Phosphinyl Acid-Based Bisubstrate Analog Inhibitors of Ras Farnesyl Protein Transferase

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The rational design, synthesis, and biological activity of phosphonyl- and phosphinyl-linked bisubstrate analog inhibitors of the enzyme Ras farnesyl protein transferase (FPT) are described. The design strategy for these bisubstrate inhibitors involved connection of the critical binding components of the two substrates of FPT (*ras* protein and farnesyl pyrophosphate, FPP) through a phosphonyl- or phosphinyl-bearing linker. Compound **14**, the first example in this series, was found to be a potent FPT inhibitor (I_{50} = 60 nM). A further 15-fold enhancement in activity was observed upon replacement of the VLS tripeptide sequence in **14** with VVM (**15**, I_{50} = 6 nM). The phosphinic acid analog **16** (I_{50} = 6 nM) was equiactive to phosphonic acid **15**. Compounds **14**–**16** afforded 1000-fold selectivity for FPT against the closely related enzyme geranylgeranyl protein transferase type I, GGT-I [**14**, I_{50}(GGT-I) = 59 μM; **15** I_{50}(GGT-I) = 10 μM; **16** I_{50}(GGT-I) = 21 μM]. Methyl and POM ester prodrugs **17**–**19** were prepared and evaluated in whole cell assays and appear to block *ras*-induced cell transformation, as well as colony formation in soft agar. A distinctive feature of this novel class of potent and selective bisubstrate FPT inhibitors is that they are non-sulfhydryl in nature.

Introduction

Ras genes play a fundamental role in cell proliferation and differentiation, and *ras* oncogenes have been implicated in the development of a variety of tumors.[1] *Ras* proteins must become membrane bound in order to exert their normal as well as oncogenic effects. This mandatory membrane binding occurs following a defined sequence of post-translational modifications, namely farnesylation, proteolysis, and carboxymethylation.[2] Inhibition of S-farnesylation, which is the first and essential step in this cascade of events, may thus be expected to block the oncogenic activity of *ras* proteins. This hypothesis has recently led to the search for inhibitors of farnesyl protein transferase (FPT)[3] as novel, rationally designed, antitumor agents.[4]

FPT catalyzes the transfer of a farnesyl group from farnesyl pyrophosphate (FPP), an intermediate in the cholesterol biosynthetic pathway, to *ras* proteins. S-Farnesylation occurs on the cysteine located at the 4th position from the C-terminus of the protein. The "CAAX" motif is a consensus sequence for farnesylation, where C is cysteine, A is an aliphatic amino acid, and X is preferably serine or methionine.[3] Since FPT catalyzes a bisubstrate reaction, it provides various opportunities for inhibitor design. Thus, inhibitors based on either of the two substrates can be prepared, and examples of both CAAX-[4,5] and FPP-based[6] inhibitors have recently appeared in the literature. An alternative and unexplored approach is the design of a collective bisubstrate inhibitor involving the incorporation of critical binding components of both the reacting partners (FPP and *ras*) in a chemically and biologically stable form. We herein report the design, synthesis, and biological activity of a new class of potent, rationally designed bisubstrate analog inhibitors of FPT.

Bisubstrate Inhibitor Design

A hypothetical "active site model" of FPT was constructed from knowledge of the substrate reaction and structural information about the enzyme (Figure 1).[6a,7] The model comprises a peptide binding domain that accommodates the critical "CAAX" motif of Ras, and a lipid binding domain in which the hydrophobic farnesyl group of FPP resides. The reaction formally proceeds by a nucleophilic attack by the thiol group of cysteine on the allylic carbon atom of FPP bearing the pyrophosphate group. An enzymic base may facilitate the reaction by abstracting the thiol proton and enhancing the nucleophilicity of the thiol group. A suitable metal ion (e.g., Mg^{2+}) may serve to complex with the PP group in order to neutralize charge and set the stage for displacement reaction. The role of Zn^{2+} ion is unclear; it may be coordinated with the thiol and/or the PP group, and/or with the free CO_2H group of Ras, or it may simply serve to stabilize the heterodimeric form of the enzyme.

The inhibitor design strategy involved hybridization of the two reacting partners into a single chemically and biologically stable entity. In our inhibitor design, the farnesyl group of FPP was retained to preserve putative hydrophobic interactions, and the *ras* C-terminal tripeptide was chosen as the peptide substrate component.[8] These two entities were connected via a phosphonic or phosphinic acid linker.[9] The phosphonic or phosphinic acid group was designed to interact with the zinc and/or magnesium metals associated with the cysteine sulfhydryl at the active site.[7b] The functional equivalence of mercaptan and phosphonyl or phosphinyl acid moieties has precedent in the area of metalloprotease inhibitors, including inhibitors of the zinc metalloproteases angiotensin-converting enzyme (ACE) and

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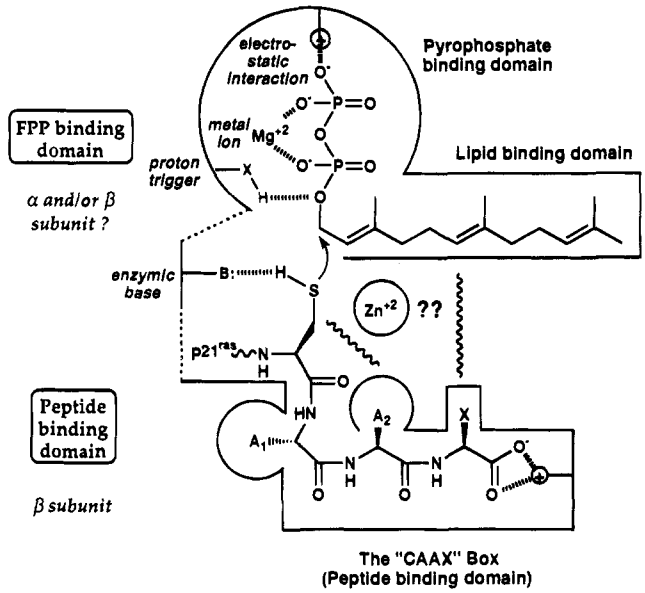


Figure 1. Hypothetical active site model of FPT.

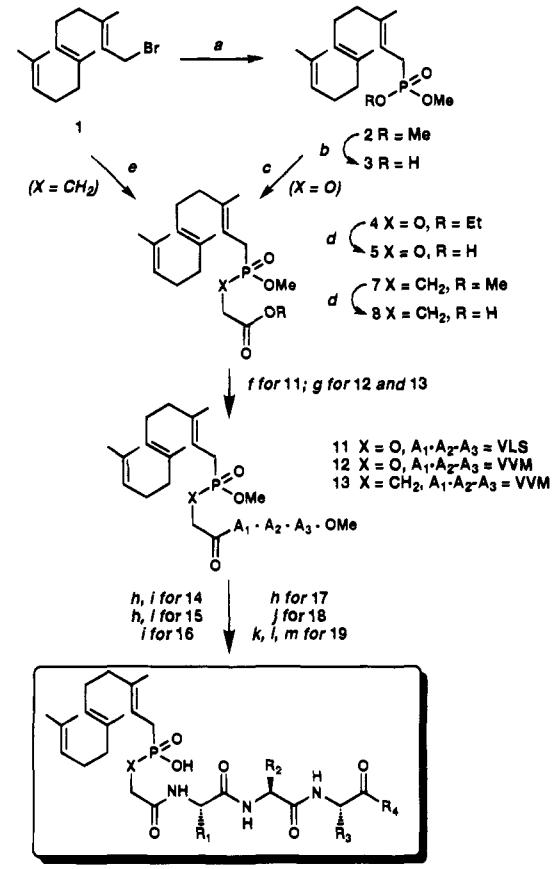
neutral endopeptidase (NEP).[9] The length of the linker was selected so as to allow conformational flexibility between the two substrate fragments, enabling them to adopt a transition state like geometry. In addition to structural novelty, incorporation of a phosphonic or phosphinic acid in place of the substrate sulfhydryl group avoids biological issues associated with a mercaptan moiety.[10] Additionally, the chemical equivalence of phosphinyl or phosphonyl oxygens avoids the introduction of an additional chiral center. This led to the proposal of evaluating phosphonic and phosphinic acid containing bisubstrate analogs **14**–**16** as FPT inhibitors.

Chemistry and Biology

Preparation of the phosphonic acid bisubstrate inhibitor **14** began with an Arbuzov reaction of trimethyl phosphite with farnesyl bromide affording the dimethyl phosphonate **2** in good yield (78%). Monohydrolysis of **2** under basic conditions followed by O-alkylation of the resulting acid **3** with ethyl bromoacetate gave **4** (100%). Conversion of diester **4** to carboxylic acid **5** was cleanly accomplished by careful hydrolysis with 1 equiv of base (91%). EDC-mediated[11] coupling of carboxylic acid **5** with the tripeptide H-VLS-OMe (**9**) gave the diester precursor 11 (50%). Selective monodemethylation at the phosphorus center by heating with sodium iodide in acetone (45%) gave intermediate **17**, which upon treatment with 1 N NaOH in dioxane afforded the desired diacid **14** (80%).[12] Coupling of **5** with the tripeptide amine H-VVM-OMe (**10**) utilizing the BOP[11] reagent, followed by similar sequence of reactions as outlined for **14**, provided the VVM analog **15**. To prepare the phosphinic acid analog of **15**, alkylation of phosphinate **6**[13] with farnesyl bromide (85%) was followed by selective monohydrolysis to carboxylic acid **8** (90%). Coupling of **8** with **10** using BOP provided the diester intermediate **13**, which was converted by one-step base hydrolysis to the desired analog **16** (Scheme 1).

Compound **14**, the first example in this series, was found to be a potent FPT inhibitor (I_{50} = 60 nM).[14] A further 15-fold enhancement in activity was observed upon replacement of the VLS tripeptide sequence in **14**

Scheme 1[a]



Cpd #	X	R_1	R_2	R_3	R_4	I_{50} (FPT)	I_{50} (GGT-I)
14	O	-CHMe$_2$	-CH$_2$CHMe$_2$	-CH$_2$OH	-OH	60 ± 30 nM	59 ± 10 uM
15	O	-CHMe$_2$	-CHMe$_2$	-(CH$_2$)$_2$SMe	-OH	6.2 ± 0.5 nM	10 ± 1.7 uM
16	CH$_2$	-CHMe$_2$	-CHMe$_2$	-(CH$_2$)$_2$SMe	-OH	6 ± 1 nM	21 ± 9.9 uM
17	O	-CHMe$_2$	-CHMe$_2$	-(CH$_2$)$_2$SMe	-OMe	*a*	*a*
18	CH$_2$	-CHMe$_2$	-CHMe$_2$	-(CH$_2$)$_2$SMe	-OMe	*a*	*a*
19	CH$_2$	-CHMe$_2$	-CHMe$_2$	-(CH$_2$)$_2$SMe	-OPOM	*a*	*a*

[a] Reagents: (a) P(OMe)$_3$, reflux, 78%; (b) 1 N NaOH, MeOH, quant; (c) K_2CO_3, DMF, $BrCH_2CO_2Et$, quant; (d) 1 N NaOH (1.0), MeOH, 91% for **5**, 90% for **8**; (e) NaH, HP(O)(OMe)CH$_2$CO$_2$Me **6**, 85%; (f) EDC, HOBt, iPr$_2$NEt, HCl·H-VLS-OMe **9**, 50%; (g) BOP, iPr$_2$NEt, HCl·H-VVM-OMe **10**, 63% for **12**, 97% for **13**; (h) NaI, acetone, reflux, 45% for **14**, 52% for **17**; (i) 1 N NaOH, dioxane, 80% for **14**, 83% for **15**, 60% for **16**; (j) TMSBr, BSTFA, 63% or Me_3N, acetone, 47%; (k) 1 N NaOH (1.0), MeOH, 73%; (l) tBuC(O)OCH$_2$I (10.0), Ag_2CO_3 (2.0), MeP(O)(OMe)$_2$ (10.0), DMF, 79%; (m) BSTFA, TMSBr, 62%. a: Compounds **17**–**19** were prepared and evaluated as prodrugs.

with VVM (**15**, I_{50} = 4 nM), a trend that parallels the structure–activity relationship observed for simple tetrapeptide-based inhibitors.[4] The phosphinic acid analog **16** (I_{50} = 6 nM) was essentially equiactive to phosphonic acid **15**. This result suggests that the phosphonic ether oxygen of **14** and **15** contributes minimally to binding, and that the binding affinity of the phosphonate moiety is primarily derived from the charged oxygen.

These inhibitors were evaluated for selectivity using the closely related enzyme geranylgeranyl protein transferase type I (GGT-I),[14] whose preferred CAAX sequences contain hydrophobic amino acids at the X position.[14] Compounds **14**–**16** were only moderately active against GGT-I [**14**, I_{50}(GGT-I) = 59 μM; **15** I_{50}(GGT-I) = 10 μM; **16** I_{50}(GGT-I) = 21 μM], thereby

affording greater than 1000-fold selectivity in favor of the targeted enzyme, FPT.

Having established the *in vitro* potency and selectivity of these inhibitors, we next tested them in cell-based models for inhibition of *ras*-mediated cell transformation.[5] Since the parent diacids have very limited cell permeability, prodrugs of the lead compounds **15** and **16** were prepared for their evaluation in whole cell assays. Carboxylic methyl ester **17**, the prodrug of phosphonic bisubstrate inhibitor **15**, was already encountered as the penultimate precursor in the synthesis of the parent diacid **15**. Carboxylic methyl ester **18** in the phosphinic bisubstrate series was prepared from diester **13** via selective demethylation at the phosphorus center by heating with trimethylamine in acetone (47%), or more efficiently by selective hydrolysis (63%) upon treatment with TMSBr in the presence of excess bis-(trimethylsilyl)trifluoroacetamide (BSTFA). Alternatively, careful treatment of diester **13** with 1.0 equiv of 1 N NaOH enabled selective hydrolysis of the carboxylic methyl ester group. The resulting acid intermediate was alkylated with pivaloyloxy methyl iodide under forcing conditions (79%) followed by treatment with BSTFA/TMSBr to provide the POM ester **19** (62%).

The effect of these inhibitors and their prodrugs in whole cells was examined in a *ras* transformation inhibition (RTI) assay. NIH-3T3 cells transfected with oncogenic *H-ras* DNA were treated with the appropriate compounds 24 h after transfection, and the effect on transformation was evaluated after 14 days. While the parent diacids **15** and **16** are essentially ineffective, the methyl ester prodrugs **17** and **18** show 75 to 80% decrease in transformed foci at *100 μM* concentration (Figure 2). No gross cytotoxicity was observed for any of the compounds at the tested concentrations. The ability of prodrugs **18** and **19**[15] to inhibit anchorage independent colony growth of *ras* transformed cells was tested by soft agar growth assay. At 100 μM concentrations, both **18** and **19**[15] almost completely suppress colony formation of *H-ras* transformed NIH 3T3 cells in soft agar (Figure 3).

Summary

In summary, *the rational design and synthesis of phosphonyl and phosphinyl acids **14–16** constitute the first examples of potent nonsulfhydryl bisubstrate analog inhibitors of FPT.*[20] *Unlike tetrapeptide-based inhibitors, a free sulfhydryl group is not a requirement for the activity of these phosphonic/phosphinic acid bisubstrate inhibitors.* The presence of a farnesyl group in these molecules may diminish their dependence on the peptide fragment for overall activity and thus enable the discovery of novel FPT inhibitors with reduced peptide character. As anticipated, these inhibitors are selective for FPT when compared to geranylgeranyl transferase GGT-I.[14] The methyl and POM ester prodrugs **17–19** appear to block *ras*-induced cell transformation in NIH3T3 cells,[15,21] as well as colony formation in soft agar. The preliminary results reported here support FPT as a target for rational discovery of antitumor agents, a conclusion that has also been derived from the independent work of other research groups.[5] Studies describing a thorough biological evaluation of these new compounds, with appropriate controls, will be reported elsewhere.[15]

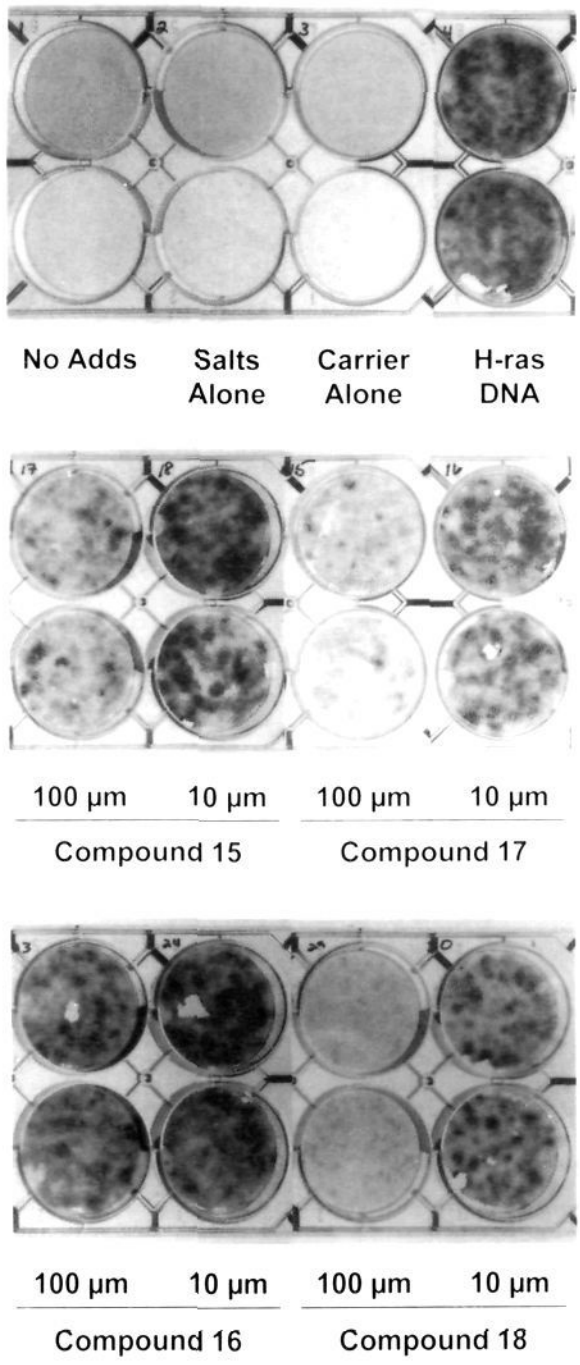


Figure 2. Ras transformation inhibition by compounds **14–18**.

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. 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–divinylbenzene copolymer purchased from Mit-

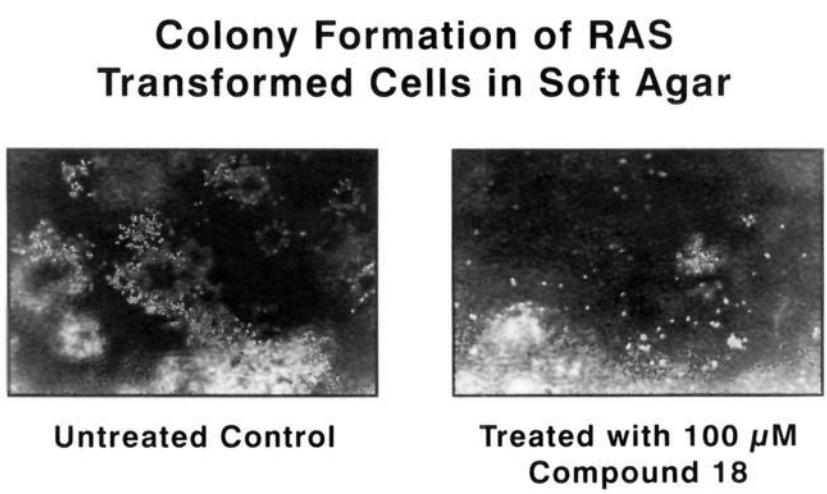


Figure 3. Inhibition of colony formation in soft agar by compound **18**.

subishi 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 Corp. (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 and JEOL FX-60Q operating at 15 MHz (^{13}C). 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.[3a] and further purified approximately 2000-fold relative to the initial crude pig brain cytosol.[16] 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.[17] 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.[17]). 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} estimations were made from percent control versus log drug concentration plots. Each compound was tested at least twice.

Geranylgeranyl Transferase (GGT-I) Inhibition Assay. GGT-I was purified from porcine brain tissue. Ras-CVLL proteins were expressed in bacteria and purified as described.[18] In order to determine the IC_{50} values for inhibition, the GGT-I *assay was adapted to a 96-well microtiter plate format as* described.[18] Briefly, 12 μM p21 rasCVLL, 0.5 μM [^{3}H]GGPP (19.3 Ci/mmol; NEN), 10 mM dithiothreitol (DTT), 5 mM $MgCl_2$, 5 μM $ZnCl_2$, 100 mM Hepes, pH 7.4, and partially purified GGTI were incubated in a total volume of 20 μL at 37 °C for 1 h, and samples were processed as described for the farnesyltransferase assay. All assays were carried out in triplicate and SEMs determined.

Ras Transformation Inhibition (RTI) Assay. The whole cell activity of FPT inhibitors was evaluated by a ras transformation inhibiton (RTI) assay. The RTI assay is based on transformation of mouse NIH 3T3 cells by oncogenic ras DNA transfection. NIH 3T3 cells (2.5×10^4 per 35 mm well) were seeded and allowed to attach overnight. The cells were transfected with linearized plasmid carrying oncogenic H-ras[19] using the calcium phosphate precipitation technique. One day following the transfection, cells were washed and inhibitors were added at the indicated concentrations into the medium. Inhibitors were replenished every 48 h for 8 days along with the change of medium, for a total of four treatments. The degree of transformation was scored after 14 days. In the absence of an inhibitor, transfected cells grow aggressively and initially give the appearance of abnormal "foci" surrounded by normal cells. In the presence of a cell permeable inhibitor, the number of foci are either reduced or transformation is completely inhibited. The percent inhibition and the gross cytotoxicity of inhibitors are evaluated visually by light microscopy.

Soft Agar Growth (SAG) Assay. The SAG assay is based on the ability of ras-transformed cells to grow in soft agar and form discrete colonies in 14–21 days. A bottom agar layer of 0.6% Noble agar (Difco) in culture medium (2 mL, Dulbecco's Modified Eagles Medium (DMEM) from Gibco supplemented with 10% calf serum from Colorado Serum Co.) was formed in 6-well culture plates and allowed to solidify at room temperature for 1–2 h. Cultures were trypsinized, and single cell suspensions were obtained. A top soft agar mixture (2 mL) containing 0.3% Noble agar, 10 000 cells, and culture medium was laid on the solidified bottom agar layer. Initially, inhibitors at the indicated concentrations were incorporated into the soft agar mixture. The cells were cultured for 2 weeks at 37 °C. FT inhibitors were replenished every 2 days for 8 days by overlaying 100 μL of 20-fold concentrated stock in PBS on the surface of the top agar layer. All the assays are carried out in parallel duplicate wells. At the end of the test period, the number of colonies larger than 0.1 mm in diameter was determined for both treated and untreated samples.

Preparation of (*E,E*)-(3,7,11-Trimethyl-2,6,10-dodecatrienyl)phosphonic Acid, Dimethyl Ester (2). A mixture of farnesyl bromide 1 (14.26 mL, 52.6 mmol) and trimethyl phosphite (6.84 mL, 57.9 mmol) was heated at reflux for 16 h. The excess trimethyl phosphite was removed *in vacuo*, and the residue was purified by flash chromatography, eluting with hexane:ethyl acetate (10:1 to 6:1) to afford **2** (8.03 g, 78%) as an oil which was homogeneous by TLC (R_f = 0.8 in hexane: ethyl acetate, 1:1, visualized by ceric ammonium sulfate) and HPLC. Mass spectrum $(M + H)^+$: calculated for $C_{17}H_{32}O_3P$ 315.2089, found 315.2099. ^1H NMR (270 MHz, $CDCl_3$): 1.58 (s, 6H), 1.66 (s, 6H), 2.06 (br m, 8H), 2.56 (dd, 2H, J = 7.6, 21.7), 3.73 (d, 6H, J = 10.5), 5.08–5.18 (m, 3H). ^{13}C NMR (68 MHz, $CDCl_3$): 15.7, 15.9, 17.4, 24.2, 25.4, 26.1, 26.2, 26.4, 39.4, 52.2, 52.3, 111.7, 111.9, 123.5, 124.0, 130.9, 135.0, 140.1, 140.3.

Preparation of (*E,E*)-(3,7,11-Trimethyl-2,6,10-dodecatrienyl)phosphonic Acid, Monomethyl Ester (3). To a solution of **2** (8.03 g, 25.6 mmol) in methanol (70 mL) was added 1 N NaOH (77 mL, 76.7 mmol), and the reaction was refluxed for 20 h. Methanol was removed *in vacuo*, and the aqueous solution was diluted with water (40 mL) and extracted with ethyl acetate (2 × 40 mL). The organic extracts were discarded, and the aqueous solution was acidified by addition of 1 N aqueous hydrochloric acid (pH = 2.0) in the presence of ethyl acetate (40 mL). After the layers were separated, the aqueous solution was reextracted with ethyl acetate (3 × 40 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **3** (7.71 g, 100%). ^1H NMR (270 MHz, $CDCl_3$): 1.59 (s, 6H), 1.67 (s, 6H), 2.06 (br m, 8H), 2.57 (dd, 2H, J = 7.6, 22.2), 3.70 (d, 3H, J = 11.1), 5.08–5.22 (m, 3H). ^{13}C NMR (68 MHz, $CDCl_3$): 15.9, 16.2, 17.6, 24.9, 25.6, 26.3, 26.4, 26.6, 27.0, 39.6, 51.7, 51.8, 111.8, 112.0, 123.7, 124.3, 131.1, 135.1, 140.5, 140.7. Anal. ($C_{16}H_{29}O_3P$) C, H.

Preparation of (*E,E*)-[[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetic Acid, Ethyl Ester (4). To a solution of **3** (1.5 g, 5 mmol) in DMF (25 mL) was added potassium carbonate (4.15 g, 30 mmol) and ethyl bromoacetate (1.66 mL, 15 mmol). The reaction was stirred at 25 °C for 4 h and filtered through Celite. The precipitate was washed with ethyl acetate, and the filtrate and the washes were combined and washed sequentially with 10% aqueous hydrochloric acid (50 mL) and saturated aqueous lithium chloride (3 × 60 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting with hexane:ethyl acetate (3:1 to 1:1) to give **3** (1.95 g, 100%), which was homogeneous by TLC (R_f = 0.37 in hexane:ethyl acetate, 1:2, visualized by ceric ammonium sulfate stain). Mass spectrum $(M + H)^+$: calculated for $C_{20}H_{36}O_5P$ 387.2247, found 387.2307. ^1H NMR (270 MHz, $CDCl_3$): 1.29 (t, 3H, J = 7.0), 1.60 (s, 6H), 1.68 (s, 6H), 2.08 (br m, 8H), 2.68 (dd, 2H, J = 7.6, 22.2), 3.77 (d, 3H, J = 11.1), 4.24 (q, 2H, J = 7.0), 4.57 (d,d, 2H, J = 2.9, 11.1), 5.09–5.22 (m, 3H). ^{13}C NMR (68 MHz, $CDCl_3$): 14.0, 15.9, 16.2, 17.6, 25.2, 25.6, 26.3, 26.4, 26.6, 27.3, 52.2, 52.3, 61.3, 62.2, 62.3, 111.6, 111.7, 123.7, 124.2, 131.2, 135.2, 140.8, 141.0, 168.4, 168.5.

Preparation of (*E,E*)-[[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetic Acid (5). To a solution of **4** (1 g, 2.59 mmol) in methanol:water (2:1) was added at 0 °C 1 N aqueous sodium hydroxide solution (2.59 mL, 2.59 mmol). After stirring for 3 h at 0 °C, methanol was removed *in vacuo*, and the aqueous solution was diluted with water (30 mL) and extracted with ethyl acetate (2 × 20 mL). The organic extracts were discarded, and the aqueous layer was acidified with 1 N hydrochloric acid (pH = 2.0) in the presence of ethyl acetate (40 mL). After the layers were separated, the aqueous solution was reextracted with ethyl acetate (3 × 30 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated *in vacuo* to give **5**. Mass spectrum $(M + H)^+$: calculated for $C_{18}H_{32}O_5P$ 359.1988, found 359.2014. ^1H NMR (270 MHz, $CDCl_3$): 1.59 (s, 6H), 1.67 (s, 6H), 2.07 (m, 8H), 2.73 (dd, 2H, J = 7.6, 22.8), 3.79 (d, 3H, J = 11.1), 4.60 (d, 2H, J = 12.3), 5.09–5.18 (m, 3H). ^{13}C NMR (68 MHz, $CDCl_3$): 16.0, 16.3, 17.7, 25.1, 25.7, 26.4, 26.5, 26.7, 27.2, 38.7, 52.9, 53.0, 62.8, 62.9, 111.1, 111.3, 123.7, 123.8, 124.3, 131.3, 135.4, 141.4, 141.6, 170.7.

Preparation of 3-(Methoxyphosphinyl)propanoic Acid, Methyl Ester (6). Compound **6** was prepred by slight modifications of the literature procedure.[13] Methyl hypophosphite (6 g, 75 mmol) was added to a solution of methyl acrylate (6.8 mL, 75.8 mmol) and diisopropylethylamine (1.34 mL, 7.73 mmol) at 0 °C, after which the mixture was warmed to room temperature and stirred for 24 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution (15 mL) and extracted with chloroform (4 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by vacuum distillation (97–100 °C at 0.7 mmHg) to afford **6** as an oil (5.0 g, 40%). Mass spectrum $(M + H)^+$: calculated for $C_5H_{12}O_4P$ 167.0474, found 167.0472. IR (neat): 3445–2999, 2957, 2373, 1738, 1441, 1244, 1044 cm^{-1}. ^1H NMR ($CDCl_3$): 2.03–2.15 (m, 2 H), 2.54–2.80 (m, 2 H), 3.72 (s, 3 H), 3.80 (d, 3 H, J = 11.7), 7.19 (d, 1 H, J = 548.2). ^{13}C NMR ($CDCl_3$): 22.9, 24.3, 25.6, 25.7, 51.8, 52.0, 52.7, 52.8, 172.1, 172.3 ppm.

Preparation of (*E,E*)-3-[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]propanoic Acid, Methyl Ester (7). Sodium hydride (60% suspension in oil, 0.36 g, 9.0 mmol) was added to a solution of **6** (1.5 g, 9.0 mmol, 1.0 equiv) in THF (20 mL) at −5 °C, and the mixture was stirred for 15 min. Farnesyl bromide (2.45 mL, 9.0 mmol) was added dropwise, and the reaction was warmed to room temperature and stirred for 16 h. The reaction was quenched by addition of saturated aqueous ammonium chloride solution (50 mL) and extracted with ethyl acetate (4 × 50 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (eluting with 3:1 hexane/acetone) to afford **7** (2.79 g, 85%). TLC: R_f = 0.59 (1:1 hexane/acetone, visualization by PMA). Mass spectrum $(M + H)^+$: 371. ^1H NMR ($CDCl_3$): 1.60 (s, 6 H), 1.66 (s, 3 H), 1.68 (s, 3 H), 1.97–2.18 (m, 10 H), 2.54–2.66 (m, 4 H), 3.70 (s, 3 H), 3.71 (d, 3 H, J = 10.6), 5.08–5.19 (m, 3 H). ^{13}C NMR ($CDCl_3$): 15.9, 16.2, 17.5, 21.6, 22.9, 25.5, 26.2, 26.3, 26.6, 27.9, 29.2, 39.6, 51.0, 51.1, 51.9, 112.1, 112.3, 123.5, 124.1, 131.2, 135.3, 140.6, 140.8, 172.5, 172.7. Anal. ($C_{20}H_{35}PO_4 \cdot 0.81H_2O$) C, H.

Preparation of (*E,E*)-3-[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]propanoic Acid (8). Sodium hydroxide (1 N, 7.54 mL, 7.54 mmol) was added to a 0 °C solution of **7** (2.79, 7.54 mmol) in methanol (21 mL) and water (10 mL) and stirred at this temperature for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue was diluted with water (50 mL) and washed with chloroform (1 × 50 mL). The organic wash was discarded, and the aqueous solution was acidified by addition of concentrated hydrochloric acid to pH 1.0 and was extracted with methylene chloride (4 × 50 mL). The combined organics extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford **8** (2.42 g, 90%), which was homogeneous by TLC (R_f = 0.63, 6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA). Mass spectrum $(M + H)^+$: 357. ^1H NMR ($CDCl_3$): 1.60 (s, 6 H), 1.66 (s, 3 H), 1.68 (s, 3 H), 1.96–2.10 (m, 10 H), 2.52–2.70 (m, 4 H), 3.72 (d, 3 H, J = 10.6), 5.06–5.30 (m, 3 H). ^{13}C NMR

($CDCl_3$): 16.3, 16.7, 18.0, 21.5, 22.8, 26.0, 26.6, 26.7, 26.8, 26.9, 27.0, 28.1, 29.4, 40.0, 51.9, 52.0, 111.9, 112.1, 123.9, 124.0, 124.6, 131.6, 135.8, 141.5, 141.7, 174.8, 175.0 ppm. Anal. ($C_{19}H_{33}O_4P \cdot 0.32H_2O$) C, H.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[[[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetyl]-L-valyl]-L-leucyl]-L-serine, Methyl Ester (11). To a solution of **5** (843 mg, 2.35 mmol) in THF (70 mL) were added sequentially hydroxybenzotriazole (369 mg, 2.35 mmol), diisopropylethylamine (408 μL, 2.35 mmol), tripeptide **9** (valylleucylserine methyl ester hydrochloride, 864 mg, 2.35 mmol), and *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (450 mg, 2.35 mmol) at 0 °C. The reaction mixture was warmed gradually to room temperature, and after stirring for 20 h, the suspension was diluted with ethyl acetate (200 mL) and washed sequentially with saturated aqueous sodium bicarbonate (100 mL), 10% sodium bisulfate (100 mL), and water (3 × 100 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography, eluting with hexane:acetone (2:1 to 1:1), to give **11** (775 mg, 50%), which was homogeneous by TLC (R_f = 0.55, acetone:hexane, 1:1, visualized by ceric ammonium sulfate). Mass spectrum $(M + H)^+$: calculated for $C_{33}H_{59}O_9N_3P$ 672.3993, found 672.3989. ^1H NMR (270 MHz, $CDCl_3$): 0.92 (m, 12H), 1.59 (s, 6H), 1.68 (s, 6H), 1.69 (br m, 3H), 2.07 (m, 9H), 2.68 (dd, 2H, J = 7.6, 21.6), 3.76 (s, 3H), 3.78 (d, 3H, J = 10.5), 3.82–4.61 (m, 7H), 5.08–5.16 (m, 3H). ^{13}C NMR (68 MHz, $CDCl_3$): 16.0, 16.4, 17.7, 18.1, 18.2, 19.2, 21.9, 22.0, 22.9, 24.7, 25.7, 26.4, 26.5, 26.6, 26.7, 30.7, 31.0, 39.7, 40.4, 40.7, 51.9, 52.0, 52.5, 53.2, 53.6, 53.7, 54.9, 58.5, 58.7, 62.6, 63.9, 110.7, 110.8, 110.9, 123.5, 124.3, 131.3, 135.5, 135.5, 141.9, 168.1, 168.2, 170.8, 171.3, 171.3, 172.1.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[[[Methoxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetyl]-L-valyl]-L-valyl]-L-methionine, Methyl Ester (12). (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.442 g, 1.0 mmol) was added to a solution of **5** (0.358 g, 1.0 mmol) and tripeptide **10** (valylvalylmethionine methyl ester hydrochloride, 0.398 g, 1.0 mmol) in acetonitrile (5 mL) and DMF (3 mL). Diisopropylethylamine (0.349 mL, 2.0 mmol) was added, and the reaction was stirred for 16 h at room temperature. The reaction was quenched by addition of hydrochloric acid (1 N, 50 mL), and the mixture was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were washed sequentially with aqueous sodium carbonate solution (10%, 50 mL) and aqueous lithium chloride (10%, 2 × 100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (eluting with 3:2 hexane/acetone) to afford **12** (0.44 g, 63%) as an oil, which was homogeneous by TLC (R_f = 0.42, 1:1 acetone/hexane, visualization by PMA). Mass spectrum $(M + H)^+$: calculated for $C_{34}H_{60}N_3O_8PS$ 702.3917, found 702.3928. $[\alpha]_D = -20.5°$ (c = 1.06, $CHCl_3$). IR (CH_2Cl_2 film): 3279, 2963, 1751, 1640, 1549 cm^{-1}. ^1H NMR ($CDCl_3$): 0.93–0.97 (m, 12 H), 1.59 (s, 6 H), 1.67 (s, 6 H), 1.97–2.19 (m, 12 H), 2.07 (s, 3 H), 2.47 (t, 2 H, J = 7.0), 2.67 (dd, 2 H, J = 7.6, 22.3), 3.73, 3.75 (s, 3 H), 3.77, 3.78 (d, 3 H, J = 10.6, 11.2, diastereomers), 4.36–4.69 (m, 5 H), 5.17–5.20 (m, 3 H), 7.08–7.42 (m, 3 H). ^{13}C NMR ($CDCl_3$): 15.4, 16.0, 16.4, 17.7, 18.1, 18.3, 19.1, 19.2, 25.7, 26.4, 26.7, 29.3, 29.9, 30.9, 31.1, 39.7, 51.5, 52.5, 58.4, 58.5, 58.7, 63.9, 64.0, 111.0, 111.1, 111.2, 123.6, 124.3, 131.3, 135.5, 167.7, 167.7, 170.9, 171.1, 172.1 ppm.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[3-[Hydroxy(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)phosphinyl]-1-oxopropyl]-L-valyl]-L-valyl]-L-methionine, Methyl Ester (13). Diisopropylethylamine (0.349 mL, 2.0 mmol) was added to a solution of **8** (0.356 g, 1.0 mmol and tripeptide **10** (valylvalylmethionine methyl ester hydrochloride, 0.398 g, 1.0 mmol) in acetonitrile (12 mL) and DMF (4 mL) at room temperature. To the mixture was added BOP (0.443 g, 1.0 mmol), and the reaction mixture was stirred an additional 16 h. The reaction was quenched by addition of 1 N hydrochloric acid (50 mL) and extracted with ethyl acetate (4 × 50 mL); the combined organic extracts were washed with 10% aqueous sodium carbonate (50 mL) and 10% aqueous lithium chloride (3 × 50 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (eluting with 2:1 acetone/hexane) to afford **13** (0.350 g, 50%) as an oil, which was homogeneous by TLC (R_f = 0.86, 2:1 acetone:hexane, visualization by PMA). Mass spectrum $(M + H)^+$: calculated for $C_{35}H_{62}N_3O_7PS$ 700.4124, found 700.4150. IR (CH_2Cl_2 film 1638 cm^{-1}. $[\alpha]_D = -38.4°$ (c = 0.19, CH_3OH). ^1H NMR (CD_3OD): 0.90–1.00 (m, 12 H), 1.60 (s, 6 H), 1.62 (s, 3 H), 1.66 and 1.69 (s, 3 H, diastereomers), 1.93–2.18 (m, 14 H), 2.06 (s, 3 H), 2.48–2.63 (m, 6 H), 3.71 (s, 3 H), 3.72 (d, 3 H, J = 10.6), 4.13 (d, 1 H, J = 8.8), 4.17 (d, 1 H, J = 7.6), 4.56–4.61 (m, 1 H), 5.08–5.11 (m, 3 H). ^{13}C NMR ($CDCl_3$): 15.0, 15.8, 16.2, 17.5, 18.0, 18.2, 18.3, 18.8, 19.0, 25.5, 26.3, 26.5, 27.3, 29.1, 29.8, 30.7, 31.7, 39.5, 51.4, 51.5, 51.6, 52.3, 53.6, 58.7, 58.9, 59.6, 69.5, 111.5, 111.6, 123.4, 124.1, 131.1, 135.3, 141.1, 141.3, 171.9, 172.0, 172.1, 172.7 ppm.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[[[Hydroxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetyl]-L-valyl]-L-leucyl]-L-serine, Disodium Salt (14). To a solution of **11** (161 mg, 0.240 mmol) in acetone (2 mL) was added sodium iodide (181 mg, 1.20 mmol). After refluxing for 8 h, the reaction was diluted with ethyl acetate (50 mL) and was washed sequentially with 10% aqueous sodium thiosulfate (30 mL) and saturated sodium chloride (30 mL). The aqueous washes were combined and extracted with chloroform:methanol (10:1, 6 × 50 mL). The ethyl acetate and chloroform:methanol extracts were combined, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography, eluting with chloroform:methanol (100:0 to 90:10), to give the phosphonic acid carboxylic methyl ester intermediate (74 mg, 45%). TLC: R_f = 0.92 (1-butanol:acetic acid:water:ethyl acetate, 1:1:1:1, visualized by ceric ammonium sulfate). ^1H NMR (400 MHz, CD_3OD): 0.96 (m, 12H), 1.58 (s, 6H), 1.66 (s, 6H), 1.58–1.73 (m, 3H), 1.94–2.17 (m, 9H), 2.47 (dd, 2H, J = 7.7, 21.8), 3.74 (s, 3H), 3.86 (m, 2H), 4.20–4.56 (m, 5H), 5.06–5.32 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): 16.2, 16.6, 17.8, 18.5, 19.9, 22.4, 23.4, 25.9, 26.0, 27.8, 32.6, 40.9, 41.1, 41.5, 48.1, 48.4, 48.7, 49.0, 49.3, 49.6, 49.9, 50.0, 52.9, 53.5, 56.3, 59.4, 62.7, 64.3, 64.4, 117.3, 117.5, 125.3, 125.4, 132.1, 136.1, 138.5, 138.7, 171.9, 172.7, 172.8, 173.6, 174.5 ppm.

To a solution of the above intermediate (74 mg, 0.109 mmol) in dioxane:water (2:1, 1.5 mL) was added 1 N sodium hydroxide (0.12 mL, 0.12 mmol). After stirring for 2 h, the dioxane was removed *in vacuo*. The aqueous solution was purified on CHP 20P (6 mL), eluting with water:acetonitrile (100:0 to 0:100). Fractions containing the major product were combined, concentrated, and lyophilized to give **14** (60 mg, 80%) as a white powder, mp 150–152 °C. TLC: R_f = 0.81 (BuOH:HOAc:H_2O:EtOAc, 1:1:1:1, visualized by ceric ammonium sulfate). Mass spectrum $(M + Na)^+$: calculated for $C_{31}H_{54}N_3O_9PNa$ 666.3501, found 666.3495. ^1H NMR (400 MHz, DMSO-d_6): 0.83 (m, 12H), 1.55 (s, 6H), 1.58 (m, 3H), 1.62 (s, 3H), 1.23–1.66 (m, 3H), 1.93–2.23 (m, 9H), 2.27 (dd, 2H, J = 6.41, 20.94), 3.62 (br m, 2H), 3.99–4.39 (5H), 5.04–5.22 (m, 3H). Anal. ($C_{31}H_{52}N_2O_9Na_2 \cdot 3.36H_2O$) C, H, N.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[[[Hydroxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetyl]-L-valyl]-L-valyl]-L-methionine, Disodium Salt. Sodium hydroxide (1 N, 0.567 mL, 0.567 mmol) was added to a solution of **17** (0.15 g, 0.218 mmol, 1.0 equiv) in dioxane (11 mL), and the mixture was stirred at room temperature for 16 h. The reaction was concentrated *in vacuo*, and the residue was purified on a column of CHP-20 gel (gradient elution 0–50% aqueous acetonitrile). Fractions containing the major product were combined and concentrated *in vacuo*, and the residue was dissolved in water (10 mL), millipore filtered, and lyophilized to afford **15** (0.13 g, 83%) as a white powder, mp > 220 °C dec. TLC: R_f = 0.70 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA). Mass spectrum $(M + Na)^+$: 696. IR (KBr): 1645 cm^{-1}. ^1H NMR (CD_3OD): 0.94–1.00 (m, 12 H), 1.59 (s, 6 H), 1.66 (s, 6 H), 1.93–2.16 (m, 12 H), 2.05 (s, 3 H), 2.41–2.51 (m, 4 H), 4.14–4.36 (m, 5 H), 5.09–5.11 (m, 2 H), 5.31–5.34 (m, 1 H). $[\alpha]_D = -19.8°$ (c = 0.52, CH_3OH). Anal. ($C_{32}H_{54}N_3O_8PSNa \cdot 1.94H_2O$) C, H, N.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[3-[Hydroxy(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)phosphinyl]-1-oxopropyl]-L-valyl]-L-valyl]-L-methionine, Disodium Salt (16). Sodium hydroxide (1 N, 0.412 mL, 0.412 mmol) was added to a solution of **13** (0.131 g, 0.187 mmol,) in dioxane (2 mL), and the mixture was stirred at room temperature for 72 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by chromatography on CHP-20P gel (eluting sequentially with water (75 mL), 2.5%, 5.0, 7.5%, and 10% aqueous acetonitrile). Fractions containing the major product were combined and concentrated *in vacuo*, and the residue was dissolved in water (10 mL), millipore filtered, and lyophilized to afford **16** (0.08g, 60%) as a white powder, mp > 195 °C dec. TLC: R_f = 0.78 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA). IR (KBr): 1638 cm^{-1}. Mass spectrum $(M + Na)^+$: 694. ^1H NMR (CD_3OD): 0.94 (m, 12 H), 1.59 (s, 6 H), 1.66 (s, 6 H), 1.72–1.75 (m, 2 H), 1.95–2.11 (m, 12 H), 2.05 (s, 3 H), 2.30–2.49 (m, 6 H), 4.15 (d, 1 H, J = 7.6), 4.19 (d, 1 H, J = 7.7), 4.29 (t, 1 H, J = 7.2), 5.08–5.12 (m, 2 H), 5.35 (br m, 1 H). $[\alpha]_D$ = −27.1° (c = 0.17, CH_3OH). Anal. ($C_{33}H_{56}N_3O_7PSNa_2 \cdot 2.71H_2O$) C, H, N.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[[[Hydroxy(3,7,11-trimethyl-2,6,10-dodecatrienyl)phosphinyl]oxy]acetyl]-L-valyl]-L-valyl]-L-methionine, Methyl Ester (17). Sodium iodide (0.915 g, 6.1 mmol) was added to a solution of **12** (0.856 g, 1.22 mmol) in acetone (20 mL), and the mixture was heated at reflux for 20 h. The reaction was concentrated *in vacuo*, the residue was dissolved in aqueous sodium thiosulfate solution (5%, pH 2, 50 mL), and the solution was saturated with sodium chloride. The aqueous solution was extracted with chloroform (10 × 100 mL), and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by successive triturations with diethyl ether (1 × 50 mL), hexane (1 × 50 mL), and ethyl acetate (1 × 50 mL) to afford **17** (0.436 g, 52%) as a solid, mp 174–178 °C. Mass spectrum $(M + H)^+$: 688. TLC: R_f = 0.79 (6:3:1 1-propanol/ammonium hydoxide/water, visualization by PMA). IR (KBr): 1640 cm^{-1}. ^1H NMR (CD_3OD): 0.92–1.00 (m, 12 H), 1.60 (s, 6 H), 1.72 (s, 3 H), 1.75 (s, 3 H), 1.94–2.13 (m, 12 H), 2.06 (s, 3 H), 2.52 (m, 4 H), 3.73 (s, 3 H), 4.12–4.75 (m, 5 H), 5.14–5.33 (m, 3 H). $[\alpha]_D$ = −49.2° (c = 1.04, $CHCl_3$). Anal. ($C_{33}H_{58}N_3O_8PS \cdot H_2O$) C, H, N.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[3-[Hydroxy(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)phosphinyl]-1-oxopropyl]-L-valyl]-L-valyl]-L-methionine, Methyl Ester, Monosodium Salt (18). Trimethylamine was bubbled into a 0 °C solution of **16** (0.50 g, 0.73 mmol) in acetone (50 mL) until the solution was saturated, and then the mixture was stirred for 0.5 h. The reaction mixture was concentrated *in vacuo*, and the water soluble trimethylammonium salt was passed through a Dowex Na^+ ion exchange column (eluting with 20% aqueous acetonitrile). The appropriate fractions were combined and concentrated *in vacuo*, and the residue was dissolved in water (1 mL), millipore filtered, and lyophilized to afford **18** (0.40 g, 78%) as a white powder, mp > 200 °C dec. TLC: R_f = 0.78 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA). IR (KBr): 1740 cm^{-1}. ^1H NMR (CD_3OD): 0.91–0.98 (m, 12 H), 1.59 (s, 6 H), 1.66 (s, 6 H), 1.71–1.76 (m, 2 H), 1.94–2.09 (m, 12), 2.06 (s, 3 H), 2.31–2.58 (m, 6 H), 3.70 (s, 3 H), 4.12 (d, 1 H, J = 8.1), 4.15 (d, 1 H, J = 7.7), 4.56 (br m, 1 H), 5.09–5.12 (m, 2 H), 5.32 (br m, 1 H). $[\alpha]_D$ = −43.3° (c = 0.145, CH_3OH). Anal. ($C_{34}H_{59}N_3O_7SPNa \cdot 2.49H_2O$) C, H, N.

Preparation of (*E,E*)-*N*-[*N*-[*N*-[3-[Hydroxy(3,7,11-trimethyl-1-oxo-2,6,10-dodecatrienyl)phosphinyl]-1-oxopropyl]-L-valyl]-L-valyl]-L-methionine, (2,2-Dimethyl-1-oxopropoxy)methyl Ester (19). Sodium hydroxide (1 N, 14.3 mL, 14.3 mmol) was added to a −26 °C solution of **16** (10.0 g, 14.3 mmol) in methanol:water (115 mL:5 mL), after which the mixture was warmed to room temperature and stirred for 48 h. Another aliquot of sodium hydroxide (1 N, 3.6 mL, 3.6 mmol) was added, and the reaction mixture was stirred at room temperature for 6 h. The reaction was quenched carefully by addition of concentrated hydrochloric acid and was extracted with methylene chloride (6 × 700 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford the methyl phosphinyl ester carboxylic acid intermediate (7.2 g, 73%, TLC R_f 0.84 (6:3:1 1-propanol:ammonium hydroxide:water). Iodomethyl pivalate (2.4 g, 10.0 mmol) was added to a mixture of the above intermediate (0.685 g, 1.0 mmol), silver carbonate (0.552 g, 2.0 mmol), and dimethyl methylphosphonate (1.1 mL, 10.0 mmol) in DMF (10 mL), and the mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of 10% aqueous lithium chloride (50 mL) and extracted with methylene chloride (4 × 50 mL). The combined organic extracts were washed sequentially with 10% aqueous lithium chloride (3 × 75 mL) and aqueous $NaHSO_4$ (2 × 150 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (eluting with 1:1 to 3:1 acetone/hexane) to afford the methyl phosphinyl pivaloyl carboxyl diester intermediate (0.632 g, 79%, TLC R_f = 0.76 (3:1 acetone/hexane). Mass spectrum $(M + H)^+$: 800. IR (CH_2Cl_2 film): 1757 cm^{-1}. ^1H NMR ($CDCl_3$): 0.86–0.94 (m, 12 H), 1.20 (s, 9 H), 1.59 (s, 6 H), 1.68 (s, 3 H), 1.68 (s, 3 H), 2.06–2.19 (m, 14 H), 2.06 (s, 3 H), 2.65 (m, 6 H), 3.68 (dd, 3 H, J = 10.6, 11.1), 4.45 (m, 1 H), 4.61 (m, 2 H), 5.08–5.18 (m, 3 H), 5.71 (d, 1H, J = 5.3), 5.85 (d, 1 H, J = 5.6), ^{13}C NMR ($CDCl_3$): 15.15, 15.92, 16.30, 17.59, 17.91, 18.00 18.43, 18.51, 19.03, 19.12, 22.58 (d, J = 90), 25.60, 26.37, 26.64, 26.75, 27.67, 29.20, 29.80, 30.72, 31.67, 38.61, 39.62, 39.68, 51.28, 51.37, 53.79, 57.04, 58.83, 69.40, 79.76, 112.20, 123.54, 124.20, 131.20, 135.34, 140.70, 170.50, 171.22, 171.31, 171.51, 210.70. Anal. ($C_{40}H_{70}N_3O_9PS$) C, H, N.

Bis(trimethylsilyl)trifluoroacetamide (1.93 mL, 7.26 mmol) was added to a solution of the above mixed diester intermediate (0.29 g, 0.363 mmol) in methylene chloride (6 mL), and the resulting mixture was stirred for 0.5 h. Trimethylsilyl bromide (0.115 ml, 0.871 mmol) was then added, and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated *in vacuo*, the residue dissolved in methanol, (5 mL), and the mixture concentrated again *in vacuo*. The residue was purified by successive triturations with diethyl ether (3 × 20 mL) and petroleum ether (4 × 20 mL), and the resulting solid was vacuum dried to afford **19** (0.17 g, 62%), mp 165–171 °C. TLC: R_f = 0.73 (6:3:1 1-propanol/ammonium hydroxide/water, visualization by PMA). Mass spectrum $(M + H)^+$: 786. IR (KBr): 1638, 1757 cm^{-1}. $[\alpha]_D$ = −47.9° (c = 0.19, MeOH). ^1H NMR (CD_3OD): 0.83–1.13 (m, 12 H), 1.18–1.28 (s, 9 H), 1.59–1.77 (m, 12 H), 1.97–2.28 (m, 14 H), 2.06 (s, 3H), 2.51–2.75 (m, 6 H), 4.17–4.31 (m, 2 H), 4.63–4.75 (m, 1 H), 5.15–5.23 (m, 3 H), 5.72 (d, 1 H, J = 5.2), 5.83 (d, 1 H, J = 5.6). Anal. ($C_{39}H_{68}N_3PS \cdot 0.22H_2O$) C, H, N.

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(12) Attempts at direct, one-step base hydrolysis of diester **11** resulted in a mixture of products, arising from competing cleavage of both the methyl group and the linker moiety attached on the phosphorous. The phosphonic monoacid **17** arising from monodealkylation of **11** is resistant to further base-catalyzed hydrolysis, thereby solving the problem of the cleavage of the linker group during the base hydrolysis of the carboxylic methyl ester group of **17** in the second step.

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