Synthesis of Protein Farnesyltransferase and Protein Geranylgeranyltransferase Inhibitors: Rapid Access to Chaetomellic Acid A and Its Analogues

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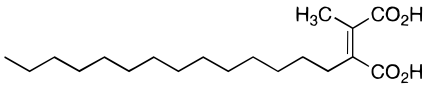
A facile two-step stereospecific synthesis of the protein farnesyltransferase inhibitor chaetomellic acid A (**1**) and its analogues was developed. Addition of organocuprates derived from Grignard reagents (e.g. tetradecylmagnesium chloride and $CuBr{\cdot}Me_2S$) to dimethyl acetylenedicarboxylate (DMAD) in tetrahydrofuran containing hexamethylphosphoramide was followed by capture of the resulting copper enolates with a variety of electrophiles (e.g. methyl iodide) to give dimethyl *cis*-butenedioate derivatives **4**−**11**. Hydrolysis with lithium hydroxide generated the corresponding lithium carboxylates, which readily closed to 2,3-disubstituted maleic anhydrides **17**−**20** upon acid treatment. Compound **16**, an analogue wherein the tetradecyl group of **1** is replaced by a farnesyl moiety, is 7-fold more potent than **1** as an inhibitor of protein farnesyltransferase from yeast and displays a 100:1 selectivity for this enzyme relative to yeast protein geranylgeranyltransferase. In contrast, analogue **15**, which contains a geranylgeranyl side chain, shows ca. 10:1 selectivity for the latter enzyme.

Introduction

Isoprenylation is a critically important post-translational modification that can control protein location and cellular activation.[1] For example, addition of the C_{15} farnesyl unit to Ras by protein farnesyltransferase[2] (PFTase) is essential for its association with cell membranes and promotion of cell-transforming activity.[3] Mutated ras genes are found in about 25% of human tumors and are believed to play an important role in human tumor growth.[4] Recently PFTase inhibitors were found to reduce the number of tumorigenic phenotypes of cells transformed by ras in both cell culture and animal models.[5] Chaetomellic acid A (**1**) is one of several



1 chaetomellic acid

dicarboxylic acid-containing natural products identified as potent inhibitors of Ras PFTase.[6−8] It is a nanomolar competitive inhibitor of farnesyl pyrophosphate binding to mammalian PFTase, presumably because the diacid moiety acts as a stable pyrophosphate mimic.[6,9] It has previously been made by two approaches. The first reported synthesis of **1** uses methyl palmitate and methyl pyruvate in a nonstereospecific aldol/elimination sequence, requiring separation of diastereoisomers, to provide the corresponding anhydride in 18% overall yield.[10] The second, more efficient, approach (three steps, 64% overall yield) relies on a cobalt-mediated radical coupling strategy.[11] We now report a convenient two-

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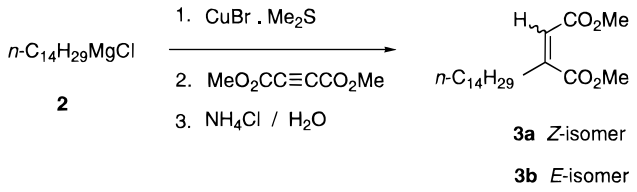

Figure 1. Addition of **2** to DMAD. See Table 1.

step stereospecific preparation of chaetomellic acid (**1**) and analogues, including **16**, which is 7-fold more potent than **1** as an inhibitor of yeast PFTase, using tandem vicinal difunctionalization[12] of dimethyl acetylenedicarboxylate (DMAD). Selective inhibitors of protein geranylgeranyltransferase-I (PGGTase-I) from yeast are also described.

Results and Discussion

Syntheses. The conjugate addition of organocuprates (obtained by treatment of Grignard and organolithium reagents with copper(I) salts) to alkynes is a widely used synthetic method.[13,14] However, α-functionalization (*via* enolate trapping) of acetylenes having electron-withdrawing substituents,[15] such as acetylene dicarboxylates,[16] has limitations imposed by the instability of copper intermediates at the higher temperatures necessary for certain α-functionalizations. For example, methyl propynoate is reported to undergo efficient conjugate addition, but attempts to alkylate the vinyl copper intermediate were often unsuccessful.[17] Initial experiments using an aqueous quench show that solvent, temperature, and reaction time influence the stereochemical outcome of the conjugate addition of Grignard-derived tetradecyl organocuprate **2** (Figure 1) to dimethyl acetylenedicarboxylate (Table 1). Reaction in THF at −78 °C for a short period (45 min) gives exclusively *cis*-addition[18] of the tetradecyl moiety and proton to generate the *Z*-diester **3a**. However, prolonged reaction time (3 h), higher temperature (−40 °C), or use of ether as a solvent[19] all lead to rapid deterioration of stereoselectivity. Addition of dimethyl sulfide as a cosolvent to ether (1:2)[20] allows some recovery of the ratio.

Table 1. Conjugate Addition of 2 to Dimethyl Acetylenedicarboxylate

	conditions			products	
entry	solvent	*T* (°C)	time[a] (h)	*Z*:*E* ratio (**3a**:**3b**)[b]	yield[c] (%)
1	THF	−78	0.45	100:0	85
2	THF	−78	3	95:5	83
3	THF	−40	3	88:12	75
4	ether	−78	1	78:22	78
5	ether	−40	1	61:39	78
6	ether−Me_2S	−40	1	85:15	60

[a] Time between addition of DMAD and quenching of reaction. [b] Determined by ^{1}H NMR. [c] Isolated yield.

Attempts to generate tetra-substituted olefins by capture of the copper enolate in THF at −78 °C with reactive methylating agents such as MeI, $(Me)_3O^+{}^-BF_4$ and MeOTf failed and gave only the *Z*-diester **3a** upon workup. At temperatures higher than −40 °C, small amounts of methylated products form along with **3a** and the isomerized product **3b**. Fortunately complexation with HMPA in THF is highly effective[21] at stabilizing the enolate adduct resulting from conjugate addition and greatly retards its isomerization or thermal decomposition, even at 20 °C. Thus, Michael addition of the organocopper reagent **2** to DMAD in the presence of HMPA, followed by capture of the resulting enolate with methyl iodide, generates chaetomellic acid A methyl ester **4** (Figure 2) in 78% yield (Table 2). Careful hydrolysis with lithium hydroxide affords chaetomellic acid A (**1**) in quantitative yield. This is the simplest and most efficient approach to this compound reported thus far.

A variety of other electrophiles also capture such copper enolates effectively. These include allylic halides (farnesyl or geranylgeranyl bromide), acylating agents (tetradecanoyl chloride), *N*-bromosuccinimide, and trimethyltin chloride to give **5**−**11** (Table 2). Reaction yields depend on the quality of the cuprous bromide−dimethyl sulfide complex, CuBr·Me_2S. Use of freshly prepared reagent[22] or recrystallization[23] of commercially available complex is usually essential for satisfactory results. Some diene **12** forms during the preparation of **10** and **11**, presumably because of thermal decomposition of the vinyl copper intermediate (R = Me) at temperatures above −40 °C. This type of dimerization also occurs to give diene **13** during attempted alkylation of DMAD by a dialkylcopper reagent, (*n*-butyl)$_2$CuLi. Symmetrical dienes of type **12** and **13** are known to be generated *via* thermal decomposition[16a] or oxidation[24] of vinylcopper

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(18) NOE experiments on **3a** and the corresponding *E*-isomer **3b** confirm the stereochemical assignment. In the case of **3a,** irradiation of the vinylic hydrogen showed 2% enhancment of the allylic hydrogens' signal while irradiation of the allylic hydrogens resulted in signal enhancment of the vinylic hydrogen by 16%. No such NOE was observed in the case of the *E*-isomer, **3b**.

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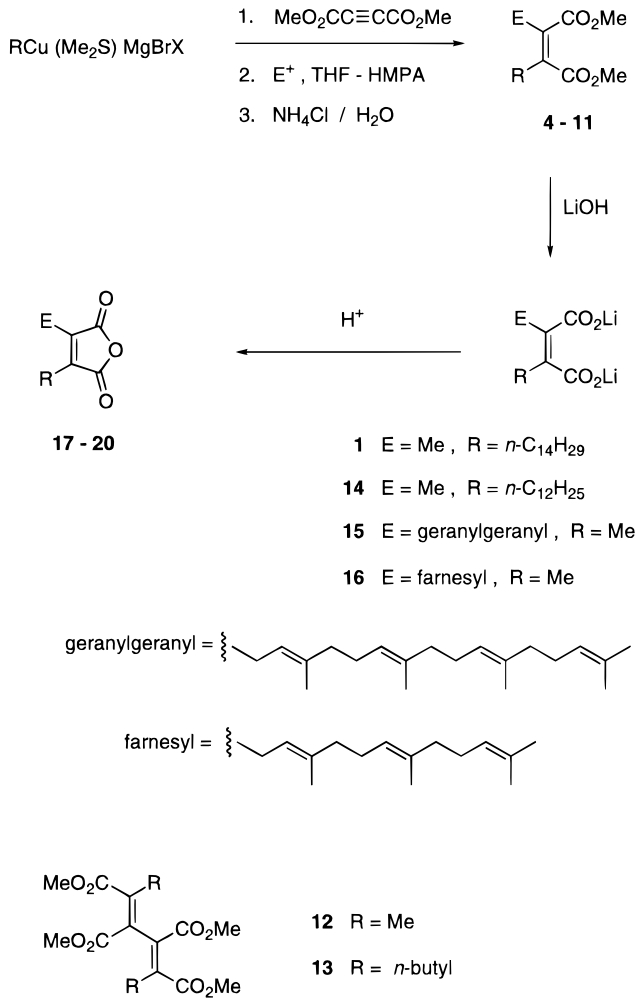

Figure 2. Tandem addition to DMAD. See Table 2.

Table 2. Tandem Addition to Dimethyl Acetylenedicarboxylate[a]

entry	R	E^+X^-	product	yield (%)[b]
1	n-$C_{14}H_{29}$	MeI	**4**	78
2	n-$C_{12}H_{25}$	MeI	**5**	77
3	Me	geranylgeranyl-Br	**6**	80
4	Me	farnesyl-Br	**7**	81
5	Me	$C_{13}H_{27}COCl$	**8**	83
6	n-$C_{14}H_{29}$	NBS[c]	**9**	67
7	Me	NBS[c]	**10**	75
8	Me	Me_3SnCl	**11**	49

[a] See Figure 2 for structures. [b] Isolated yields. [c] *N*-Bromosuccinimide; E^+ = Br.

reagents. Hydrolysis of **5**−**7** under conditions similar to those used for **4** generates the corresponding lithium salts **14**−**16**, which cyclize rapidly to the corresponding anhydrides **18**−**20** upon exposure to acidic conditions, as has previously been observed for chaetomellic acid (**1**) (i.e. formation of **17**).

The tin adduct **11** can also be coupled to farnesyl bromide under modified Stille conditions[25] using Pd/Cu in 85% yield, thereby establishing yet another route to the potent inhibitor **16**. Presumably this approach could also be extended to a variety of vinyl halides to generate further analogues of **1** for biological testing. In short, this very simple approach affords rapid, stereochemically-controlled access to a host of dicarboxylic acid analogues which can mimic pyrophosphates in biological systems.

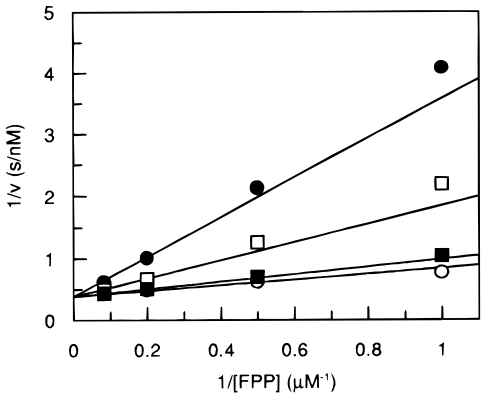

Figure 3. Inhibition of PFTase with analog **16**. Double-reciprocal plot with FPP as the varied substrate at fixed concentrations of **16**. Concentrations of analog **16** were 0.5 (○), 1 (■), 4 (□), and 10 (●) μM. FPP was present at concentrations of 1−12 μM. Dansyl-Gly-Cys-Val-Ile-Ala was held constant at 2.4 μM. PFTase (1.1 nM) was used to initiate the reactions.

Table 3. Inhibition of Protein Prenyltransferases from Yeast with Chaetomellic Acid (1) and Analogues 14−16

	IC_{50} (μM)	
inhibitor	PFTase	PGGTase I
1	17 ± 3	>300
14	4 ± 0.1	112 ± 3
15	96 ± 16	11.5 ± 0.6
16	2.4 ± 0.08	277 ± 21

Enzyme Inhibition. Chaetomellic acid (**1**) and its analogs **14**−**16** were evaluated for inhibition of yeast protein farnesyltransferase (PFTase) and yeast protein geranylgeranyl-transferase (PGGTase-I) using the continuous fluorescence assay of Pompliano *et al.*[26] The results are summarized in Table 3. Chaetomellic acid (**1**) inhibited PFTase with an IC_{50} of 17 μM but did not inhibit PGGTase-I at all (>300 μM). Compound **16**, containing a farnesyl side chain, was the most potent inhibitor of PFTase and exhibited a good selectivity for PFTase over PGGTase-I (100:1). In contrast, analog **15**, containing a geranylgeranyl side chain, was a fairly good inhibitor of PGGTase-I (IC_{50} = 11.5 μM), although the level of selectivity for PGGTase-I over PFTase was lower (~10:1).

Compound **16** was shown to be a competitive inhibitor of PFTase (Figure 3) against FPP with a $K_I = 1.1 \pm 0.1$ μM. This pattern of inhibition is similar to that found for chaetomellic acid when tested with PFTase from bovine brain.[2c] It is interesting to note the difference in potency of chaetomellic acid as an inhibitor of PFTase from bovine brain and from yeast. Chaetomellic acid has an IC_{50} of 55 nM with the enzyme from bovine brain and an IC_{50} of 17 μM with the yeast enzyme. This difference parallels the difference in the K_D values for FPP by the two enzymes. FPP binds much tighter to PFTase from bovine brain (K_D = 12 nM)[27] than to the yeast enzyme (K_D = 75 nM).[28] An inhibitor which competes with FPP for binding might well be expected to reflect this differ-

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ence. For example, α-(hydroxyfarnesyl)phosphonic acid, another competitive inhibitor with respect to FPP, has a reported IC_{50} of 30 nM with PFTase from bovine brain,[27] but a much higher IC_{50} (290 nM) with the yeast enzyme (unpublished results).

The present study offers rapid synthetic access to protein prenyl transferase inhibitors and demonstrates that modification of the side chain can greatly assist both enhanced potency and control of selectivity for specific enzymes of this type. Additional investigations on design of improved inhibitors are on-going.

Experimental Procedures[28,29]

Conjugate Addition to Dimethyl Acetylenedicarboxylate (DMAD). Dimethyl (*Z*)-2-Tetradecylbutenedioate (3a) and Its (*E*)-Isomer 3b. Tetradecylmagnesium chloride (1.20 mL of 1.00 M solution in THF, 1.2 mmol) is added dropwise to a suspension of cuprous bromide−dimethyl sulfide complex ($CuBr·Me_2S$, 0.25 g, 1.20 mmol) in THF (6 mL) at −40 °C. The resulting yellow suspension is stirred at −40 °C for 2 h and then cooled to −78 °C, and freshly distilled DMAD (0.14 g, 1.00 mmol) in THF (2 mL) is added dropwise to give a dark red-brown mixture. After 1 h, the reaction mixture is quenched with saturated aqueous NH_4Cl solution (2 mL, adjusted to pH 8 with 10% ammonia) and allowed to warm to room temperature. After 30 min, the mixture is partitioned between ether and water. The aqueous layer is extracted with ether (3 × 5 mL), and the combined organic extracts are washed with additional saturated aqueous NH_4Cl solution (20 mL), and brine (20 mL). Drying over Na_2SO_4 and concentration *in vacuo* gives 332 mg of crude product. Purification by flash column chromatography (SiO_2, petroleum ether/ether 9:1) gives **3a** (289 mg, 85%) as a white solid: mp 50.5−51.0 °C; IR ($CDCl_3$) 2916, 2849, 1729, 1717 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.81 (t, 1H, J = 1.4 Hz), 3.83 (s, 3H), 3.72 (s, 3H), 2.35 (dt, 2H, J = 1.4, 8.3 Hz), 1.5 (quintet, 2H), 1.3 (br s, 22H), 0.88 (t, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4 (s), 165.4 (s), 151.1 (s), 119.0 (d), 52.3 (q), 51.8 (q), 34.4 (t), 31.9 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 28.9 (t), 26.9 (t), 22.7 (t), 14.1 (q); HRMS (EI) calcd for $C_{20}H_{36}O_4$ 340.2614, found 340.2614. Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.55; H, 10.66. Found: C, 70.63; H, 10.71.

The (*E*)-isomer **3b** had the following spectroscopic data: IR ($CHCl_3$) 2924, 2853, 1727, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.72 (s, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 2.78 (dt, not resolved, 2H, J = 7.5 Hz), 1.45 (quintet, 2H), 1.25 (br s, 22H), 0.88 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (s), 148.6 (s), 52.5 (q), 51.7 (q), 31.9 (t), 29.7 (t), 29.6 (t), 29.4 (t), 29.3 (t), 28.0 (t), 22.7 (t), 14.1 (q); HRMS (EI) calcd for $C_{20}H_{36}O_4$ 340.2614, found 340.2617. Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.55; H, 10.66. Found: C, 70.80; H, 10.68.

General Procedure for Conjugate Addition−Enolate Capture. Chaetomellic Acid A Dimethyl Ester (4). The procedure for **3a** was followed with the following modifications: after addition of DMAD at −78 °C, the reaction mixture was stirred for 40 min, then HMPA−THF solution (1:1, 2 mL) was added, which resulted in the heterogeneous mixture becoming nearly homogeneous. Subsequently, the electrophile, MeI (2.5 mmol, 0.36 g, 0.16 mL) in THF (2 mL), was added and stirring was continued for 5 min at −78 °C. After warming the mixture to room temperature overnight, saturated aqueous NH_4Cl (2 mL, adjusted to pH 8 with 10% ammonia) was added to the yellow reaction mixture at −20 °C. The mixture was stirred at 20 °C for 30 min and then partitioned between ether and water. The aqueous layer was extracted with ether (3 × 10 mL), and the combined organic extracts were successively washed with additional aqueous NH_4Cl (20 mL), water (2 × 20 mL) and brine (20 mL). Drying (Na_2SO_4) and concentration *in vacuo* gave 365 mg of a yellow oil. Purification by flash column chromatography (SiO_2; petroleum ether/ether 8:2) gave **4**[10] (277 mg, 78%) as a colorless oil: IR ($CHCl_3$) 2924, 2853, 1725, 1644, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.74 (s, 3H), 3.73 (s, 3H), 2.31 (t, 3H, J = 7.5 Hz), 1.93 (s, 3H), 1.42 (quintet, 2H), 1.24 (br s, 22H), 0.86 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.6 (s), 169.1 (s), 139.7 (s), 131.5 (s), 52.1 (q), 52.0 (q), 31.9 (t), 30.1 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 27.7 (t), 22.6 (t), 14.9 (q), 14.0 (q); HRMS (EI) calcd for $C_{21}H_{38}O_4$ 354.2770, found 354.2763. Anal. Calcd for $C_{21}H_{38}O_4$: C, 71.15; H, 10.80. Found: C, 71.13; H, 10.77.

Depending on the conditions, a small amount of the (*E*)-isomer of **4** could also be isolated: IR ($CDCl_3$) 2925, 2854, 1726, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.78 (s, 3H), 3.77 (s, 3H), 2.43 (dt, 2H, J = 0.8, 7.6 Hz), 1.99 (s, 3H), 1.40 (quintet, 2H), 1.25 (br s, 22H), 0.87 (t, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5 (s), 169.3 (s), 139.1 (s), 131.9 (s), 51.8 (q), 51.7 (q), 31.9 (t), 31.4 (t), 29.7 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 22.7 (t), 17.6 (q) 14.1 (q); HRMS (EI) calcd for $C_{21}H_{38}O_4$ 354.2770, found 354.2768. Anal. Calcd for $C_{21}H_{38}O_4$: C, 71.15; H, 10.80. Found: C, 71.28; H, 10.76.

Dimethyl (*Z*)-2-Dodecyl-3-methylbutenedioate (5). The general procedure for **4** was used with dodecylmagnesium bromide (1.20 mL of 1.00 M in ether, 1.20 mmol) as the Grignard reagent to give **5** (250 mg, 77%), which was obtained as an oil: IR ($CHCl_3$) 2925, 2854, 1725, 1643, 1459, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.73 (s, 3H), 3.71 (s, 3H), 2.30 (t, 3H, J = 7.3 Hz), 1.91 (s, 3H), 1.38 (quintet, 2H), 1.23 (br s, 18H), 0.85 (t, 3H, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.6 (s), 169.0 (s), 139.7 (s), 131.5 (s), 52.1 (q), 52.0 (q), 31.9 (t), 30.1(t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 27.7 (t), 14.9 (q), 14.0 (q); HRMS (EI) calcd for $C_{19}H_{34}O_4$ 326.2457, found 326.2457. Anal. Calcd for $C_{19}H_{34}O_4$: C, 69.90; H, 10.50. Found: C, 70.47; H, 10.83.

Dimethyl (*Z*)-2-(Geranylgeranyl)-3-methylbutenedioate (6). The general procedure for **4** was employed using $CuBr·Me_2S$ (0.30 g, 1.46 mmol), methyl magnesium bromide (0.49 mL of 3.00 M in ether, 1.46 mmol), DMAD (0.20 g, 1.40 mmol), and freshly prepared[30] geranylgeranyl bromide (0.97 g, 2.91 mmol) to give **6** (481 mg, 80%) as a colorless oil: IR ($CHCl_3$) 2948, 2921, 1725, 1642, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 5.08 (m, 4H), 3.74 (s, 3H), 3.73 (s, 3H), 3.05 (d, 2H, J = 7.1 Hz), 2.07−1.96 (m, 12H), 1.95 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.2 (s), 169.0 (s), 138.1 (s), 138.0 (s), 135.2 (s), 134.8 (s), 132.0 (s), 131.1 (s), 124.4 (d), 124.2 (d), 123.9 (d), 118.4 (d), 52.1 (q), 52.0 (q), 39.7 (t), 28.9 (t), 26.7 (t), 26.6 (t), 26.5 (t), 25.6 (q), 17.6 (q), 16.1 (q), 15.9 (q), 15.1 (q); HRMS (EI) calcd for $C_{27}H_{42}O_4$ 430.3083, found 430.3069. Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.53; H, 10.07.

Depending on the conditions, a small amount of the (*E*)-isomer of **6** (32 mg, 5%) was obtained as an oil: IR ($CHCl_3$, cast) 2946, 2919, 1726 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.09 (m, 4H), 3.77 (s, 3H), 3.74 (s, 3H), 3.20 (d, 2H, J = 7.2 Hz), 2.06−1.94 (m, 12H), 2.01 (s, 3H), 1.67 (s, 3H), 1.61 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.3 (s), 169.0 (s), 138.0 (s),137.7 (s), 135.1 (s), 134.9 (s), 131.7 (s), 131.2 (s), 124.4 (d), 124.2 (d), 124.0 (d), 119.6 (d), 51.9 (q), 51.7 (q), 39.7 (t), 30.2 (t), 26.7 (t), 26.6 (t), 23.4 (q), 17.6 (q), 17.5 (q), 16.0 (q), 15.9 (q); HRMS (EI) calcd for $C_{27}H_{42}O_4$ 430.3083, found 430.3071. Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.63; H, 10.20.

Dimethyl (*Z*)-2-Farnesyl-3-methylbutenedioate (7). The general procedure for **4** was employed using $CuBr·Me_2S$ (0.41 g, 2.00 mmol), methyl magnesium bromide (0.67 mL of 3.00 M in ether, 2.00 mmol), DMAD (0.27 g, 1.90 mmol), and farnesyl bromide (1.14 g, 4.00 mmol) to give **7** (560 mg, 81%) as an oil: IR (neat) 2949, 2920, 1725, 1643, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.1 (m, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 3.03 (d, 2H, J = 7.1 Hz), 2.0 (m, 8H), 1.93 (s, 3H), 1.65 (s, 3H), 1.56 (s, 6H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 169.2 (s), 169.0 (s), 138.2 (s), 138.1 (s), 135.1 (s), 132.0 (s), 131.2 (s), 124.1 (d),

(29) General procedures and instrumentation have recently been published; see: Witter, D. A.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 2613−2623.

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123.8 (d), 118.2 (d), 52.1 (q), 52.0 (q), 39.6 (t), 28.9 (t), 26.5 (t), 26.3 (t), 25.7 (t), 17.6 (q), 16.1 (q), 16.0 (q), 15.1 (q); HRMS (EI) calcd for $C_{22}H_{34}O_4$ 362.2457, found 362.2449. Anal. Calcd for $C_{22}H_{34}O_4$: C, 72.98; H, 9.45. Found: C, 72.92; H, 9.31.

Palladium-Mediated Cross-Coupling Between 11 and Farnesyl Bromide To Give 7. To a degassed solution of farnesyl bromide (57 mg, 0.20 mmol) in dry DMF (1 mL) were sequentially added **11** (64 mg, 0.20 mmol) and $Pd(Ph_3)_4$ (23 mg, 0.020 mmol). Copper(I) iodide (29 mg, 0.15 mmol) was then added in one portion and the resulting yellow mixture was stirred under Ar at 20 °C for 12 h. The mixture was then diluted with ether (15 mL) and filtered over Celite. The filterate was stirred for 30 min with a large excess of a saturated aqueous NH_4Cl solution, and the resultant organic phase was seperated and concentrated *in vacuo*. The residue was diluted with ether (15 mL), stirred for 1 h with an excess of a 50% aqueous KF solution, and filtered over Celite, and the filterate was extracted with ether (5 × 25 mL). The organic extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give an orange oil. Purification by flash chromatography (SiO_2, petrolum ether/ether 8.5:1.5) gave **7** (62 mg, 85%) as a colorless oil.

Dimethyl (*Z*)-3-Methyl-2-(1-oxotetradecyl)butenedioate (8). The general procedure for **4** was employed using $CuBr{\cdot}Me_2S$ (0.30 g, 1.46 mmol), methylmagnesium bromide (0.49 mL of 3.00 M in ether, 1.46 mmol), DMAD (0.20 g, 1.40 mmol), and myristoyl chloride (0.72 g, 2.92 mmol) to give **8** (427 mg, 83%) as a wax: IR ($CHCl_3$) 2924, 2853, 1736, 1705, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.80 (s, 3H), 3.77 (s, 3H), 2.59 (t, 2H, J = 7.3Hz), 2.01 (s, 3H), 1.60 (m, 2H), 1.42 (br s, 20H), 0.87 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.2 (s), 168.6 (s), 164.6 (s), 141.5 (s), 135.7 (s), 52.6 (q), 52.5 (q), 43.0 (t), 31.9 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.0 (t), 23.3 (t), 22.6 (t), 17.0 (q), 14.1 (q); HRMS (EI) calcd for $C_{21}H_{36}O_5$ 368.2563, found 368.2564. Anal. Calcd for $C_{21}H_{36}O_5$:C, 68.45; H, 9.85 Found: C, 68.34; H, 9.95.

Dimethyl 2-Bromo-3-tetradecylbutenedioate (9). The general procedure for **4** was employed except that after the addition of the electrophile, *N*-bromosuccinimide (0.39 g, 2.19 mmol), the mixture was allowed to warm to −40 °C and stirred for 1 h before quenching. Workup and purification in the usual way gave **9** (280 mg, 67%) as an oil: IR ($CHCl_3$) 1739, 1616 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.80 (s, 3H), 3.77 (s, 3H), 2.51 (dt, not resolved, 2H, J = 7.5 Hz), 1.48 (quintet, 2H), 1.24 (br s, 22H), 0.86 (t, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.8 (s), 163.9 (s), 143.7 (s), 119.1 (s), 53.4 (q), 52.7 (q), 34.2 (t), 31.9 (t), 29.7 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 26.8 (t), 22.7 (t), 14.1 (q); HRMS (EI) calcd for $C_{20}H_{35}{}^{81}BrO_4$ 420.1698, found 420.1688; calcd for $C_{20}H_{35}{}^{79}BrO_4$ 418.1719, found 418.1712. Anal. Calcd for $C_{20}H_{35}BrO_4$: C, 57.28; H, 8.41. Found: C, 57.51; H, 8.56.

Dimethyl 2-Bromo-3-methylbutenedioate (10) and Dimethyl 3,4-Dicarbomethoxy-2,3-dimethyl-2,4-hexadiene-1,6-dioate (12). The general procedure for **4** was employed using $CuBr{\cdot}Me_2S$ (0.84 g, 4.10 mmol), methylmagnesium bromide (1.37 mL of 3.00 M in ether, 4.10 mmol), DMAD (0.57 g, 4.00 mmol), and NBS (1.42 g, 8.00 mmol) to give **10** (711 mg, 75%) as an oil: IR ($CHCl_3$) 2954, 1739, 1622, 1435 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.74 (s, 3H), 3.69 (s, 3H), 2.05 (s, 3H); MS (CI) m/z 254 $(M + NH_4)^+$. Anal. Calcd for $C_7H_9BrO_4$: C, 35.47; H, 3.38. Found: C, 35.63; H, 3.51.

Trituration of the crude product (before chromatography) with ether−petrolum ether (4:6) gave diene **12** (31 mg, 5%) as a white crystalline solid: mp 88−89 °C; IR ($CHCl_3$) 1725, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.83 (s, 3H), 3.73 (s, 3H), 1.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4 (s), 165.1 (s), 143.5 (s) 127.1 (s), 52.6 (q), 52.5 (q), 17.8 (q); HRMS (EI) calcd for $C_{14}H_{18}O_8$ 314.1002, found 314.0996. Anal. Calcd for $C_{14}H_{18}O_8$: C, 53.50; H, 5.77. Found: C, 53.28; H, 5.57.

Dimethyl 3-Methyl-2-(trimethyltin)butenedioate (11). The general procedure for **4** was employed using $CuBr{\cdot}Me_2S$ (0.82 g, 4.00 mmol), methylmagnesium bromide (1.35 mL of 3.0 M solution in ether), DMAD (0.50 g, 3.50 mmol), and trimethyltin chloride (8.00 mL of 1.00 M solution in THF, 8.00 mmol). After the addition of trimethyltin chloride, the reaction mixture was warmed to −40 °C and stirred for 4 h. Isolation in the usual way gave **11** as a colorless liquid: IR ($CHCl_3$) 1713, 1613, 1433 cm^{-1}; ^1H NMR (CD_2Cl_2, 360 MHz) δ 3.68 (s, 3H), 3.67 (s, 3H), 2.01 (s, J_{Sn-H} = 4.3 Hz, 3H), 0.30 (s, J_{Sn-H} = 27.3 Hz, 9H); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 172.6 (s), 166.5 (s), 149.7 (s), 139.1 (s), 52.3 (q), 51.6 (q), 20.8 (q), −8.0 (q); MS (POSFAB) m/z 349 $(M + Na)^+$.

Dimethyl 2,3-Dibutyl-3,4-dicarbomethoxy-2,4-hexadiene-1,6-dioate (13). The procedure for **3a** was followed using $CuBr{\cdot}Me_2S$ (0.21 g, 1.00 mmol), *n*-BuLi (1.25 mL of 1.6 M in hexane, 2.00 mmol), and DMAD (0.11 g, 0.80 mmol). After isolation as before, **13** (121 mg, 38%)[16b] was obtained as a colorless oil: IR (CH_2Cl_2) 2934, 1728, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 3.80 (s, 3H), 3.70 (s, 6H), 2.3−2.2 (m, 4H), 1.40−1.23 (m, 8H), 0.85 (t, 6H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 169.2 (s), 165.2 (s), 148.8 (s), 125.8 (s), 31.9, 28.8, 22.5, 13.7 (CH_3); HRMS (EI) calcd for $C_{20}H_{30}O_8$ 398.1941, found 398.1935.

General Procedure for Basic Hydrolysis of Esters 4−11 to Lithium Salts 1 and 14−16 and Formation of Anhydrides 17−20. To the ester (50 mg) in THF−H_2O (2 mL, 1:1 in case of **4** and **5**, or 2:1 in case of **6** and **7**) was added 1.0 N LiOH (2 equiv) and the mixture was stirred at room temperature until the starting material was consumed as indicated by TLC. The solvent was removed in *vacuo* and the remaining solid was dissolved in H_2O (3 mL). Freeze-drying of this solution gives a white fluffy solid of the respective lithium salts **1** and **14**−**16**. Alternatively, acidification of the aqueous solution with 1.0 N HCl at 0 °C and extraction with ether give corresponding anhydrides **17**−**20**.

Lithium salt of chaetomellic acid A (1): 99% yield; IR (KBr) 3440, 2921, 2851, 1555, 1438 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 2.24 (dt, not resolved, 2H, J = 7.8 Hz), 1.83 (s, 3H), 1.47 (m, 2H), 1.28, br s, 22H), 0.89 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.2 (s), 179.9 (s), 139.6 (s), 132.8 (s), 33.1 (t), 31.6 (t), 31.1 (t), 30.5 (t), 30.4 (t), 30.3 (t), 29.5 (t), 23.7 (t), 16.3 (q), 16.2(q); MS (POSFAB) m/z 339 (MH^+). Anal. Calcd for $C_{19}H_{32}Li_2O_4-H_2O$: C, 64.04; H, 9.62. Found: C, 63.65; H, 9.36.

(*Z*)-2-Dodecyl-3-methylbutenedioic acid, dilithium salt (14): 99% yield; IR (KBr) 2923, 2852, 1593, 1578, 1542, 1433 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 2.24 (t, 2H, J = 7.6 Hz), 1.83 (s, 3H), 1.47 (m, 2H), 1.28 (br s, 18H), 0.89 (t, 3H, J = 6.6 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 182.7 (s), 182.2 (s), 141.4 (s), 134.3 (s), 34.4(t), 32.6 (t), 32.2 (t), 32.1 (t), 32.0 (t), 31.9 (t), 31.8 (t), 30.7 (t), 25.2 (t), 17.8 (q), 16.4 (q); MS (POSFAB) m/z 311 (MH^+).

(*Z*)-2-(Geranylgeranyl)-3-methylbutenedioic acid, dilithium salt (15): 85% yield; IR (CH_3OH) 2912, 1590, 1540, 1438, 1385 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.23 (dt, 1H, J = 1.0, 6.8 Hz), 5.08 (m, 3H), 3.00 (d, 2H, J = 6.7 Hz), 2.12−1.92 (m, 12H), 1.84 (s, 3H), 1.67 (s, 3H), 1.66 (s, 3H), 1.58 (s, 9H); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.0 (s), 178.9 (s), 137.5 (s), 135.9 (s), 135.8 (s), 134.5 (s), 132.1 (s), 125.7 (d), 125.5 (d), 123.4 (d), 41.0 (t), 40.9 (t), 40.8 (t), 30.4 (t), 27.8 (t), 27.6 (t), 25.9 (q), 17.8 (q), 16.6 (q), 16.4 (q), 16.1 (q); MS (CI) m/z 432 $(M + NH_4)^+$.

(*Z*)-2-Farnesyl-3-methylbutenedioic acid, dilithium salt (16): 99% yield; IR ($CHCl_3$) 2920, 1590, 1543, 1435, 1401 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 5.23 (dt, 1H, J = 1.0, 6.8 Hz), 5.08 (m, 2H), 2.99 (d, 2H, J = 6.7 Hz), 2.11−1.93 (m, 8H), 1.83 (s, 3H), 1.66 (s, 3H), 1.65 (s, 3H), 1.58 (s, 6H); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.5 (s), 179.5 (s), 137.5 (s), 136.2 (s), 135.9 (s), 134.0 (s), 132.4 (s), 125.5 (d), 125.2 (d), 123.1 (d), 40.7 (t), 40.6 (t), 30.1 (t), 27.6 (t), 27.5 (t), 25.9 (q), 17.8 (q), 16.4 (q), 16.1 (q); MS (CI) m/z 364 $(M + NH_4)^+$.

Chaetomellic anhydride (17):[6b] 99% yield; IR ($CHCl_3$) 2924, 2853, 1767 cm^{-1}; ^1H NMR (CD_3OD, 360 MHz) δ 2.46 (dt, not resolved, 2H, J = 7.5 Hz), 2.03 (s, 3H), 1.57 (quintet, 2H), 1.34−1.28 (m + br s, 22H), 0.89 (t, 3H, J = 6.6 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.8 (s), 167.6 (s), 145.4 (s), 141.9 (s), 33.1 (t), 30.8 (3 t), 30.7 (t), 30.6 (t), 30.5 (2 t), 30.4 (t), 30.3 (t), 28.5 (t), 25.1 (t), 23.7 (t), 14.4 (q), 9.3 (q); HRMS (EI) calcd for $C_{19}H_{32}O_3$ 308.2351, found 308.2355. Anal. Calcd for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 73.67; H, 10.37.

2-Dodecyl-3-methylmaleic anhydride (18): 99% yield; IR (CH_2Cl_2) 2925, 2854, 1767, 1466 cm^{-1}; ^1H NMR (CD_3OD,

360 MHz) δ 2.46 (dt, 2H, J = 0.5, 7.7 Hz), 2.03 (s, 3H), 1.57 (quintet, 2H), 1.34−1.28 (m + br s, 18H), 0.89 (t, 3H, J = 6.7 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 167.8 (s), 167.5 (s), 145.4 (s), 141.9 (s), 33.1 (t), 30.7 (3 t), 30.6 (t), 30.4 (2 t), 28.9 (t), 28.5 (t), 25.0 (t), 23.7 (t), 14.4 (q), 9.3 (q); HRMS (EI) calcd for $C_{17}H_{28}O_3$ 280.2039, found 280.2031. Anal. Calcd for $C_{17}H_{28}O_3$: C, 72.82; H, 10.06. Found: C, 72.46; H, 10.24.

2-(Geranylgeranyl)-3-methylmaleic anhydride (19): 75% yield; IR (CD_2Cl_2) 2945, 1769 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.11 (m, 4H), 3.16 (d, 2H, J = 7.3 Hz), 2.10−1.95 (m, 12H), 2.05 (s, 3H), 1.72 (s, 3H), 1.67 (s, 3H), 1.60 (s, 9H); ^{13}C NMR (CD_2Cl_2, 75 MHz) δ 166.9 (s), 166.3 (s), 143.7 (s), 140.5 (s), 140.4 (s), 135.8 (s), 135.3 (s), 131.6 (s), 124.7 (d), 124.5 (d), 124.1 (d), 116.6 (d), 40.1 (t), 40.0 (t), 39.9 (t), 27.2 (t), 27.0 (t), 26.7 (t), 25.8 (q), 23.8 (t), 17.7 (q), 16.5 (q), 16.1 (q), 9.7 (q); HRMS (EI) calcd for $C_{25}H_{36}O_3$ 384.2665, found 384.2660. Anal. Calcd for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.21; H, 9.31.

2-Farnesyl-3-methylmaleic anhydride (20): 76% yield; IR (CD_2Cl_2) 2941, 1768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 5.11 (m, 3H), 3.16 (d, 2H, J = 7.3 Hz), 2.13−1.95 (m, 8H), 2.07 (s, 3H), 1.72 (s, 3H), 1.67 (s, 3H), 1.59 (s, 6H); ^{13}C NMR (CD_2Cl_2, 100 MHz) δ 166.9 (s), 166.3 (s), 143.7 (s), 140.5 (s), 140.4 (s), 135.8 (s), 131.6 (s), 124.6 (d), 124.1 (d), 116.6 (d), 40.1 (t), 39.9 (t); 27.1 (t), 26.7 (t), 25.8 (q), 23.7 (t), 17.7 (q), 16.0 (q), 9.7 (q); HRMS (EI) calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2033. Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.94; H, 9.14.

Prenyltransferase Assays. Recombinant yeast PFTase[31] and recombinant yeast PGGTase-I[32] were produced in *Escherichia coli* and purified by immunoaffinity chromatography as previously described. Catalytic rate constants (k_{cat}) were measured using a fluorescence assay that continuously monitored farnesylation of dansylated pentapeptide[26,33] using a Spex FluoroMax model spectrofluorimeter with λ_{ex} = 340 (slit width = 5.1 nm) and λ_{em} = 486 nm (slit width = 5.1 nm) and 3 mm square cuvettes. For PFTase, assays (250 μL) were conducted at 30 °C in 50 mM TrisHCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.0. Dansyl-Gly-Cys-Val-Ile-Ala was the peptide substrate. PFTase (1.0−2.0 nM) was used to initiate the reactions. For PGGTase-I, assays (220 μL) were conducted at 30 °C in 50 mM TrisHCl, 1 mM $MgCl_2$, 11 μM $ZnCl_2$, 5 mM DTT, 0.02% (w/v) *n*-dodecyl-β-D-maltoside, pH 7.5. Dansyl-Gly-Cys-Ile-Ile-Leu was the peptide substrate. PGGTase-I (47 nM) was used to initiate the reactions. Initial rates were measured from the linear region of each run, and all measurements were made in duplicate. Rates were measured in counts/second per second and converted to units of s^{-1} using a conversion factor calculated from the slope of a line generated in a plot of concentration of synthetic dansyl-Gly-((*S*)-farnesyl)Cys-Val-Ile-Ala or dansyl-Gly-((*S*)-geranylgeranyl)Cys-Ile-Ile-Leu versus fluorescence intensity.

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Supporting Information Available: NMR spectra for compounds **1** and **14**−**16** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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