was cooled, neutralized with 25% aqueous H_2SO_4 to pH 2–3, and saturated with solid NaCl. After extraction with 250 mL of Et_2O, the organic layers were combined, dried ($MgSO_4$), and concentrated *in vacuo* to yield 3.21 g (70%) of **2** as a colorless oil: TLC silica gel (8:1:1 nPrOH/concentrated NH_3/H_2O) R_f = 0.35, I_2 and PMA, homogeneous; IR (CH_2Cl_2 film) 2986, 1738, 1231, 1148 cm^{-1}; ^{1}H NMR ($CDCl_3$, 270 MHz) 1.30 (t, 3H, J = 7 Hz), 1.33 (t, 6H, J = 7 Hz), 2.43 (dd, 2H, J = 7, 18 Hz, 3.67 (dt, 1H, J = 7, 12 Hz), 4.18 (m, 6H) 10.23 (br s, 1H); HRMS (M + H)$^+$ calcd 283.0947, found 283.0960 (δ = 4.6).

Preparation of (*E,E*)-3-Oxo-2-(phosphonomethyl)-3-[(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]propanoic Acid (3). To a stirred solution of 0.310 g (1.10 mmol) of **2**

in 3 mL of CH_2Cl_2 at 0 °C was added 2 drops of DMF, followed by the dropwise addition of 190 μL (2.2 mmol, Aldrich) of oxalyl chloride. The solution was warmed to 25 °C, stirred for 2 h, and concentrated *in vacuo*. To a solution of 312 mg (1.21 mmol) of 1 in 3 mL of CH_2Cl_2 at 0 °C was added iPr_2NEt (570 uL, 2.7 mmol), the mixture was cooled to 0 °C, and the acid chloride formed from above in CH_2Cl_2 (3 mL) at 0 °C was added dropwise via cannula. The resulting solution was stirred for 45 min, and then 30 mL of Et_2O was added. The organic layer was washed successively with 15 mL each of 1 N aqueous HCl, H_2O, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to yield a crude yellow-white oil. Purification via flash chromatography on 60 mL of Merck silica gel eluting with 2:1 EtOAc/petroleum ether, followed by filtration through Act III neutral alumina eluted with 3:1 EtOAc/petroleum ether, afforded 0.335 g (65%) of the triester penultimate precursor of **3** as a yellow oil: TLC silica gel (3:1 EtOAc/petroleum ether) $R_f = 0.18$; MS (CI-CH_4/N_2O, + ions) *m/e* 526 (M + C_2H_5), 486 (M + H); IR ($CHCl_3$) 3680, 3437, 3016, 2996, 2980, 2928, 2872, 2856, 1742, 1680, 1245, 1209, 1110, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) 1.27 (t, 3H, J = 7 Hz), 1.30 (t, 3H, J = 7 Hz), 1.31 (t, 3H, J = 7 Hz), 1.59 (s, 6H), 1.67 (t, 6H), 2.03 (m, 8H), 2.44 (dd, 2H, J = 7, 14 Hz), 3.55 (dt, 1H, J = 7, 14 Hz), 3.87 (br m, 2H), 4.07 (2 overlapping quint, 4H, J = 7 Hz), 4.21 (q, 2H, J = 7 Hz), 5.09 (t, 2H, J = 7 Hz), 5.19 (t, 1 H, J = 7 Hz), 6.33 (br, 1H).

To a solution of 0.355 g (0.70 mmol) of the above intermediate in 2 mL of distilled CH_2Cl_2 at room temperature was added dropwise 185 μL (1.4 mmol, Aldrich, distilled) of collidine, followed by 370 μL (2.8 mmol, Aldrich) of bromotrimethylsilane. The reaction was stirred at 25 °C for 2 h, 15 min, and then concentrated *in vacuo*. Benzene was added to the residue, the solution was concentrated *in vacuo*, and the remainder was pumped at high vacuum. The residue was dissolved in 5.6 mL of 1 N aqueous NaOH (8 equiv) solution and stirred for 16 h. The solution was lyophilized, and the lyophilate was purified by chromatography on a 2.5 cm diameter × 20 cm high column of HP-20 packed in water. The column was eluted first with H_2O to an eluant pH of 9–10 followed by a 10% aqueous CH_3CN, collecting 10–12 mL fractions. The appropriate fractions were combined, evaporated, lyophilized, and pump-dried to provide 0.310 g (80%) of **3** as a very hygroscopic white lyophilate. A 1% solution of **3** had a pH of 8.95: TLC silica gel (6:3:1 *n*PrOH/concentrated NH_3/H_2O) $R_f = 0.35$, I_2 and PMA; MS (FAB, + ions) *m/e* 490 (M + Na), 468 (M + H), 446 (M + 2H − Na), 424, (M + 3H − 2Na), 402 (M + 4H − 3Na); IR (KBr) 3410, 3316, 2966, 2922, 2854, 1639, 1587, 1384, 1079, 979 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) 1.60 (s, 6H), 1.66 (s, 6H), 1.92 (m, 2H), 2.07 (m, 8H), 2.03 (m, 8H, 3.33 (ddd, 1H, J = 5.5, 7.5, 13 Hz), 3.75 (dd, 1H, J = 7, 15 Hz), 3.81 (dd, 1H, J = 7, 15 Hz), 5.17 (2 overlapping triplets, 2H, J = 7 Hz), 5.24 (t, 1H, J = 6 Hz). Anal. Calcd for $C_{19}H_{29}NO_6P{\cdot}Na_3$ (MW 467.379): C, 41.01; H, 7.04; N, 2.52. Found: C, 40.75; H, 6.89; N, 2.75. Calcd for 16% water, corrected MW 556.39.

Preparation of 3-(Dimethoxyphosphinyl)propanoic Acid 4. Dimethyl trimethylsilyl phosphite (19.5 g, 0.107 mol, 1.2 equiv) and ethyl acrylate (9.67 mL, 0.089 mol, 1.0 equiv) were heated neat for 2 h at 117 °C. The reaction mixture was then cooled to room temperature and diluted with diethyl ether (200 mL), and the reaction was slowly quenched with water (10 mL). The mixture was stirred for 15 min, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by vacuum distillation (115–120 °C, 1.5 mmHg) to afford the triester penultimate precursor of **4** (9.0 g, 40%): MS (M + H)$^+$ 211; HRMS (M + H)$^+$ calcd 211.0735, found: 211.0736 (δ = 0.5 ppm); IR (CH_2Cl_2 film) 2961, 1736, 1377, 1284 cm^{-1}; ^1H ($CDCl_3$) 1.21 (t, 3H, J = 7.04), 1.97–2.10 (m, 2H), 2.47–2.57 (m, 2H), 3.69 (d, 6H, J = 10.55), 4.09 (q, 2H, J = 7.04); ^{13}C ($CDCl_3$) 13.7, 18.5, 20.6, 26.9, 27.0, 52.0, 52.1, 60.4, 171.3, 171.6. Sodium hydroxide (1 N, 4.76 mL, 1.0 equiv) was added to a solution of the above intermediate (1.0 g, 4.76 mmol, 1.0 equiv) in methanol (5 mL) at 0 °C, allowed to warm to room temperature, and stirred for 16 h. The reaction mixture was concentrated under vacuum, the residue was dissolved in water (10 mL) and extracted with dichloromethane (3 × 50 mL), the organic extracts were discarded, and the aqueous layer was acidified to pH 2.0 (1 N HCl) and concentrated under vacuum. The residue was dissolved in ethyl acetate (25 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum to afford **4** (0.78 g, 90%): TLC R_f = 0.67 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by I_2/anisaldehyde); MS (M + H)$^+$ 183; HRMS (M + H)$^+$ calcd 183.0422, found 183.0425 (δ = 1.6 ppm); IR (CH_2Cl_2) 2926, 2855, 1736, 1443 cm^{-1}; ^1H ($CDCl_3$) 2.07–2.19 (m, 2H), 2.56–2.67 (m, 2H), 3.76 (d, 6H, J = 11.1); ^{13}C ($CDCl_3$) 18.5, 20.6, 26.8, 52.7, 52.8, 174.3, 174.6.

Preparation of 2-(Diethoxyphosphinyl)acetic Acid (5). A solution of 5.00 g (22.2 mmol) of triethyl phosphonoacetate in 11 mL (22.5 mmol, 1.0 equiv) of 1 M NaOH was stirred at room temperature under nitrogen for 3 h. The EtOH was evaporated, and the aqueous phase was acidified with 1 N $KHSO_4$ and then extracted with five 70 mL portions of CH_2Cl_2. The combined organic phase was washed with 50 mL of brine, dried over $MgSO_4$, and evaporated to provide 3.49 g (95%) of **5** as a colorless oil: TLC silica gel (8:1:1 *n*-PrOH/concentrated NH_3/H_2O) $R_f = 0.26$; IR (CH_2Cl_2, film) 2986, 1738, 1231, 1148 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) 1.35 (t, 6H, J = 7.0 Hz, OCH_2CH_3), 3.00 (d, 2H, J = 11.5 Hz, PCH_2), 4.19 (quint, 4H, J = 7.0 Hz), 8.90 (br, 4H, CO_2H); HRMS (M + H)$^+$ calculated for $C_{10}H_{19}O_7P$ 283.0947, found 283.0960 (δ = 4.6).

Preparation of (*E,E*)-[3-Oxo-3-[(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]propyl]phosphonic Acid (6). 1,1′-Carbonyldiimidazole (0.126 g, 0.777 mmol, 1.0 equiv) was added to a solution of **4** (0.141 g, 0.777 mmol, 1.0 equiv) in THF (2.5 mL), and the resultant mixture was stirred for 15 min at 0 °C and 1 h at 20 °C. Farnesylamine hydrochloride 1 (0.2 g, 0.777 mmol, 1.0 equiv) was added followed by diisopropylethylamine (0.162 mL, 0.93, 1.2 equiv), and the mixture was stirred for 16 h. The reaction was quenched with HCl (1N, 50 mL), the mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were washed with Na_2CO_3 (10%, 50 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 25:1 chloroform/methanol) to afford the diester penultimate precursor of **6** (0.194 g, 65%): TLC R_f = 0.25 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 386; IR (CH_2Cl_2 film) 2963, 1655, 1449, 1233 cm^{-1}; ^1H ($CDCl_3$) 1.60 (s, 6H), 1.67 (s, 6H), 2.00–2.17 (m, 10H), 2.39–2.50 (m, 2H), 3.73 (d, 6H, J = 10.6), 3.84 (d, 2H, J = 6.5), 5.09 (br m, 2H), 5.18 (br m, 1H). Bis(trimethylsilyl)trifluoroacetamide (0.59 mL, 2.2 mmol, 4.5 equiv) was added to a solution of the above intermediate (0.19 g, 0.494 mmol, 1.0 equiv) in dichloromethane (4 mL), and the mixture was stirred for 1 h at room temperature. Bromotrimethylsilane (0.163 mL, 1.24 mmol, 2.5 equiv) was then added, and the mixture was stirred for 16 h. The reaction mixture was concentrated under vacuum, the residue dissolved in methanol (5 mL) and NaOH (1N, 1.2 mL), and the mixture stirred for 15 min and concentrated under vacuum. The residue was purified by CHP-20P gel (eluting sequentially with water (500 mL) and acetonitrile (40%, 500 mL)) to afford **6** (0.104 g, 53%): mp 195 °C dec; TLC R_f = 0.64 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by anisaldehyde); MS (M + Na)$^+$ 380; IR (KBr) 1628 cm^{-1}; ^1H (CD_3OD) 1.58 (s, 6H), 1.64 (s, 3H), 1.66 (s, 3H), 1.67–1.75 (m, 2H), 1.93–2.10 (m, 8H), 2.41–2.46 (m, 2H), 3.74 (d, 2H, J = 6.9), 5.06–5.10 (m, 2H), 5.20 (br m, 1H). Anal. ($C_{18}H_{30}NO_4PNa_2{\cdot}0.11H_2O$) Calcd: C, 53.61; H, 7.55; N, 3.47. Found: C, 53.91; H, 8.00; N,3.34.

Preparation of (*E,E*)-[2-Oxo-2-[(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]ethyl]phosphonic Acid (7). Following the CDI coupling protocol described for preparation of **6**, acid **5** (562 mg, 2.87 mmol, 1.0 equiv) was reacted with farnesylamine **1** (700 mg, 3.16 mmol, 1.1 equiv) in THF (5 mL) to afford the diester precursor of **7** (737.1 mg, 58%): TLC silica gel (EtOAc) $R_f = 0.14$; MS (CI-CH_4/N_2O + ions) *m/e* 428 (M + C_2H_5), 400; IR (film) 3400, 3289, 2979, 2970, 2927, 2877, 2858, 1658, 1552, 1295, 1243, 1057, 1029, 972 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) 1.34 (t, 6H, J = 7.0 Hz), 1.60 (s, 6H), 1.68 (s, 6H), 1.9–2.2 (m, 8H), 2.83 (d, 2H, J = 10.5 Hz), 3.87 (t, 2H, J = 7.0 Hz), 4.14 (quint, 4H, J_{HH} = 7.0 Hz, J_{HP} = 7.0 Hz), 5.09 (m, 2H), 5.20 (t, 1H, J = 7 Hz), 6.60 (br, 1H).

A solution of 737.2 mg (1.85 mmol) of above intermediate and 490 μL (3.7 mmol, 2.0 equiv) of collidine in 12 mL of dry CH_2Cl_2 at 0 °C under argon was treated with 980 μL (7.4 mmol, 4.0 equiv) of bromotrimethylsilane. The resulting solution was stirred for 1 h at 0 °C and for 20 h at room temperature, and the solvent was evaporated. The residue was dissolved in a mixture of 1.03 mL (7.4 mmol, 4.0 equiv) of triethylamine and 4 mL of MeOH, stirred 15 min, and evaporated. The organic phase formed on addition of 75 mL of EtOAc was washed with 15 mL of 10% HCl, 15 mL of 1:1 H_2O/brine, and 15 mL of brine, dried over $MgSO_4$, and evaporated. The so-obtained oil was dissolved in a mixture of 4.6 mL (4.6 mmol, 2.5 equiv) of 1 M KOH and 4 mL of MeOH. After evaporating the methanol, the water was removed by lyophilization and the lyophilizate was dissolved in 4 mL of H_2O and loaded into a 2.5 cm diameter × 16 cm length column of HP-20, packed in water. The column was eluted with a forerun of 300 mL of water, followed by a gradient created by the gradual addition of acetonitrile to water. Approximately 15 mL fractions were collected every two minutes. Fractions 28–32 were combined and lyophilized, and the resulting white powder was further dried at high vacuum over P_2O_5 to provide 488.7 mg (63%) of **7**. A 1% solution of **7** in water has a pH of 8.25: TLC silica gel (6:3:1 *n*PrOH/concentrated NH_3/H_2O) R_f = 0.34; MS (FAB, + ions) *m/e* 420 (M + K), 382 (M + H); IR (KBr) 3371, 3103, 3083, 2928, 1631, 1552, 1143, 1073, 978 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) 1.58 (s, 6H), 1.67 (s, 3H), 1.9–2.2 (m, 8H), 2.52 (d, 2H, J = 18.5 Hz), 3.81 (d, 2H, J = 6.5 Hz), 5.09 (m, 2H), 5.26 (t, 1H, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 67.8 MHz) 16.1, 16.4, 17.8, 25.9, 27.5, 27.8, 38.6, 40.2 (d, J_{CP} = 112.0 Hz), 40.7, 40.8, 122.0, 125.2, 125.4, 132.1 136.2, 139.5, 173.5 (d, J_{CP} = 4.0 Hz); ^{31}P NMR (CD_3OD) 12.5 ppm (s). Anal. Calcd for $C_{17}H_{20}KNO_4P$ (MW 381.502) C, 48.66; H, 6.73; N, 3.34; P, 7.38. Found: C, 48.63; H, 7.15; N, 3.28; P, 7.0.

Preparation of (*E,E*)-3-Oxo-3-[(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]propanoic Acid (8). Malonyl chloride ethyl ester (0.099 mL, 0.777 mmol, 1.0 equiv) was added to a solution of farnesylamine hydrochloride **1** (0.2 g, 0.777 mmol, 1.0 equiv) in THF (2.0 mL) at 0 °C. To the reaction mixture was added diisopropylethylamine (0.338 mL, 1.94 mmol, 2.5 equiv), and the reaction mixture was stirred at room temperature for 16 h. The reaction was quenched with water (20 mL), the mixture was extracted with ethyl acetate (3 × 40 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 4:1 hexane/ethyl acetate) to afford the ethyl ester penultimate precursor of **8** (0.153 g, 59%): TLC R_f = 0.66 (1:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 336; ^1H ($CDCl_3$) 1.29 (t, 3H, J = 7.2), 1.60 (s, 6H), 1.68 (s, 6H), 1.96–2.18 (m, 8H), 3.30 (s, 2H), 3.93 (d, 1H, J = 6.4), 3.95 (d, 1H, J = 5.9), 4.21 (q, 2H, J = 7.2), 5.08–5.20 (m, 2H), 5.23 (br m, 1H), 7.00 (br m, 1H); ^{13}C ($CDCl_3$) 14.3, 16.3, 16.6, 18.0, 26.0, 26.6, 27.0, 37.9, 39.8, 40.0, 41.5, 61.8, 119.9, 124.0, 124.6, 131.5, 135.7, 140.4, 165.1, 169.8. Sodium hydroxide (1 N, 0.640 mL, 0.640 mmol, 1.5 equiv) was added to a solution of the above intermediate (0.143 g, 0.427 mmol, 1.0 equiv) in methanol (1 mL), and the mixture was stirred at room temperature. After 16 h the reaction mixture was concentrated under vacuum and the residue chromatographed on a column of CHP-20 gel (eluting sequentially with water (300 mL), methanol (50%, 200 mL), and methanol (100 mL)) to afford **8** (0.100 g, 71%) as the sodium salt: mp 190–193 °C dec; TLC R_f = 0.37 (9:1:0.05 chloroform/methanol/acetic acid); MS (M + H)$^+$ 308, (M + Na)$^+$ 330; IR (KBr, NHCO) 1616 cm^{-1}; ^1H (CD_3OD) 1.60 (s, 6H), 1.66 (s, 3H), 1.69 (s, 3H), 1.95–2.14 (m, 8H), 3.09 (s, 2H), 3.82 (d, 2H, J = 6.4), 5.06–5.13 (m, 2H), 5.24 (br m, 1H). Anal. Calcd for $C_{18}H_{28}O_3NNa{\cdot}0.50H_2O$: C, 63.89; H, 8.64; N, 4.14. Found: C, 63.88; H, 8.65; N,4.08.

Preparation of (*E,E*)-4-Oxo-4-[(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]butanoic Acid (9). Following the procedure described for preparation of **8** but using carbomethoxypropionyl chloride (0.099 mL, 0.777 mmol, 1.0 equiv), farnesylamine hydrochloride **1** (0.2 g, 0.777 mmol, 1.0 equiv) was converted to the methyl ester penultimate precursor of **9** (0.254 g, 98%): TLC R_f = 0.59 (1:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 336; ^1H ($CDCl_3$) 1.65 (s, 6H), 1.72 (s, 3H), 1.73 (s, 3H), 2.03–2.23 (m, 8H), 2.54 (t, 2H, J = 6.5), 2.72 (t, 2H, J = 7.0), 3.74 (s, 3H), 3.89 (d, 1H, J = 6.5), 3.91 (d, 1H, J = 5.8), 5.14 (t, 2H, J = 6.5), 5.25 (t, 1H, J = 7.0), 5.86 (br m, 1H); ^{13}C ($CDCl_3$) 15.9, 16.2, 16.3, 17.6, 20.4, 21.1, 25.6, 26.2, 26.6, 29.2, 30.8, 37.4, 39.4, 39.6, 51.6, 119.8, 123.6, 124.2, 131.2, 135.2, 139.7, 170.9, 173.4. Sodium hydroxide hydrolysis (as described in preparation of **8**) of the above intermediate (0.193 g, 0.576 mmol, 1.0 equiv) afforded **9** (0.18 g, 91%) as the sodium salt: mp 175–180 °C dec; TLC R_f = 0.61 (9:1:0.05 chloroform/methanol/acetic acid); MS (M + H)$^+$ 322, (M + Na)$^+$ 344; IR (KBr, NHCO) 1641 cm^{-1}; ^1H (CD_3OD) 1.59 (s, 6H), 1.66 (s, 3H), 1.67 (s, 3H), 1.93–2.13 (m, 8H), 2.43 (br m, 4H), 3.76 (d, 2H, J = 6.9), 5.07–5.18 (m, 2H), 5.21 (m, 1H). Anal. Calcd for $C_{19}H_{30}O_3NNa{\cdot}0.25H_2O$: C, 65.58; H, 8.84; N, 4.03. Found: C, 65.51; H, 9.11; N, 3.92.

Preparation of (*E,E*)-2-[(Diethoxyphosphinyl)methyl]-3-oxo-3-[(4,8,12-trimethyl-3,7,11-tridecatrienyl)amino]-propanoic Acid, Ethyl Ester (11). Following the CDI coupling protocol described for preparation of **6**, acid **2** (0.21 g, 1.1 mmol, 1.0 equiv) was reacted with homofarnesylamine hydrochloride **10** (0.3 g, 1.1 mmol, 1.0 equiv) to afford triester **11** (0.493 g, 90%): TLC R_f = 0.58 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 500; HRMS (M + H)$^+$ calcd 500.3141, found 500.3141 (δ = 0 ppm); IR (CH_2Cl_2 film) 3082, 2965, 2926, 1742 cm^{-1}; ^1H ($CDCl_3$) 1.29 (t, 3H, J = 7.0), 1.31 (t, 6H, J = 7.1), 1.60 (s, 6H), 1.63 (s, 3H), 1.68 (s, 3H), 2.00–2.23 (m, 10H), 2.43 (dd, 2H, J = 7.0, 17.6), 3.20–3.32 (m, 2H), 3.51–3.56 (m, 1H), 4.02–4.14 (m, 4H), 4.20 (q, 2H, J, 7.0), 5.10 (br m, 3H), 6.42 (m, 1H); ^{13}C ($CDCl_3$) 14.4, 16.4, 16.6, 16.7, 16.8, 18.1, 23.8, 25.9, 26.1, 27.0, 27.2, 28.3, 29.7, 40.1, 40.3, 47.8, 54.3, 62.1, 62.3, 120.6, 124.3, 124.8, 131.7, 135.6, 138.8, 166.9, 167.1, 169.9, 170.1.

Preparation of (*E,E*)-3-Oxo-2-(phosphonomethyl)-3-[(4,8,12-trimethyl-3,7,11-tridecatrienyl)amino]propanoic Acid (12). Bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.478 mL, 1.8 mmol, 4.5 equiv) was added to a solution of **11** (0.2 g, 0.4 mmol, 1.0 equiv) in dichloromethane (5 mL), and the mixture was stirred for 1 h at room temperature. Bromotrimethylsilane (TMSBr, 0.131 mL, 1.0 mmol, 2.5 equiv) was then added, and the mixture was stirred for 18 h. The reaction was concentrated under vacuum, the residue dissolved in methanol (5 mL) and NaOH (1N, 3.8 mL, 3.8 mmol, 9.5 equiv), and the mixture stirred for 18 h. The reaction mixture was concentrated under vacuum, and the residue purified by SP-207 gel (eluting sequentially with water (500 mL) and CH_3CN (250 mL)) to afford **12** (0.136 g, 71%): mp 210 °C dec; TLC R_f = 0.44 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by anisaldehyde); MS (M − H)$^-$ 414 free acid; IR (KBr) 1593, 1647 cm^{-1}; ^1H (CD_3OD) 1.64 (s, 6H), 1.69 (s, 3H), 1.71 (s, 3H), 2.01–2.31 (m, 12H), 3.15–3.25 (m, 2H), 3.42–3.55 (m, 1H), 5.00–5.21 (m, 3H). Anal. Calcd for $C_{20}H_{31}NPO_6Na_3{\cdot}0.64H_2O$: C, 48.74; H, 6.60; N, 2.84. Found: C, 49.02; H, 6.95; N, 2.68.

Preparation of 2-[(Diethoxyphosphinyl)methyl]-3-(dodecylamino)-3-oxopropanoic Acid, Ethyl Ester (13). Following the CDI coupling protocol described for preparation of **6**, acid **2** (0.5 g, 1.77 mmol, 1.0 equiv) was reacted with dodecylamine (Aldrich, 0.33 g, 1.77 mmol, 1.0 equiv) to afford triester **13** (0.48 g, 61%): TLC R_f = 0.86 (4:1 acetone/hexane, visualization by I_2/PMA); MS (M + H)$^+$ 450; HRMS (M + H)$^+$ calcd 450.2984, found 450.2986 (δ = 0.4 ppm); IR (CH_2Cl_2 film) 2926, 1742, 1667, 1559 cm^{-1}; ^1H ($CDCl_3$) 0.88 (t, 3H, J = 6.5), 1.21–1.50 (m, 29H), 2.43 (dd, 2H, J = 7.6, 17.6), 3.18–3.33 (m, 2H), 3.50–3.60 (m, 1H), 4.02–4.09 (m, 4H), 4.20 (q, 2H, J = 7.6), 6.44 (br m, 1H); ^{13}C ($CDCl_3$) 14.0, 14.1, 16.2, 16.3, 22.6, 23.3, 25.4, 26.8, 29.2, 29.3, 29.5, 29.6, 31.9, 40.0, 47.4, 60.9, 61.7, 61.9, 62.0, 166.5, 166.6, 169.5, 169.7.

Preparation of 3-(Dodecylamino)-3-oxo-2-(phosphonomethyl)propanoic Acid (14). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **13** (0.2 g, 0.499 mmol, 1.0 equiv) to triacid **14** (0.07 g, 36%): mp 245 °C dec; TLC R_f = 0.82 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by I_2/anisaldehyde); MS (M + H)$^+$ 366;

IR (KBr) 1643 cm^{-1}; ^1H (CD_3OD) 0.89 (t, 3 H, J = 6.4), 1.24–1.37 (m, 18 H), 1.48–1.53 (m, 2 H), 1.89–1.98 (m, 1 H), 2.14–2.24 (m, 1 H), 3.12–3.19 (m, 2 H), 3.45–3.50 (m, 1 H). Anal. Calcd for $C_{16}H_{30}NO_6PNa_2 \cdot H_2O$: C, 44.97; H, 7.55; N, 3.28. Found: C, 44.99; H, 7.62; N, 3.35.

Preparation of (*E,E*)-3,7,11-Trimethyl-2,6,10-dodecatrienal (15). A solution of dimethyl sulfoxide (7.9 mL, 83.25 mmol, 1.85 equiv) in CH_2Cl_2 (30 mL) was added to a solution of oxalyl chloride (4.7 mL, 53.96 mmol, 1.2 equiv) in CH_2Cl_2 (120 mL) at −65 °C and stirred 10 min with a mechanical stirrer. A solution of farnesol (Aldrich, 10 gm, 45.0 mmol, 1.0 equiv) in CH_2Cl_2 (30 mL) was added dropwise over 20 min, maintaining a temperature of −63 °C, and then stirred an additional 30 min at −65 °C. Triethylamine (38 mL, 270 mmol, 6 equiv) was added dropwise over 20 min, and the mixture was stirred an additional 15 min at −65 °C. After the reaction mixture was warmed to room temperature, the reaction was quenched with water (500 mL), the mixture was extracted with CH_2Cl_2 (2 × 200 mL), and the combined organic extracts were washed sequentially with with HCl (1N, 4 × 100 mL) and Na_2CO_3 (1 × 100 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum to afford **15** (9.9 g, 100%): TLC R_f = 0.63 (4:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 221; ^1H ($CDCl_3$) 1.61 (s, 3H), 1.67 (s, 3H), 1.68 (s, 3H), 1.96–2.07 (m, 4H), 2.17–2.26 (m, 4H), 2.17 (s, 3H), 5.07–5.10 (m, 2H), 5.87–5.90 (m, 1H), 9.85 (m 1H).

Preparation of (*E*)-3,3,7,11-Tetramethyl-6,10-dodecadienal (16). Methyllithium (1.4 M, 145 mmol, 4.0 equiv) was added dropwise to a mechanically stirred solution of CuI (14.6 g, 76.4 mmol, 2.1 equiv) in THF (200 mL) while maintaining a temperature below −50 °C. The reaction mixture was then stirred at −78 °C for 15 min, 0 °C for 15 min, and room temperature for 5 min and recooled to −78 °C. TMEDA (27.5 mL, 182 mmol, 5.0 eqiuv.) was added dropwise, keeping the temperature below −60 °C, and then stirred at −78 °C for 45 min. Trimethylsilyl chloride (23.1 mL, 182 mmol, 5.0 equiv) was added dropwise, and the mixture was stirred for 30 min at −78 °C. A solution of farnesaldehyde **15** (8 g, 36.4 mmol, 1.0 equiv) in THF (30 mL) was added dropwise, and the mixture was then stirred for 3.5 h at −78 °C. The reaction was quenched at −78 °C with HCl (1N, 500 mL), and the mixture was warmed to room temperature and extracted with diethyl ether (4 × 150 mL). The combined organic extracts were washed with KOH (1N, 300 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (Mallinckrodt Silicar silica gel CC7, 100–200 mesh, 5% solution pH 6.5, eluting with 9:1 petroleum ether/diethyl ether) to afford aldehyde **16** (5.7 g, 66%), which was characterized by MS and ^1H NMR: TLC R_f = 0.75 (9:1 petroleum ether/diethyl ether, visualization by PMA); MS (M + H)$^+$ 237; ^1H($CDCl_3$) 1.13 (s, 6H), 1.42–1.45 (m, 2H), 1.66 (s, 6H), 1.74 (s, 3H), 2.00–2.11 (m, 6H), 2.34 (d, 2H, J = 1.76), 5.16 (br m, 2H), 9.91 (t, 1H, J = 3.52); ^{13}C ($CDCl_3$) 15.8, 17.6, 22.6, 25.6, 26.6, 27.4, 33.4, 39.6, 42.6, 54.6, 124.1, 124.2, 135.0, 203.3. Extensive storage of **16** was avoided, and it was normally prepared just prior to use.

Preparation of (*E*)-3,3,7,11-Tetramethyl-6,10-dodecadien-1-amine (17). Ammonium acetate (1.62 g, 21.0 mmol, 10.0 equiv) was added to a solution of aldehyde **16** (0.5 g, 2.1 mmol, 1.0 equiv) and 4 Å powdered molecular sieves (0.50 g) in methanol (8 mL). Sodium cyanoborohydride (0.133 g, 2.1 mmol, 1.0 equiv) was added to the mixture which was stirred at room temperature for 16 h. The reaction was quenched with water (1 mL), stirrred for 0.5 h, and filtered through Celite (eluting with methanol), and the filtrate was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (10 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 4:1 hexane/ethyl acetate, then 5:1 $CHCl_3$/methanol) to afford **17** (0.137 g, 27%): TLC R_f = 0.13 (9:1 chloroform/methanol, visualization by PMA); MS (M + H)$^+$ 238; ^1H ($CDCl_3$) 0.94 (s, 6H), 1.25 (br m, 2H), 1.54 (br m, 2H), 1.60 (s, 6H), 1.68 (s, 3H), 1.93–2.11 (m, 6H), 3.06 (br m, 2H), 5.09 (br m, 2H), 6.74 (br m, 2H). Amine **17** was prepared just prior to use and utilized directly in the subsequent coupling reaction.

Preparation of (*E*)-3-Oxo-2-(phosphonomethyl)-3-[(3,3,7,11-tetramethyl-6,10-dodecadienyl)amino]propanoic Acid (18). "BOP" reagent (0.256 g, 0.578 mmol, 1.0 equiv) was added to a solution of **17** (0.137 g, 0.578 mmol, 1.0 equiv) and **2** (0.163 g, 0.578 mmol, 1.0 equiv) in acetonitrile (3.0 mL) and DMF (1.0 mL) at room temperature. Diisopropylethylamine (0.403 mL, 2.31 mmol, 4.0 equiv) was added, and the reaction mixture was stirred for 3.5 h at room temperature. The reaction was quenched with HCl (1N, 50 mL), the mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were washed sequentially with Na_2CO_3 (10%, 2 × 50 mL) and LiCl (10%, 3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (3:1 hexane/acetone) to afford the penultimate triester precursor of **18** (0.10 g, 34%): TLC R_f = 0.53 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 502; ^1H ($CDCl_3$) 0.91 (s, 6H), 1.19–1.34 (m, 11H), 1.43 (t, 2H, J = 8.2), 1.60 (s, 6H), 1.68 (s, 3H), 1.97–2.04 (m, 6H), 2.44 (dd, 2H, J = 7.0, 17.0), 3.25 (br m, 2H), 3.56 (br m, 1H), 4.02–4.14 (m, 4H), 4.20 (q, 2H, J = 7.1), 5.09 (m, 2H), 6.54 (m, 1 H); ^{13}C ($CDCl_3$) 13.9, 14.1, 15.8, 16.2, 16.3, 17.6, 22.5, 23.3, 25.4, 25.6, 26.6, 26.9, 32.2, 36.3, 39.6, 40.8, 42.0, 47.3, 61.8, 62.0, 124.3, 124.6, 131.2, 134.7, 166.5, 166.6, 169.4, 169.6. BSTFA (0.850 mL, 3.2 mmol, 16.0 equiv) was added to a solution of the above intermediate (0.100 g, 0.20 mmol, 1.0 equiv) in dichloromethane (5 mL), and the mixture was stirred for 1 h at room temperature. Bromotrimethylsilane (0.237 mL, 1.8 mmol, 9.0 equiv) was added, and the mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated under vacuum, and the residue was dissolved in methanol (5 mL) and NaOH (1 N, 2.4 mL), stirred for 16 h, and concentrated under vacuum. The residue was purified by CHP-20P gel (eluting sequentially with water (500 mL) and 50% aqueous acetonitrile (200 mL)), and the appropriate fractions were concentrated under vacuum. The residue was dissolved in water (10 mL), millipore filtered, and lyophilized to afford **18** (0.084 g, 88%): mp 220 °C dec; TLC R_f = 0.32 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by anisaldehyde); MS (M + H)$^+$ 418, free acid 417; IR (KBr) 1597, 1642 cm^{-1}; ^1H (CD_3OD) 0.92 (s, 6H), 1.19–1.25 (m, 2H), 1.44–1.53 (m, 2H), 1.60 (s, 6H), 1.66 (s, 3H), 1.90–2.19 (m, 8H), 3.15–3.23 (m, 2H), 3.41–3.48 (m, 1H), 5.06–5.13 (m, 2H). Anal. Calcd for $C_{20}H_{33.3}NO_6PNa_{2.7} \cdot 0.15H_2O$: C, 50.09; H, 7.06; N, 2.92. Found: C, 50.01; H, 7.40; N, 3.09.

Preparation of (*E,E*)-*N*-Methyl-*N*-(3,7,11-trimethyl-2,6,-10-dodecatrienyl)-4-methylbenzenesulfonamide (19). Triphenylphosphine (35.38 g, 134.9 mmol, 3.0 equiv) was added to a solution of *N*-methyl-*p*-toluenesulfonamide (20.89 g, 112.4, 2.5 equiv) in THF (150 mL). To the stirring mixture was then added farnesol (Aldrich, 10 g, 4.97 mmol, 1.0 equiv), and the reaction mixture was cooled to 0 °C. Diethyl azodicarboxylate (17.7 mL, 112.4 mmol, 2.5 equiv) was added dropwise, and the reaction mixture was warmed to room temperature and stirred for 3 h. The mixture was concentrated under vacuum, the residue passed through a silica gel plug (eluting with 1:1 ethyl acetate/hexane), and the eluent concentrated under vacuum. The residue was purified by flash chromatography (eluting with 8:1 hexane/ethyl acetate) to afford **19** (14.45 g, 83%): TLC R_f = 0.56 (4:1 hexane/ethyl acetate, visualization by PMA); MS (M − H)$^-$ 388; HRMS (M − H)$^-$ calcd 388.2310, found 388.2321 (δ = 2.8 ppm); ^1H ($CDCl_3$) 1.58 (s, 3H), 1.60 (s, 6H), 1.68 (s, 3H), 1.96–2.07 (m, 8H), 2.43 (s, 3H), 2.63 (s, 3H), 3.61 (d, 2H, J = 7.03), 5.06–5.09 (m, 3H), 7.32 (d, 2H, J = 8.2), 7.67 (d, 2H, J = 8.21); ^{13}C ($CDCl_3$) 15.9, 16.1, 17.6, 21.4, 25.6, 26.1, 26.6, 33.8, 39.5, 39.6, 47.5, 118.1, 123.5, 124.2, 127.5, 129.5, 141.2; IR (CH_2Cl_2 film) 2920, 1452, 1346, 1163 cm^{-1}.

Preparation of (*E,E*)-2-[(Diethoxyphosphinyl)methyl]-3-[methyl(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]-3-oxopropanoic Acid, Ethyl Ester (20). Sodium (0.22 g, 9.6 mmol, 4.5 equiv) was added to a solution of naphthalene (2.1 g, 9.6 mmol, 4.5 equiv) in 1,2 dimethoxyethane (12.8 mL), and the mixture was stirred at room temperature for 1.5 h. A solution of **19** (0.83 mg, 2.13 mmol, 1.0 equiv) in 1,2-dimethoxyethane (1 mL) was added in one portion, and stirring was then continued for 1 h at room temperature. The reaction

was quenched with water (2 mL) followed by sodium bicarbonate (10%, 40 mL), the mixture was extracted with diethyl ether (3 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under vacuum to afford *N*-farnesyl-*N*-methylamine, which was used immediately without purification: TLC R_f = 0.19 (9:1:0.05 chloroform/methanol/acetic acid, visualization by PMA); MS (M + H)$^+$ 236. 1,1'-Carbonyldiimidazole (0.345 g, 2.13 mmol, 1.0 equiv) was added to a solution of acid **2** (0.60 g, 2.13 mmol, 1.0 equiv) in THF (8 mL), and the resultant mixture was stirred for 15 min at 0 °C and 1 h at 20 °C. The reaction was cooled to 0 °C, and a solution of the above amine (0.5 g, 2.13 mmol, 1.0 equiv) in THF (2 mL) was added, after which it was warmed to room temperature and stirred for 16 h. The reaction was quenched with sodium bicarbonate (10%, 50 mL), the mixture was extracted with diethyl ether (3 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 3:1 hexane/acetone) to afford **20** (0.44 g, 42%): TLC R_f = 0.57 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 500; HRMS (M + H)$^+$ calcd 500.3141, found 500.3142 (δ = 0.2 ppm); IR (CH_2Cl_2 film) 2930, 1746, 1653, 1445 cm^{-1}; ^{1}H ($CDCl_3$) 1.18–1.27 (m, 9H), 1.52 (s, 6H), 1.52, 1.60, 1.62, 1.64 (s, 6H total, rotamers), 1.89–2.03 (m, 8H), 2.39 (dd, 2H, J = 7.0, 17.0), 2.85, 2.90, 3.00, 3.09 (s, 3 H total, rotamers), 3.91–4.15 (m, 9H), 5.00–5.18 (m, 3H); ^{13}C ($CDCl_3$) 13.9, 15.8, 16.1, 16.2, 17.5, 23.5, 23.9, 25.5, 25.6, 26.0, 26.2, 26.5, 33.2, 33.4, 34.6, 34.8, 35.7, 36.0, 36.1, 39.5, 42.8, 43.0, 45.3, 47.5, 47.9, 49.4, 61.6, 61.7, 110.8, 118.7, 119.1, 123.3, 123.5, 124.1, 131.1, 135.1, 135.3, 139.8, 140.1, 146.2, 167.2, 167.3, 168.5, 168.6, 168.7, 168.8.

Preparation of (*E,E*)-3-[Methyl(3,7,11-trimethyl-2,6,10-dodecatrienyl)amino]-3-oxo-2-(phosphonomethyl)propanoic Acid (21). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **20** (0.180 g, 0.361 mmol, 1.0 equiv). The crude product was purified by SP-207 gel (eluting sequentially with water (500 mL) and methanol (50%, 150 mL)) to afford **21** (0.116, 67%): mp 200 °C dec; TLC R_f = 0.50 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA); MS (M − H)$^-$ 414, acid; IR (KBr) 1624 cm^{-1}; ^{1}H (CD_3OD) 1.59, 1.64, 1.65, 1.68, 1.69, 1.70, 1.72 (s, 12H, total, rotamers), 1.95–2.20 (m, 10H), 2.80, 2.82, 2.90, 2.93, 3.04, 3.06, 3.11, 3.15 (s, 3H total, rotamers), 3.95–4.20 (m, 3H), 5.07–5.39 (m, 3H). Anal. Calcd for $C_{20}H_{31}NO_6PNa_3 \cdot 0.68H_2O$: C,48.66; H, 6.61; N, 2.84. Found: C, 48.83; H, 6.37; N, 2.67.

Preparation of (*R*)-(2-Oxo-3-oxetanyl)carbamic Acid, 1,1-Dimethylethyl Ester (24). A THF solution (20 mL) of DEAD (3.8 mL, 24.4 mmol, 1.0 equiv) was added dropwise to a solution of PPh_3 (6.4 g, 24.4 mmol, 1.0 equiv) in THF (45 mL) at −78 °C until a thick precipitate formed (15 min). *N*-Boc-D-serine (5 g, 24.4 mmol, 1.0 equiv) in THF (25 mL) was then added dropwise at −78 °C, and the mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was concentrated under vacuum and the residue purified by flash chromatography (eluting with 3:1 hexane/ethyl acetate) to afford **24** (2.4 g, 54%): mp 118–120 °C; TLC R_f = 0.46 (3:2 hexane/ethyl acetate, visualization by Rydon); MS (M + H)$^+$ 188; IR (KBr) 1844 cm^{-1}; $[\alpha]_D$ = +26.2° (c = 0.99, CH_3CN); ^{1}H ($CDCl_3$) 1.46 (s, 9H), 4.44 (br m, 2H), 5.11 (m, 1H), 5.60 (d, 1H, J = 8.2); ^{13}C ($CDCl_3$) 28.1, 59.4, 66.5, 81.2, 154.6, 169.6. Anal. Calcd for $C_8H_{13}O_4N$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.59; H, 6.88; N, 7.43.

Preparation of *N*-Boc-3-(dimethoxyphosphinyl)-L-alanine, Methyl Ester (25). Trimethyl phosphite (10.0 mL, 84.8 mmol, 12.0 equiv) was added to lactone **23** (1.3 g, 7.1 mmol, 1.0 equiv), and the mixture was stirred for 48 h at 70 °C. The reaction mixture was concentrated under vacuum and the residue purified by flash chromatography (eluting with 3:2 hexane/ethyl acetate) to afford **25** (1.96 g, 89%): TLC R_f = 0.06 (3:2 hexane/ethyl acetate, visualization by Rydon); MS (M + H)$^+$ 312; HRMS (M + H)$^+$ calcd 312.1212, found 312.1209 (δ = 1.0 ppm); IR (CH_2Cl_2 film) 2936, 1717, 1516, 1252 cm^{-1}; $[\alpha]_D$ = +10.9° (c = 2.3, $CHCl_3$); ^{1}H ($CDCl_3$) 1.45 (s, 9H), 2.38 (dd, 2H, J = 5.1, 17.1), 3.75–3.80 (m, 9H), 4.55 (m, 1H), 5.65 (br m, 1H); ^{13}C ($CDCl_3$) 26.0, 28.2, 48.9, 52.4, 52.5, 52.6, 54.2, 54.3, 80.1, 171.2, 171.4.

Preparation of *N*-Boc-3-(dimethoxyphosphinyl)-D-alanine, Methyl Ester (26). Trimethyl phosphite (17.4 mL, 147.6 mmol, 12.0 equiv) was added to lactone **24** (2.3 g, 12.3 mmol, 1.0 equiv), and the mixture was stirred for 48 h at 70 °C. The reaction mixture was concentrated under vacuum and the residue purified by flash chromatography (eluting with 1:1 hexane/ethyl acetate) to afford triester **26** (3.25 g, 86%): TLC R_f = 0.1 (1:1 hexane/ethyl acetate, visualization by Rydon); MS (M + H)$^+$ 312; IR (CH_2Cl_2 film) 1713 cm^{-1}; $[\alpha]_D$ = −11.1° (c = 2.3, $CHCl_3$); ^{1}H ($CDCl_3$) 1.45 (s, 9H), 2.34–2.42 (m, 2H), 3.74 (d, 6H, J = 10.6), 3.77 (s, 3H), 4.56 (br m, 1H), 5.74 (d, 1H, J = 7.6); ^{13}C ($CDCl_3$) 25.9, 28.0, 28.1, 48.8, 80.0, 155.0, 171.2, 171.3. Anal. Calcd for $C_{11}H_{22}NO_7P \cdot 0.14H_2O$: C, 42.11; H, 7.16; N, 4.46. Found: C, 41.88; H, 7.26; N, 4.69.

Preparation of 3-(Dimethoxyphosphinyl)-L-alanine, Methyl Ester, Hydrochloride (27). Anhyrous HCl in dioxane solution (Aldrich, 4 M, 6.2 mL, 24.4 mmol, 4.0 equiv) was added to a solution of **25** (1.9 g, 6.1 mmol, 1.0 equiv) in ethyl acetate (30 mL), and the mixture was stirred at room temperature for 5 h. The reaction was concentrated under vacuum to afford **27** (1.5 g, 100%): TLC R_f = 0. 09 (4:1:1 1-butanol/acetic acid/water, visualization by Rydon); MS (M + H)$^+$ 212, free amine; IR (CH_2Cl_2 film) 2959, 1750, 1715, 1516 cm^{-1}; ^{1}H ($CDCl_3$) 2.35 (dd, 2 H, J = 5.87, 17.59), 3.37 (s, 3 H), 3.70 (d, 6 H, J = 12.9), 4.30–4.60 (m, 1 H); ^{13}C ($CDCl_3$) 25.8, 27.9, 48.8, 50.2, 52.6, 80.1, 155.1, 171.9, 172.0.

Preparation of 3-(Dimethoxyphosphinyl)-D-alanine, Methyl Ester, Hydrochloride (28). Anhyrous HCl in dioxane solution (Aldrich, 4 M, 5.8 mL, 23.2 mmol, 4.0 equiv) was added to a solution of **26** (1.8 g, 5.8 mmol, 1.0 equiv) in methanol (20 mL), and the mixture was stirred at room temperature for 5 h. The reaction was concentrated under vacuum to afford amine hydrochloride **28** (1.43 g, 100%): TLC R_f = 0.2 (4:1:1 1-butanol/acetic acid/water, visualization by Rydon); MS (M + H)$^+$ 212, free amine; HRMS (M + H)$^+$, free amine, calcd 212.0688, found, 212.0690 (δ = 0.9 ppm); IR (CH_2Cl_2 film) 3399–2959, 1751, 1607, 1445, 1240 cm^{-1}; $[\alpha]_D$ = −10° (c = 1.01, CH_3OH); ^{1}H (CD_3OD) 2.53–2.62 (m, 2H), 3.81 (d, 3H, J = 2.1), 3.83 (d, 3H, 2.1), 3.88 (s, 3H), 4.39 (br m, 1 H).

Preparation of (*E,E*)-3-(Dimethoxyphosphinyl)-*N*-(3,7,-10-trimethyl-1-oxo-2,6,10-dodecatrienyl)-L-alanine, Methyl Ester (29). "BOP" reagent (0.446 g, 1.0 mmol, 1.0 equiv) was added to a solution of farnesoic acid **22** (0.236 g, 1.0 mmol, 1.0 equiv) and amine **27** (0.250 g, 1.0 mmol, 1.0 equiv) in acetonitrile (5 mL) and DMF (1.7 mL) at room temperature. Diisopropylethylamine (0.520 mL, 3.0 mmol, 3.0 equiv) was added, and the reaction mixture was stirred for 16 h at room temperature. The reaction was quenched with HCl (1N, 100 mL), the mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were washed sequentially with Na_2CO_3 (10%, 50 mL) and LiCl (10%, 2 × 100 mL), dried filtered, and concentrated under vacuum. The residue was purified by flash chromatography (4: 1 hexane/acetone) to afford **29** (0.223 g, 52%): TLC R_f = 0.6 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 430; HRMS (M + H)$^+$ calcd 430.2358, found 430.2371 (δ = 3.0 ppm); IR (CH_2Cl_2 film) 2957, 1748, 1522, 1246 cm^{-1}; $[\alpha]_D$ = +14.5° (c = 1.25, $CHCl_3$); ^{1}H ($CDCl_3$) 1.60 (s, 6H), 1.68 (s, 3H), 2.00–2.16 (m, 8H), 2.16 (s, 3H), 2.37–2.48 (m, 2H), 3.71–3.77 (m, 9H), 4.75–4.95 (m, 1H), 5.09 (br m, 2H), 5.66 (s, 1H), 6.82 (d, 1H, J = 7.6); ^{13}C ($CDCl_3$) 15.8, 17.5, 18.3, 21.9, 25.5, 25.9, 26.5, 28.1, 39.5, 40.7, 47.1, 47.2, 52.3, 52.5, 117.1, 122.8, 123.5, 131.1, 135.7, 155.8, 166.4, 171.2.

Preparation of (*E,E*)-3-(Dimethoxyphosphinyl)-*N*-(3,7,-10-trimethyl-1-oxo-2,6,10-dodecatrienyl)-D-alanine, Methyl Ester (30). Following the BOP coupling protocol described for preparation of **29**, farnesoic acid **22** (0.354 g, 1.5 mmol, 1.0 equiv) and amine **28** (0.371 g, 1.5 mmol, 1.0 equiv) were reacted to afford **30** (0.27 g, 42%): TLC R_f = 0.52 (1:1 hexane/acetone, visualization by PMA); MS (M + H)$^+$ 430; HRMS (M + H)$^+$ calcd 430.2359, found 430.2356 (δ = 0.7 ppm); IR (CH_2Cl_2 film) 2959, 2924, 1734, 1462 cm^{-1}; $[\alpha]_D$ = −15.2° (c = 1.21, $CHCl_3$); ^{1}H ($CDCl_3$) 1.61 (s, 6H), 1.68 (s, 3H), 1.98–2.17 (m, 8H), 2.16 (s, 3H), 2.36–2.47 (m, 2H), 3.73 (d, 6H, J = 10.6),

3.77 (s, 3H), 4.81–5.00 (m, 1H), 5.09 (br m, 2H), 5.64 (s, 1H), 6.67 (d, 1H, J = 7.6); ^{13}C ($CDCl_3$) 16.0, 17.6, 18.4, 25.6, 26.0, 26.6, 27.7, 39.6, 40.8, 47.3, 52.4, 52.5, 52.6, 117.1, 123.0, 124.2, 131.3, 136.0, 156.1, 166.6, 171.1, 171.2.

Preparation of [*S*-(*E*,*E*)]-3-Phosphono-2-[(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]propanoic Acid (31). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **29** (0.158 g, 0.368 mmol, 1.0 equiv) to triacid **31** (0.077 g, 46%): mp 210 °C dec; TLC R_f = 0.52 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by UV); MS (M + 3Na − 2H)$^+$ 454; IR (KBr) 1601 cm^{-1}; $[\alpha]_D$ = +4.3° (c = 0.91, CH_3OH); ^{1}H (CD_3OD) 1.59 (s, 3H), 1.61 (s, 3H), 1.66 (s, 3H), 1.96–2.17 (m, 10H), 2.10 (s, 3H), 4.42–4.46 (m, 1H), 5.06–5.15 (m, 2H), 5.78 (s, 1 H). Anal. Calcd for $C_{18}H_{28}NO_6PNa_2{\cdot}0.5H_2O$: C, 49.09; H, 6.64; N, 3.18. Found: C, 49.08; H, 6.44; N, 3.00.

Preparation of [*R*-(*E*,*E*)]-3-Phosphono-2-[(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]propanoic Acid (32). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **30** (0.165 g, 0.385 mmol, 1.0 equiv) to triacid **32** (0.12 g, 69%): mp 210 °C dec; TLC R_f = 0.53 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by UV); MS (M − H)$^-$ 386; IR (KBr) 1601 cm^{-1}; $[\alpha]_D$ = −4.5° (c = 0.92, CH_3OH); ^{1}H (CD_3OD) 1.60 (s, 3H), 1.61 (s, 3H), 1.66 (s, 3H), 1.96–2.18 (m, 10H), 2.11 (s, 3H), 4.44 (br m, 1H), 5.08–5.13 (m, 2H), 5.78 (s, 1H). Anal. Calcd for $C_{18}H_{27}NO_6PNa_3$: C, 47.69; H, 6.00; N, 3.09. Found: C, 48.04; H, 6.38; N, 3.03.

Preparation of (*E*,*E*)-*N*-(3,7,10-Trimethyl-1-oxo-2,6,10-dodecatrienyl)-L-glutamic Acid, Diethyl Ester (35). Following the BOP coupling protocol described for preparation of **29**, farnesoic acid **22** (0.30 g, 1.27 mmol, 1.0 equiv) and L-glutamic acid diethyl ester hydrochloride **33** (Aldrich, 0.305 g, 1.27 mmol, 1.0 equiv) were reacted to afford diester **35** (0.47 g, 88%): TLC R_f = 0.32 (4:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 422; HRMS (M + H)$^+$ calcd 422.2907, found 422.2909 (δ = 0.5 ppm); IR (CH_2Cl_2 film) 3368–2930, 1736, 1552, 1385 cm^{-1}; $[\alpha]_D$ = +14.4° (c = 2.9, $CHCl_3$); ^{1}H ($CDCl_3$) 1.25 (t, 3H, J = 7.0), 1.29 (t, 3H, J = 7.0), 1.61 (s, 3H), 1.68 (s, 6H), 1.99–2.32 (m, 10H), 2.15 (s, 3H), 2.36–2.45 (m, 2H), 4.12 (q, 2H, J = 7.04), 4.20 (q, 2H, J = 7.0), 4.6–4.69 (m, 1H), 5.10 (m, 2H), 5.60 (s, 1H), 6.14 (d, 1H, J = 7.6); ^{13}C ($CDCl_3$) 14.17, 16.04, 17.68, 18.49, 25.69, 26.12, 26.72, 27.65, 30.47, 39.68, 40.72, 51.43, 60.65, 61.57, 117.40, 123.08, 124.26, 131.37, 136.01, 155.68, 166.70, 172.26, 172.95.

Preparation of (*E*,*E*)-*N*-(3,7,10-Trimethyl-1-oxo-2,6,10-dodecatrienyl)-D-glutamic Acid, Diethyl Ester (36). Following the BOP coupling protocol described for preparation of **29**, farnesoic acid **22** (0.250 g, 1.06 mmol, 1.0 equiv) and D-glutamic acid diethyl ester hydrochloride **34** (0.253 g, 1.06 mmol, 1.0 equiv) were reacted to afford diester **36** (0.180 g, 40%): TLC R_f = 0.70 (2:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 422; HRMS (M + H)$^+$ calcd 422.2907, found 422.2896 (δ = 2.6 ppm); IR (CH_2Cl_2 film) 2982, 1734, 1667, 1530 cm^{-1}; $[\alpha]_D$ = −14.2° (c = 1.35, $CHCl_3$); ^{1}H ($CDCl_3$) 1.15–1.31 (m, 6H), 1.61 (s, 6H), 1.68 (s, 3H), 1.98–2.20 (m, 10H), 2.16 (s, 3H), 2.37–2.45 (m, 2H), 4.12 (q, 2H, J = 7.6), 4.20 (q, 2H, J = 7.6), 4.62–4.69 (m, 1H), 5.06–5.10 (m, 2H), 5.61 (s, 1H), 6.24 (d, 1H, J = 3.6); ^{13}C ($CDCl_3$) 13.96, 15.84, 17.51, 18.28, 25.51, 25.92, 26.52, 27.41, 30.29, 40.69, 51.23, 60.44, 61.36, 117.23, 122.90, 124.05, 131.17, 135.80, 155.44, 166.53, 172.09, 172.78.

Preparation of [*S*-(*E*,*E*)]-2-[(3,7,11-Trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]pentanedioic Acid (37). Sodium hydroxide (1 N, 1.34 mL, 1.34 mmol, 2.1 equiv) was added to a solution of **35** (0.27 g, 0.64 mmol, 1.0 equiv) in ethanol (5 mL), and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under vacuum, dissolved in water (3 mL), and purified through a CHP-20P gel column (eluting sequentially with water (150 mL) and 30% aqueous acetonitrile (250 mL)). The appropriate fractions were combined, concentrated under vacuum, millipore filtered, and lyophilized to afford **37** (0.176 g, 67%): mp 240 °C dec; TLC R_f = 0.68 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA); MS (M + 2Na − H)$^+$ 410; IR (KBr) 1589 cm^{-1}; $[\alpha]_D$ = +14.8° (c = 1.21, CH_3OH); ^{1}H (CD_3OD) 1.59 (s, 3H), 1.61 (s, 3H), 1.65 (s, 3H), 1.96–2.26 (m, 12H), 2.08 (s, 3H), 4.25–4.28 (m, 1H), 5.08–5.13 (m, 2H), 5.77 (s, 1H). Anal. Calcd for $C_{20}H_{29}NO_5Na_2{\cdot}0.42H_2O$: C, 57.61; H, 7.21; N, 3.36. Found: C, 57.57; H, 7.17; N, 3.40.

Preparation of [*R*-(*E*,*E*)]-2-[(3,7,11-Trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]pentanedioic Acid (38). Following the hydrolysis procedure described for preparation of **37**, sodium hydroxide (1 N, 0.899 mL, 0.899 mmol, 2.1 equiv) treatment of diester **36** (0.180 g, 0.428 mmol, 1.0 equiv) afforded **38** (0.170 g, 97%): mp 200 °C dec; TLC R_f = 0.79 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA); MS (M + Na)$^+$ 388; IR (KBr) 1589 cm^{-1}; $[\alpha]_D$ = −14.9° (c = 1.14, CH_3OH); ^{1}H (CD_3OD) 1.59 (s, 3H), 1.62, (s, 3H), 1.66 (s, 3H), 1.96–2.28 (m, 12H), 2.09 (s, 3H), 4.25–4.28 (m, 1H), 5.08–5.14 (m, 2H), 5.78 (s, 1H). Anal. Calcd for $C_{20}H_{29}NO_5Na_2{\cdot}0.51H_2O$: C, 57.39; H, 7.23; N, 3.35. Found: C, 57.12; H, 7.11; N, 3.62.

Preparation of (*E*,*E*)-*N*-(3,7,10-Trimethyl-1-oxo-2,6,10-dodecatrienyl)-L-aspartic Acid, Dimethyl Ester (41). Following the BOP coupling protocol described for preparation of **29**, farnesoic acid **22** (0.472 g, 2.0 mmol, 1.0 equiv) and L-aspartic acid methyl ester hydrochloride **39** (Aldrich, 0.417 g, 2.0 mmol, 1.0 equiv) were reacted to afford diester **41** (0.65 g, 86%): TLC R_f = 0.51 (2:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 380; HRMS (M + H)$^+$ calcd 380.2437, found 380.2449 (δ = 3.2 ppm); IR (CH_2Cl_2 film) 2955, 1744, 1667, 1439 cm^{-1}; $[\alpha]_D$ = +38.1° (c = 3.51, $CHCl_3$); ^{1}H ($CDCl_3$) 1.60 (s, 6H), 1.67 (s, 3H), 1.9–2.17 (m, 8H), 2.16 (s, 3H), 2.88 (dd, 1H, J = 4.7, 17), 3.05 (dd, 1H, J = 4.7, 17.0), 3.68 (d, 3H, J = 1.8), 3.76 (d, 3H, J = 1.8), 4.89–4.94 (m, 1H), 5.09 (m, 2H), 5.63 (s, 1H), 6.51 (d, 1H, J = 7.6); ^{13}C ($CDCl_3$) 15.81, 17.45, 18.28, 25.45, 25.86, 26.49, 36.08, 39.45, 40.66, 48.00, 51.69, 52.47, 117.20, 122.87, 124.05, 131.08, 135.77, 155.56, 166.27, 171.31, 171.42.

Preparation of (*E*,*E*)-*N*-(3,7,10-Trimethyl-1-oxo-2,6,10-dodecatrienyl)-D-aspartic Acid, Dimethyl Ester (42). Following the BOP coupling protocol described for preparation of **29**, farnesoic acid **22** (0.472 g, 2.0 mmol, 1.0 equiv) and D-aspartic acid methyl ester hydrochloride **40** (Aldrich, 0.417 g, 2.0 mmol, 1.0 equiv) were reacted to afford diester **42** (0.588 g, 78%): TLC R_f = 0.51 (2:1 hexane/ethyl acetate, visualization by PMA); MS (M + H)$^+$ 380; HRMS (M + H)$^+$ calcd 380.2437, found 380.2445 (δ = 2.1 ppm); IR (CH_2Cl_2 film) 2955, 1742, 1667, 1516 cm^{-1}; $[\alpha]_D$ = − 41.4° (c = 4.63, $CHCl_3$); ^{1}H ($CDCl_3$) 1.60 (s, 6H), 1.67 (3H), 1.85–2.16 (m, 8H), 2.15 (s, 3H), 2.87 (dd, 1H, J = 4.1, 17), 3.05 (dd, 1H, J = 4.1, 17), 3.68 (d, 3H, J = 1.2), 3.75 (d, 3H, J = 1.2), 4.89–4.95 (m, 1H), 5.08–5.10 (m, 2H), 5.62 (s, 1H), 6.48 (d, 1H, J = 8.2); ^{13}C ($CDCl_3$) 15.84, 17.48, 18.31, 25.48, 25.89, 26.52, 36.11, 39.51, 40.69, 48.03, 51.72, 52.49, 117.23, 122.90, 124.08, 131.14, 135.83, 155.59, 166.30, 171.31, 171.42.

Preparation of [*S*-(*E*,*E*)]-2-[(3,7,11-Trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]butanedioic Acid (43). Following the hydrolysis procedure described for preparation of **37**, sodium hydroxide (1 N, 1.39 mL, 1.39 mmol, 2.1 equiv) treatment of **41** (0.25 g, 0.66 mmol, 1.0 equiv) afforded diacid **43** (0.205 g, 79%): mp 240 °C dec; TLC R_f = 0.57 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA); MS (M + Na)$^+$ 374; IR (KBr) 1599 cm^{-1}; $[\alpha]_D$ = +24.3° (c = 1.17, CH_3OH); ^{1}H (CD_3OD) 1.59 (s, 3H), 1.61 (s, 3H), 1.66 (s, 3H), 1.96–2.17 (m, 8H), 2.09 (s, 3H), 2.66–2.69 (m, 2H), 4.50–4.53 (m, 1H), 5.07–5.14 (m, 2H), 5.75 (s, 1H). Anal. Calcd for $C_{19}H_{27}NO_5Na_2{\cdot}0.23H_2O$: C, 57.11; H, 6.93; N, 3.50. Found: C, 57.20; H, 7.17; N, 3.41.

Preparation of [*R*-(*E*,*E*)]-2-[(3,7,11-Trimethyl-1-oxo-2,6,10-dodecatrienyl)amino]butanedioic Acid (44). Following the hydrolysis procedure described for preparation of **37**, sodium hydroxide (1 N, 1.60 mL, 1.60 mmol, 2.1 equiv) treatment of **42** (0.288 g, 0.76 mmol, 1.0 equiv) afforded diacid **44** (0.286 g, 95%): mp 240 °C dec; TLC R_f = 0.57 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA); MS (M + Na)$^+$ 374; IR (KBr) 1599 cm^{-1}; $[\alpha]_D$ = −26.01° (c = 1.43, CH_3OH); ^{1}H (CD_3OD) 1.59 (s, 3H), 1.61 (s, 3H), 1.66 (s, 3H), 1.96–2.17 (m, 8H), 2.09 (s, 3H), 2.66–2.69 (m, 2H), 4.50–4.53 (m, 1H), 5.07–5.11 (m, 2H), 5.75 (s, 1H). Anal. Calcd

for $C_{19}H_{27}NO_5Na_2 \cdot 0.21H_2O$: C, 57.18; H, 6.92; N, 3.51. Found: C, 57.17; H, 6.51; N, 3.52.

Preparation of (*E,E*)-4,8,12-Trimethyl-3,7,11-tridecatrienenitrile (45). Tetrabutylammonium cyanide (0.591 g, 2.2 mmol, 1.0 equiv) was added to a solution of *trans,trans*-farnesyl bromide (Aldrich, 0.587 mL, 2.2 mmol, 1.0 equiv) in acetonitrile (1.1 mL), and the mixture was stirred at room temperature for 3 h. The reaction was concentrated under vacuum and the residue purified by flash chromatography (eluting with 20:1 petroleum ether/diethyl ether) to afford **45** (0.47 g, 94%): TLC R_f = 0.45 (9/1 hexane/ethyl acetate); MS $(M + H)^+$ 232; IR (CH_2Cl_2 film) 777, 831,920,984, 1078, 1107, 1152, 1225, 1331, 1381, 1447, 1505, 1520, 1539, 1576, 1620, 1636, 1651, 1667, 2249, 2855, 2918, 2967, 3430 cm^{-1}; ^1H ($CDCl_3$) 1.60 (s, 6H), 1.67 (s, 6H), 1.97–2.17 (m, 8H), 3.03 (d, 2H, J = 6.45), 5.05–5.13 (m, 2H), 5.16 (t, 1H, J = 6.45); ^{13}C ($CDCl_3$) 15.8, 16.0, 16.2, 17.5, 25.5, 25.9, 26.5, 39.0, 39.5, 111.5, 118.4, 123.1, 124.1, 131.1, 135, 142.1; HRMS $(M + H)^+$ calcd 232.2065, found 232.2067 (δ = 0.9 ppm). Anal. Calcd for $C_{16}H_{25}N \cdot 0.2H_2O$: C, 81.78; H, 10.90; N, 5.96. Found: C, 81.63; *H, 10.89; N, 5.80.*

Preparation of (*E,E*)-4,8,12-Trimethyl-3,7,11-tridecatrienoic Acid (46). Potassium hydroxide (1.251 g, 22.3 mmol, 5.7 equiv) in water (2 mL) was added to a solution of farnesyl cyanide **45** (0.91 g, 3.9 mmol, 1.0 equiv) in ethanol (10 mL), and the mixture was heated to reflux for 3 h. The reaction mixture was concentrated under vacuum, dissolved in water (75 mL), and extracted with diethyl ether (2 × 75 mL). The organic layers were discarded, the aqueous layer was cooled to 0 °C, and the pH was lowered to 4 with 20% aqueous H_2SO_4. The solution was extracted with diethyl ether (3 × 70 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under vacuum. The residue was purified by flash chromatography (eluting with 2:1 hexane/acetone) to afford **46** (0.765 g, 78%): TLC R_f = 0.62 (2:1 hexane/acetone, visualization by PMA); MS $(M + NH_4)^+$ 268; HRMS $(M + H)^+$ calcd 251.2011, found 251.2020 (δ = 3.6 ppm); IR (CH_2Cl_2 film), 3418–2972, 2928, 1715, 1659 cm^{-1}; ^1H ($CDCl_3$) 1.60 (s, 6H), 1.63 (s, 2H), 1.68 (s, 3H), 1.74 (s, 1H), 1.97–2.18 (m, 8H), 3.05 (d, 2H, J = 7.0), 5.09 (m, 2H), 5.31 (t, 1H, J = 7.0); ^{13}C ($CDCl_3$) 15.95, 16.30, 17.62, 23.38, 25.48, 25.66, 26.20, 26.43, 26.69, 32.08, 33.66, 33.95, 39.56, 39.68, 115.47, 116.11, 123.62, 123.88, 124.28, 124.34, 131.20, 131.25, 135.17, 135.54, 139.17, 139.29, 179.34.

Preparation of (*E,E*)-3-(Dimethoxyphosphinyl)-*N*-(4,8,12-trimethyl-1-oxo-3,7,11-tridecatrienyl)-L-alanine, Methyl Ester (47). Following the BOP coupling protocol described for preparation of **29**, homofarnesoic acid **46** (0.232 g, 0.93 mmol, 1.0 equiv) and amine **39** (0.230 g, 0.93 mmol, 1.0 equiv) were reacted to afford triester **47** (0.19 g, 46%): TLC R_f = 0.32 (1:1 hexane/acetone, visualization by PMA); MS $(M + H)^+$ 444; ^1H ($CDCl_3$) 1.44, 1.60, 1.67, 1.79, (s, 12H, *E/Z* mixture), 1.99–2.17 (m, 8H), 2.35–2.42 (m, 2H), 3.02 (d, 2H, J = 7.6), 3.73 (m, 9H), 4.45–4.90 (m, 1H, *E/Z* mixture), 5.11 (m, 2H), 5.35 (m, 1H), 6.96 (m, 1H); ^{13}C ($CDCl_3$) 15.7, 16.1, 17.4, 23.3, 25.3, 25.4, 25.8, 26.0, 26.3, 26.5, 27.4, 28.0, 29.1, 31.8, 35.2, 35.4, 39.5, 47.3, 47.4, 48.7, 52.4, 115.7, 116.3, 123.33, 123.6, 124.1, 131.0, 135.1, 135.4, 140.9, 141.0, 155.0, 170.6, 170.8, 171.1, 171.2.

Preparation of (*E,E*)-3-(Dimethoxyphosphinyl)-*N*-(4,8,12-trimethyl-1-oxo-3,7,11-tridecatrienyl)-D-alanine, Methyl Ester (48). Following the BOP coupling protocol described for preparation of **29**, homofarnesoic acid **46** (0.20 g, 0.80 mmol, 1.0 equiv) and amine **40** (0.198 g, 0.80 mmol, 1.0 equiv) were reacted to afford **48** (0.20 g, 56%): TLC R_f = 0.46 (1:1 hexane/acetone, visualization by PMA); MS $(M + H)^+$ 444; HRMS $(M + H)^+$ calcd 444.2515, found 444.2516. (δ = 0.2 ppm); ^1H ($CDCl_3$) 1.50, 1.51, 1.52, 1.53, 1.56, 1.58, 1.67, 1.68 (s, 12H, *E/Z* mixture), 1.85–2.09 (m, 8H), 2.25–2.38 (m, 2H), 2.90 (d, 2H, J = 7.3), 3.62–3.67 (m, 9H), 4.59–4.62 (m, 1H), 4.97–5.04 (m, 2H), 5.21–5.25 (m, 1H); ^{13}C ($CDCl_3$) 16.26, 16.63, 17.93, 23.90, 26.03, 26.39, 27.40, 27.59, 27.84, 27.88, 33.08, 36.08, 36.22, 40.83, 40.90, 53.42, 53.60, 53.63, 53.66, 117.57, 118.11, 125.13, 125.25, 125.51, 132.18, 132.24, 136.34, 141.15, 172.49, 172.62, 174.49.

Preparation of [*S*-(*E,E*)]-3-Phosphono-2-[(4,8,12-trimethyl-1-oxo-3,7,11-tridecatrienyl)amino]propanoic Acid (49). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **47** (0.180 g, 0.41 mmol, 1.0 equiv) to afford **49** (0.10 g, 53%): mp 215 °C dec; TLC R_f = 0.58 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by anisaldehyde); MS $(M + Na)^+$ 424, free acid 401; IR (KBr) 1605 cm^{-1}; $[\alpha]_D$ = +17.5° (c = 0.4, CH_3OH); ^1H (CD_3OD) 1.55, 1.60, 1.63, 1.66, 1.75, 1.76 (s, 12H, *E/Z* mixture), 1.92–2.13 (m, 10H), 3.00 (d, 2H, J = 7.6), 4.38–4.43 (m, 1H), 5.10–5.15 (m, 2H), 5.38 (br m, 1H). Anal. Calcd for $C_{19}H_{30}NO_6PNa_2 \cdot 1.4H_2O$: C, 48.49; H, 7.02; N, 2.98. Found: C, 48.49; H, 6.96; N, 2.92.

Preparation of [*R*-(*E,E*)]-3-Phosphono-2-[(4,8,12-trimethyl-1-oxo-3,7,11-tridecatrienyl)amino]propanoic Acid (50). The protocol utilizing BSTFA/TMSBr followed by NaOH treatment as described for preparation of **12** was used for deprotection of triester **48** (0.190 g, 0.43 mmol, 1.0 equiv) to afford **50** (0.02 g, 10%): mp 175 °C dec; TLC R_f = 0.35 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by anisaldehyde); MS $(M + Na)^+$ 424, free acid 401; IR (KBr) 1603 cm^{-1}; $[\alpha]_D$ = −3.5° (c = 0.4, CH_3OH); ^1H (CD_3OD) 1.59, 1.61, 1.62, 1.63, 1.64, 1.66, 1.74, 1.75 (s, 12H), 1.91–2.12 (m, 10H), 3.02 (d, 2H, J = 6.4), 4.32 (m, 1H), 5.07–5.14 (m, 2H), 5.38–5.40 (m, 1H). Anal. Calcd for $C_{19}H_{29}NO_6PNa_3 \cdot 2.05H_2O$: C, 45.25; H, 6.62; N, 2.78. Found: C, 45.56; H, 6.77; N, 2.47.

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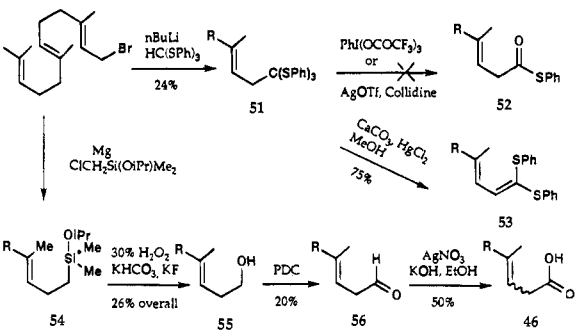

Displacement of farnesyl bromide with anion of $HC(SPh)_3$ as carboxylic acid synthon gave the desired ortho thioester **51** only in modest yields (24%). Attempts at hydrolyzing **51** to the corresponding carboxymethyl ester yielded only the conjugated diene **53**, which resisted further hydrolysis even under extended reaction times. Attempts at converting **51** to the thioester **52** were also unrewarding. Next, we explored the preparation of *E*-**46** via oxidation of homofarnesol **55**. Thus, treatment of farneysl bromide with the hydroxylmethylating (isopropoxydimethylsilyl)methyl grignard reagent[34] followed by oxidative cleavage of the crude product **54** gave homofarnesol **55** in 26% overall yield after chromatographic purification. Surprisingly, oxidation of **55** to **56** was very inefficient with TPAP/NMO or under Swern conditions and proceeded slowly but cleanly with PDC to afford homofarnesaldehyde **56** in modest yields. Further oxidation of **56** to the acid **46** under basic conditions using $AgNO_3$ once again gave the *E/Z* mixture.

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