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Novel route to chaetomellic acid A and analogues: Serendipitous discovery of a more competent FTase inhibitor

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ABSTRACT

A new practical route to chaetomellic acid A (**ACA**), based on the copper catalysed radical cyclization (RC) of (*Z*)-3-(2,2-dichloropropanoyl)-2-pentadecylidene-1,3-thiazinane, is described. Remarkably, the process entailed: (i) a one-pot preparation of the intermediate *N*-α-perchloroacyl-2-(*Z*)-alkyliden-1,3-thiazinanes starting from *N*-(3-hydroxypropyl)palmitamide, (ii) a two step smooth transformation of the RC products into **ACA** and (iii) only one intermediate chromatographic purification step. The method offers a versatile approach to the preparation of **ACA** analogues, through the synthesis of an intermediate maleic anhydride with a vinylic group at the end of the aliphatic tail, a function that can be transformed through a thiol–ene coupling. Serendipitously, the disodium salt of 2-(9-(butylthio)nonyl)-3-methylmaleic acid, that we prepared as a representative sulfurated **ACA** analogue, was a more competent FTase inhibitor than **ACA**. This behaviour was analysed by a molecular docking study.

1. Introduction

Prenylation of proteins with polyisoprenoids is an important post-translational modification, which plays a major role in cell proliferation of both normal and cancerous cells.[1] Proteins, which undergo prenylation, are all characterized by a CAAX motif at their carboxy terminus, where C is a cysteine residue, A is any aliphatic amino acid, and X could be variable (usually alanine, serine, methionine, or glutamine).[1,2] Prenylation can occur via the covalent attachment of either a 15-carbon farnesyl moiety or a 20-carbon geranylgeranyl moiety to the cysteine of CAAX motif-containing proteins, the process being catalysed by farnesyltransferase (FTase) or geranylgeranyltransferase (GGTase) enzymes.[1d] Geranylgeranylation is found to be responsible for the prenylation of 80–90% of prenylated proteins;[3] whereas, lamins A and B,[1b,1c,4] the fungal mating factor,[2a] γ-transducin,[2e,2f] the γ subunit of heterotrimeric G-proteins[4] and Ras-proteins[2c,4] are farnesylated.

Farnesylation is required for the subcellular localization and transformation activity of the oncogenic variants of Ras.[1a,2c,5] Consequently, the inhibition of farnesylation prevents localization of these proteins at the cell membrane, thereby prohibiting cell transformation. For this reason, the development of farnesyltransferase (FTase) inhibitors have emerged as novel class of pharmaceutical agents in noncytotoxic anticancer therapy.[1a,6] Enzymatic and crystallographic techniques have explained the mechanism of FTase catalysis, demonstrating that farnesylation proceeds via an ordered mechanism with farnesyl pyrophosphate (FPP) binding first, followed by the CAAX moiety of the Ras-protein and then by the farnesyl transfer to the cysteine residue C.[1d,6,7]

Inhibitors design is largely based on the CAAX motif, the farnesyl moiety, or both (in the latter case inhibitors mimic the transition state).[1a,1d,6a,8] Also several natural products are able to inhibit the FTase.[1a,9] These substances have been generally

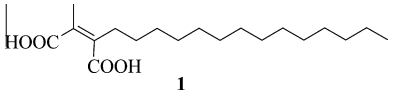

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Figure 1. Chaetomellic acid A (**1**).

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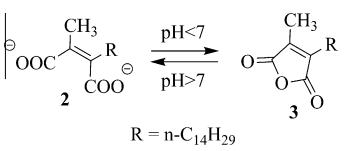
identified through screening program of microbial natural products.[9] It was just during one of these screening tests that a Merck's team isolated, from extracts of the coelomycete *Chaetomella acutiseta*, chaetomellic acid A (**ACA**) **1** (Fig. 1),[10] a dicarboxylic acid that inhibited, in its dianionic form **2**, the recombinant human FTase, with an IC_{50} value of 55 nM.[11] Chaetomellic acid A (**1**) has a high tendency to cyclize and, in fact, it was isolated as anhydride **3**.[10a] The cyclic form, however, is unstable under mild basic conditions (pH 7.5) and is readily hydrolysed to the dicarboxylate anion **2**, the biologically active component (Scheme 1).[10a]

FTase activity of the diacid anion of **1** is noncompetitive towards the acceptor peptide Ras, but is highly competitive with respect to farnesyl pyrophosphate (FPP).[11] This may be explained by the structural similarity between the dicarboxylate anion **2** and FPP, since both possess a hydrophilic head group bound to a hydrophobic tail (Fig. 2).[10a] The maleate unit aligns well with the corresponding diphosphate moiety, since the negatively charged oxygen atoms can achieve a spacing within 0.1 Å,[12] while the difference in the distance between the carboxylic carbons and between the phosphorus atoms is only 0.4 Å.[10a] The flexible nature of the aliphatic chain, instead, permits it to fill the same space, as the hydrophobic end of FPP, upon binding to the enzyme.

Unfortunately **1** did not inhibit Ras processing in *ras*-transformed NIH3T3, perhaps because of poor penetration in these cells or its untimely enzymatic transformation.[11] However, Bach, on studying the capacity of branch-specific inhibitors of the cytosolic isoprenoid pathway (downstream from mevalonic acid) to block cell cycle progression in tobacco BY-2 cells, observed that **ACA** (**1**) behaved like a true cell cycle inhibitor, in that it led to a specific arrest in the cell cycle.[13] Vederas, instead, in an effort to characterize the FPP binding site of rubber transferase showed that **1** is able to inhibit, in vitro, rubber biosynthesis promoted by rubber transferase from *Hevea Brasiliensis*.[14] Besides, the same author found that **1** was also able to reduce the activity of the PBP1b (penicillin-binding protein 1b), albeit with modest potency.[15] Recently, Sabbatini has shown that the inhibition of the Ras/ERK1/2 pathway by **1** resulted in a beneficial effect on acute ischemia-reperfusion injury in rats, preserving either renal function and histology.[16] These results were the consequence of the reduced apoptosis/necrosis of renal cells observed after oxidative stress in rats, as also shown both in human tubular and endothelial cells in culture. **ACA** (**1**) selectively inhibited the membrane-bound Ha-Ras (a pro-apoptotic pathway), since it did not alter the membrane-bound Ki-Ras (an antiapoptotic pathway), nor different prenylated intracellular proteins like Rab.[16] Similar results have been also described in an experimental murine model of ischemic stroke (exocytoxic lesion), in which **ACA** (**1**) administration increased the intracellular concentration of inactive Ha-Ras, significantly reducing the production of superoxide anion and the volume of cerebral necrotic tissue, following the improved survival of hypoxic neuronal cells.[17]

Because of its biological activity[10a,11,13–17] and potential employment as therapeutic agents,[18] chaetomellic acid A (**1**) has been the subject of considerable synthetic efforts. In general, the synthesis of anhydride **3** has been targeted since this compound is easier to manipulate/isolate than diacid **1**. The synthetic strategies investigated can be roughly grouped into two general categories:



Scheme 1. pH dependent equilibrium between the active dianionic open form **2** and the anhydride chaetomellic A (**3**).

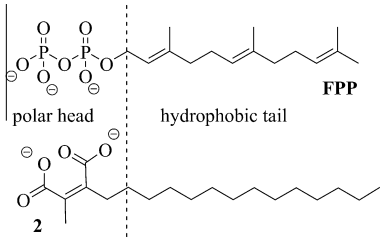


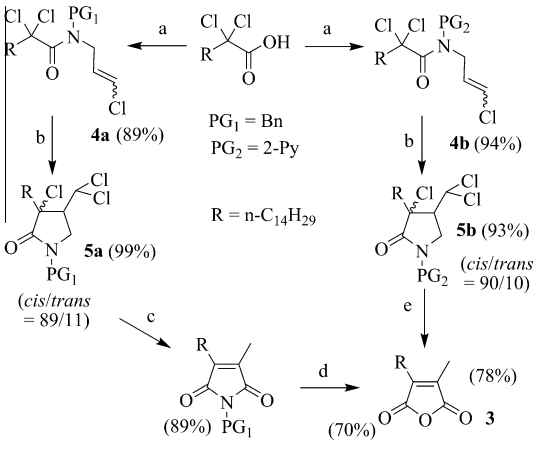
Figure 2. Structural similarity between the dianionic form of chaetomellic acid A **2** and farnesyl pyrophosphate (FPP).

(i) alkylation of maleic precursors[18a,19] and (ii) assembly of the pivotal 1,4-dicarbonyl group.[20]

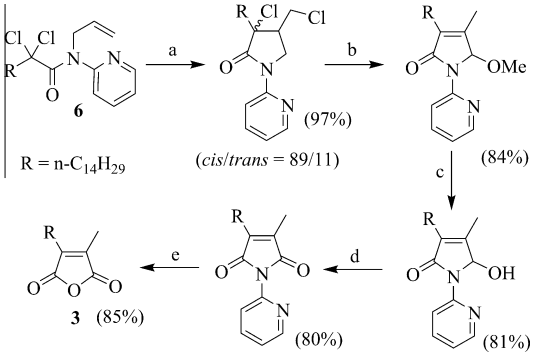
Singh prepared a number of analogues of **1**, characterized by a modified hydrophobic tail (having one C=C double bond or a shorter chain length) or by the replacement of the methyl in the polar head with H, hydroxymethyl or larger groups.[20c] No modified **ACA**, however, showed more potent inhibition than **2** against the recombinant human FTase. Also the groups of Vederas and Poulter synthesized some **ACA** analogues, namely those with R = *n*-$C_{12}H_{25}$, farnesyl, or geranylgeranyl.[19a] These substances were evaluated for inhibition of the yeast FTase and, unlike Singh's observations, the compounds with the shorter tail showed greater activity than **ACA** (**1**).

In spite of the variety of known methods, all of the reported synthetic procedures to **3** suffer from one or more of the following disadvantages: (i) low yields, (ii) costly reagents, (iii) unstable precursors and/or reactants, (iv) harmful solvents, (v) unwieldy protocols, or (vi) lack of versatility.

We tried to overpass these disadvantages and a few years ago devised an interesting way to prepare **3**, where the anhydride moiety was constructed through the atom-transfer radical cyclization (ATRC) of *N*-(benzyl)- or better *N*-(2-pyridyl)-*N*-(3-chloro-2-propenylamino)-2,2-dichlorohexadecanamides **4**, followed by the functional rearrangement (FR) of the intermediate trichloro γ-lactams **5** (Scheme 2).[21a,21b] The same strategy was then applied to *N*-(2-pyridyl)-*N*-allyl-2,2-dichlorohexadecanamide **6** (Scheme 3), the preparation of which uses a more accessible secondary allylamine. The lack of the Cl atom in the allyl moiety, in this case, called for the introduction of an oxidative extra step in the synthetic route.[21c]



Scheme 2. Reagents and conditions: (a) (1) $(COCl)_2$, CH_2Cl_2, DMF, 23 °C, 2 h; (2) *N*-benzyl-3-Cl-2-propenylamine (PG_1 = Bn) or 2-(3-chloro-2-propenylamino)pyridine (PG_2 = 2-Py), Py, 23 °C, 1 h. (b) CuCl-TMEDA, CH_3CN, argon, 60 °C, 20 h. (c) (1) Na^0, CH_3OH/diethyl ether, 25 °C, 20 h; (2) H^+/H_2O. (d) (1) KOH, CH_3OH/THF, reflux, 2 h; (2) H^+/H_2O. (e) (1) Na^0, CH_3OH/diethyl ether, 25 °C, 20 h; (2) H_2SO_4 4 N, 110 °C, 2 h.



Scheme 3. Reagents and conditions: (a) CuCl–TMEDA, toluene, argon, 60 °C, 24 h. (b) Na^0, toluene/MeOH, 25 °C, 24 h. (c) H_2SO_4/H_2O, 110 °C, 3 h. (d) One-pot procedure: (1) Na^0, toluene/MeOH, 25 °C, 24 h; (2) H_2SO_4/H_2O, 110 °C, 3 h. (e) MnO_2, CH_2Cl_2, 25 °C, 20 h. (f) H_2SO_4/H_2O, 110 °C, 4 h.

Both of these procedures have the same two important drawbacks. The former is the use of long chain α-perchlorocarboxylic acids (these starting materials are not very easy to prepare), while the latter is the lack of a perfect control of the ATRC stereoselectivity. This is a critical event for the economy of the process since the FR reaction is viable only in the case of *cis*-lactams,[21b,22] and the *cis*/*trans* ratio of the γ-lactams, delivered by the ATRC step, is acceptable only when these lactams carry a bulky substituent at C-3 and a chloro- or a dichloro-methyl group at C-4. In fact, when we tested the viability of the complementary strategy, in which the methyl substituent is introduced through the acid reagent while the long aliphatic chain is added through the allylamino moiety, the stereoselectivity of the ATRC lactam was disappointing (the dichloro-γ-lactam from the cyclization of *N*-benzyl-*N*-[(*E*)-2-hexenyl)]-2,2-dichloropropanamide was recovered as a 66/34 *cis*/*trans* mixture of four diastereomers).[21c] Since a practical route to functionalized 2,2-dichloro acids appeared not viable, the ATRC-FR approach to chaetomellic acid A analogues lost any appeal.

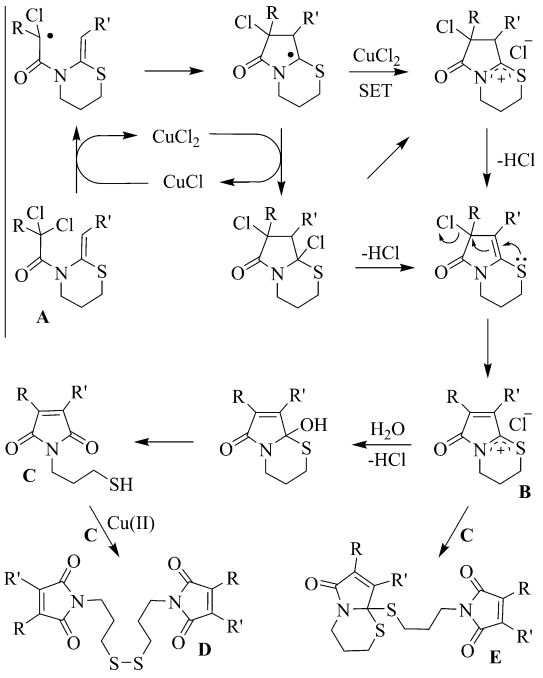
Now as part of a project about the acute kidney injury (AKI) from ischaemia-reperfusion in rat, we were asked to develop a versatile way to chaetomellic acid A (**1**), and analogues, for the prevention of the ischemic damage, through the inhibition of the pathway Ras/ERK1/2. Here we describe the new synthetic method and the serendipitous discovery of an ACA analogue having a higher affinity for the FTase than the natural product.

2. Results and discussion

2.1. Synthesis of ACA

To solve the intrinsic problems of the ATRC-FR paths to maleic anhydrides, we have recently studied the copper catalyzed radical cyclization (RC) of *N*-α-perchloroacyl cyclic ketene-*N*,*X*(X = O, NR, S)-acetals. The RC of *N*-α-perchloroacyl-2-(*Z*)-alkyliden-1,3-thiazinane **A** was shown to be, by far, the most efficient and selective reaction (Scheme 4).[23] The catalytic cycle begins with the formation of a carbamoyl methyl radical, which leads to a cascade of reactions, including a radical polar crossover step, culminating in the formation of the maleimide nucleus (**D** and **E**), or of a direct precursor of it (**E**) (Scheme 4). The typical large prevalence of **E** over **D** likely means that the quenching of the acyliminium cation **B** by **C** is kinetically favoured in comparison with the oxidative homocoupling of **C**.

The disulfide **D** and thioacetal **E**, without being isolated, were subjected to an oxidative treatment to reveal the C=O group, masked as an *S*,*S*-acetal in **E**. The crude products from this step were then hydrolyzed according to Argade's method,[19c] affording



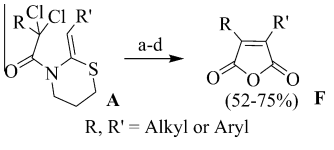
Scheme 4. Possible mechanism for the RC of *N*-α-perchloroacyl six membered cyclic ketene-*N*,*S*-acetals **A**.

the expected anhydrides **F** in a more than acceptable overall yield from the starting enamides **A** (Scheme 5).[23b]

The new process bypasses the stereoselectivity problem of the ATRC step in the ATRC-FR method. Moreover its versatility is no longer reliant on the somewhat exotic functionalized 2,2-dichloroacyl chlorides, but can rely on the more accessible functionalized carboxylic acids. In addition we have recently developed an easy, cheap and efficient preparation of short-chain α-perchloroacyl chlorides, from α-perhalogenation of the corresponding unfunctionalized acyl halides with Cl_2, using tetraalkylammonium chloride as a catalyst.[24]

However, the new method is still unpractical and unsuited for large-scale preparation. The starting enamides **A** are secured through acylation of 2-alkyl-5,6-dihydro-4*H*-1,3-thiazines, substrates that can be obtained following a literature procedure,[25] involving thionation of *N*-(3-hydroxypropyl)-carboxyamides with the Lawesson's reagent (LR). In our hands the preparation of 5,6-dihydro-4*H*-1,3-thiazines was too expensive and characterized by a susceptible and too lengthy work-up; furthermore LR has also a repulsive high equivalent weight. In addition, the deprotection of the thioacetal group in **E** (following the method of Hajipour)[26] was exceedingly long (around 2 d) and used a large amount of solid reagents (Scheme 5, step c).[23]

After a search of literature, looking for a more appropriate method for thionation of amides, our attention was attracted to the use of cheap Berzelius reagent (P_4S_{10}). This reagent is



Scheme 5. Preparation of the 3,4-disubstituted-2,5-furandiones. Reagents and conditions: (a) CuCl (5 mol %), TMEDA (10 mol %), CH_3CN, Na_2CO_3, argon, 17 h, 30 °C; (b) silica–sulfuric acid, $NaNO_3$, SiO_2/H_2O 3:2, CH_2Cl_2, 45 °C, 40 h; (c) KOH, CH_3OH–THF, reflux 2 h; (d) HCl (10%), rt.

characterized by an attractive low equivalent weight,[27] which, when combined with hexamethyldisiloxane (HMDO), issues better (or comparable) results than LR.[27,28] The combination of P_4S_{10}-HMDO is known as the Curphey reagent (CR). HMDO has the function to scavenge the reactive electrophilic polythiophosphates (thionation by-products), before they cause any side reaction.

Thionation of **7** was carried out with the CR in CH_2Cl_2 at reflux (Scheme 6). For an effective transformation, however, the amount of water present in the reaction mixture must be kept low. As **7** is recovered by filtration from water suspensions, it is the major source of humidity. Thus it is recommended to use a dry starting hydroxyamide (e.g. re-crystallized material from diethyl ether). Otherwise the H_2S, which comes from hydrolytic processes, causes the [N,O]-migration of the thioacyl or acyl moieties.[29] The problems associated with the presence of H_2O can be also downsized by working in open vessels, since the volatile H_2S has the chance to escape from the reaction mixture through the condenser.

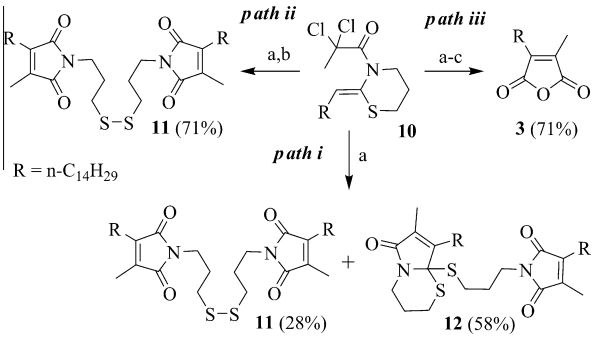
Astonishingly the MS-spectra of the unique peak, observed during the GC-MS analysis of the final reaction mixture, was peculiar of the cyclic thioimidate **9** and not of the expected hydroxythioamide **8**. Soon we realized that this was indeed an instrumental fake. We speculated that the thermal stress, to which **8** was subjected in the injection chamber, resulted in cyclization to **9**.[30] In fact on heating at 150 °C (2–3 h), under vacuum and a slight flow of argon, the crude reaction mixture from the thionation step, after evaporation of the volatiles, gave **9**, also free of the silylated side-products. The intermediate thioimidate is, however, somewhat sensitive to hydrolysis, and cannot be chromatographed on silica-gel. But it was indeed quite clean and was acylated, as such, using 2,2-dichloropropanoyl chloride, affording the *N*-acyl-ketene-*N*,*S*-acetal **10** (a relatively robust molecule, that was purified by chromatography) in high yield (80–90%) (Scheme 6). Because analogues of *N*-acyl-ketene-*N*,*S*-acetals typically have a *Z* configured C=C bond,[23b] the same geometry was assigned to **10**, and to the other enamides we prepared in this work.

Using a reasonably pure sample of **10** (a condition that has to be maintained also with the other enamides we prepared), the radical cyclization proceeded smoothly giving, as expected, the disulfide **11** and the thioacetal **12** (Scheme 7, path i).

The delatentization of the C=O function, hidden as an *S*,*S*-acetal can be generally achieved by heavy metals coordination (such as Hg^{2+} or Cu^{2+}), by alkylation or by oxidative methods.[31] While we were studying a more appropriate protocol than that of Hajipour, we incidentally observed that a crude extract from the RC of **10**, left on the laboratory bench for 3 weeks, contained only the symmetric dimer **11**. Evidently, residual copper and air promoted the oxidative deprotection of **12** and its transformation into **11**. We were unable to repeat this excellent result, but a large conversion of **12** into **11** was always experienced.

To make the deprotection of **12** reproducible and achievable in reasonable times, we considered that an oxidative method based on I_2, a reagent effectively employed for the regeneration of the carbonyl group from *S*,*S*-acetals,[32] could be helpful. Particularly attractive is the possibility to generate iodine (or I^+ reagents) in situ, through the oxidation of the iodide anion.[33] After a number of tests, we found it convenient to add KI (100 mg/5 mmol of **10**) and a few drops of water (5–10/5 mmol of **10**) to the open Schlenk tube, once the RC reaction was completed (Scheme 7, path ii). A possible mechanism for the conversion of **12** into **11** is outlined in Scheme 8. The process is intramolecular, as shown in the scheme, when the '*endo*' sulfur is attacked, otherwise it could be intermolecular.

Finally, the hydrolysis of chromatographed **11**, adapting Argade's protocol, gave the targeted **3a** in good yield (94%).[19c] More appropriately, it was possible to carry out the hydrolytic step directly on the crude extract of **11** (Scheme 7, path iii). In this way, since a purification step is spared, the process gives a higher global yield (71% against 66%).

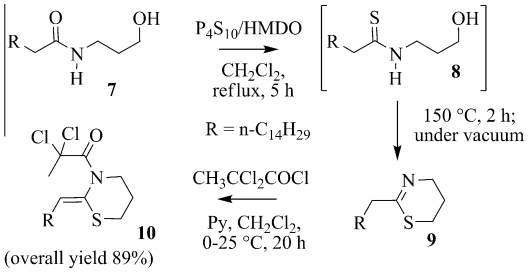


Scheme 7. Reactions of the *N*-2,2-dichloropropanoyl ketene-*N*,*S*-acetal **10**. Reagents and conditions: (a) CuCl (10 mol %), TMEDA (20 mol %), CH_3CN/toluene (3:2), Na_2CO_3, argon, 30 °C, 19–24 h; (b) KI, H_2O, rt, 24 h; (c) NaOH, THF/H_2O, rt, 12 h, acidic work-up.

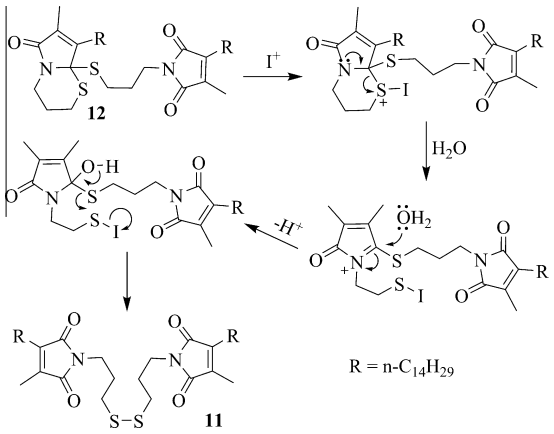
2.2. A route for the synthesis of tailor made ACA analogues

Aiming to develop an intrinsically versatile process, capable of delivering tailor-made **ACA** analogues, we targeted the construction of **18** (Scheme 9), a chaetomellic anhydride with a vinyl group at the end of a shorter aliphatic chain. An intermediate of this type is particularly valuable, since on the terminal $CH{=}CH_2$ one can add thiols through a radical addition. This reaction, also known as thiol–ene coupling, is a useful 'click' methodology.[34]

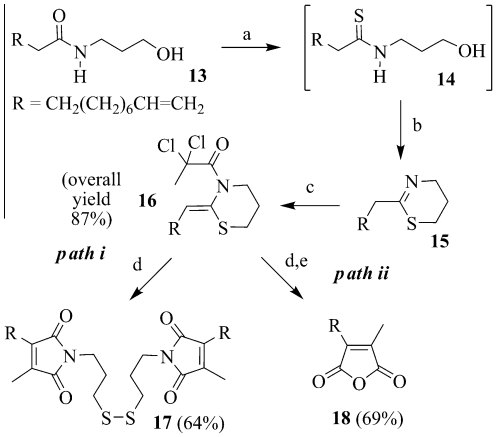
The starting *N*-(3-hydroxypropyl)undec-10-enamide **13** can be easily obtained by manipulation of undecylenic acid, an interesting renewable resource,[35] industrially produced on heating ricinoleic



Scheme 6. Preparation of the *N*-2,2-dichloropropanoyl ketene-*N*,*S*-acetal **10**.



Scheme 8. Possible mechanism for deprotection of the thioacetal moiety in **12**.



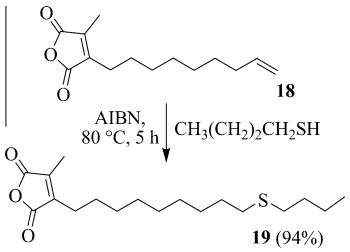
Scheme 9. Preparation of anhydride **18**. Reagents and conditions: (a) P_4S_{10}/HMDO, CH_2Cl_2, reflux, 5 h; (b) 150 °C, 2 h, under vacuum; (c) CH_3CCl_2COCl, Py, CH_2Cl_2, 0–25 °C, 20 h; (d) CuCl (10 mol %), TMEDA (20 mol %), CH_3CN/toluene (3:2), Na_2CO_3, argon, 30 °C, 19–24 h; (e) (i) KI, H_2O, 24 h, (ii) NaOH, THF/H_2O, rt, 12 h, acidic work-up.

acid, the main component of castor oil.[36] Hydroxyamide **13**, treated as usual, gave the enamide **16** in high yield (Scheme 9). Enamide **16** was next subjected to the RC, which, after the addition of KI during the work-up procedure, afforded the symmetric disulfide **17** or, once it was hydrolyzed, the targeted anhydride **18** (Scheme 9, path i and ii, respectively).

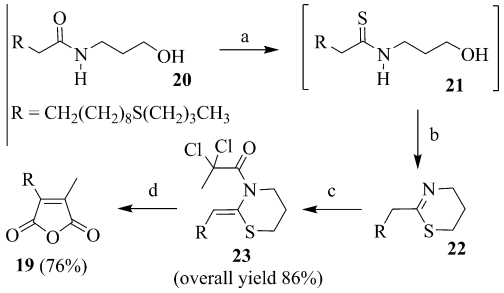
With the anhydride **18** in our hand, we were ready to test the thio-click reaction. We focused on the preparation of **19**, an isosteric **ACA** analogue. Thus, the radical addition of butanethiol to **18** had to be realized. Since anhydride **18** carried two olefinic functions a problem of chemoselectivity could raise. The two C=C bonds are, however, quite different: one is electron-poor and tetrasubstituted, whereas the other is electron rich and monosubstituted. As the thiyl radical is electrophilic[37] and the rate of radical attack controlled by steric and polar factors,[38] we anticipated that attack at the apical methylene carbon should be favored.[34d]

At the beginning we tried the initiation of the radical chain at room temperature, using organoboranes (such as triethylborane or 2-ethylbenzo[*d*][1,3,2]dioxaborole),[39] but procedure and results were unappealing. We then resorted to AIBN, and gratifyingly an almost quantitative addition of butanethiol to the apical C=C was smoothly attained. No involvement of the maleic end, as foreseen, was noted (Scheme 10).

Alternatively **19** was also prepared, always in good yields, from the 11-(butylthio)-*N*-(3-hydroxypropyl)-undecanamide **20** (Scheme 11), by bringing forward the thiol–ene coupling on the starting hydroxyamide **13**. It is noted that the thioether function in the aliphatic chain survived the oxidative deprotection of the thioacetal intermediate (not shown), and was not converted into a sulfoxide group.



Scheme 10. Radical addition of butanethiol to the terminal C=C group of **18**.



Scheme 11. Preparation of anhydride **19**. Reagents and conditions: (a) P_4S_{10}/HMDO, CH_2Cl_2, reflux, 5 h; (b) 150 °C, 2 h, under vacuum; (c) CH_3CCl_2COCl, Py, CH_2Cl_2, 0–25 °C, 20 h; (d) (i) CuCl (10 mol %), TMEDA (20 mol %), CH_3CN/toluene (3:2), Na_2CO_3, argon, 30 °C, 19–24 h, (ii) KI, H_2O, rt, 24 h, (iii) NaOH, THF/H_2O, rt, 12 h, acidic work-up.

2.3. FTase assays

Taking in mind the previously pointed out pharmacological activity of ACA, we then examined the efficacy of the new chaetomellic analogue against the FTase, to see if the replacement of the tenth CH_2 in the aliphatic tail with sulfur hadn't impaired the inhibition power. This required the preliminary conversion of the anhydride **19** into the corresponding disodium salt **27** (Fig. 3). The conversion was efficiently achieved in four easy steps: (i) reaction of the anhydride with a stoichiometric amount of NaOH in THF/H_2O, (ii) evaporation of the solution under vacuum, (iii) re-dissolution of the remaining material in water, and finally (iv) freeze-drying.[19a] Using this procedure we also prepared the sodium maleates **2** and **26**, from the anhydrides **3** and **18** secured in this work, and **28**, from 3-hexadecyl-4-methylfuran-2,5-dione[21c] that we had in stock (Fig. 3).

These substances were evaluated for inhibition against yeast and rat FTases, using the continuous fluorescence assay.[40] In both assays we used the same dansylpeptide (dansyl-Gly-Cys-Val-Ile-Ala). As far as we know, there is no reported IC_{50} value with dansyl-GCVIA for inhibition of rat FTase. For example, peptidomimetic inhibitors were tested with dansyl-Gly-Cys-Val-Leu-Ser for inhibition of rat FTase, showing submicromolar activities.[41] We believe that there is no major difference between dansyl-GCVIA and dansyl-GCVLS as a peptide substrate for inhibition of rat FTase. Since FTases are promiscuous with regard to the CAAX box, the exact sequence should not be a problem. Indeed Singh tried the same Ras-CVLS with both FTases, and he did not raise any doubt about the results of his tests.[20c] Interestingly Fierke, during his study on substrate recognition by wild FTase,[42] observed that dansyl-GCVIA and dansyl-GCVLS were similarly recognized, respectively: $k_{cat}/K_M^{peptide}$ 120 and 170 ($mM^{-1}\ s^{-1}$), and $k_{cat} > 0.2$ and 0.4 (s^{-1}). In our IC_{50} measurements, the concentration of rat FTase was 10 times increased (15 nM against 1.5 nM) to compensate a possible lower affinity of dansyl-GCVIA for the enzyme and

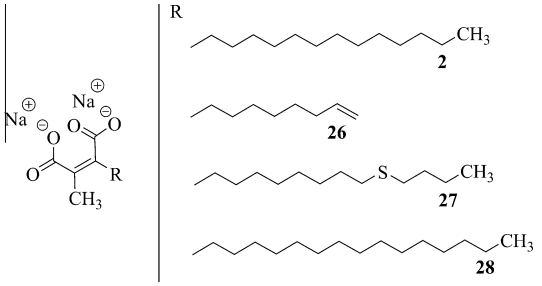


Figure 3. Sodium maleates used in the FTase assays.

Table 1
Inhibition of yeast and rat FTases by **ACA** and analogues[a]

No.	Inhibitor	Yeast FTase IC_{50} (μM ± SD)	Rat FTase IC_{50} (μM ± SD)
1	**2**	16.7 ± 0.9 (17 ± 3)[18a]	0.91 ± 0.08
2	**26**	13.4 ± 1.1	0.49 ± 0.05
3	**27**	3.5 ± 0.3	0.19 ± 0.01
4	**28**	65.6 ± 16.7	2.6 ± 0.3

[a] For conditions see 'FTase assays' in the Section 4.

to increase the rate at which the bound substrate is converted to product. The IC_{50} values are summarized in Table 1.

The value we determined for the sodium salt of chaetomellic acid A (**2**) against yeast FTase, compared very favorably to that previously measured (Table 1, no. 1).[19a] Astonishingly the thia-analogue **27** was approximately 5 times more potent than **ACA** (Table 1 no. 1 and 3), showing an IC_{50} value (3.5 μM) near that of chaetomellic acid C **29** (4 μM) and of the farnesylated **ACA** analogue **30** (2.4 μM) (Fig. 4).[19a] This result confirms that it is unnecessary to retain the hydrophobic farnesyl group for having potent FPP analogues inhibitors, contrary to what was previously claimed.[43] Since FPP has more affinity for mammalian FTases,[20c] inhibition, as expected,[20c] was better against the rat Ftase (Table 1); above all, this second round of assays stressed the same order of activity between the four sodium maleates tested.

The size of the tail appears critical,[20c] in fact with chains ⩾16 the inhibition power collapses (Table 1, no. 4). Unexpectedly the intermediate **26**, notwithstanding it shorter C-9 chain (which, however, implement a terminal vinyl group), gave an IC_{50} at least analogous to that of the natural inhibitor **2**.

2.4. Molecular modelling

A posteriori computational analysis has been carried out on the interaction of thia-analogue **27** with FTase in order to get insights into its moderate increased inhibition potency with respect to the parent compound **2**.

After an extensive analysis of the X-ray structures of FTase available in the PDB data bank, the X-ray structure of rat FTase complexed with farnesyl pyrophosphate (FPP) (PDB ref code 1FT2)[44] and of the ternary complex in which the rat FTase interacts with the FPT-II FPP analog and the substrate peptide CVLS (PDB ref code 1TN8)[45] were selected. Superposition of the two 3D structures by alignment of all enzyme Cα atoms shows that the structures of the enzyme in these complexes are essentially identical, and the location and conformation of the isoprenoid and nonreactive isoprenoid analogs are very similar.

In fact, only a few minor side chain rearrangements are observed in the proximity of the anionic head binding sites of the isoprenoid analogs, and of the C-terminal carboxylate residues of the CVLS peptide.

The choice of the conformation of the thia-analogue **27** (among the many low-energy quasi-extended conformations it can assume) to be considered for docking experiments was based on: (a) the best alignment with the isoprenoid analogs, taken as references; (Fig. 5a and b) the best fit of the molecular volume of **27** and the volume of the supermolecule formed by FPP and FPT-II FPP, which can be considered to reflect the overall shape and the conformational flexibility of the enzyme binding site (Fig. 5b).

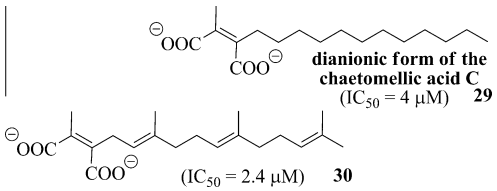


Figure 4. Structure of the **ACA** analogues **29** and **30**, and their IC_{50} values against yeast FTase.[19a]

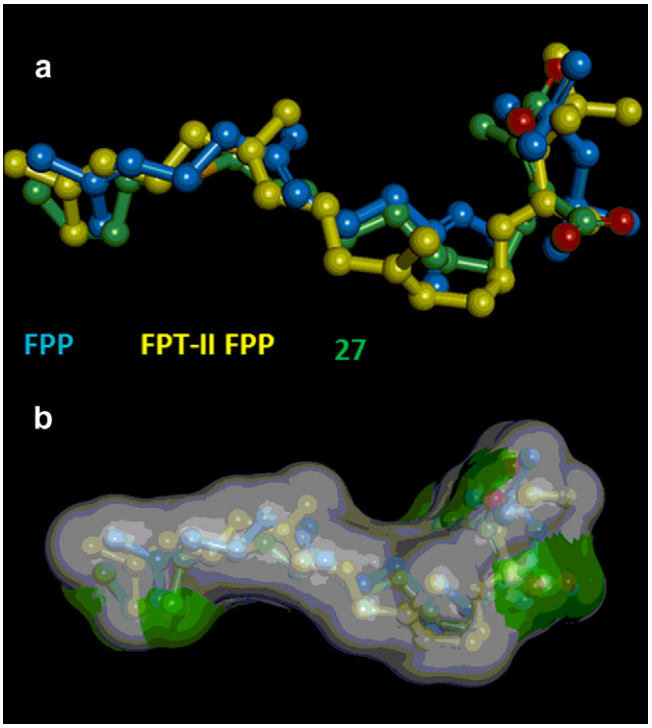


Figure 5. (a) Alignment of FPP, in the conformation assumed in the 1FT2 pdb structure (blue), FPT-II FPP, in the conformation assumed in the 1TN8 pdb structure (yellow), and of the thia-analogue **27** in the quasi-extended conformation chosen (atom colors: carbon atoms are in green, oxygen atoms in red, and sulfur atom in orange). (b) Superposition of the molecular volume of **27** (green) and the volume of the supermolecule (white) formed by FPP and FPT-II FPP. In the figure the hydrogen atoms are omitted for clarity.

The structural motif of hydrophilic head group of **27** is well accommodated into the highly positively charged pocket, located near the subunit interface and adjacent to the catalytic zinc ion, which constitutes the site of the diphosphate moiety of farnesyl diphosphate (FPP) in the crystal structures of the binary and ternary complexes.[7c,44,46] This pocket is formed by amino acid residues K164, Y200, and H201 from the α-subunit of the enzyme and Y300, K294, R291, H248 from the β-subunit (Fig. 6, top).

The hydrophobic tail of inhibitor **27** perfectly fits the length (14 Å) of the hydrophobic funnel-shaped groove of the enzyme, extended along one side of the cavity and it interacts with a number of conserved aromatic residues (Fig. 6, bottom and inset). These are: Y154, W102, and Y205 of the β-subunit; they are located at the bottom of the hydrophobic cavity and are considered to constitute the discriminants for the maximum length of the ligands,[44] C254, G250, Y251, W303, Y361 and the aliphatic portion of the side-chain of R202 of the β-subunit; Y166, H201, and aliphatic portion of the side-chain of K164 of the α-subunit.

The CVLS peptide sandwiches compound **27** against the wall of the hydrophobic cavity, sequestering the 20% of the solvent accessible surface, still available after the establishment of the binary complex, from the solvent permeating the cavity (Fig. 6). A direct van der Waals contact between the Leu residue of the CVLS peptide and the aliphatic tail of **27** is observed. It is worth noting that the same interaction is achieved by Ile, uponmutation of the CAAX peptide used in the docking experiments into the one used in the experimental measurements (CVIA).

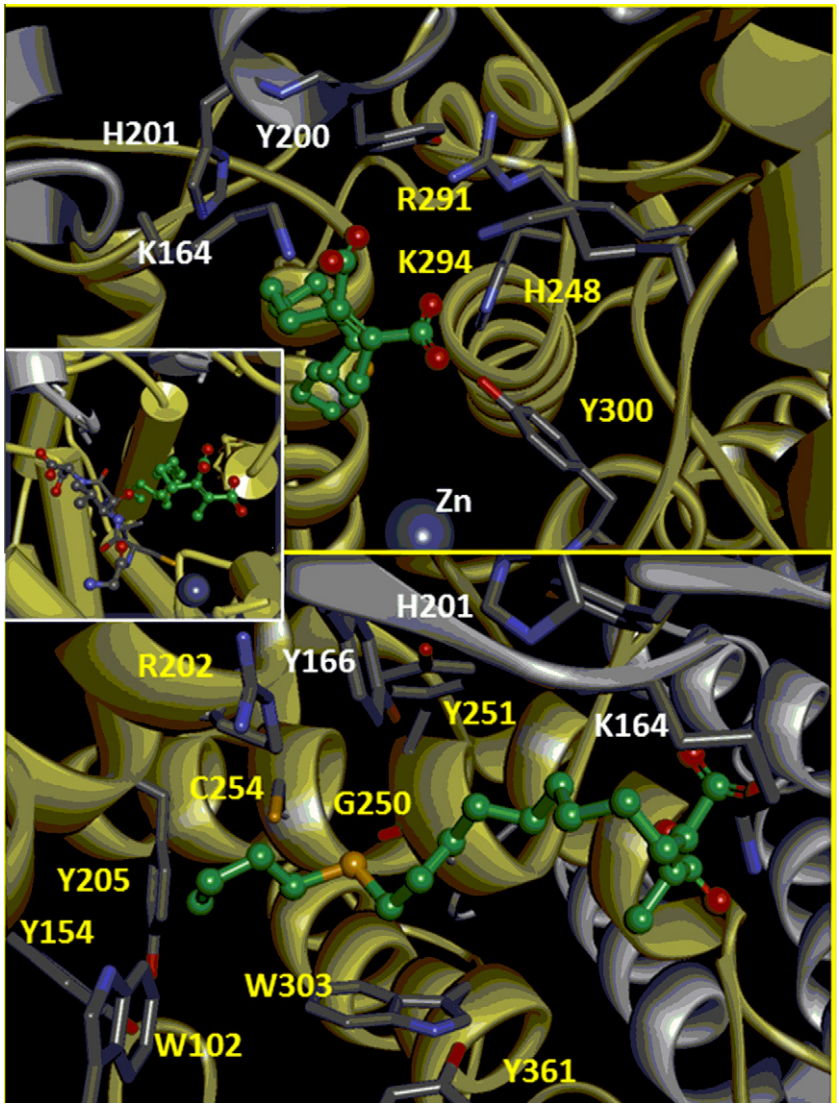


Figure 6. The interaction of inhibitor **27** and the FTase binding site. The enzyme α-subunit is represented in grey, the β-subunit is represented in yellow. Aminoacid residues involved in the interations are colored by element type (grey: carbon, blue: nitrogen, red: oxygen). Compound **27** is represented according to the color code of Figure 5. The hydrogen atoms are not displayed for clarity. Top: focus on the interactions of the anionic head of **27** with the FTase binding site; bottom: focus on the interactions established by the hydrophobic tail of compound **27**; inset: an overall view of the ternary complex FTase-**27**-peptide (CVLS).

Interestingly, the sulfur atom in the aliphatic chain is found surrounded by three aromatic residues (W303, Y251, and Y156), R202, C254, and in direct contact with the oxygen atom of the backbone chain of G250. Thus, the high versatility of the divalent sulfur atom towards both electron-rich and electron-poor atoms is perfectly satisfied in this environment.[47] In particular, the clear orientation preference for sulfur relative to oxygen atom of G250 could be a reason of the improved inhibitory power observed with respect to the parent ligand (compound **2**) and it is worth of further investigations.

3. Conclusion

In this article we have described a new route to chaetomellic acid A (**1**). The process implements our recent method for the preparation of maleic anhydrides, which is based on the copper catalyzed radical cyclization of *N*-α-perchloroacyl-2-(*Z*)-alkyliden-1,3-tiazinanes. To make the process more appealing two critical adjustments had to be introduced: (i) the preparation of the intermediate enamide was realized with a practical, cheap and efficient one-pot procedure, thanks to the use of the Curphey reagent in the thionation step, and (ii) the required deprotection of the intermediate thioacetal was easily achieved through the addition of KI, during the work-up procedure of the radical cyclization reaction. Remarkably, conversion of the starting *N*-(3-hydroxypropyl)palmitamide into **ACA** (**1**) entailed only one intermediate chromatographic purification step.

Since the process uses carboxylic acid as starting materials, this allowed us to develop a versatile approach to the preparation of **ACA** analogues, through the synthesis of an intermediate maleic anhydride with a vinylic group at the end of the aliphatic tail, a function that can be transformed through a thiol–ene coupling. Alternatively, the same thia-analogue can be also efficiently secured, by bringing forward the thio-click reaction on the starting *N*-(3-hydroxypropyl)-unsaturated amide. This more rigid alternative well matches with the large-scale preparation of ACA analogues, as it avoids the use of a valuable maleic anhydride intermediate.

Finally, we serendipitously observed that the sodium salt **27**, prepared from the representative sulfurated anhydride **19**, was a

more competent FTase inhibitor than **ACA** (**1**). On the ground of a molecular modeling study we attributed the improved inhibitory power of **27** to the direct contact of the sulfur atom with the oxygen atom of the backbone chain of G250. This result and the capability to assemble tailor made thia-analogues of **ACA** favorably meet and will be further investigated to identify competent molecules for the prevention of the ischemic damage.

4. Experimental part

4.1. General

Reagents and solvents were standard grade commercial products, purchased from Aldrich or Acros, and generally used without further purification. CH_2Cl_2 was dried over 3 Å sieves (5% w/v). Hexamethyldisiloxane was commercial or obtained following a literature procedure.[48] 2,2-Dicloropropanoyl chloride was prepared by chlorination with Cl_2 of propionyl chloride in the presence of tetrabutylammonium chloride.[24] The starting *N*-(3-hydroxypropyl)palmitamide[49] **7** and *N*-(3-hydroxypropyl)undec-10-enamide[50] **13** were secured in high yields (on the scale of 100–200 g) from the corresponding acids, after methylation and ensuing amino-de-methoxylation with 3-aminopropanol. The substitution is helped by the addition of K_2CO_3 10 mol % and of THF to solubilize the mixture of reagents (heating at reflux was required with methyl palmitate).[29,51] The 11-(butylthio)-*N*-(3-hydroxypropyl)undecanamide **20** was prepared in quantitative yield by standard radical addition of butanethiol to **13**, using AIBN as initiator.[34d,52] The silica gels used for flash chromatography was Silica Gel 60 Merck 0.040–0.063 mm. TLC were performed on silica coated plates Merck 60 F_{254}, using UV light (254 nM) or cerium molybdate solutions to visualize the spots.

^{1}H NMR and ^{13}C NMR spectra were recorded on 'Varian 500 MHz' or Jeol GSX 400. ^{13}C NMR were obtained with full proton decoupling. ^{1}H NMR and ^{13}C NMR signals attribution was based on gradient-enhanced ^{1}H,^{1}H-DQF-COSY, ^{1}H,^{13}C-Edited-HSQC, and ^{1}H,^{13}C-HMBC experiments, run with standard pulses programmes. IR spectra were recorded on a 'FT-IR Perkin Elmer 1600 Series' while MS spectra on a 'HP G1800C GCD System Series II'. High resolution mass spectra (HRMS) were obtained on an 'Agilent 6520 Accurate-Mass Q-TOF LC/MS' and ESI-MS were run on a Bruker Esquire 4000 instrument.

Recombinant yeast FTase was produced in *Escherichia coli* and purified over HisTrap affinity column (HisTrap™ HP, GE Healthcare) as previously described.[53] Rat FTase was purchased from Jena Bioscience (Germany). *n*-Dodecyl-β-D-maltoside was purchased from ACROS (Geel, Belgium) and dansyl-Gly-Cys-Val-Ile-Ala was synthesized from HSC Core Research Facilities at University of Utah. Fluorescent signals for yeast FTase assays were recorded on a SpexFluoroMax spectrofluorimeter (Jobin Yuan Spex, Edison, NJ).

4.2. Preparation of ACA and analogues

4.2.1. Preparation of (*Z*)-3-(2,2-dichloropropanoyl)-2-pentadecylidene-1,3-thiazinane (10)

In a one necked 100 mL round bottom flask, fitted with a condenser, dry *N*-(3-hydroxypropyl)palmitamide **7** (20.0 mmol, 6.27 g) and P_4S_{10} (3.68 mmol, 1.63 g) were weighed. Then dry CH_2Cl_2 (20 mL) and hexamethyldisiloxane (33.4 mmol, 7.16 mL) were added. The mixture, under stirring, was than heated at reflux (5 h). Afterwards the solvent was evaporated, and the remaining oil was stirred at 150 °C (2–3 h) under vacuum (8–9 mm Hg), while argon, delivered through a capillary, bubbled inside the liquid (this helps the removal of silylphosphate, issued as side-product). Next the crude 2-pentadecyl-5,6-dihydro-4*H*-1,3-thiazine **9** was dissolved in dry CH_2Cl_2 (15 mL) and pyridine (50 mmol, 4.03 mL) added. The stirred solution was then cooled at 0 °C (ice bath) and 2,2-dichloropropanoyl chloride (30 mmol, 4,84 g), diluted in CH_2Cl_2 (5 mL), was slowly dropped (10 min), through a dropping funnel. Subsequently the ice-bath was removed. The mixture was thermostatted at 25 °C (20 h), afterwards it was diluted with water (40 mL). When all the solid, which developed during the acylation, was dissolved in the added water, the mixture was poured in a separation funnel, further diluted with other water (60 mL) and extracted with CH_2Cl_2 (4 × 20 mL) (the separation of the phases is rather slow). The organic extracts were collected and evaporated. The crude mixture was purified by flash-chromatography on silica gel, eluting with a petroleum ether (PE, bp 40–70 °C)/diethyl ether (Et_2O) gradient (from 100/0 to 90/10). The enamide **10** was recovered as a yellow oil (7.77 g, yield 89%); [HRMS found 436.2198. $C_{22}H_{40}Cl_2NOS$ $(M+H)^+$ requires 436.2202]; R_f (95% PE/Et_2O) 0.55; ν_{max} (neat) 2924, 2853, 1663 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.89 (t, *J* 7.1 Hz, 3H, C*H*$_3$CH$_2$), 1.27 (br s, 20H, (CH$_2$)$_{10}$), 1.35 (m, 2H, CH$_2$), 1.44 (m, 2H, C*H*$_2$CH$_2$CH=C), 2.11 (br, 2H, SCH$_2$C*H*$_2$CH$_2$N), 2.29 (m, 2H, C*H*$_2$CH=C), 2.34 (s, 3H, CH$_3$C), 2.87 (br s, 2H, CH$_2$S), 4.17 (br, 2H, CH$_2$N), 6.10 (br, 1H, CH=C); δ_C (125 MHz, $CDCl_3$) 14.1, 22.6, 26.3 (broad), 28.6 (broad), 28.9, 29.3, 29.35, 29.4, 29.6, 29.65 (4 overlapped CH$_2$), 29.7, 31.9, 36.9, 50.1, 80.2, 131.6 (broad and weak, C=), 136.2 (broad and weak, CH=), 163.4; ESI-MS: 436.6 $[M+H]^+$.

4.2.2. Radical cyclization of (*Z*)-3-(2,2-dichloropropanoyl)-2-pentadecylidene-1,3-thiazinane (10)

CuCl (0.42 mmol, 0.042 g), Na_2CO_3 (4.6 mmol, 0.488 g) and the substrate **10** (4.2 mmol, 1.84 g) were weighed into an oven dried Schlenk tube, then CH_3CN/toluene 1:1 (6.3 mL) and TMEDA (0.2 mmol, 126 μL) were added under argon. The mixture was stirred at 30 °C and after 24 h diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were concentrated under vacuum. Flash chromatography of the recovered material on silica gel, eluting with a PE/Et_2O gradient (from 100/0 to 20/80) gave the disulfide **11** (0.45 g, 28%) as a white wax, [HRMS found 761.5324. $C_{44}H_{77}N_2O_4S_2$ $(M+H)^+$ requires 761,5319], R_f (60% PE/Et_2O) 0.42, and the thioacetal **12** (0.92 g, 58%) as a white solid [HRMS found 745.5380. $C_{44}H_{77}N_2O_3S_2$ $(M+H)^+$ requires 745,5370], R_f (60% PE/Et_2O) 0.20, mp 63–64 °C.

4.2.2.1. 1,1′-[3,3′-Disulfanediylbis(propane-3,1-diyl)]bis(3-methyl-4-tetradecyl-1*H*-pyrrole-2,5-dione) (11). ν_{max} (Nujol) 1768 and 1708 cm^{-1}; δ_H (500 MHz, $CDCl_3$): 0.88 (t, *J* 7.0 Hz, 6H, C*H*$_3$CH$_2$), 1.26 (br s, 36H, 2 × (CH$_2$)$_9$), 1.29 (8H, (CH$_2$)$_4$), 1.52 (bm, 4H, 2 × C*H*$_2$CH$_2$C=C), 1.96 (s, 6H, 2 × CH$_3$), 1.97 (quint, *J* 6.9 Hz, 4H, 2 × SCH$_2$C*H*$_2$CH$_2$N), 2.36 (t, *J* 7.3 Hz, 4H, 2 × CH$_2$-C=C), 2.68 (t, *J* 6.9 Hz, 4H, 2 × CH$_2$S), 3.57 (t, *J* 6.9 Hz, 4H, 2 × CH$_2$N); δ_C (125 MHz, $CDCl_3$): 8.5, 13.9, 22.5, 23.5, 28.0, 28.1, 29.1, 29.2, 29.35, 29.40, 29.5, 31.8, 35.4, 36.5, 136.9, 141.2, 171.9, 172.2; ESI-MS: 783.8 $[M+Na]^+$.

4.2.2.2. 3-Methyl-1-{3-(8-methyl-6-oxo-7-tetradecyl-3,4,6,8*a*-tetrahydro-2*H*-pyrrolo[2,1-*b*][1,3]thiazin-8*a*-ylthio)propyl}-4-tetradecyl-1*H*-pyrrole-2,5-dione (12). ν_{max} (neat) 1706, 1681 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 0.89 (2t, *J* 7.0 Hz each, 6H, 2 × C*H*$_3$CH$_2$), 1.26 (br, 43H), 1.38 (m, 2H, CH$_2$), 1.51 (m, 2H, CH$_2$), 1.60 (m, 2H, C*H*$_{ax}$H+CH), 1.65 (quint, *J* 7.0 Hz, 2H, CH$_2$C*H*$_2$CH$_2$), 1.83 (t, *J* 7.0 Hz, 2H, CH$_2$S), 1.91 (s, 3H, CH$_3$), 1.96 (s, 3H, CH$_3$), 1.99 (bdt, 1H, C*H*$_{eq}$H), 2.37 (m, 3H, CH$_2$-C=C+C*H*H-C=C), 2.49 (m, 1H, CH*H*-C=C), 2.67 (dt, *J* 13.2, 1.3 Hz, 1H, C*H*$_{eq}$HS), 3.08 (dt, *J* 13.2, 3.0 Hz, 1H, C*H*$_{ax}$HN,), 3.34 (dt, *J* 13.2, 1.3 Hz, 1H, C*H*$_{ax}$HS), 3.45 (m, 2H, CH$_2$N), 4.30 (br dt, *J* 13.2, 1.3 Hz, 1H, C*H*$_{eq}$HN); δ_C (125 MHz, $CDCl_3$) 8.66, 8.98, 14.10, 22.67, 23.69, 25.75, 25.88, 26.80, 27.80, 27.82, 28.20, 28.83, 29.26, 29.33, 29.34, 29.35,

29.50, 29.54, 29.56, 29.62, 29.63, 29.65, 29.67, 29.68, 30.14, 31.90, 31.91, 35.71, 37.04, 74.37, 129.69, 136.93, 141.20, 154.38, 168.12, 171.81, 172.10; ESI-MS: 767.8 $[M+Na]^+$.

4.2.3. Preparation of 1,1′-[3,3′-disulfanediylbis(propane-3,1-diyl)]bis(3-methyl-4-tetradecyl-1*H*-pyrrole-2,5-dione) (11)

CuCl (0.50 mmol, 0.050 g), Na_2CO_3 (5.5 mmol, 0.590 g) and the substrate **10** (5.0 mmol, 2.19 g) were weighed into an oven dried Schlenk tube, then CH_3CN/toluene 3:2 (5 mL) and TMEDA (0.2 mmol, 151 μL) were added under argon. The mixture was stirred at 30 °C and after 19 h the tube was open and KI (100 mg) and a few drops of water were added. The mixture was vigorously stirred in the open air for 24 h, after which it was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were concentrated under vacuum. Flash chromatography of the recovered material on silica gel, eluting with a PE/Et_2O gradient (from 100/0 to 40/60) gave the disulfide **11** (1.35 g, 71%).

4.2.4. Hydrolysis of 1,1′-(3,3′-disulfanediylbis(propane-3,1-diyl))bis(3-methyl-4-tetradecyl-1*H*-pyrrole-2,5-dione) (11)

In a one-necked 25 mL Erlenmeyer flask **11** (2.5 mmol, 1.903 g), THF (2.5 mL) and a solution of NaOH 5 M (2.5 mL) were added. The reaction mixture was stirred for 12 h, after which it was treated with HCl 36% (2 mL), diluted with brine/water 1:1 and extracted with CH_2Cl_2 Flash chromatography of the recovered material on silica gel, eluting with a PE/Et_2O gradient (from 100/0 to 20/80) gave the chaetomellic anhydride A **3** (1.45 g, 94%); spectroscopic data are in agreement with those reported in the literature.[21b]

4.2.5. Preparation of chaetomellic anhydride A (3)

CuCl (2.00 mmol, 0.200 g), Na_2CO_3 (22.0 mmol, 2.332 g) and the substrate **10** (20.0 mmol, 8.73 g) were weighed into an oven dried Schlenk tube, then CH_3CN/toluene 3:2 (20 mL) and TMEDA (4.0 mmol, 604 μL) were added under argon. The mixture was stirred at 30 °C and after 19 h the tube was open and KI (400 mg) and a few drops of water were added. The mixture was vigorously stirred in the open air for 24 h, after which it was diluted with water (50 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were concentrated under vacuum. The recovered material was diluted with THF (10 mL). Next a solution of NaOH 5 M (10 mL) was added. The reaction mixture was stirred for 12 h, after which it was treated with HCl 36% (8 mL), diluted with brine/water 1:1 and extracted with CH_2Cl_2. Flash chromatography of the recovered material on silica gel, eluting with a PE/Et_2O gradient (from 100/0 to 20/80) gave the chaetomellic anhydride A **3** (4.40 g, 71%).

4.2.6. Preparation of (*Z*)-3-(2,2-dichloropropanoyl)-2-(dec-9-enylidene)-1,3-thiazinane (16)

Following the same procedure used to prepare **10**, *N*-(3-hydroxypropyl)undec-10-enamide **13** (40 mmol, 9.66 g) gave **16** as a yellow oil (12.69 g, yield 87%); [HRMS found 364.1240. $C_{17}H_{28}Cl_2NOS$ $(M+H)^+$ requires 364.1263]; R_f (95% PE/Et_2O) 0.50; ν_{max} (neat) 3075, 2925, 2854 and 1664 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 1.32, 1.38, 1.44 (overlapped multiplets, 10H, $(CH_2)_5$), 2.05 (dq, 2H, *J* 7.1, 1.2 Hz, CH_2CH=CH_2), 2.11 (br s, 2H, $SCH_2CH_2CH_2N$), 2.29 (q, 2H, *J* 7.5 Hz, CH_2CH=CNS), 2.34 (s, 3H, CH_3), 2.87 (br s, 2H, CH_2S), 4.17 (br s, 2H, CH_2N), 4.94 (part A of an AMXY2 system, J_{cis} 10.2, 2.1, 1.2 Hz, 1H, CH*H*=), 5.00 (part M of an AMXY2 system, J_{trans} 16.9, J_{gem} 2.1, $^{1,4}J$ 1.2 Hz, 1H, CH*H*=), 5.82 (part X of an AMXY2, J_{trans} 16.9, J_{cis} 10.2, ^3J 6.8 Hz, 1H, C*H*=CH_2), 6.21 (broad, 1H, CH=CNS); δ_C (125 MHz, $CDCl_3$) 26.1, 28.3, 28.7, 28.8 (2 overlapped CH_2), 28.9, 29.0, 29.6, 33.5, 36.7, 50.0, 80.1, 114.1, 131.6 (broad and weak, C=) 135.0 (s, broad and weak, *C*H=C), 139.1, 163.5; ESI-MS: 364.5 $[M+H]^+$, 386.4 $[M+Na]^+$.

4.2.7. Preparation of 1,1′-(3,3′-disulfanediylbis(propane-3,1-diyl))bis(3-methyl-4-(non-8-enyl)-1*H*-pyrrole-2,5-dione) (17)

Following the same procedure used to prepare **11**, (*Z*)-3-(2,2-dichloropropanoyl)-2-(dec-9-enylidene)-1,3-thiazinane **16** (5.0 mmol, 1.83 g) gave **17** as a pale yellow oil (0.99 g, 64%); [HRMS found 617.3450. $C_{34}H_{53}N_2O_4S_2$ $(M+H)^+$ requires 617.3441]; R_f (70% PE/Et_2O) 0.52; ν_{max} (neat) 3455, 3074, 1768, 1703, 1640 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.28 (br s, 12H, 2 × $(CH_2)_3$), 1.35 (4H), 1.50 (bm, 4H, 2 × CH_2CH_2C=C), 1.94 (s, 6H, 2 × CH_3), 1.94 (m, overlapped, 4H, 2 × $SCH_2CH_2CH_2N$), 2.02 (br q, *J* 7.9 Hz, 4H, 2 × CH_2CH=C), 2.35 (t, *J* 7.7, 4H, 2 × CH_2C=C), 2.62 (t, *J* 7.3 Hz, 4H, 2 × CH_2S), 3.55 (t, *J* 7.3 Hz, 4H, 2 × CH_2N), 4.91 (part A of an AMXY2 system, J_{cis} 10.2, J_{gem} 2.1, $^{1,4}J$ 1.2 Hz, 2H, CH*H*=), 4.97 (ddq, part M of an AMXY2 system, J_{trans} 16.9, J_{gem} 2.1, $^{1,4}J$ 1.2 Hz, 2H, 2 × C*H*H=), 5.78 (part X of an AMXY2, J_{trans} 16.9, J_{cis} 10.3, J_{XY} 6.8 Hz, 2H, 2 × CH=C); δ_C (100.1 MHz, $CDCl_3$) 8.7, 23.6, 28.1, 28.2, 28.8, 28.9, 29.1, 29.4, 33.7, 36.0, 36.6, 114.2, 136.9, 139.0, 141.1, 171.9, 172.2; ESI-MS: 639.1 $[M+Na]^+$.

4.2.8. Preparation of 3-methyl-4-(non-8-enyl)furan-2,5-dione (18)

Following the same procedure used to prepare **3**, (*Z*)-3-(2,2-dichloropropanoyl)-2-(dec-9-enylidene)-1,3-thiazinane **16** (20 mmol, 7.29 g) gave **18**, as a pale yellow oil (3.26 g, overall yield 69%); [HRMS found 237.1465. $C_{14}H_{21}O_3$ $(M+H)^+$ requires 237.1485]; R_f (90% PE/Et_2O) 0.30. ν_{max} (neat) 3076, 1853, 1821 (w), 1767 (str), 1673 (w) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.28 (br s, 6H, $(CH_2)_3$), 1.35 (2H, CH_2), 1.56 (bm, 2H, CH_2), 2.01 (m, 2H, CH_2CH=C), 2.05 (s, 3H, CH_3), 2.43 (t, *J* 7.9, 2H, CH_2C=C), 4.91 (part A of an AMXY2 system, J_{cis} 10.2, J_{gem} 2.1, $^{1,4}J$ 1.2 Hz, 1H, CH_2=), 4.97 (ddq, part M of an AMXY2 system, J_{trans} 17.8, J_{gem} 2.1, $^{1,4}J$ 1.2 Hz, 1H, CH_2=), 5.78 (part X of an AMXY2, J_{trans} 17.8, J_{cis} 10.3, J_{XY} 6.8 Hz, 1H, CH=C); δ_C (100.1 MHz, $CDCl_3$) 9.5, 24.4, 27.5, 28.7, 28.8, 29.0, 29.3, 33.6, 114.2, 138.9, 140.4, 144.7, 165.8, 166.2; *m*/*z* (EI, 70 eV) 236 (2, M^+), 191 (100), 163 (21), 126 (97%).

4.2.9. Addition of butane-1-thiol to 3-methyl-4-(non-8-enyl)furan-2,5-dione (18)

AIBN (0.2 mmol, 0.033 g) and anhydride **18** (10.0 mmol, 2.36 g) were weighed into an oven dried Schlenk tube, then 1-butanethiol (20.0 mmol, 2.1 mL) were added under argon. The mixture was stirred at 80 °C (5 h). Next the unreacted thiol was evaporated under vacuum. Flash chromatography of the recovered material on silica gel, eluting with a PE/Et_2O gradient (from 100/0 to 20/80) gave **19**, as a pale yellow oil (3.07 g, 94%); [HRMS found 327.1980. $C_{18}H_{31}O_3S$ $(M+H)^+$ requires 327.1989]; R_f (90% PE/Et_2O) 0.26); ν_{max} (neat) 1855, 1821, 1766 (str), 1673 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.89 (t, *J* 7.5 Hz, 3H, CH_3CH_2), 1.28 (bm, 8H, CH_2), 1.37 (m, 4H, 2 × CH_2) 1.54 (m, 6H, 3 × CH_2), 2.04 (s, 3H, CH_3), 2.34 (t, *J* 7.8 Hz, 2H, CH_2C=), 2.47 (t, *J* 7.4 Hz, 2H, CH_2S), 2.48 (t, *J* 7.6 Hz, 2H, CH_2S); δ_C (100.1 MHz, $CDCl_3$) 9.5, 13.7, 220, 24.4, 27.5, 28.8, 29.1, 29.12, 29.2, 29.4, 29.6, 31.8, 31.82, 32.1, 140.4, 144.7, 165.8, 166.2; *m*/*z* (EI, 70 eV) 326 (33, M^+), 283 (8), 269 (52), 201 (74), 191 (32), 126 (38), 61 (100%).

4.2.10. Preparation of (*Z*)-3-(2,2-dichloropropanoyl)-2-[10-(butylthio)decylidene] -1,3-thiazinane (23)

Following the same procedure used to prepare **10**, 11-(butylthio)-*N*-(3-hydroxypropyl)undecanamide **20** (20 mmol, 6.64 g) gave **23** as a pale yellow oil (7.83 g, overall yield 86%); [HRMS found 454.1780. $C_{21}H_{38}Cl_2NOS_2$ $(M+H)^+$ requires 454.1766]; R_f (90% PE/Et_2O) 0.85; ν_{max} (neat) 2926, 2850, 1700, 1667 cm^{-1}; δ_H

(400 MHz, $CDCl_3$) 0.89 (t, *J* 7.1 Hz, 3H, CH_3CH_2), 1.26 (broad, 12H), 1.39 (m, 2H, CH_2CH_3), 1.55 (m, 4H), 2.09 (broad, 2H, $SCH_2CH_2CH_2N$), 2.26 (m, 2H, $CH_2CH{=}C$), 2.31 (s, 3H, CH_3), 2.48 (t, *J* 7.9 Hz, 2H, CH_2S), 2.49 (t, *J* 7.9 Hz, 2H, CH_2S), 2.84 (broad, 2H, CH_2S), 4.21 (broad, 2H, CH_2N), 6.23 (broad, 1H, CH=C); δ_C (100.1 MHz, $CDCl_3$) 13.7, 22.0, 26.2 (broad), 28.6 (broad), 28.9, 29.2, 29.22, 29.3, 29.4, 29.7, 31.8, 31.9, 32.2, 36.8, 50.2 (broad), 80.2 (CCl_2), 131.5 (broad and weak, C=), 136.4 (broad and weak, CH=), 163.4 (CO); ESI-MS: 454.6 $[M+H]^+$.

4.2.11. Preparation of 3-(9-(butylthio)nonyl)-4-methylfuran-2,5-dione (19) from 23

Following the same procedure used to prepare **3**, (*Z*)-3-(2,2-dichloropropanoyl)-2-[10-(butylthio)decylidene]-1,3-thiazinane **23** (10 mmol, 4.55 g) gave **19**, as a pale yellow oil (2.49 g, overall yield 76%).

4.3. FTase assays

4.3.1. Preparation of the sodium salts from the chaetomellic anhydrides: general procedure

In a one-necked 25 mL Erlenmeyer flask chaetomellic anhydride A or analogues (50–70 mg), THF/H_2O 1:1 (2 mL/50 mg of anhydride) and a solution of NaOH 1 M (2 equiv) were added. After overnight stirring at room temperature (16 h), the solvent was removed, the remaining material was dissolved in H_2O (3–4 mL). Freeze-drying of this solution [−13 °C (48 h) and then 25 °C (24 h) at 0.4–0.5 mm Hg] gave the sodium salts as a white solid.

4.3.2. Yeast and Rat Farnesyl Transferase assays[40]

Assays were conducted in 50 mM Tris, pH 7.0, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM DTT, 0.04% (w/v) *n*-dodecyl-β-D-maltoside and 2.4 μM dansyl-Gly-Cys-Val-Ile-Ala (dansyl-GCVIA). Reactions mixtures were preincubated at 30 °C for 5 min before farnesylation was initiated by addition of recombinant yeast FTase (1.5 nM) or rat FTase (15 nM). The fluorescence λ emission at 486 nM (slit width = 5.1 nM) was measured at λ excitation = 340 nM (slit width = 5.1 nM) for dansyl-GCVIA, using 3 mm square quartz cuvettes. The fluorescence intensity was measured for 300 sec. All measurements were made in triplicate.

4.4. Molecular modelling methods

The inhibitor **27** was constructed using 3D sketcher module of the Discovery Studio software from Acclerys (Accelrys Inc., San Diego, CA. (http://accelrys.com/products/) and was subjected to conformational analysis and energy minimization by means of the AM1 molecular orbital hamiltonian.

The X-ray structures of rat FTase complexed with farnesyl pyrophosphate (FPP)[44] and of the ternary complex in which the rat FTase interacts with the FPT-II FPP analog and the substrate peptide CVLS[45] were retrieved from the the Protein Data Bank (PDB: their pdb ref code are 1FT2 and 1TN8, respectively).[54]

The binary FTase-**27**, and ternary FTase-**27**-peptide (CVLS) complexes were built by docking into the enzyme the conformation of compound **27** which satisfies contemporaneously the two following requirements: (1) minimum the molecular volume exceeding the volume of the supermolecule formed by the alignment of FPP and FPT-II FPP, and (2) maximum difference of the energy of the conformation chosen with respect the energy of the molecule in its extended conformation (assumed to be the global minimum) of 2 kcal/mol.

The complexes were minimized by 10,000 steps of steepest descent followed by conjugate gradient until the system energy converged to 1e−05 kJ/mol using the CHARMM force-field.[55] Standard protonation states, corresponding to pH 7, were assigned to the amino acid residues.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.034. These data include MOL files and InChiKeys of the most important compounds described in this article.

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