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Efficient Syntheses, Human and Yeast Farnesyl-Protein Transferase Inhibitory Activities of Chaetomellic Acids and Analogues

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Abstract—Chaetomellic acids are a class of alkyl dicarboxylic acids that were isolated from *Chaetomella acutiseta*. They are potent and highly specific farnesyl-pyrophosphate (FPP) mimic inhibitors of Ras farnesyl-protein transferase. We have previously described the first biogenetic type aldol condensation-based total synthesis of chaetomellic acid A. Modification of the later steps of that synthesis resulted in the efficient syntheses of chaetomellic acids A and B in three steps with 75–80% overall yield. In this report, details of the original total syntheses of chaetomellic acids A, B and C, the new syntheses of acids A and B and structure–activity relationship of these compounds against various prenyl transferases including human and yeast FPTase and bovine and yeast GGPTase I are described. Chaetomellic acids are differentially active against human and yeast FPTase. Chaetomellic acid A inhibited human and yeast FPTase activity with IC_{50} values of 55 nM and 225 μM, respectively. In contrast, chaetomellic acid C showed only a 10-fold differential in inhibitory activities against human versus yeast enzymes. In keeping with molecular modeling-based predictions, the compounds with shorter alkyl side chains (C-8) were completely inactive against FPTase.

Introduction

The *ras* oncogenes are mutated in about 25% of human tumors.[1] These genes encode 21kDa proteins called p21 or Ras. The normal and oncogenic activity of this protein is dependent on its ability to associate with the inner leaflet of the plasma membrane that occurs as a result of post-translational modification. Out of several post-translational steps, farnesylation of the cysteine residue of the C-terminal CaaX motif of Ras is essential and obligatory[2] and is catalyzed by the heterodimeric enzyme farnesyl–protein transferase (FPTase). Recently, numerous potent inhibitors of this enzyme have been reported from various laboratories.[3,4] These inhibitors were derived from various sources including rationally designed peptidomimetics and screening of natural products and has led to the confirmation, at least in animal models, that inhibition of FPTase is a viable target for the development of drugs for the treatment of cancer.[5,6] In fact, a number of FPTase inhibitors are in human clinical trials by several companies including Merck.

In 1993 we reported the isolation of chaetomellic acids A (**1a**) and B (**2a**; Fig. 1), as potent inhibitors of FPTase.[7,8] We also subsequently reported the first synthesis of chaetomellic acid A based on an aldol condensation approach.[9] These acids were characterized as alkyl *cis*-dicarboxylates and appear to mimic FPP (**3**; Fig. 1) at the active site of the enzyme.[7] Structurally, these acids resemble FPP as demonstrated by molecular modelling.[7] Among the chaetomellic acids, **1a** (IC_{50} = 55 nM) is 3 times more active than **2a** (IC_{50} = 185 nM) against recombinant human FPTase.[7] These classes of natural products have a propensity to cyclize and most members were isolated in the anhydride form.[7]

There have been six new reports of syntheses of chaetomellic acid A from a number of groups. These syntheses include a cobaloxime-mediated synthesis reported[10,11] by Branchaud and Slate from the University of Oregon; a succinate to maleate oxidation method reported by Kates and Schauble[12] from Villanova University; an elegant two-step synthesis by the groups of Vederas and Poulter[13] from the University of Alberta and the University of Utah involving alkyl organocuprates; three syntheses by the Argade's group[14–16] from the National Chemical Laboratory of India; and a Barton radical

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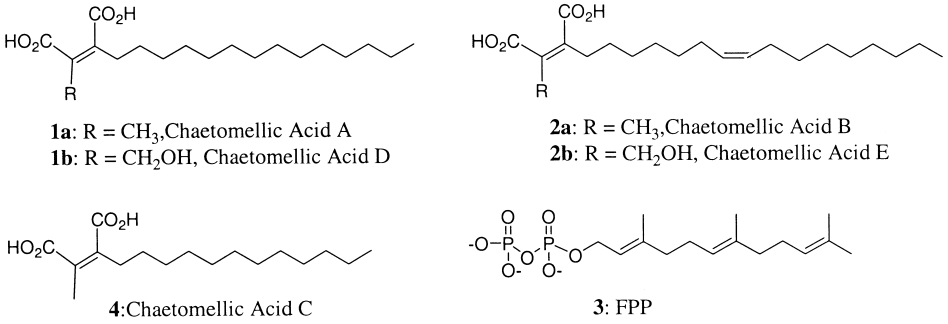


Figure 1.

decarboxylation-based synthesis reported by the Samadi group[17] from CNRS, France.

Our original synthesis was designed to generate olefinic isomers to examine the structure activity relationships. A simple modification of our initial synthesis resulted in >80% overall yield of the *cis*-dicarboxylates in three steps. In the present paper we describe the details of our original aldol condensation based total syntheses of chaetomellic acids A and B, the modified synthesis of both acids, synthesis of chaetomellic acid C, the isolation of new chaetomellic acid anhydrides D–E, and the biological evaluation of these compounds and isomers in human and yeast FPTase and GGPTase I assays.

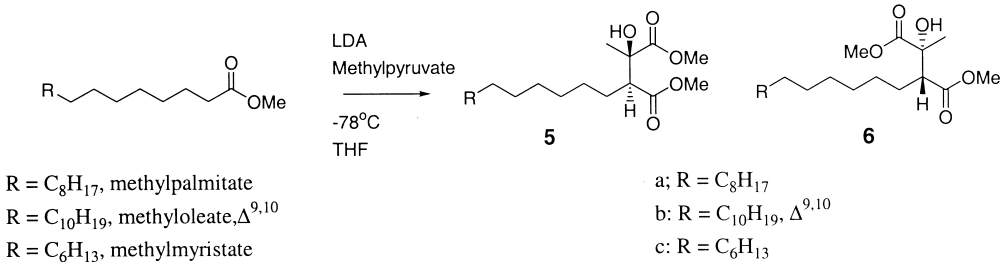
Synthesis of chaetomellic acids

The synthetic strategy of chaetomellic acids involved a biomimetic-type aldol condensation of the appropriate fatty acid ester with pyruvate followed by elimination of an equivalent of water and hydrolysis of the ester groups. Details of synthesis follows.

Aldol condensation. Aldol condensations of the appropriate fatty acid esters with methylpyruvate would give an aldol product that would have the carbon skeleton of chaetomellic acids. The aldol reactions in the standard conditions — addition of methylpyruvate to enolate — resulted in very poor yields due to the insolubility of the enolate at −78 °C. This problem could not be circumvented by raising the temperature due to the base catalyzed polymerization of methyl pyruvate at room temperature. However, performing the aldol reaction in the reverse order easily overcame this problem. The reaction of lithium diisopropylamide with methyl palmitate gave an enolate that was slowly added to the cooled (−78 °C) solution of methylpyruvate. This gave a 1:1 diastereomeric mixture of aldol products **5a** and **6a** in greater than 80% yield (Scheme 1). These products were readily separated by silica gel chromatography and their stereochemistry was determined based on the products of β-elimination reactions (vide infra).

The aldol reaction of methyl oleate with methyl pyruvate was performed in a similar manner to give a 1:1 diastereomeric mixture of **5b** and **6b** with a greater than 80% yield. Operationally this reaction was easier due to the increased solubility of the unsaturated enolate at lower temperature. A similar reaction of methyl myristate with methyl pyruvate gave **5c** and **6c**.

β-Elimination reaction. The direct acid catalyzed elimination of water molecules from the aldol products to generate the tetra-substituted olefin failed due to the difficulty of formation of a carbonium ion α- to the carboxymethyl group. However, it was possible to apply a base catalyzed β-elimination approach to generate the necessary olefin. Thus, separate reactions of **5a** and **6a** with four molar equivalents of *p*-toluenesulfonic anhydride, two molar equivalents of 2,6-di-*tert*-butyl-4-methylpyridine in a mixture of CH_2Cl_2 and pyridine (1.5:1) gave tosylates **7a** and **8a**, respectively, which were used in the next reaction without purification. Methylene chloride was removed from the reaction mixture and the tosylate **7a** was heated at 130 °C after addition of DBU to give the citraconate diester **9a** (*Z*-isomer), the itaconate diester **10a** and the mesaconate diester **11a** (*E*-isomer) in 4:3:0.5 ratio, respectively. The combined two step yield starting from **5a** to **9a–11a** was greater than 85%. Similar reactions with tosylate **8a** afforded predominantly the *E*-isomer **11a** with minor amounts of



Scheme 1. Aldol condensation of fatty acid esters with methyl pyruvate.

9a and **10a**. The elimination reaction of isomer **7a** was relatively slower than that of isomer **8a**. The stereochemistry of aldol products **5a** and **6a** was assigned as S^*,R^* and S^*,S^*, respectively, based on the product distribution of the elimination reactions just described. The assumption was made that the *anti*-periplanar elimination would be the predominant pathway whenever possible and would result in a faster rate of reaction. This would be consistent with the formation of the E-olefin **11a** from **8a** (S^*,S^*) via *anti*-periplanar elimination and formation of **9a** via *syn* or periplanar elimination from **7a** (S^*,R^*). Formation of the terminal olefin **10a** was suppressed to less than 10% when the elimination reaction was performed in refluxing toluene with 2 equivalent of DBU using purified tosylate (Scheme 2).

Aside from stereochemical assignment, the purification of the aldol product was not necessary for the preparation of olefins. In fact, the mixture of aldol products **5b**/**6b** and **5c**/**6c** reacted readily with *p*-toluenesulfonic anhydride to give a mixture of tosylate **7b**/**8b** and **7c**/**8c** which were heated in toluene in the presence of DBU to give the corresponding mixtures of olefins **9b**, **10b**, **11b** and **9c**, **10c**, **11c**, respectively. These olefins were readily separated by silica gel chromatography.

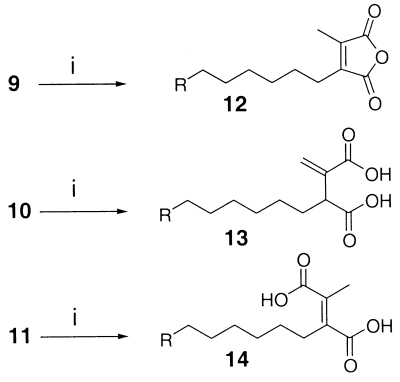
Hydrolysis of esters

Methyl esters were hydrolyzed by refluxing with 1 N sodium hydroxide in a mixture of methanol–THF–H_2O to give the corresponding acids or anhydrides in 90-95% yield. Hydrolysis of *Z*-diesters **9a**, **9b** and **9c** was rapid and gave the anhydrides of chaetomellic acid A **12a**, chaetomellic acid B **12b**, and chaetomellic acid C **12c**, respectively. We have demonstrated earlier that the anhydrides can be converted to chaetomellic acid A (**1a**) at pH 7.5 in solution. Refluxing of **11a** with 1 N NaOH overnight gave the *trans*-diacid **14a**. Hydrolysis of **10a** accordingly produced predominantly **13a** with minor amounts of **12a** and **14a** (Scheme 3).

Similar separate hydrolyses of diesters **10b**, **10c** and **11b**, **11c** gave the corresponding terminal olefinic derivatives of chaetomellic acids B and C, **13b** and **13c** and E-diacids **14b**, **14c**, respectively.

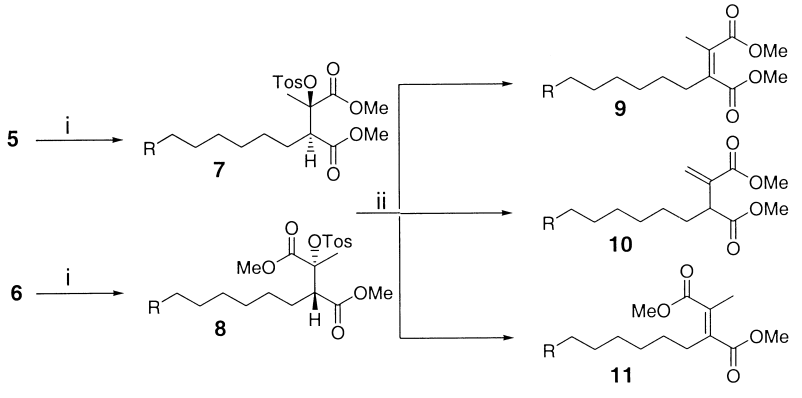
Modified synthesis of chaetomellic acids A and B

The mixtures of aldol products **5a**/**6a** and **5b**/**6b** were hydrolyzed by refluxing in either 4 N NaOH (**5a**/**6a**) or LiOH (**5b**/**6b**) to give 92 and 99% yields of diacids **15a** and **15b**, respectively (Scheme 4). These acids were not purified and refluxed in acetic anhydride for about 2 h to give anhydrides of chaetomellic acids A (**12a**) and B (**12b**) in almost quantitative yields. This modification produced chaetomellic acids A and B in >75 and >80% overall yields, respectively, and is one of the most efficient synthesis of these compounds reported to date.



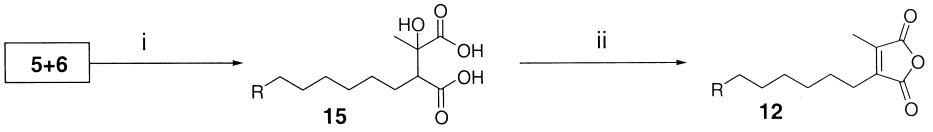
a; R = C_8H_{17}
b: R = $C_{10}H_{19}$,$\Delta^{9,10}$
c: R = C_6H_{13}

Scheme 3. Hydrolysis of esters. Reagents: (i) a. 1 N NaOH, CH_3OH–THF–H_2O. 80 °C; b. 4 N HCl.



a; R = C_8H_{17}
b: R = $C_{10}H_{19}$, $\Delta^{9,10}$
c: R = C_6H_{13}

Scheme 2. β-Elimination reactions of tosylated aldol products. Reagents: (i) *p*-toluenesulfonic anhydride, CH_2Cl_2, C_5H_5N, 2,6-di-*tert*-butyl-4-methylpyridine, 40 °C; (ii) toluene, DBU, reflux.



a: R = C_8H_{17}
b: R = $C_{10}H_{19}$, $\Delta^{9,10}$

Scheme 4. Modified synthesis of chaetomellic acids A and B. Reagents: (i) LiOH, THF–H_2O; (ii) Ac_2O, reflux.

Isolation and structure of chaetomellic acids D (1b) and E (2b)

Gel permeation chromatography of a methyl ethyl ketone extract of an unidentified sterile mycelial broth followed by reverse-phase HPLC yielded minor amounts chaetomellic acids D (**1b**) and E (**2b**) in addition to significant amounts of chaetomellic acids A and B. High resolution EI mass spectral analysis of **1b** and **2b** showed molecular formulae of $C_{19}H_{34}O_5$ and $C_{21}H_{36}O_5$, respectively. The comparison of these formulae with that of chaetomellic acids A and B indicated that these compounds contained an additional oxygen atom. ^{1}H NMR spectrum of both compounds showed the absence of the olefinic methyl group in each of the two compounds and the presence of a hydroxymethyl signal at ~δ 4.62 ppm. The remainder of the ^{1}H NMR spectrum of **1b** and **2b** was identical to that of **1a** and **2a**. Based on these observations structures **1b** and **2b** were assigned to chaetomellic acids D and E, respectively (see Fig. 1). In contrast to chaetomellic acids A and B, these compounds tended to exist in the diacid form under non acidic conditions as indicated by mass spectral analysis that could be due to the presence of the polar hydroxymethyl group.

Structure–activity relationship of chaetomellic acids

Chaetomellic acids A and B are potent, selective and specific inhibitors of recombinant human FPTase (Table 1). We have demonstrated earlier that these reversible inhibitors are competitive with respect to FPP for their inhibition and are selective for FPTase since they do not inhibit other prenyl transferases such as GGPTase-I or other FPP-utilizing enzymes such as squalene synthase. Chaetomellic acid A is the most potent (IC_{50} = 55 nM) compound of the series while the longer chain acid B was less potent (IC_{50} = 155 nM). The molecular modelling experiments with chaetomellic acid A and FPP indicated that a *cis*-diacid and a C-12 chain would be optimal for binding assuming an extended conformation of the side chain. To evaluate this prediction the isomeric diacids (**13a**, **13b**, **14a**, **14b**) of chaetomellic acids A and B and C-12 chain compound chaetomellic acid C (**4**) and its isomers (**13c**, **14c**) were synthesized and results are summarized below.

The vinyl diacid analogue of chaetomellic acid A (**13a**) is 36 times less active when compared to chaetomellic acid A and its *trans* isomer (**14a**) is completely inactive up to 100 μM. Both vinyl (**13b**) and *trans* (**14b**) isomers of chaetomellic acid B were completely inactive up to 100 μM. Surprisingly, the prediction of chain length by molecular modelling did not hold up and chaetomellic acid C (**4**), the acid that was predicted to be the most active, exhibited an IC_{50} value of 500 nM and was approximately 10-fold less active than chaetomellic acid A. However, the activity pattern of the vinyl (**13c**) and *trans* isomers (**14c**) of chaetomellic acid C (**4**) was significantly different from the corresponding isomers of

Table 1. Prenyl transferase activities of chaetomellic acids and analogues

Compounds	rHFPTase[a]	GGPTase I[b]	ScFPTase[c]	ScGGPTase[d]
Chaetomellic acid A (**1a**)	55 nM	92 μM	17 μM[e] 225 μM[f]	> 300 μM[e]
Chaetomellic acid B (**2a**)	185 nM	54 μM	300 μM*	—
Chaetomellic acid C (**4**)	500 nM	—	4 μM[e] 3 μM[f]	112 μM[e]
Chaetomellic acid D (**1b**)	250 nM	—	—	—
Chaetomellic acid E (**2b**)	270 nM	—	—	—
Vinyl acid A (**13a**)	2 μM	—	—	—
Vinyl acid B (**13b**)	> 100 μM	—	—	—
Vinyl acid C (**13c**)	4 μM	—	—	—
Trans acid A (**14a**)	> 100 μM	—	—	—
Trans acid B (**14b**)	> 100 μM	—	—	—
Trans acid C (**14c**)	5 μM	—	—	—
Aldol diacid (**15a**)	> 100 μM	—	—	—
C-8 acid (**16**)	> 100 μM	—	—	—
Farnesyl diacid (**17**)	—	—	2.4 μM[e]	277 μM[e]
Geranylgeranyl diacid (**18**)	—	—	96 μM[e]	11.5 μM[e]

[a]Recombinant human FPTase.
[b]Bovine brain GGPTase I.
[c]*Saccharomyces cerevisiae* FPTase.
[d]*Saccharomyces cerevisiae* GGPTase.
[e]Data from University of Utah, continuous fluorescence assay results.
[f]Data from Merck Research Laboratories, SPA assay results.

acids A and B. Compounds **13c** and **14c** showed IC_{50} values of 4 and 5 μM, respectively. They showed only 8- and 10-fold lower activity when compared to the corresponding *cis*-isomer chaetomellic acid C.

The weaker potency of acid C, compared to acids A and B, contradicts the prediction made by the modeling experiments. This prediction was obviously based on the assumption that the respective alkyl side chains exists in an extended conformation at the active site of the enzyme. It is obvious now that this assumption may not be correct and it is possible that the alkyl side chain could assume alternative conformations within the active sites, one of which could be coiled conformation. This observation is further supported by the complete lack of inhibitory activity of C-8 acid (**16**).[18]

X-ray co-crystal structure of rat-FPTase with FPP indicates a bend in extended conformation of FPP at the active site.[19] It is possible that a similar bend may occur for chaetomellic acids thereby facilitating and maximizing the hydrophobic interactions in the binding pocket. For chaetomellic acids to exhibit potent inhibitory activities against FPTase the dicarboxylate groups must be in register with the divalent zinc atom present at the active site. It appears that binding of the dicarboxylate group of chaetomellic acid A coordinate best with the zinc atom. Chaetomellic acids, with longer or shorter chains, forces the dicarboxylate away from the zinc register and leads to poor inhibitor binding and, hence, inhibitory activity. These results are consistent with the molecular ruler hypothesis of substrate binding and specificity.[19]

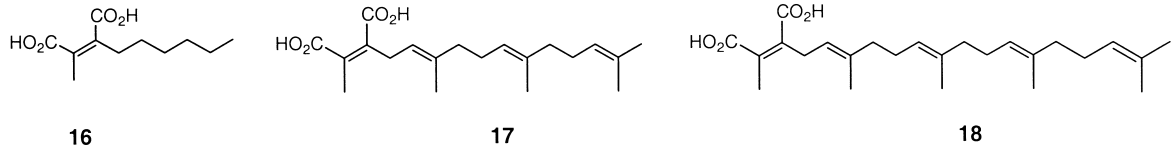
Substitutions of the olefinic methyl group with smaller (for example, H) or larger group (for example, Ph) in the head unit of chaetomellic acid A resulted in a significant reduction in inhibitory activity (Singh and Graham, unpublished results). However, the hydroxymethyl group substitution did not have any significant effect on the potency of these compounds. For example, chaetomellic acid D (**1b**) and chaetomellic acid E (**2b**) (Fig. 1) displayed IC_{50} values of 250 and 270 nM, respectively. There was no significant difference in the inhibitory activities of chaetomellic acids when tested against other mammalian prenyl transferases such as bovine brain FPTase.[8,20] The esterification of the carboxyl groups of chaetomellic acids caused the complete loss of inhibitory activities.

These compounds were evaluated against yeast (*Saccharomyces cerevisiae*) FPTase and GGPTase I enzymes and the comparative activity profiles are presented in Table 1. The data is compared to that reported by Poulter's group of the University of Utah. The groups of Vederas and Poulter originally reported that chaetomellic acid A was significantly less active against ScFPTase (IC_{50} = 17 μM) when compared to mammalian FPTase (IC_{50} = 55 nM). The activity of chaetomellic acid A against ScFPTase was even weaker (IC_{50} = 225 μM) when tested in our laboratories. The reasons for this difference in potency are not readily apparent but could be due to differences in the method of measurement of yeast FPTase activity in the two laboratories. The University of Utah group used a continuous fluorescence assay[21] while the Merck group made use of Scintillation Proximity Assay (SPA). Furthermore, the apparent lack of activity of acid A against yeast FPTase is surprising given that the yeast and mammalian enzymes share a common α-subunit.

The C-12 chain acid (**3**)[10] is 10 times less active against human FPTase when compared to the C-14 chain acid (**1a**) vide supra. However, it is more active (IC_{50} = 3 to 4 μM) than chaetomellic acid A (IC_{50} = 17 or 225 μM) when tested against yeast FPTase. Aside from the synthesis and activity of chaetomellic acid C, the Poulter and Vederas groups also reported[13] the preparation and the activities of chaetomellic acid analogues consisting of farnesyl (**17**) and geranylgeranyl (**18**) chains (Fig. 2). As expected, the farnesyl acid was active against yeast FPTase (IC_{50} = 2.4 μM) and was 100-fold selective over yeast GGPTase. In contrast, the geranylgeranyl acid (**18**) was less active against yeast GGPTase activity (IC_{50} = 11.5 μM) and was also less selective (10-fold over yeast FPTase).

The observation of differences in the potency of chaetomellic acids between mammalian and fungal (or yeast) FPTase is noteworthy. Chaetomellic acid A is more active against both recombinant human and bovine brain FPTase (IC_{50} = 55 nM) and is less active against yeast FPTase (IC_{50} = 17–225 μM), depending on the assay used. These differences in the IC_{50} values are similar to the differences in the affinity of FPP for the two enzymes. FPP has a higher affinity for mammalian FPTase (K_D = 12 nM,[22,23] than yeast FPTase (K_D = 75 nM).[24,25] Inhibitors that compete with FPP for binding for their inhibitory activity may be expected to reflect this difference of the K_D values in their IC_{50} values.

In conclusion, we have described the efficient synthesis of chaetomellic acids A, B, C and several isomers. These compounds were evaluated in various prenyl transferase assays including human and yeast FPTase, and bovine and yeast GGPTase. The activity profiles of these compounds in these assays were surprisingly different but not beyond reasonable explanation. The *cisoid* orientation of the dicarboxylic acids along with an alkyl side chain of proper length are critical for binding and hence inhibitory activities. The *trans*-, and *vinyl*-diacids are



16 17 18

Figure 2. Structures of synthetic and natural chaetomellic acid analogues (diacid form).

significantly less active in human FPTase. *cis*-Diacids consisting of shorter alkyl chain were inactive against human FPTase as predicted by molecular modeling experiments. All of the chaetomellic acids are significantly less active against yeast FPTase as would be expected due to the differences in the K_D values of the two enzymes.

Experimental

General procedure

All commercial reagents were obtained from Sigma–Aldrich and were used without any further purification. Biotinylated peptides were synthesized by Synpep and the molecular weights verified by MS by the supplier. [^{3}H]-Farnesylpyrophosphate was purchased from either Amersham or NEN; the specific radioactivity varied (15–22 C_i/mM) depending on the lot and supplier. Non-radioactive farnesylpyrophosphate was purchased from ARC, Inc. Strepavidin–SPA beads were supplied by Amersham. Hepes buffer, dithiothreitol, skim milk, bovine serum albumin (BSA), and dodecylmaltoside were from Sigma.

Mass spectra were recorded on Finnigan–MAT model MAT212 (electron impact, EI, 90 eV), MAT 90 (Fast Atom Bombardment, FAB), TSQ70B (FAB, EI), and Finnigan FTMS mass spectrometers. Exact mass measurements were performed by either high resolution (HR-EI) using perfluorokerosene (PFK) or perfluoropolypropylene oxide (Ultramark U1600F) or PEG600 (FTMSESI) as an internal standard. Trimethyl silyl derivatives were prepared with a 1:1 mixture of BSTFA:pyridine at room temperature. ^{13}C NMR spectra were recorded in CD_2Cl_2 or CD_3OD or $CDCl_3$ at 75 MHz on a Varian XL-300 spectrometer or at 100 MHz on a Varian Unity-400 spectrometer. Chemical shifts are given in ppm relative to TMS at zero using the solvent peak at 53.8 ppm (CD_2Cl_2), 49.00 ppm (CD_3OD) and 77.00 ppm ($CDCl_3$), respectively, as an internal standard. ^{1}H NMR spectra were recorded in CD_2Cl_2 or CD_3OD or $CDCl_3$ at 300 MHz on a Varian XL-300 spectrometer or at 400 MHz on a Varian Unity-400 spectrometer. Chemical shifts are given in ppm relative to TMS at zero using the solvent peak at 5.32 ppm (CD_2Cl_2), 3.30 ppm (CD_3OD) and 7.26 ppm ($CDCl_3$), respectively, as an internal standard.

Aldol condensation of methylpalmitate and methylpyruvate. *n*-Butyl lithium (1.6 M in hexane, 8.5 mL, 13.6 mmol) was added to a cooled (−78 °C) solution of diisopropyl amine (2.2 mL, 15 mmol) in THF (10 mL). The solution was stirred under N_2 at −78 °C for 10 min followed by at 0 °C for 10 min. After cooling the LDA solution at −78 °C a THF (20 mL) solution of methyl palmitate (2.7 g, 10 mmol) was added via a syringe over a 10-min period and stirring was continued for 10 min. The reaction mixture was slowly allowed to warm to 0 °C and stirred for 30 min resulting in to a faint yellow color. This enolate solution was added drop wise (total addition time 20 min) via a dropping funnel to a thoroughly cooled (−78 °C) and stirred solution of methyl pyruvate (1.2 mL, 12 mmol) in THF (10 mL). The progress of the reaction was monitored on TLC (hexane:EtOAc, 4:1). The solution was stirred for 2 h and then quenched with 10% aqueous citric acid at −78 °C and poured on to 300 mL EtOAc. The layers were separated and organic phase was washed with 100 mL water, dried over sodium sulfate, concentrated under reduced pressure and filtered through a bed of silica gel. Elution with 5% EtOAc in hexane gave 3.1 g (81.8%) of a 1:1 diastereomeric mixture of aldol products as a gum. A small portion of the mixture was chromatographed on a flash silica gel (200 cc) column packed in hexane. The column was eluted with 5% EtOAc in hexane to afford the first diastereomer (**5a**, HPLC $t_R = 7.0$ min, Whatman ODS-3, C-18 (4.6×250 mm), CH_3OH:H_2O, 92:8, at a flow rate of 1.5 mL/min), followed by the mixture and then the second diastereomer (**6a**, $t_R = 6.4$ min). Both compounds were obtained as solids. **5a:** ^{1}H NMR ($CDCl_3$, δ): 3.75 (3H, s), 3.67 (3H, s), 2.76 (1H, dd, $J = 10.5$, 3.9 Hz), 1.68 (2H, m), 1.41 (3H, s), 1.25 (24H, brm), 0.87 (3H, t, $J = 6.3$ Hz); FABMS (*m*/*z*): 373 (M + H), 340, 322, 313 (100%). **6a:** ^{1}H NMR ($CDCl_3$, δ): 3.79 (3H, s), 3.71 (3H,s), 2.75 (1H, dd, $J = 11.7$, 3.3 Hz), 1.83 (2H, m), 1.42 (3H, s), 1.23 (24H, m), 0.87 (3H, t, $J = 6.3$ Hz); FABMS (*m*/*z*): 373 (M + H), 340, 322, 313 (100%).

Aldol condensation of methyloleate and methylpyruvate. Methyl oleate (5.29 g, 10 mmol) was reacted with 12 mmol of methylpyruvate in a manner identical to the reaction just described. Silica gel filtration gave 6.52 g (82.3%) of 1:1 mixture of diastereomeric aldol products as oil. A small portion of this mixture was chromatographed on a flash silica gel (200 cc) column packed in hexane. The column was eluted with 5% EtOAc in hexane to afford the first diastereomer (**5b**), a mixture of both and the second diastereomer (**6b**), all as oil. **5b:** ^{1}H NMR ($CDCl_3$, δ): 5.34 (2H, m), 3.76 (3H, s), 3.68 (3H, s), 3.65 (1H, brs), 2.76 (1H, dd, $J = 10.2$, 3.9 Hz), 2.0 (4H, m), 1.66 (2H, m), 1.42 (3H,s), 1.27 (20H, brm), 0.88 (3H, t, $J = 6.8$ Hz); HRESI-FTMS (*m*/*z*): 399.3093 [M + H] (calcd for $C_{23}H_{43}O_5$: 399.3111). **6b:** ^{1}H NMR ($CDCl_3$, δ): 5.34 (2H, m), 3.80 (3H, s), 3.72 (3H,s), 3.51 (1H, brs), 2.72 (1H, dd, $J = 11.7$, 3.3 Hz), 2.0 (4H, m), 1.84 (2H, m), 1.43 (3H,s), 1.27 (20H, m), 0.88 (3H, t, $J = 6.6$ Hz). HRESI-FTMS (*m*/*z*): 399.3096 [M + H] (calcd for $C_{23}H_{43}O_5$: 399.3111).

Elimination reaction of methylpalmitate aldol products. To a solution of **5a** (160 mg, 0.43 mmol) in CH_2Cl_2 (1.5 mL) and pyridine (1.0 mL) was added 2,6-di-*tert*-butyl-4-methylpyridine (200 mg, 0.97 mmol) followed by *p*-toluenesulfonic anhydride (570 mg, 1.74 mmol) and the solution was heated at 50 °C under N_2 overnight. Progress of the reaction was monitored on TLC (hexane:EtOAc, 85:15). The tosylate was less polar than starting alcohol. A small portion of the tosylate was purified on a silica gel column using 2 to 5% EtOAc in hexane to give **7a** (^{1}H NMR ($CDCl_3$, δ): 7.76 (2H, d, $J = 8.4$ Hz), 7.32 (2H, d, $J = 8.4$ Hz), 3.83 (3H, s), 3.67 (3H, s), 2.95 (1H, dd, $J = 11.7$, 3.0 Hz), 2.44 (3H, s), 1.81 (3H, s), 1.60 (2H, m), 1.24 (24H, m), 0.88 (3H, t, $J = 6.6$ Hz), FABMS (*m*/*z*): 527 (M + H)). DBU (0.5 mL) was added and the reaction mixture was heated at 130–

140 °C for 3 h to give citraconic acid diester (HPLC, Whatman ODS-3 (C-18), 4.6×250 mm, CH_3OH:H_2O (92:8), flow rate 1.5 mL/min; t_R = 8.8 min, ~50%), itaconic acid diester analogue (t_R = 9.4 min, 40%) and mesaconic acid diester analogue (t_R = 9.8 min, ~10%).

Similarly, the reaction of **6a** (90 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL), pyridine (1 mL), 2,6-di-*tert*-butyl-4-methylpyridine (100 mg) with *p*-toluenesulfonic anhydride (270 mg) at 50 °C followed by heating with DBU for 20 min gave major mesaconic acid diester with minor amounts of citraconate and itaconate isomers. Both reaction mixtures were cooled to room temperature, combined and worked up together, they were poured on to EtOAc (200 mL) and washed sequentially with 4 N aqueous HCl (3×50 mL), water, 10% aq $NaHCO_3$ (3×50 mL) followed by water. The EtOAc extract was dried (Na_2SO_4), evaporated under reduced pressure. The product mixture was chromatographed on a flash silica gel column (100 cc) packed in hexane and eluted with 2% followed by 3% EtOAc to give mesaconate **11a** (25 mg), itaconate **10a** (88 mg) and citraconate **9a** (80 mg) in order of their elution, a combined yield of 81%. The mixtures of aldol products could be used for the elimination reaction without any problem. **9a**: ^{1}H NMR ($CDCl_3$, δ): 3.75 (3H, s), 3.74 (3H, s), 2.32 (2H, t, J = 7.2 Hz), 1.94 (3H, brs), 1.43 (2H, m), 1.24 (22H, m), 0.86 (3H, t, J = 6.6 Hz); HREIMS (m/z): 354.2741 (calcd for $C_{21}H_{38}O_4$: 354.2808). **10a**: ^{1}H NMR ($CDCl_3$, δ): 6.35 (1H, s), 5.74 (1H, s), 3.76 (3H, s), 3.67 (3H, s), 3.49 (1H, t, J = 7.2 Hz), 1.86 (1H, m), 1.65 (1H, m), 1.24 (24H, m), 0.87 (3H, t, J = 6.3 Hz); EIMS (m/z): 354 $(M)^+$. **11a**: ^{1}H NMR ($CDCl_3$, δ): 3.78 (3H,s), 3.77 (3H, s), 2.43 (2H, t, J = 8.5 Hz), 1.99 (3H, brs), 1.40 (2H, m), 1.24 (22H, m), 0.87 (3H, t, J = 7.8 Hz); EIMS (m/z): 354 $(M)^+$.

Elimination reaction of methyloleate aldol products. To a solution of the diastereomeric mixture of **5b** and **6b** (2.2 g, 5.5 mmol) in CH_2Cl_2 (10 mL) and pyridine (5 mL) was added 2,6-di-*tert*-butyl-4-methylpyridine (2.3 g, 11 mmol) followed by *p*-toluenesulfonic anhydride (5.4 g, 16.5 mmol). The solution was stirred at room temperature overnight under N_2. The progress of the reaction was examined by TLC (hexane:EtOAc, 85:15). After completion of the reaction DBU (4 mL) was added and CH_2Cl_2 was removed under vacuum and the mixture was heated at 130–140 °C for 6 h. The reaction mixture was cooled to room temperature and poured on to EtOAc (400 mL) and washed sequentially with 4 N aq HCl (3×100 mL), water, 10% aq $NaHCO_3$ (3×100 mL) followed by water. The EtOAc extract was dried (Na_2SO_4), evaporated under reduced pressure and the product was chromatographed on a flash silica gel column (300 cc) packed in hexane. Elution with 1, 2% followed by 3% EtOAc in hexane gave first mesaconate (*trans*) diester **11b** (70 mg), itaconate diester **10b** (1.56 g) and citraconate (*cis*) diester **9b** (160 mg) all as oil. **9b**: ^{1}H NMR ($CDCl_3$, δ): 5.34 (2H, m), 3.76 (3H, s), 3.74 (3H, s), 2.32 (2H, t, J = 7.5 Hz), 2.00 (4H, m), 1.94 (3H, brs), 1.57 (2H, m), 1.42 (2H, m), 1.31–1.24 (18H, m), 0.88 (3H, t, J = 6.0 Hz); HREIMS (m/z): 380.2928 (calcd for $C_{23}H_{40}O_4$: 380.2966). **10b**: ^{1}H NMR ($CDCl_3$, δ): 6.36 (1H, s), 5.75 (1H, s), 3.77 (3H, s), 3.68 (3H, s), 3.50 (1H, t, J = 7.2 Hz), 2.0 (4H, m), 1.90 (1H, m), 1.66 (1H, m), 1.27 (20H, m), 0.88 (3H, t, J = 6.8 Hz); HRESI-FTMS (m/z): 381.3003 [M + H] (calcd for $C_{23}H_{41}O_4$: 381.3005). **11b**: ^{1}H NMR ($CDCl_3$, δ): 5.34 (2H, m), 3.78 (3H, s), 3.77 (3H, s), 2.44 (2H, t, J = 7.4 Hz), 2.00 (7H, brs and m), 1.40 (2H, m), 1.27 (18H, m), 0.88 (3H, t, J = 6.8 Hz); HRESI-FTMS (m/z): 381.3012 [M + H] (calcd for $C_{23}H_{41}O_4$: 381.3005).

Elimination reaction of methylmyristate aldol products

The aldol reaction of methylmyristate with methylpyruvate gave aldol products **5c**/**6c**, which were tosylated to give **7c**/**8c**, and subjected to elimination reaction just described to give **9c**, **10c** and **11c**. **9c**: ^{1}H NMR ($CDCl_3$, δ): 3.76 (3H, s), 3.75 (3H, s), 2.32 (2H, t, J = 7.2 Hz), 1.94 (3H, brs), 1.43 (2H, m), 1.25 (18H, m), 0.88 (3H, t, J = 6.6 Hz); HRESI-FTMS (m/z): 327.2553 [M + H] (calcd for $C_{19}H_{35}O_4$: 327.2535). **10c**: ^{1}H NMR ($CDCl_3$, δ): 6.35 (1H, s), 5.74 (1H, s), 3.76 (3H, s), 3.67 (3H, s), 3.49 (1H, t, J = 7.2 Hz), 1.86 (1H, m), 1.65 (1H, m), 1.24 (20H, m), 0.87 (3H, t, J = 6.3 Hz); HRESI-FTMS (m/z): 327.2552 [M + H] (calcd for $C_{19}H_{35}O_4$: 327.2535). **11c**: ^{1}H NMR ($CDCl_3$, δ): 3.78 (3H, s), 3.77 (3H, s), 2.44 (2H, t, J = 8.5 Hz), 1.99 (3H, brs), 1.40 (2H, m), 1.25 (18H, m), 0.88 (3H, t, J = 7.8 Hz); HRESI-FTMS (m/z): 327.2553 [M + H] (calcd for $C_{19}H_{35}O_4$: 327.2535).

Hydrolysis of dimethyl esters

Chaetomellic acid A anhydride (12a). A solution of citraconate dimethyl ester **9a** (40 mg) in THF (1 mL), methanol (1 mL), and water (1 mL) was added 4 N NaOH (0.5 mL) and the mixture was heated at reflux overnight. The progress of the reaction was monitored on HPLC (Whatman C-18, 4.6×250 mm, CH_3CN:H_2O, 90:10, flow rate 1.5 mL/min). After completion of the reaction it was cooled to 0 °C and acidified with 4 N HCl to pH 2. The product was extracted with CH_2Cl_2 (2×25 mL), washed with water, dried (Na_2SO_4) and evaporated to give colorless anhydride (t_R = 8.9 min) which solidified upon cooling. This product was identical with the natural product (HPLC, ^{1}H NMR).

Chaetomellic acid B anhydride (12b). A solution of *cis*-dimethyl ester analogue **9b** (55 mg) hydrolyzed accordingly and the product was extracted with EtOAc (3×50 mL). The EtOAc solution was washed with water, dried (Na_2SO_4) and evaporated to give colorless product anhydride as an oil. This product was identical with natural chaetomellic acid B anhydride (HPLC, ^{1}H NMR).

Chaetomellic acid C anhydride (12c). Hydrolysis of **9c** in an analogous manner gave the anhydride, ^{1}H NMR ($CDCl_3$, δ): 2.45 (2H, t, J = 7.5 Hz), 2.07 (3H, s), 1.57 (2H, m), 1.26 (18 H, m), 0.88 (3H, t, J = 7 Hz); HRESI-FTMS of diacid (m/z): 297.2072 [M–H] (calcd for $C_{17}H_{29}O_4$: 297.2066).

***trans*-Chaetomellic acid A (14a).** Using conditions described above the mesaconate (*trans*) diester **11a** (4.4 mg) was refluxed overnight with 4 N NaOH in methanol (0.5 mL), THF (0.5 mL) and water (0.5 mL). The

mixture was cooled to room temperature and acidified to pH 2 by addition of 4 N HCl. The diacid was extracted with ethyl acetate (3×20 mL), washed with 20 mL water, dried (Na_2SO_4) and evaporated under reduced pressure to give 4 mg of colorless powder of **14a**. ^{1}H NMR ($CDCl_3$, δ): 2.58 (2H, t, J=7.8 Hz), 2.14 (3H, s), 1.52 (2H, m), 1.26 (22H, m), 0.89 (3H, t, J=6.6 Hz); HRESI-FTMS (m/z): 325.2385 [M–H] (calcd for $C_{19}H_{33}O_4$: 325.2379).

***trans*-Chaetomellic acid B (14b).** Using conditions described above mesaconate (*trans*) diester analogue **11b** (45 mg) was refluxed overnight to give 21 mg of diacid **14b** after purification by HPLC (Whatman C-18, 22×250 mm, 60 to 80% aq CH_3CN containing 0.1% TFA, at a flow rate of 10 mL/min) as a powder. **14b**: ^{1}H NMR ($CDCl_3$, δ): 5.34 (2H, m), 2.58 (2H, t, J=7.8 Hz), 2.15 (3H, s), 2.00 (4H, m), 1.52 (2H, m), 1.32, 1.26 (18H, m), 0.88 (3H, t, J=6.6 Hz); ESIMS (m/z): 375 (M + Na).

***trans*-Chaetomellic acid C (14c).** A similar hydrolysis of **11c** gave diacid **14c** in quantitative yield as a powder. **14c**: ^{1}H NMR ($CDCl_3$ + CD_3OD, δ): 2.38 (2H, t, J=8.5 Hz), 1.93 (3H, s), 1.33 (2H, m), 1.13 (18H, m), 0.76 (3H, t, J=7 Hz); HRESI-FTMS (m/z): 297.2073 [M-H] (calcd for $C_{17}H_{29}O_4$: 297.2066).

Vinyl-chaetomellic acid A (13a). Vinyl diester **10a** (50 mg) was hydrolyzed following the conditions described for *cis*- diester. The reaction was analyzed by HPLC (Whatman C-18 (4.6×250 mm), CH_3CN–H_2O + 0.08% TFA, and flow rate 1.5 mL/min). The reaction produced a mixture of three products, **13a** (72%, t_R=7.7 min), **12a** (20%, t_R=12.9 min) and **14a** (8%, t_R=5.9 min) in an over all yield of ~80%. These acids were purified by HPLC (Whatman C-18, 22×250 mm, gradient elution with CH_3CN–H_2O + 0.1% TFA, 65 to 75% CH_3CN over 60 min followed by 90% at a flow rate of 10 mL/min) to give **13a** as an amorphous powder. **13a**: ^{1}H NMR (CDCl3, δ): 6.52 (1H, s), 5.82 (1H,s), 3.37 (1H, t, J=7 Hz), 1.93 (1H, m), 1.73 (1H, m), 1.25 (24 H, m), 0.88 (3H, t, J=6.6 Hz); HRESI-FTMS (m/z): 325.2390 [M–H] (calcd for $C_{19}H_{33}O_4$: 325.2379).

Vinyl-chaetomellic acid B (13b). A similar hydrolysis of 50 mg of **10b** and purification by HPLC (Whatman C-18, 22×250 mm, gradient elution with CH_3CN:H_2O (containing 1% TFA) 65:35 to 75:25 over 60 min followed by 90:10 at a flow rate 10 mL/min) gave **13b** as major product as a gum. **12b** and **14b** were also isolated as minor products. **13b**: ^{1}H NMR ($CDCl_3$, δ): 6.51 (1H, s), 5.81 (1H, s), 5.35 (2H, m), 3.36 (1H, m), 2.62 (1H, m), 2.01 (4H, m), 1.75 (1H, m), 1.26 (20H, m), 0.88 (3H, t, J=7 Hz), HRESI-FTMS (m/z): 351.2530 [M–H] (calcd for $C_{21}H_{35}O_4$: 351.2535).

Vinyl-chaetomellic acid C (13c). Prepared from **10c** as an amorphous powder, ^{1}H NMR ($CDCl_3$, δ): 6.54 (1H, s), 5.83 (1H, s), 3.36 (1H, m), 1.95 (1H, m) 1.75 (1H, m), 1.26 (20H, m), 0.88 (3H, t, J=7 Hz), HRESI-FTMS (m/z): 297.2076 [M–H] (calcd for $C_{17}H_{29}O_4$: 297.2066).

Hydrolysis of 5a/6a. To a solution of a mixture of **5a**/**6a** (284 mg) in methanol (2 mL), THF (2 mL) and water (2 mL) was added 1 mL of 4 N NaOH. The solution was stirred at room temperature for overnight followed by heating at reflux for 18 h. The reaction was cooled to room temperature, acidified to pH 2 by addition of concd HCl and extracted with ethyl acetate (3×50 mL). The organic layer was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure to give 240 mg (91.6%) of chromatographically homogeneous diastereomeric diacid **15a** as colorless powder. ^{1}H NMR (CD_3OD, δ): 2.73 (1H, m), 1.82 (1H, m), 1.66 (1H, m), 1.44, 1.41 (3H, s), 1.29 (24H, m), 0.89 (3H, t, J=7.2 Hz); HRESI-FTMS (m/z): 343.2499 [M–H] (calcd for $C_{19}H_{35}O_5$: 343.2485).

Hydrolysis of 5b/6b. To a solution of 540 mg of the diastereomeric mixtures of **5b**/**6b** in THF (6 mL) was added a 2 mL aqueous solution of 230 mg of lithium hydroxide. The solution was stirred overnight at room temperature followed by heating at 80 °C for 4 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool down to ambient temperature and acidified to pH 2 by addition of concentrated hydrochloric acid, poured on to EtOAc (100 mL), washed with water (2×100 mL), and dried (Na_2SO_4). EtOAc was removed by concentration under reduced pressure and dried in a vacuum desiccator to give 500 mg (99.6%) of chromatographically homogeneous diacid **15b** as a gum. ^{1}H NMR ($CDCl_3$, δ): 5.36 (2H, m), 2.87, 2.86 (1H, each t, J=9.6 Hz), 2.03 (4H, m), 1.55, 1.49 (3H, s), 1.34-126 (22H, m), 0.90 (3H, t, J=7.2 Hz), HRESI-FTMS (m/z): 370.2719 (calcd for $C_{21}H_{38}O_5$: 370.2757).

Chaetomellic acid B anhydride (12b). The diastereomeric diacids **15b** (460 mg) in 3 mL acetic anhydride was heated at reflux for 2 h. The progress of the reaction was followed up by HPLC (Zorbax RX C-8, 4.6×250 mm, CH_3CN–H_2O + 0.1% TFA, 85/15, 1 mL/min). After completion of the reaction, acetic anhydride was distilled off under reduced pressure, product was filtered through a small bed of silica gel and eluted with ethyl acetate to give 405 mg (97.6%) homogeneous chaetomellic acid B anhydride (**12b**) as a gum.

Chaetomellic acid A anhydride (12a). The diastereomeric mixture of diacids **15a** (12.2 mg) was similarly heated in 1 mL acetic anhydride for 2 h to give 10.9 mg (>99%) of chaetomellic acid A anhydride **12a**.

Isolation of chaetomellic acids D (1b) and E (2b). A 1.2 L regrowth of sterile mycelia grown on liquid media was extracted with methyl ethyl ketone (1.2 L) by shaking at room temperature for 30 min. Methyl ethyl ketone was evaporated under reduced pressure and residual water was removed via lyophilization. The mixture was chromatographed on a 2.0 L Sephadex LH 20 column and eluted with methanol. The FPTase active fractions were pooled to give 600 mg of gum. This material was suspended in 5 mL 1:1 mixture of CH_3OH:CH_3CN and filtered. The filtrate was chromatographed, in three equal aliquots, on a Whatman C-18 column (22×250 mm). Gradient elution with 70 to 80% aq CH_3CN containing 0.2% TFA in 60 min at a flow rate of 10 mL/

min gave two fractions eluting from 31 to 44 min and 45 to 58 min. The former fraction weighing 100 mg contained polar chaetomellic acids D and E whereas the latter fraction was major and possessed about 300 mg of 1:1 mixture of chaetomellic acids A and B. The polar fraction containing compounds **1b** and **2b** was chromatographed again on a similar HPLC column. Elution with a gradient of 50 to 90% aq CH_3CN +0.2% TFA over 60 min gave 6.4 mg of pure chaetomellic acid D **1b** (elution time 46–47 min) and 2.8 mg of chaetomellic acid E (elution time 49 min), both as gums. **1b**: ^{1}H NMR ($CDCl_3$, δ): 4.62 (2H, s), 2.60 (2H, t, J=8 Hz), 1.63 (2H, m), 1.36–1.29 (22H, m), 0.91 (3H, t, J=7.5 Hz); HREIMS (m/z): 342.2370 (calcd for $C_{19}H_{34}O_5$: 342.2406). **2b**: ^{1}H NMR ($CDCl_3$, δ): 5.38 (2H, m), 4.62 (2H, s), 2.60 (2H, t, J=8 Hz), 2.04 (4H, m), 1.63 (2H, m), 1.36–1.29 (18H, m), 0.91 (3H, t, J=7.5 Hz); HREIMS (m/z): 368.2535 (calcd for $C_{21}H_{36}O_5$: 368.2562).

Recombinant human FPTase assay. Human FPTase was prepared as described by Omer et al.[26] FPTase assays were performed as described[8] and contained the following: 2 nM FPTase, 50 nM [3H]-farnesyl diphosphate (FPP) and 100 nM Ras-CVIM or 400 nM Ras-CVLS. All substrates were used at concentrations that corresponded to Km levels. Reactions were kept at at 31 °C for 60 min. Reactions were initiated with FPTase and terminated with one mL of 1.0 N HCl in ethanol. Precipitates were collected onto filtermats using a TomTec Mach II cell harvestor, washed with 100% ethanol, dried and counted in a LKB β-plate counter. The assay was linear with respect to both substrates, FPTase levels and time; less than 10% of the [^{3}H]-FPP was utilized during the reaction period. Fermentation extracts or pure compounds were dissolved in 100% DMSO and diluted 20-fold into the assay to give a final solvent concentration of 5%.

Yeast FPTase assay. Reaction mixtures for the determination of IC_{50} values with rScFPTase (pGP114 was obtained from Dr. C. Dale Poulter of the University of Utah, rScFPTase was expressed in *Esterichia coli* from this plasmid and purified as described[27]) contained the following (in a total volume of 0.05 mL): sodium Hepes buffer, pH 7.5 (50 mM), biotinyl-YRASNRSCVLS (1.0 μM), rSc FTase (0.05 activity units), [^{3}H]-farnesyl pyrophosphate (0.94 μM; 3 Ci/mmol), $MgCl_2$ (1 mM), $ZnCl_2$ (50 μM), dodecylmaltoside (0.05%), dithiothreitol (5 mM), DMSO (5%) and test compound. Reaction mixtures were prepared in 96-well microtiter plates (Microfluor W Flat Bottom plates; Dynex Technologies Inc). Reaction was initiated by the addition of enzyme. Incubation was for 15 min at 30 °C. Reaction was terminated by addition to each well of the following solution (in a total volume of 0.1 mL): phosphoric acid, pH 3.5 (80 mM), $MgCl_2$ (600 mM), sodium azide (0.03%), skim milk (1.25%), BSA (2.5%), and Strepavidin SPA beads (0.75 mg; 2-fold molar excess over the peptide concentration). The contents of the wells were mixed by rotary shaking and the plates were allowed to stand at room temperature for 2–24 h before counting. Radioactivity was determined with a Packard TopCount. Counting efficiency for ^{3}H was 25%.

Bovine GGPTase I assay. Partially purified geranylgeranyl protein transferase type 1 (GGPTase-1) was prepared from bovine brain as described by Moores et al.[28] GGPTase-1 was assayed in a volume of 100 μL containing 100 mM Hepes pH 7.4, 5 mM $MgCl_2$, 5 mM dithiothreitol, 100 nM [^{3}H]-geranylgeranyl diphosphate (15 C_i/mmol, American Radiolabeled Chemicals), 650 nM Ras-CAIL and 1 mg/mL GGPTase at 37 °C for 30 min. Assay tubes were processed as described above for farnesyl transferase.

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