Bioorganic & Medicinal Chemistry 18 (2010) 543–556

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Towards the synthesis of bisubstrate inhibitors of protein farnesyltransferase: Synthesis and biological evaluation of new farnesylpyrophosphate analogues

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ARTICLE INFO

*Article history:*
Received 4 September 2009
Accepted 4 December 2009
Available online 11 December 2009

*Keywords:*
Farnesyltransferase inhibitors
Imidazole
FPP analogues
Trypanosoma
Malaria

ABSTRACT

Protein farnesyltransferase (FTase) has recently appeared as a new target of parasitic diseases, a field poor in drugs in development. With the aim of creating new bisubstrate inhibitors of FTase, new farnesyl pyrophosphate analogues have been studied. Farnesyl analogues with a malonic acid function exhibited the best inhibitory activity on FTase. This group was introduced into our imidazole-containing model leading to new compounds with submicromolar activities. Kinetic experiments have been realized to determine their binding mode to the enzyme.



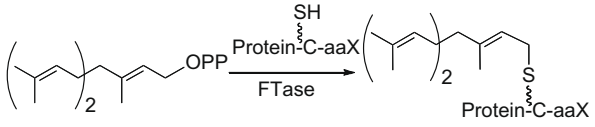
## 1. Introduction

New drugs are desperately needed for diseases caused by protozoan parasites, such as African sleeping sickness, Chagas disease and leishmaniasis. These tropical diseases represent major health problems. The World Health Organization estimates that 300,000 cases of African sleeping sickness occur annually in 36 countries in sub-Saharan Africa, that 16–18 million people in Latin America are chronically suffering from Chagas disease, and that 12 million people worldwide are infected with *Leishmania* spp.[1] Besides, between one and two million people die annually from malaria and a further 600 million cases occur each year.[2] The currently used drugs for these diseases are generally highly toxic and often ineffective, and drug resistance has developed in some cases. New and vulnerable protein targets within the cellular processes of the parasite are needed in the search of novel therapeutics.

Protein prenylation represents a critical post-translational modification as it plays a crucial role in intracellular signal transduction, cell proliferation, and apoptosis. The process of prenylation includes the reaction of farnesylation. Since its identification, the protein farnesyltransferase (FTase) has emerged as a promising target in cancer therapy.[3–7] FTase is an heterodimeric zinc metalloenzyme[8] that catalyses the transfer of a farnesyl group ($C_{15}$) from farnesylpyrophosphate (FPP) to the free thiol group of a cysteine residue embedded in the C-terminal CaaX sequence motif of proteins where C is a cysteine, a an aliphatic amino acid, X a serine, a methionine, an alanine or a glutamine (Scheme 1).

Farnesyltransferase inhibitors (FTIs) have been extensively developed for anticancer therapy, and diverse compounds with drug-like properties are available.[9,10] Recently, FTase has also appeared as a potential target for the treatment of parasitic diseases as it has been shown that protein farnesylation occurs in trypanosomatids and in the malaria parasite.[11] A variety of FTIs that are well tolerated in man have shown growth inhibition activity and killing of *Trypanosoma brucei* and *Plasmodium falciparum*.[12–15] Most of FTIs are FPP or CaaX competitive inhibitors.[16–22] However, only a few bisubstrate compounds, that is, able to bind both FPP and CaaX sites, have been reported. This kind of compound is expected to exhibit better specificity and affinity for the enzyme as it blocks both active sites.

In the course of our research on bisubstrate inhibitors, we have designed new potential FTIs based on an imidazole ring known to realize a strong interaction with the zinc atom in the FTase catalytic binding site thanks to its basic nitrogen. Moreover, this imidazole ring is a common moiety of potent FTIs.[23] We chose to introduce an acidic function and a hydrophobic chain to mimic




**Scheme 1.** Protein farnesylation.

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doi:10.1016/j.bmc.2009.12.017

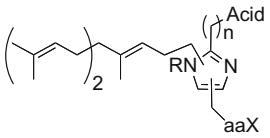



**Figure 1.** General structure of potential bisubstrate analogues.

the FPP and a tripeptide in order to connect the CaaX-binding site (Fig. 1).

The early first derivatives we synthesized were substituted imidazole rings bearing an acidic moiety and either a hydrophobic or a peptidic chain. Series of succinic acid–FPP analogues where the lipophilic chain length has been modified **1**[24] and of imidazole derivatives functionalized by different tripeptides and acidic chains **2**[25] were synthesized and their inhibitory activity was evaluated on FTase (Fig. 2).

Among these first analogues, compound **1c** bearing a farnesyl chain was the most active derivative on human FTase ($IC_{50}$ = 12 μM) and proved to be competitive to FPP and uncompetitive to CaaX motif. Compound **2c** with the valine–phenylalanine–methionine (VFM) tripeptide at the C-5 position of the imidazole[26] and an acidic chain with $n = 3$ exhibited the best inhibitory activity ($IC_{50}$ = 60 μM) among the imidazole-containing derivatives. Contrary to compound **1c**, this derivative is competitive to the CaaX motif and not to FPP.

Therefore our aim was to combine all these elements to synthesize more powerful FTIs. We present in this article the preparation of new FPP analogues **3** (Fig. 3) with different acidic functions in order to find a good mimic of the FPP pyrophosphate moiety as well as the synthesis and biological activities of potential bisubstrate inhibitors based on the general structure depicted in Figure 1.

## 2. Results and discussion

### 2.1. Synthesis of new FPP analogues with various pyrophosphate surrogates

The FPP pyrophosphate moiety plays two roles: it participates to FPP binding through its acidic functions and it is postulated to be activated by $Mg^{2+}$ to facilitate its departure during the enzymatic farnesylation.[27,28] Therefore we chose to mimic pyrophosphate by other acidic functions or by groups able to bind $Mg^{2+}$. Most of the FPP analogues described in the literature contained various mono/di-acidic phosphoryl mimics like phosphonic acid or phosphonate. The importance of this acidic part was demonstrated as crucial for the inhibition mechanism.[29–31] Because the phosphoryl mimic has been largely studied and displayed some bioavailability problems, we explored the synthesis and the biological evaluation of FPP analogues with various other functions like carboxylic, hydroxamic or sulfamic acids, amide, or sulfonamide. Each analogue was designed to evaluate three parameters: the nature of the acidic moiety, the length and the nature of the chain linking the farnesyl group to the acidic moiety (Fig. 3).

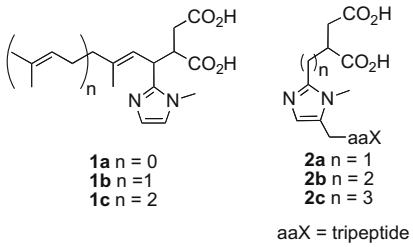



**Figure 2.** Structure of previously synthesized imidazole-containing FTIs.

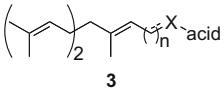



**Figure 3.** Model of new FPP analogues X = C, O, N, carbonyl, amide.

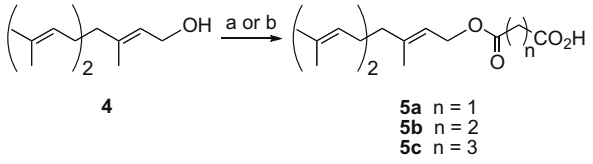
We began our investigation with monocarboxylic acid analogues. In this series, derivatives where the carboxylic acid is linked to the farnesyl moiety by a C–C bond has already been synthesized.[24] Therefore we intend to change this linkage to an ester or a keto bond. Compounds with an ester bond were synthesized by nucleophilic attack of *trans–trans*-farnesol **4** on Meldrum's acid ($n = 0$), succinic ($n = 1$) or glutaric anhydrides ($n = 2$) to give the products **5a–c**, respectively (Scheme 2).

As previously described for the synthesis of substituted FPP analogues,[32] the deprotonation with *n*-BuLi of sodium ethyloxalacetate followed by the addition of *trans–trans*-farnesyl bromide **6** led to the common ester intermediate **7** of the two keto analogues **11a** and **11b**. The attempt to hydrolyze the ester moiety of **7** under different basic conditions did not allow us to form the keto derivative **8**. We isolated either the corresponding decarboxylated product or the starting material depending on the reaction temperature. The chain length was modified by α-alkylation of **7** with ethyl 2-bromoacetate **9a** ($n = 1$) or ethyl 3-chloropropionate **9b** ($n = 2$) and gave, respectively, the keto diesters **10a** and **10b**.[33] The one step saponification–decarboxylation of **10a–b** at 70 °C provided the desired compounds **11a–b** (Scheme 3).

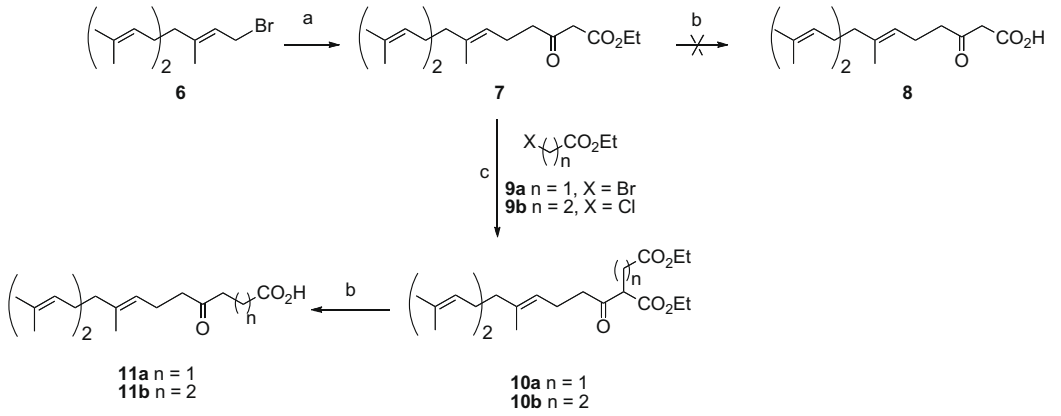
In our previous work, we chose succinic acid as a FPP pyrophosphate mimic because it is present in several potent natural FPP competitive inhibitors like chaetomellic acid A ( Fig. 4).[34,35] A stereoselective synthesis[26] of these analogues has shown that the (*R*) or (*S*) configuration of the succinic moiety have little influence on the FTase inhibition. As malonic acid was already used as replacement of the pyrophosphate moiety[36] and to avoid any stereoselective synthesis, we decided to build up malonic acid analogues connected to the farnesyl moiety by a unsaturated or saturated C–C bond, an ether or an amide linkage.

*trans–trans*-Farnesal **12**, easily obtained from *trans–trans*-farnesol **4**,[37] was submitted to Knoevenagel reaction using Yamamoto and co-workers[38] procedure and subsequent saponification afforded compound **14** in low yield due to the instability of this derivative. Starting from the commercially available dimethylmalonate, compound **16** was readily obtained in two steps by nucleophilic substitution of the *trans–trans*-farnesyl bromide **6** and ester cleavage by lithium hydroxide. Finally, the nucleophilic attack of hydroxymethylmalonate on *trans–trans*-farnesyl bromide **6** led to malonic acid **18** after ester hydrolysis. The synthesis of the amidomalonic acid derivatives **21a–b** (unsaturated and saturated compounds, respectively) was realized by pseudopeptidic coupling of diethylaminomalonate with two carboxylic acid analogues **19a–b** which have previously been described[24] and by final deprotection of the diethylesters by lithium hydroxide (Scheme 4).

Hydroxamate derivatives have already been shown to bind to $Mg^{2+}$, for instance in yeast enolase.[39] Therefore, in the series of metal chelating FPP analogues, we decided to prepare hydroxamic




**Scheme 2.** Reagents and conditions: (a) Meldrum's acid, toluene, reflux, 4 h (**5a**, 100%); (b) succinic or glutaric anhydride, DMAP, $CH_2Cl_2$, rt (**5b**, 90%; **5c**, 70%).




**Scheme 3.** Reagents and conditions: (a) sodium oxalacetate, *n*-BuLi, THF, 0 °C (89%); (b) NaOH, EtOH, 70 °C, 1 h (**11a**, 100%; **11b**, 85%); (c) KHMDS, THF, −78 °C, 1 h then **9a–b**, −78 °C to rt, 8 h (**10a**, 66%; **10b**, 45%).

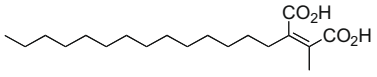
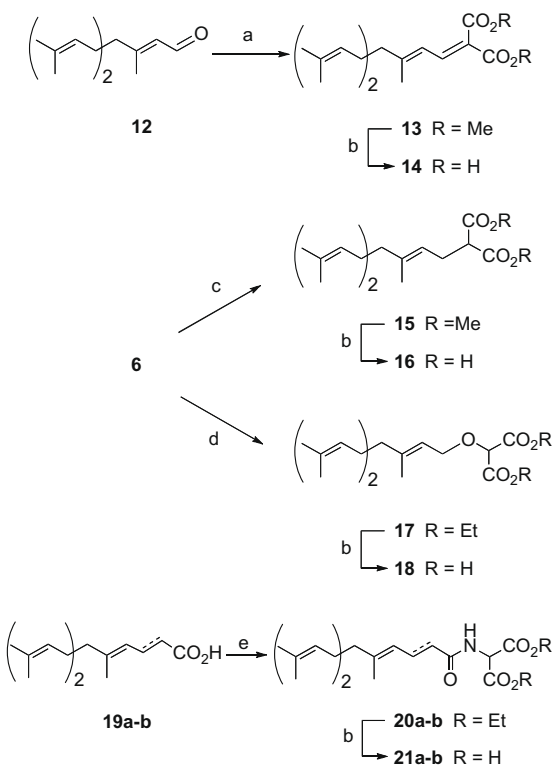
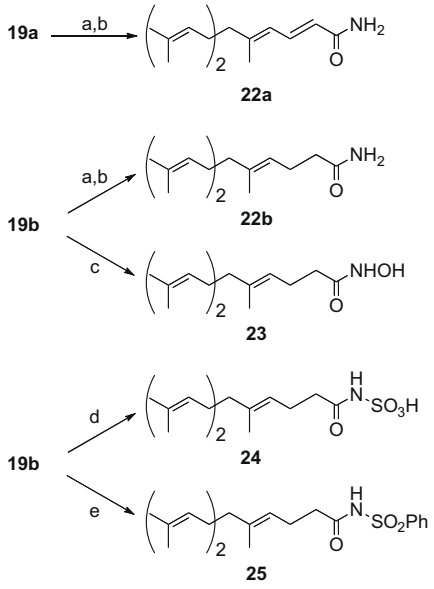



**Figure 4.** Chaetomellic acid A.




**Scheme 4.** Reagents and conditions: (a) dimethylmalonate, KHMDS, THF, 0 °C then rt, 5 h (**13**, 92%); (b) LiOH, THF/$H_2O$ 1:1, rt, 2 h (**14**, 22%; **16**, 100%; **18**, 100%; **21a**, 100%; **21b**, 37%); (c) dimethylmalonate, $TiCl_4$, THF, 0 °C, 1 h then pyridine, rt, 2 h (**15**, 63%) (d) diethylhydroxymalonate, NaH 60% in oil, THF, rt, 14 h (**17**, 42%); (e) diethylaminomalonate, NMM, EDCI, HOBt, $CH_2Cl_2$, rt, 4 h/18 h (**20a**, 98%; **20b**, 93%).

type analogues and their close amide derivatives. Compounds **22a–b** were successfully obtained using an activation-nucleophilic attack sequence[40] on carboxylic acids **19a–b** with ethyl chloroformate and ammoniac. Classical coupling conditions between carboxylic acid **19b** and hydroxylamine afforded **23** in a quantitative yield (Scheme 5).




**Scheme 5.** Reagents and conditions: (a) ethylchloroformate, $NEt_3$, THF, 0 °C, 30 min; (b) $NH_4Cl$, KOH/MeOH, rt, 3 h (**22a**, 54%; **22b**, 100%); (c) $NH_2OH$, NMM, EDCI, HOBt, $CH_2Cl_2$, rt, 3 h (**23**, 100%); (d) $NH_2SO_3H$, pivaloyl chloride, $NEt_3$, $CH_2Cl_2$, −78 °C then 0 °C, 1 h (**24**, 8%); (e) benzenesulfonamide, NMM, EDCI, HOBt, $CH_2Cl_2$, rt, 18 h (**25**, 77%).

The sulfamic moiety has been used as a surrogate of a phosphate moiety in the design of asparagine synthetase inhibitors.[41,42] We first tried to connect the sulfamic moiety to the farnesyl group through a sulfonate bond but the instability of the obtained compound prompted us to change our strategy. We decided to synthesize the *N*-acyl sulfamic acid derivative **24** starting from the carboxylic acid **19b**. After different acid activation assays, compound **24** was finally obtained with the mixed anhydride methodology but in poor yield. These results led us to wonder if the free sulfamic moiety was stable enough. We investigated the synthesis of the phenyl analogue **25** because phenylsulfonamide derivatives were efficient inhibitors of the zinc containing carbonic anhydrase.[43,44] Starting from carboxylic acid **19b** and benzenesulfonamide, compound **25** was obtained in 77% yield (Scheme 5).

## 2.2. Synthesis of imidazole-containing analogues

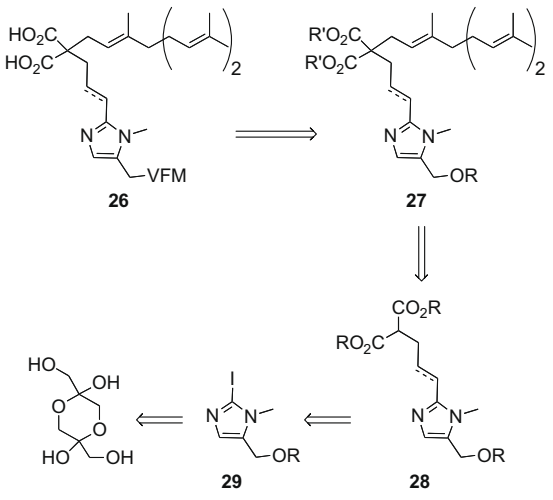
Previous work in our group led to the synthesis of imidazole-containing analogues with a succinic acid moiety and with either

a farnesyl or a peptidyl chain (compounds **1** and **2**, Fig. 2). Based on the biological evaluation of our FPP analogues (see below), we intended to synthesize a new imidazole-containing analogue bearing a malonic acid moiety and a farnesyl chain attached on the α-position of the carboxylic acids. We chose this position for the farnesyl moiety because previous results have shown that a close proximity between the farnesyl chain and the imidazole ring was detrimental to the activity.[24] We planned the retrosynthetic route depicted in Scheme 6. The peptidyl chain could be introduced at the end of the synthesis by reductive amination of the aldehyde obtained after deprotection and oxidation of the C-5 hydroxymethylimidazole derivative **27**. The key step of the synthesis relies on a Suzuki C–C cross coupling on the 2-iodoimidazole derivative **29**.[26]

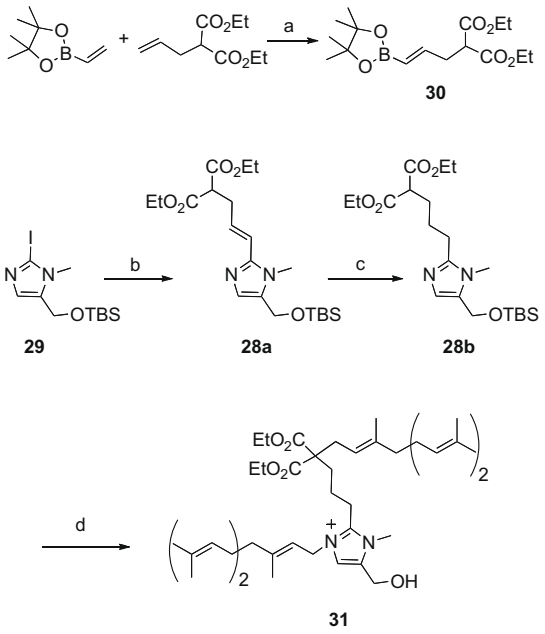
To apply this reaction, it was necessary to first synthesize the aliphatic vinyl boronate **30** that was successfully obtained by cross-coupling metathesis between diethylallylmalonate and vinylpinacolborane (Scheme 7). After optimization of the Suzuki cross-coupling reaction, compound **28a** was obtained in 80% yield. Hydrogenation of the double bond afforded the desired product **28b** in 64% yield (Scheme 7).

The first attempt to introduce the farnesyl chain on **28b** in the presence of sodium hydroxide led to the introduction of two farnesyl moieties together with the loss of the TBS group. Farnesylation occurred between the two carboxylic esters as expected and on the second nitrogen of the imidazole to form the imidazolium **31** (Scheme 7). The attempts to avoid the formation of this secondary product by using weak bases or no base at all were unsuccessful. This peculiar reactivity of the imidazole ring is due to the presence of two protonable nitrogens that stabilizes the positive charge by an equilibrated form. This reactivity has been exploited for ionic liquids or as stable carben precursor in particular. To avoid this imidazolium formation, we changed our strategy by carrying out first the oxidation of the alcohol in order to destabilize the imidazolium (Scheme 8). Compound **32** was then submitted to farnesylation in the presence of sodium hydride and provided the expected compound **33** in a quantitative yield. The aldehyde **32** and VFM–OMe[25] were coupled by a reductive amination followed by saponification of the ester functions. Although the saponification step was undertaken during a long reaction time, only two of the three esters were hydrolyzed. Other conditions using stronger bases or higher reaction temperature were tempted but none of them were conclusive, leading to degradation or leaving starting material unchanged (Scheme 8).

To evaluate the actual influence of the peptidyl and farnesyl chains, we needed compounds bearing only one of these chains.




**Scheme 6.** Retrosynthetic pathway.



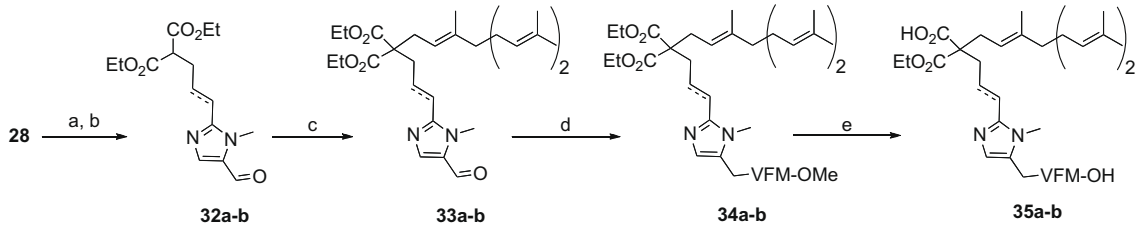

**Scheme 7.** Reagents and conditions: (a) Grubbs I catalyst 10 mol %, $CH_2Cl_2$, reflux, 3 h (**30**, 92%); (b) **30**, $Pd(PPh_3)_4$, $Na_2CO_3$, DMF, 130 °C, 5 h (**28a**, 80%); (c) $H_2$, Pd/C, AcOEt, rt, 18 h (**28b**, 64%); (d) NaH, **6**, THF, 0 °C then rt, 3 h (**31**, 50%).

Compound **2c** represents the derivative without the farnesyl group and we synthesized compound **36** bearing no tripeptide.[45] The synthesis was carried out as for compound **35** starting from 2-iodo-1-methylimidazole. The Suzuki cross-coupling with compound **30** afforded **37** in 85% yield that was further hydrogenated under Pd catalyst. As for compound **28b** farnesylation occurred both at the imidazole ring and at the malonyl carbon. Therefore, to minimize the imidazole ring farnesylation, the reaction with farnesyl bromide **6** was carried out at low temperature leading to major formation of the desired compound **38**. As previously observed in this series, ester hydrolysis was difficult and the desired diacid **36** was obtained in only 27% yield (Scheme 9).

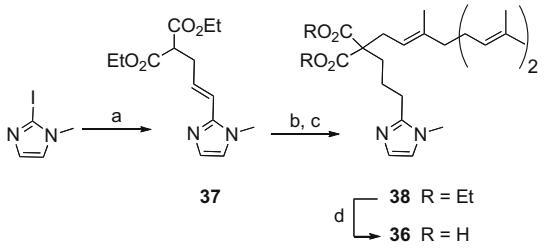
## 3. Biological evaluation

FPP analogues were evaluated for their inhibitory activity against recombinant yeast and human FTases[46] using a fluorescent based assay[47,48] and adapted to 96-well plate format. The $IC_{50}$ were determined and compared to the $IC_{50}$ of chaetomellic acid A (Fig. 4) measured under our conditions (Table 1).

For the monocarboxylic acids, the nature of the linkage does not seem to be important. However the distance between the farnesyl and acidic groups slightly modifies the activity. The best results were obtained with short chains with C–C bond linkage (**19a–b**) but only in the case of yeast FTase or with longer chain either with an ester or a keto linkage (**5c** and **11b**, respectively) for both enzymes. However all these activities are low compared to chaetomellic acid A. As previously observed,[24] the introduction of a second carboxylic acid greatly enhances the activity whatever the enzyme is concerned. On the contrary to C or O-linkage (**16** and **18**, respectively), the presence of an amide (**21a–b**) seems detrimental to the activity. The other non-carboxylic functions do not lead to any advantage since, in almost all cases, compounds **22a–b**, **23–25** expressed an inhibitory activity superior to 200 μM. The most active compounds **16** and **18** showed interesting activities in the submicromolar range. When compared to its succinic analogue ($IC_{50}$ = 2.5 and 5.4 μM on yeast and human FTase, respectively),[24] compound **16** showed the same activity against yeast




**Scheme 8.** Reagents and conditions: (a) TBAF, THF, rt, 2 h; (b) $MnO_2$, $CH_2Cl_2$, 40 °C, 18 h (**32a**, 85%; **32b**, 64%) (c) NaH, **6**, THF, 0 °C, 2 h (**33a**, 100%; **33b**, 77%) (d) (i) $NH_2$–VFM–OMe, 4 Å MS, $CH_2Cl_2$–MeOH, rt, 10 min then aldehyde **33a** or **33b**, (ii) $NaBH_3CN$, MeOH/AcOH (10:1), rt, 18 h (**34a**, 34%; **34b**, 46%) (e) LiOH, THF/MeOH/$H_2O$ (1:1:1), rt, seven days (**35a**, 100%; **35b**, 27%).



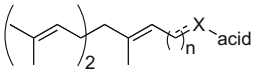

**Scheme 9.** Reagents and conditions: (a) **30**, $Pd(PPh_3)_4$, $Na_2CO_3$, DMF, 130 °C, 5 h (85%); (b) $H_2$, Pd/C, AcOEt, rt, 18 h (**37**, 93%); (c) NaH, 0 °C, 1 h then **6**, THF, 0 °C, 1 h 30 min (**38**, 64%); (d) LiOH, THF/MeOH/$H_2O$ (1:1:1), rt, eight days (**39**, 27%).

FTase but a fivefold increase against the human enzyme. Before introducing the malonic function into our potential bisubstrate inhibitors, it was necessary to precise the binding mode of these malonyl FPP analogues. As expected, the kinetic experiments, realized on compound **16**, showed that it binds to the FPP site and not to the peptide one as it revealed to be a FPP competitive inhibitor ($K_i^{app}$ = 0.26 μM) and to be uncompetitive toward the peptidyl substrate Dns-GCVLS.

Generally, the esters of peptide-containing derivatives are much less active than their acid analogues in the enzymatic assay and are usually employed as prodrugs in cell-based assays.[49] This was verified with compounds **34a–b** that were unable to inhibit human FTase. It is to be noted that the presence of all chains greatly increased the activity of our imidazole-containing inhibitors with $IC_{50}$ going from 60 μM for **2c** (no farnesyl chain) or 21 μM for **36** (no peptidyl chain) to 0.64 μM and 0.24 μM for **35a** and **35b**, respectively. Compound **35b** bearing the saturated acid chain revealed to be the best inhibitor of our imidazole-containing inhibitor series with a submicromolar range activity. To explain these results and determine if our compounds were bisubstrate inhibitors, kinetic studies were realized on both compounds **35a** and **35b**. These experiments showed that these derivatives were FPP competitive inhibitors with $K_i^{app}$ = 0.5 and 0.23 μM for **35a** and **35b**, respectively. However, these compounds were non-competitive CaaX inhibitors with $K_i^{app}$ = 17 and 7.7 μM and $K_i'^{app}$ = 15 and 5.6 μM, respectively.[50] It is to be noted that $K_i^{app}$ for FPP inhibition are very similar for compounds **16**, **35a** and **35b** (0.26, 0.5 and 0.23 μM, respectively) whereas the CaaX inhibition goes from uncompetitive to non-competitive inhibition. Therefore, the better $IC_{50}$ obtained for **35b** compared to **16** and **35a** must result from the differences observed in their $K_i^{app}$ for CaaX inhibition.

With the aim of finding new active compounds against some protozoan parasites, we evaluated the activity of our imidazole-containing compounds against *P. falciparum* and *T. brucei* proliferation.

**Table 1**
Activity of FPP analogues on recombinant yeast and human FTases




| Compounds | *n* | X | Acid | $IC_{50}$ (μM) yeast FTase | $IC_{50}$ (μM) human FTase |
|---|---|---|---|---|---|
| Chaetomellic acid[a] | | | | 0.34 | 0.175 |
| **19a**[b] | 2[c] | –CH– | –COOH | 40 | 130 |
| **19b**[b] | 2 | $-CH_2-$ | –COOH | 42 | 170 |
| **5a** | 1 | $-OCO-CH_2-$ | $-CO_2H$ | 80 | 160 |
| **5b** | 1 | $-OCO-(CH_2)_2-$ | $-CO_2H$ | 100 | 130 |
| **5c** | 1 | $-OCO-(CH_2)_3-$ | $-CO_2H$ | 35 | 60 |
| **11a** | 2 | $-CO-(CH_2)_2-$ | $-CO_2H$ | 80 | >200 |
| **11b** | 2 | $-CO-(CH_2)_3-$ | $-CO_2H$ | 15 | 60 |
| **14** | 1[c] | — | $HO_2C-C-CO_2H$ | nd[d] | Inactive |
| **16** | 1 | — | $HO_2C-CH-CO_2H$ | 4 | 0.90 |
| **18** | 1 | –O– | $HO_2C-CH-CO_2H$ | 5 | 0.88 |
| **21a** | 2[c] | –CONH– | $HO_2C-C-CO_2H$ | nd | 10 |
| **21b** | 2 | –CONH– | $HO_2C-CH-CO_2H$ | 200 | 60 |
| **22a** | 2[c] | $-CONH_2$ | — | Inactive | Inactive |
| **22b** | 2 | $-CONH_2$ | — | Inactive | >200 |
| **23** | 2 | –CONH–OH | — | Inactive | nd |
| **24** | 2 | –CONH– | $-SO_3H$ | >200 | 85 |
| **25** | 2 | $-CONH-SO_2Ph$ | — | >200 | >200 |

[a] $IC_{50}$ measured under our conditions.
[b] See Ref. 24.
[c] Unsaturated derivative.
[d] Not determined.

As expected, the ester derivatives were more active on parasites than their acidic forms. We observed the same behavior on *P. falciparum* as on the free enzyme, that is, the activity goes from $IC_{50}$ = 17.7 μM (without the tripeptide) or 4 μM (without the farnesyl group) to 1 μM when all chains are present on the imidazole ring. Our derivatives **34a–b** and **38** showed promising activity against *T. brucei*, compound **34a** being more active on the bloodstream form than on the procyclic form. As such agents should not be toxic for humans, we also evaluated their cytotoxicity on both normal (MRC5) and cancer (KB) human cell lines. As shown in Table 2 all our compounds are weakly or not cytotoxic, what is encouraging for antiparasitic purpose.

## 4. Conclusions

In a first part, we have synthesized sixteen new FPP analogues with different functions introduced on the farnesyl chain in order to find a good mimic of the FPP pyrophosphate moiety. This study revealed that a malonic acid attached to the farnesyl group could properly interact with FTase at the FPP binding site and was accordingly chosen as the acidic part for the new FTase inhibitors we aimed to synthesize. In a second part, we performed the synthesis of new imidazole-containing derivatives composed by two main moieties, a farnesyl chain with an acidic function and a peptidyl chain linked by an imidazole ring in order to occupy both FTase substrate binding sites. The key reaction of these syntheses was a Suzuki cross coupling, a new C–C coupling methodology on 2-iodoimidazole optimized in our laboratory with aliphatic vinyl boronates.[26] Biological profile evaluation of our imidazole analogues has shown that the simultaneous presence of potential FPP and CaaX mimics enhanced the inhibitory activity going from an $IC_{50}$ value around 60 μM to a submicromolar range. Kinetic experiments have demonstrated that our compounds do not express bisubstrate properties; they have a good affinity for the FPP but not for the CaaX-binding site. Our results also showed that an increased inhibition of the CaaX motif greatly improved the overall activity. These results as well as the encouraging activities against *P. falciparum* and *T. brucei* and the weak cytotoxicity upon mammalian cells prompt us to carry on our investigation on potential bisubstrate FTIs with an imidazole ring as central motif. The relative position of the farnesyl and peptidyl chains in our imidazole-containing inhibitors is probably not appropriate enough to fit both CaaX and FPP binding sites. Therefore, the synthesis of new imidazole-containing analogues substituted on different positions by the farnesyl and peptidyl chains is currently under investigation.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. General method

Unless otherwise indicated, all reactions were carried out with magnetic stirring and in case of air-sensitive compounds reactions were carried out in oven-dried glassware under argon. Commercial compounds were used without any further purification. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane ($CH_2Cl_2$), triethylamine ($NEt_3$), diisopropylamine and toluene were distilled over calcium hydride. *N,N′*-Dimethylformamide (DMF) was dried over $MgSO_4$ followed by distillation under reduced pressure. Pyridine was stored over KOH and other solvents over 4 Å molecular sieves. Analytical thin-layer chromatography was carried out on precoated silica gel aluminum plates (SDS TLC plates, Silica Gel $60F_{254}$). Column chromatography was performed with silica gel SDS 60 A CC (40–63 μm) or with prepacked Redisep® columns. Preparative TLC (PLC) was performed on Merck TLC with Silica Gel $60F_{254}$.

NMR spectra ($^1H$ and $^{13}C$) were recorded on a Brucker Avance 300 (300 MHz) and Avance 500 (500 MHz). Chemical shifts ($\delta$) are given in ppm relative to $CDCl_3$ (7.27 ppm; 77.14 ppm), $CD_3OD$ (3.34 ppm; 49.9 ppm). Splitting patterns are designed as: s, singlet; d, doublet; t, triplet; q, quartet; qi, quintuplet; m, multiplet; br, broad and combinations thereof. Coupling constants *J* are reported in hertz (Hz). IR spectra were recorded on a Perkin–Elmer Spectrum BX. Mass spectra were recorded on Thermofinningan Automass with a quadripole detection (IE) and on Thermoquest AQA Navigator with a TOF detection (ESI-HRMS).

#### 5.1.2. General procedure A: saponification of FPP analogues

To a solution of ester **13**, **15**, **17** or **20** in THF/$H_2O$ (1:1), lithium hydroxide (3.3 equiv) was added. After being stirred at room temperature for 0.5–72 h, the reaction mixture was neutralized by HCl 10% and extracted by dichloromethane. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The purification was realized when necessary by column chromatography to afford the target compounds **14**, **16**, **18** or **21**.

#### 5.1.3. General procedure B: pseudopeptidic coupling

To a solution of acid **19** in dichloromethane, *N*-methylmorpholine (2 equiv), EDCI (1.2 equiv), amine (1 equiv) and HOBt (1.2 equiv) were added. After being stirred 4–18 h at room temperature, the reaction mixture was neutralized by HCl 10% and extracted by dichloromethane. The organic layer was dried over

**Table 2**
Inhibition of recombinant human FTase and of proliferation of *T. brucei*, *P. falciparum*, KB and MRC5 cells by imidazole-containing analogues

| Compound | $IC_{50}$ (μM) *h*FTase | % Inh. at 10 μM *T. brucei brucei* | | $IC_{50}$ (μM) *P. falciparum* | % Inh. at 10 μM KB | % Inh. at 10 μM MRC5 |
|---|---|---|---|---|---|---|
| | | Bloodstream | Procyclic | | | |
| **2c**[a] | 60 | nd[b] | nd | 17.7[c] | nd | nd |
| **34a** | Inactive | 92[d] | 28[e] | 1.1 | 60[e] | Inactive |
| **35a** | 0.640 | Inactive | Inactive | 17.2 | Inactive | Inactive |
| **34b** | Inactive | nd | 54[e] | 1.0 | 44[e] | Inactive |
| **35b** | 0.240 | Inactive | Inactive | 31.4 | Inactive | Inactive |
| **36** | 21 | nd | nd | nd | nd | nd |
| **38** | nd | 89[f] | nd | 4 | nd | nd |

[a] From Ref. 25.
[b] Not determined.
[c] Triester of compound **2c**.
[d] 64% at 1 μM.
[e] Inactive at 1 μM.
[f] 24% at 1 μM.

$Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel to afford desired compounds **20**, **23** or **25**.

#### 5.1.4. General procedure C: amine analogues formation

Ethyl chloroformate (1.1 equiv) and triethylamine (1.1 equiv) were added to a solution of **19** in anhydrous THF at 0 °C under argon. After being stirred 30 min at 0 °C, the solution was filtered and concentrated. A solution of KOH, 0.69 M in methanol, (1.4 equiv) and ammonium chloride (1.4 equiv) was prepared at room temperature under argon. After being stirred for 30 min, the reaction residue in anhydrous THF was added to the ammonium hydroxide suspension. After being stirred for 3 h at room temperature, the solvent was removed under reduced pressure. The resulting white powder was dissolved in $CH_2Cl_2$, filtered and concentrated. The residue was purified by preparative TLC ($CH_2Cl_2$/MeOH 9:1) to afford compound **22**.

#### 5.1.5. General procedure D: TBS deprotection–oxidation

Tetrabutylammonium fluoride in solution 1 M in THF (2 equiv) was added to a solution of **28** in anhydrous THF at room temperature. After being stirred for 2–3 h at room temperature, the reaction was allowed to cool at room temperature and some drops of acetone were added. The reaction mixture was extracted with ethyl acetate and washed with $H_2O$. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography to afford the free alcohol derivative. This latter was added to a suspension of activated manganese dioxide(IV) (6 equiv) in dichloromethane at room temperature. The reaction mixture was stirred for 18 h at 40 °C, then, after cooling, filtered on a pad of Celite (EtOAc) and concentrated.

#### 5.1.6. General procedure E: farnesylation

Sodium hydride, 95% (1 equiv) was added to a solution of **32** or **37** in anhydrous THF at 0 °C. After being stirred for 1 h at 0 °C, *trans–trans*-farnesyl bromide **6** (1 equiv) was added at 0 °C. The reaction mixture was stirred 1.5–2 h at 0 °C then the reaction was stopped by addition of $H_2O$ and extracted with ethyl acetate. The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified if necessary by column chromatography to afford compounds **33** or **38**.

#### 5.1.7. General procedure F: reductive amination

A solution of trifluoroacetic acid (1.6 mL, 75%) in dichloromethane was added to the tripeptide (1.2 equiv) under anhydrous conditions. After being stirred at 0 °C for 20 min, diethyl ether was added to the reaction mixture and the solvent was removed under reduced pressure. This process was repeated until a white solid is obtained. To this solid in $CH_2Cl_2$/MeOH 8:2 (1.9 mL) were added at room temperature molecular sieves and distilled triethylamine (1 equiv). The reaction mixture was stirred for 10 min at room temperature then aldehyde **33** (1 equiv) dissolved in $CH_2Cl_2$/MeOH 8:2 (1 mL) was added. The reaction mixture was stirred at room temperature for 6 h then sodium cyanoborohydride (1.2 equiv) dissolved in a solution of 1% acetic acid in methanol (0.12 mL) was added. After being stirred for 15 h at room temperature, the reaction was stopped by addition of $H_2O$ and the reaction mixture was extracted with dichloromethane. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography to afford compound **34**.

#### 5.1.8. General procedure G: saponification of imidazole-containing analogues

Lithium hydroxide (20 equiv) was added to a solution **34** or **38** in THF/$H_2O$/MeOH 1:1:1 (2 mL). The reaction mixture was stirred at room temperature for 7–8 days then stopped by neutralization with a solution of HCl 10% and the reaction mixture was extracted with dichloromethane. The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure to afford compound **35** or **36**.

#### 5.1.9. 3-Oxo-3((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)propanoic acid (5a)

To a solution of commercial Meldrum's acid (67 mg, 0.47 mmol) in anhydrous toluene (2.5 mL) under argon at room temperature was added commercial *trans–trans*-farnesol **4** (113 μL, 0.45 mmol). After being stirred 4 h at 110 °C, the solvent was removed under reduced pressure to afford **5a** (138 mg, 100%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.60 (s, 3H), 1.60 and 1.68 (s, 6H), 1.72 (s, 3H), 1.92–2.18 (m, 8H), 3.44 (s, 2H), 4.70 (d, 2H, $J$ = 7.0 Hz), 5.09 (m, 2H), 5.35 (t, 1H, $J$ = 7.0 Hz), 9.56 (br s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.3, 26.0, 16.8, 18.0, 26.5, 27.0, 39.8, 40.0, 41.0, 63.2, 117.7, 123.8, 124.6, 131.6, 135.9, 143.9, 167.4, 171.5. IR ($CH_2Cl_2$) 2965, 2919, 2857, 1739, 1731, 1443, 1150 cm$^{-1}$. MS (ESI$^+$) *m/z* 331 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{25}O_4$ [M+Na]$^+$: 331.1885, found 331.1877.

#### 5.1.10. 4-Oxo-4((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyloxy)butanoic acid (5b)

To a solution of DMAP (497 mg, 4.1 mmol) and succinic anhydride (94 mg, 0.94 mmol) in anhydrous $CH_2Cl_2$ (2.5 mL) under argon at room temperature was added commercial *trans–trans*-farnesol **4** (113 μL, 0.45 mmol). After being stirred for 2 h, the solvent was evaporated. The reaction mixture was extracted with ethyl acetate, washed with a 10% HCl solution and twice with brine. The organic layer was dried over $Na_2SO_4$ and concentrated to afford **5b** (131 mg, 90%) as a yellowish oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.60 (s, 3H), 1.60 and 1.68 (s, 6H), 1.70 (s, 3H), 1.93–2.17 (m, 8H), 2.58–2.73 (m, 4H), 4.62 (d, 2H, $J$ = 7.0 Hz), 5.09 (m, 2H), 5.34 (td, 1H, $J$ = 7.0 Hz and $J'$ = 1.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.3, 26.0, 16.8, 18.0, 26.0, 27.0, 29.2, 29.3, 39.8, 40.0, 62.1, 118.3, 123.9, 124.6, 131.6, 135.8, 142.9, 172.5, 178.5. MS (ESI$^+$) *m/z* 345 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{19}H_{30}O_4Na$ [M+Na]$^+$: 345.2042, found 345.2054.

#### 5.1.11. 5-Oxo-5-((2*E*,6*E*)-3,7,11-trimethyl-dodeca-2,6,10-trienyl)pentanoic acid (5c)

Commercial *trans–trans*-farnesol **4** (0.157 mL, 0.6 mmol) was added to a solution of DMAP (667 mg, 5.5 mmol) and glutaric anhydride (148 mg, 1.3 mmol) in dichloromethane (3.3 mL) at room temperature. After being stirred for 1 h, the reaction mixture was hydrolyzed by a saturated $NaHCO_3$ solution. The organic layer was neutralized with a 10% HCl solution then dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on $C_{18}$ silica (gradient $H_2O$/$CH_3CN$ 8:2 v/v to $CH_3CN$ in 20 min) to give pure compound **5c** as a colorless oil (137 mg, 70%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 (s, 6H), 1.68 (s, 3H), 1.71 (s, 3H), 1.93 (q, 2H, $J$ = 4.0 Hz), 1.93–1.99 (m, 4H), 2.0–2.08 (m, 4H), 2.40–2.47 (m, 4H), 4.60 (d, 2H, $J$ = 7.0 Hz), 5.09 (m, 2H), 5.34 (td, 1H, $J$ = 7.0 Hz and $J'$ = 2.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.0, 16.5, 17.7, 20.2, 25.7, 33.0, 33.4, 39.5, 39.7, 61.5, 118.2, 123.6, 124.3, 131.3, 135.5, 142.5, 171.4, 178.6. MS (ESI$^+$) *m/z* 359 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{20}H_{32}O_4Na$ [M+Na]$^+$: 359.2198, found 359.2196.

### 5.1.12. (6*E*,10*E*)-Ethyl 7,11,15-trimethyl-3-oxohexadeca-6,10,14-trienoate (7)

*n*-BuLi, 1,6 M in hexane, (2 mL, 3.2 mmol) was added to a solution of commercial sodium ethyl acetoacetate (480 mg, 3.10 mmol) in anhydrous THF (14.5 mL) at 0 °C under argon. After being stirred 45 min at 0 °C, commercial *trans–trans*-farnesyl bromide **6** (271 μL, 1 mmol) was added dropwise and the reaction mixture was stirred 30 min at 0 °C. The mixture was poured into a solution of saturated $K_2HPO_4$ and extracted three times with $CH_2Cl_2$. The organic layer was dried over $Na_2SO_4$ and concentrated. The crude product was then purified by column chromatography (heptane/$CH_2Cl_2$ 1:1) to afford **7** (300 mg, 89%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.27 (t, 3H, $J$ = 7.0 Hz), 1.59 and 1.67 (s, 6H), 1.59 (s, 3H), 1.61 (s, 3H), 1.92–2.11 (m, 8H), 2.30 (t, 2H, $J$ = 7.5 Hz), 2.56 (t, 2H, $J$ = 7.5 Hz), 3.42 (s, 2H), 4.18 (q, 2H, $J$ = 7.0 Hz), 5.03–5.14 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.4, 16.3, 17.9, 26.0, 22.5, 26.8, 27.0, 39.9, 40.0, 43.3, 49.7, 61.6, 122.4, 124.3, 124.7, 131.5, 135.3, 137.0, 167.5, 202.8. MS (ESI$^+$) $m/z$ 357 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{34}O_3Na$ [M+Na]$^+$: 357.2406, found 357.2361.

### 5.1.13. Diethyl 2-((4*E*,8*E*)-5,9,13-trimethyltetradeca-4,8,12-trienoyl)succinate (10a)

To a solution of KHMDS (181.8 mg, 0.91 mmol) in anhydrous THF (0.9 mL) was added **7** (201 mg, 0.6 mmol) in anhydrous THF (2.5 mL) at −78 °C under argon. After being stirred for 1 h, commercial ethyl bromoacetate was added dropwise at −78 °C. The reaction mixture was stirred 35 min at −78 °C and then allowed to warm to room temperature. After being stirred for 7 h at room temperature, the reaction was quenched by water and a 10% HCl solution and extracted with ethyl acetate. The organic layer was dried over $Na_2SO_4$ and concentrated. The residue was purified by column chromatography (heptane/AcOEt 95:5) to afford **10a** (166 mg, 66%) as a colorless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.25 (t, 3H, $J$ = 7.0 Hz); 1.27 (t, 3H, $J$ = 7.0 Hz); 1.60 and 1.69 (s, 6H); 1.61 (s, 3H); 1.62 (s, 3H); 1.95–2.01 (m, 4H); 2.03–2.10 (m, 4H); 2.30 (q, 2H, $J$ = 7.5 Hz); 2.65 (dt, 1H, $J$ = 7.5 Hz and $J'$ = 17.5 Hz); 2.76 (dt, 1H, $J$ = 7.5 Hz and $J'$ = 17.5 Hz); 2.83 (dd, 1H, $J$ = 6.5 Hz and $J'$ = 17.5 Hz); 2. 96 (dd, 1H, $J$ = 8.0 Hz and $J'$ = 17.5 Hz); 3.97 (t, 1H, $J$ = 7.0 Hz); 4.13 (q, 2H, $J$ = 7.0 Hz); 4.20 (q, 2H, $J$ = 7.0 Hz); 5.09 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.4, 16.3, 18.0, 26.0, 22.4, 26.9, 27.1, 32.7, 40.0, 43.1, 54.4, 61.2, 62.0, 122.6, 124.4, 124.7, 131.6, 135.4, 136.9, 168.8, 171.7, 204.0. IR ($CH_2Cl_2$) 2979, 2933, 1730, 1715, 1160 cm$^{-1}$. MS (ESI$^+$) $m/z$ 443 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{25}H_{40}O_5Na$ [M+Na]$^+$: 443.2773, found 443.2787.

### 5.1.14. Diethyl 2-((4*E*,8*E*)-5,9,13-trimethyl-tetradeca-4,8,12-trienoyl)pentanedioate (10b)

Compound **7** (0.300 g, 0.90 mmol) in anhydrous THF (3.2 mL) was added to a solution of KHMDS (0.268 g, 1.34 mmol) in THF (1.3 mL) at −78 °C. After being stirred for 1 h at −78 °C, ethyl-3-chloropropionate (0.1335 mL, 1.08 mmol) was added dropwise. The reaction mixture was stirred for 35 min at −78 °C then allowed to warm to room temperature. After being stirred for 8 h, the reaction mixture was neutralized by 10% HCl and extracted by ethyl acetate and $H_2O$. The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (heptane then heptane/AcOEt 9:1) to give pure compound **10b** as a colorless oil (177.3 mg, 45%) $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.28 (t, 6H, $J$ = 7.0 Hz), 1.60 and 1.61 (s, 6H), 1.62 (s, 3H), 1.69 (s, 3H), 1.95–2.01 (m, 4H), 2.03–2.10 (m, 4H), 2.17 (t, 2H, $J$ = 7.0 Hz), 2.30 (q, 2H, $J$ = 7.5 Hz), 2.36 (t, 2H, $J$ = 7.5 Hz), 2.55 and 2.62 (t, 1H, $J$ = 7.0 Hz), 3.58 (t, 1H, $J$ = 7.0 Hz), 4.13 (q, 2H, $J$ = 6.0 Hz), 4.20 (q, 2H, $J$ = 7.0 Hz), 5.10 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.2, 16.0, 17.7, 22.1, 23.0, 25.7, 26.6, 26.7, 31.6, 39.7, 42.2, 57.6, 60.5, 61.4, 122.1, 124.0, 124.4, 131.4, 135.1, 137.0, 170.0, 173.0, 204.5. MS (ESI$^+$) $m/z$ 457 [M+Na]$^+$.

### 5.1.15. (7*E*,11*E*)-8,12,16-Trimethyl-4-oxoheptadeca-7,11,15-trienoic acid (11a)

An aqueous solution of 2 M NaOH (0.35 mL, 0.70 mmol) was added to a solution of **10a** (90.4 mg, 0.23 mmol) in ethanol at room temperature. After being stirred 1 h at 70 °C, the reaction was cooled to room temperature then hydrolyzed by 1 M HCl. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over $Na_2SO_4$. The expected compound **11a** was obtained after solvent removal (73.0 mg, 100%) as a colorless oil. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.62 (3s, 9H), 1.65 (s, 3H), 1.96–2.16 (m, 8H), 2.24–2.34 (m, 2H), 2.54 (m, 4H), 2.75 (m, 2H), 5.13 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.9, 18.8, 26.8, 24.3, 28.4, 28.7, 29.6, 39.0, 41.7, 44.4, 125.0, 126.3, 133.0, 136.9, 138.1, 177.4, 212.4. IR ($CH_2Cl_2$) 2916, 1712 cm$^{-1}$. MS (ESI$^-$) $m/z$ 319 [M−H]$^-$. HRMS (ESI$^-$) calcd for $C_{20}H_{31}O_3$ [M−H]$^-$: 319.2273, found 319.2275.

### 5.1.16. (8*E*, 12*E*)-9,13,17-Trimethyl-5-oxo-octadeca-8,12,16-trienoic acid (11b)

An aqueous solution of 2 M NaOH (0.612 mL, 1.22 mmol) was added to a solution of **10b** (177.3 mg, 0.41 mmol) in ethanol (3.2 mL) at room temperature. After being stirred for 1 h at 70 °C, the reaction was cooled to room temperature and acidified by 1 M HCl. The reaction mixture was extracted by ethyl acetate. The organic layer was washed by brine, dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by recrystallisation of the calcium salt in dichloromethane to give pure compound **11b** (116.4 mg, 85%) $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 and 1.62 (s, 6H), 1.63 (s, 3H), 1.69 (s, 3H), 1.93 (qi, 2H, $J$ = 7.0 Hz), 1.96–2.0 (m, 4H), 2.06–2.1 (m, 4H), 2.29 (q, 2H, $J$ = 7.0 Hz), 2.40 (t, 2H, $J$ = 7.0 Hz), 2.45 (t, 2H, $J$ = 8.0 Hz), 2.51 (t, 2H, $J$ = 7.0 Hz), 5.08 (m, 3H). $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 16.0, 17.7, 23.4, 25.7, 26.8, 29.7, 39.7, 39.73, 42.9, 116.4, 122.6, 124.4, 133.0, 136.9, 141.1, 173.4, 212.4. MS (ESI$^+$) $m/z$ 357 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{34}O_3Na$ [M+Na]$^+$: 357.2406, found 357.2424.

### 5.1.17. Dimethyl 3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienylidene)malonate (13)

To a solution titanium tetrachloride(IV) (0.299 mL, 2.72 mmol) in anhydrous THF (10 mL) were added dimethylmalonate (0.234 mL, 2.04 mmol) and farnesal **12** (0.300 g, 1.36 mmol) in THF (2 mL) at 0 °C under argon. The reaction mixture was stirred 1 h at 0 °C then pyridine (0.440 mL, 5.44 mmol) was added. After being stirred 2 h at room temperature, $H_2O$ was added and the reaction was extracted by ethyl acetate. The organic layer was dried over $Na_2SO_4$ then the solvent was removed under reduced pressure. The crude product was purified by preparative TLC ($CH_2Cl_2$) to afford pure compound **13** as a colorless oil (288 mg, 63%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 (s, 6H), 1.68 (s, 3H), 1.94 (s, 3H), 1.97–2.40 (m, 8H), 3.79 and 3.85 (s, 6H), 5.08 (m, 2H), 6.28 (d, 1H, $J$ = 13.0 Hz), 7.69 (dd, 1H, $J$ = 13.0 Hz and $J'$ = 6.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.1, 17.7, 25.7, 26.2, 26.7, 39.6, 40.9, 52.2, 120.5, 122.9, 124.3, 131.6, 136.4, 141.3, 156.2, 167.3. IR (neat) 1740 cm$^{-1}$.

### 5.1.18. 3-((2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trienylidene)-malonic acid (14)

Prepared according to general procedure A on **13** (0.233 g, 0.698 mmol) in 3 mL solvent with lithium hydroxide (0.097 g, 2.30 mmol) for 72 h. The crude product was purified by HPLC on $C_{18}$ silica gel to afford pure compound **14** as a white solid (213 mg, 100%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 (s, 6H), 1.68 (s, 3H), 1.89 (s, 3H), 1.99–2.54 (m, 8H), 5.09 (m, 2H), 7.50 (d, 1H,

$J$ = 14.0 Hz), 8.48 (m, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.5, 17.9, 25.7, 27.3, 40.9, 121.9, 123.3, 124.2, 131.6, 135.7, 137.9, 141.4. IR (neat) 2920, 1740, 1700 cm$^{-1}$. Mp 139 °C. MS (ESI$^+$) *m/z* 329 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{18}H_{26}O_4Na$ [M+Na]$^+$: 329.1728, found 329.1728.

### 5.1.19. Dimethyl 2-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)malonate (15)

To a mixture of KHMDS (2.14 mL, 2.16 mmol) and dimethylmalonate (0.248 mL, 2.16 mmol) in anhydrous THF (6 mL), a solution of *trans–trans*-farnesyl bromide **6** (0.391 mL, 1.44 mmol) in THF (3 mL) was added at 0 °C. After being stirred 5 h at room temperature, the reaction mixture was poured into $H_2O$ then extracted by ethyl acetate. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (heptane then heptane/AcOEt 9:1) to give pure compound **15** as colorless oil (0.462 g, 92%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.61 (s, 3H), 1.62 (s, 3H), 1.65 (s, 3H), 1.70 (s, 3H), 1.97–2.01 (m, 4H), 2.02–2.10 (m, 4H), 2.63 (t, 2H, $J$ = 8.0 Hz), 3.39 (t, 1H, $J$ = 8.0 Hz), 3.74 (s, 6H), 5.09 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.0, 17.7, 25.7, 26.5, 26.8, 27.6, 39.7, 51.9, 52.4, 119.4, 123.9, 124.4, 131.3, 135.2, 138.8, 169.6. IR (neat) 1740 cm$^{-1}$. MS (ESI$^+$) *m/z* 359 [M+Na]$^+$ HRMS (ESI$^+$) calcd for $C_{20}H_{32}O_4Na$ [M+Na]$^+$: 359.2198, found 359.2192.

### 5.1.20. 2-(3,7,11-Trimethyl-dodeca-2,6,10-trienyl)malonic acid (16)

Prepared according to general procedure A on **15** (0.100 g, 0.30 mmol) in 2.7 mL solvent with lithium hydroxide (0.041 g, 0.98 mmol) for 2 h. The crude product was purified by column chromatography on $C_{18}$ silica (gradient $H_2O/CH_3CN$ 7:3 to $CH_3CN$ in 15 min) to afford **16** as a colorless oil (0.020 g, 22%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.61 (s, 3H), 1.62 (s, 3H), 1.66 (s, 3H), 1.70 (s, 3H), 1.97–2.01 (m, 4H), 2.02–2.10 (m, 4H), 2.69 (t, 2H, $J$ = 7.0 Hz), 3.46 (t, 1H, $J$ = 7.0 Hz), 5.11 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.1, 17.7, 25.7, 26.5, 26.8, 27.6, 39.7, 51.4, 118.7, 123.8, 124.4, 131.3, 135.3, 139.6, 173.9. MS (ESI$^+$) *m/z* 331 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{18}H_{28}O_4Na$ [M+Na]$^+$: 331.1885, found 331.1896.

### 5.1.21. Diethyl 2-(3,7,11-trimethyldodeca-2,6,10-trienyloxy)malonate (17)

Diethylhydroxy malonate (0.062 g, 0.35 mmol) was added to a suspension of sodium hydride (60% in oil, 0.017 g, 0.42 mmol), in anhydrous THF (10 mL) under argon then *trans–trans*-farnesyl bromide **6** (0.095 mL, 0.35 mmol) was added. After being stirred 14 h at room temperature, the reaction mixture was neutralized by 10% HCl and extracted with dichloromethane. The organic layer was washed with brine then dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The purification of the crude product was realized by column chromatography (heptane/AcOEt 95:5) to afford pure compound **17** as a colorless oil (55.5 mg, 42%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.28 (t, 6H, $J$ = 7.0 Hz), 1.58 (s, 3H), 1.60 (s, 3H), 1.65 (s, 3H), 1.68 (s, 3H), 1.96–2.07 (m, 8H), 2.76 (d, 2H, $J$ = 8.0 Hz), 3.71 (s, 1H), 4.24 (q, 4H, $J$ = 7.0 Hz), 5.09 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.0, 16.2, 16.7, 25.9, 26.5, 26.7, 39.9, 51.4, 65.4, 95.3, 116.1, 123.9, 124.4, 131.5, 139.5, 140.7, 172.8. IR (neat) 1730 cm$^{-1}$. MS (ESI$^+$) *m/z* 403 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{22}H_{36}O_5Na$ [M+Na]$^+$: 403.2460, found 403.2450.

### 5.1.22. 2-(3,7,11-Trimethyldodeca-2,6,10-trienyloxy)malonic acid (18)

Prepared according to general procedure A on **17** (55.5 mg, 0.146 mmol) in 3 mL solvent with lithium hydroxide (20.2 mg, 0.482 mmol) for 4 h. The product **18** was obtained as a pure colorless oil (47 mg, 100%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 (s, 3H), 1.61 (s, 3H), 1.66 (s, 3H), 1.69 (s, 3H), 1.97–2.08 (m, 8H), 2.79 (d, 2H, $J$ = 8.0 Hz), 3.51 (s, 1H), 5.12 (m, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.2, 17.3, 25.5, 27.2, 27.4, 39.9, 63.5, 79.7, 118.5, 124.0, 124.2, 131.6, 134.5, 138.4, 174.2. IR (neat) 1730 cm$^{-1}$. MS (ESI$^-$) *m/z* 323 [M−H]$^-$. HRMS (ESI$^-$) calcd for $C_{18}H_{28}O_5$ [M−H]$^-$: 323.1858, found 323.1873.

### 5.1.23. Diethyl 2-(5,9,13-trimethyltetradeca-2,4,8,12-tetraenoylamino)-malonate (20a)

Prepared according to general procedure B on **19a** (0.060 g, 0.229 mmol) 2 mL solvent for 18 h with *N*-methylmorpholine (0.050 mL, 0.457 mmol), EDCI (0.053 g, 0.274 mmol), diethylaminomalonate (0.048 g, 0.229 mmol) and HOBt (0.037 g, 0.274 mmol). After work up, the purification was realized by column chromatography ($CH_2Cl_2$ then $CH_2Cl_2$/MeOH 9:1) to afford pure compound **20b** as a colorless oil (89.6 mg, 93%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.30 (t, 6H, $J$ = 7 Hz), 1.59 (s, 6H), 1.66 (s, 3H), 1.87 (s, 3H), 1.95–2.15 (m, 8H), 4.23–4.33 (m, 4H), 5.08 (m, 2H), 5.23 (d, 1H, $J$ = 8.0 Hz), 5.84 (d, 1H, $J$ = 15.0 Hz), 5.97 (d, 1H, $J$ = 12.0 Hz), 6.42 (d, 1H, $J$ = 6.0 Hz), 7.56 (dd, 1H, $J$ = 15.0 Hz and $J'$ = 12.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.0, 16.0, 17.4, 17.7, 25.7, 26.2, 26.7, 39.7, 40.3, 56.6, 62.6, 119.8, 123.1, 124.2, 131.5, 135.9, 139.0, 149.9, 166.1, 166.5. IR (neat) 3350, 1740, 1670, 1520 cm$^{-1}$. MS (ESI$^+$) *m/z* 442 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{24}H_{37}NO_5Na$ [M+Na]$^+$: 442.2569, found 442.2563.

### 5.1.24. 2-(5,9,13-Trimethyltetradeca-2,4,8,12-tetraenoylamino)-malonic acid (21a)

Prepared according to general procedure A on **20a** (0.096 g, 0.23 mmol) in 6 mL solvent with lithium hydroxide (0.032 g, 0.76 mmol) for 30 min. After work up, the crude product was purified by column chromatography ($CH_2Cl_2$/MeOH 8:2) to afford pure compound **21a** as a colorless oil (30.3 mg, 37%). $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 1.61 and 1.63 (s, 6H), 1.67 (s, 3H), 1.89 (s, 3H), 1.91–2.20 (m, 8H), 3. 75 (s, 1H), 5.11 (m, 2H), 6.07 (m, 2H), 7.55 (dd, 1H, $J$ = 17.0 Hz and $J'$ = 11.0 Hz). $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 16.2, 17.3, 17.8, 25.9, 27.2, 27.9, 40.8, 41.3, 68.9, 121.8, 124.7, 125.4, 131.9, 138.8, 139.4, 150.2, 169.9. IR (neat) 3340, 2920, 1750, 1640, 1520 cm$^{-1}$. MS (ESI$^-$) *m/z* 362 [M−H]$^-$. HRMS (ESI$^-$) calcd for $C_{20}H_{28}NO_5$ [M−H]$^-$: 362.1952, found 362.1967.

### 5.1.25. Diethyl 2-(5,9,13-trimethyltetradeca-4,8,12-trienoylamino)-malonate (20b)

Prepared according to general procedure B on **19b** (0.100 g, 0.39 mmol) 5 mL solvent for 4 h with *N*-methylmorpholine (0.085 mL, 0.77 mmol), EDCI (0.0887 g, 0.46 mmol), diethylaminomalonate (0.0816 g, 0.39 mmol) and HOBt (0.0625 g, 0.46 mmol). After work up and purification by column chromatography (heptane/AcOEt 8:2) pure compound **20b** was obtained as a colorless oil (157.9 mg, 98%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.32 (t, 6H, $J$ = 7.0 Hz), 1.61 (s, 6H), 1.65 (s, 3H), 1.70 (s, 3H), 1.90–2.03 (m, 4H), 2.06–2.12 (m, 4H), 2.31–2.39 (m, 4H), 4.23–4.34 (m, 4H), 5.12 (m, 4H), 6.48 (d, 1H, $J$ = 6.5 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.2, 16.1, 17.9, 23.9, 25.5, 26.9, 36.1, 40.1, 56.4, 62.8, 122.3, 124.5, 128.6, 137.2, 144.3, 166.5, 172.7. IR 1730, 1640, 1540 cm$^{-1}$. MS (ESI$^+$) *m/z* 444 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{24}H_{39}NO_5Na$ [M+Na]$^+$: 444.2726, found 444.2728.

### 5.1.26. 2-(5,9,13-Trimethyltetradeca-4,8,12-trienoylamino)malonic acid (21b)

Prepared according to general procedure A on **20b** (0.1579 g, 0.38 mmol) in 4.5 mL solvent with lithium hydroxide (0.0521 g, 1.24 mmol) for 1 h. Compound **21a** was obtained as a pure white solid (0.137 g, 100%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.60 (s, 9H), 1.67 (s, 3H), 1.95–2.07 (m, 8H), 2.32–2.40 (m, 4H), 5.10 (m, 3H), 5.17 (d, 1H, $J$ = 7.0 Hz), 7.22 (d, 1H, $J$ = 7.0 Hz), 9.17 (br s, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 16.0, 16.1, 17.7, 23.8, 25.7, 26.7, 26.8, 35.

8, 39.7, 39.7, 56.6, 121.7, 124.0, 124.4, 131.3, 135.1, 137.7, 168.3, 175.4. IR (neat) 3320, 1730, 1640, 1540 $cm^{-1}$. MS ($ESI^+$) *m/z* 388 $[M+Na]^+$. HRMS ($ESI^+$) calcd for $C_{20}H_{31}NO_5Na$ $[M+Na]^+$: 388.2100, found 388.2137.

### 5.1.27. (2*E*,4*E*,8*E*) 5,9,13-Trimethyltetradeca-2,4,8,12-tetraenamide (22a)

Prepared according to general procedure C on **19a** (50 mg, 0.19 mmol) with triethylamine (31 μL, 0.21 mmol) and ethyl chloroformate (21 μL, 0.21 mmol) in 0.4 mL solvent. The ammonium hydroxide solution was prepared with KOH, 0.69 M in methanol (0.4 mL, 0.27 mmol) and ammonium chloride (14 mg, 0.27 mmol). After work up, the residue was purified by preparative TLC ($CH_2Cl_2$/MeOH 9:1) to afford **22a** as a white powder (26.4 mg, 50%). $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 1.62, 1.64 and 1.69 (s, 9H), 1.93 (s, 3H), 1.97–2.16 (m, 4H), 2.19–2.24 (m, 4H), 5.07–5.18 (m, 2H), 5.95 (dl, 1H, $J$ = 14.5 Hz), 6.04 (d, 1H, $J$ = 11.5 Hz), 7.53 (dd, 1H, $J$ = 11.5 Hz and $J'$ = 14.5 Hz). $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 17.0, 18.1, 18.6, 26.8, 28.1, 28.6, 41.7, 42.1, 123.2, 125.4, 125.6, 126.2, 133.0, 137.6, 140.1, 150.5, 172.7. IR 3332, 3189, 2968, 2930, 1660, 1593, 1397. MS ($ESI^+$) *m/z* 284 $[M+Na]^+$.

### 5.1.28. (4*E*,8*E*)-5,9,13-Trimethyltetradeca-4,8,12-trienamide (22b)

Prepared according to general procedure C on **19b** (52 mg, 0.19 mmol) with triethylamine (31 μL, 0.21 mmol) and ethyl chloroformate (21 μL, 0.21 mmol) in 0.4 mL solvent. The ammonium hydroxide solution was prepared with KOH, 0.69 M in methanol (0.4 mL, 0.27 mmol) and ammonium chloride (14 mg, 0.27 mmol). After work up, the residue was purified by preparative TLC ($CH_2Cl_2$/MeOH 95:5) to afford **22b** as a white powder (30 mg, 54%). $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.60, 1.61, 1.64 and 1.69 (s, 12H), 1.95–2.12 (m, 8H), 2.26 (t, 2H, $J$ = 7.0 Hz), 2.34 (td, 2H, $J$ = 6.5 Hz and $J'$ = 7.0 Hz), 5.05 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 16.4, 18.0, 24.3, 26.0, 26.8, 27.1, 36.3, 40.0, 40.1, 122.8, 124.3, 131.7, 135.5, 137.5, 175.7. IR (neat) 3339, 3189, 2966, 2919, 2853, 1659, 1445, 1380. MS ($ESI^+$) *m/z* 286 $[M+Na]^+$.

### 5.1.29. (4*E*,8*E*)-*N*-Hydroxy-5,9,13-trimethyltetradeca-4,8,12-trienamide (23)

To a solution of **19a** (0.064 g, 0.24 mmol) in dichloromethane (1 mL), *N*-methylmorpholine (0.058 mL, 0.53 mmol), EDCI (0.056 g, 0.29 mmol), hydroxylamine (0.017 g, 0.24 mmol) and HOBt (0.039 g, 0.29 mmol) were added. After being stirred for 3 h at room temperature, the reaction mixture was hydrolyzed by $H_2O$ then extracted by dichloromethane. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. La purification of the crude product was realized by column chromatography (heptane/AcOEt 9:1) to give pure compound **33** as colorless oil (67 mg, 100%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.61 (s, 6H), 1.65 (s, 3H), 1.70 (s, 3H), 1.90–2.10 (m, 8H), 2.29–2.54 (m, 4H), 5.12–5.16 (m, 4H), 8.87 (br s, 1H). MS ($ESI^+$) *m/z* 302 $[M+Na]^+$.

### 5.1.30. (4*E*, 8*E*)-5,9,13-Trimethyltetradeca-4,8,12-trienoylsulfamic acid (24)

To a solution of **19a** (0.100 g, 0.38 mmol) in dichloromethane (1 mL) at −78 °C, pivaloyl chloride (0.047 mL, 0.38 mmol) was added and the reaction mixture was stirred for 1 h at −78 °C. In another flask, acetic acid (0.002 mL, 0.034 mmol) and distilled triethylamine (0.057 mL, 0.40 mmol) were introduced successively to a solution of sulfamic acid (0.033 g, 0.34 mmol) in dichloromethane (1 mL). The mixture was warmed to 30 °C for 10 min then cooled to 0 °C. The first solution was added to the second one. After being stirred for 1 h at 0 °C, $H_2O$ was added and the reaction mixture was extracted by dichloromethane. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (heptane/AcOEt 98:2 then 95:5) to afford pure compound **24** as a colorless oil (10.5 mg, 8%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.62 (s, 6H), 1.66 and 1.70 (s, 6H), 1.98–2.12 (m, 8H), 2.38 (m, 2H), 2.50 (m, 2H), 5.13 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 16.4, 18.1, 23.2, 26.1, 26.9, 27.2, 35.9, 40.0, 121.7, 124.4, 124.8, 131.7, 135.6, 137.9, 169.5. IR (neat) 1709, 1438 $cm^{-1}$. MS ($ESI^-$) *m/z* 342 $[M-H]^-$. HRMS ($ESI^-$) calcd for $C_{17}H_{28}NO_4S$ $[M-H]^-$: 342.1739, found 342.1737.

### 5.1.31. (4*E*,8*E*)-5,9,13-Trimethyl-*N*-(phenylsulfonyl)tetradeca-4,8,12-trienamide (25)

Prepared according to general procedure B on **19a** (0.035 g, 0.134 mmol) 2 mL solvent with *N*-methylmorpholine (0.029 mL, 0.268 mmol), EDCI (0.031 g, 0.161 mmol), benzenesulfonamide (0.021 g, 0.134 mmol) and HOBt (0.022 g, 0.161 mmol) for 18 h. The purification of the crude product was realized by preparative TLC ($CH_2Cl_2$/MeOH 95:5) to afford pure compound **25** as a colorless oil (38 mg, 77%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.58 and 1.61 (s, 6H), 1.62 and 1.70 (s, 6H), 1.96–2.10 (m, 8H), 2.30 (m, 4H), 5.04 (m, 1H), 5.09 (m, 2H), 7.57 (t, 2H, $J$ = 7.0 Hz), 7.57 (t, 1H, $J$ = 8.0 Hz), 8.08 (t, 2H, $J$ = 8.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 16.0, 16.1, 17.7, 23.0, 25.7, 26.5, 26.7, 36.5, 39.6, 39.7, 121.2, 123.8, 124.3, 128.4, 128.9, 131.7, 133.9, 135.3, 138.4, 138.9, 170.2. MS ($ESI^+$) *m/z* 426 $[M+Na]^+$. HRMS ($ESI^+$) calcd for $C_{23}H_{33}NO_3SNa$ $[M+Na]^+$: 426.2079, found 426.2091.

### 5.1.32. (*E*)-Diethyl 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allylmalonate (30)

Pinacol vinyl boronate (3.123 mL, 18.42 mmol) was added to a solution of diethylallylmalonate (1.218 mL, 6.14 mmol) in dichloromethane (60 mL). The reaction mixture was warmed to 40 °C. Grubbs I catalyst (0.758 g, 0.91 mmol) was added in three times every hour. After being stirred 3 h at 40 °C, the reaction mixture was cooled to room temperature and the catalyst was removed by filtration on silica gel. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (heptane/AcOEt 9:1) to afford pure compound **30** as an oil (1.8367 g, 92%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.24 (m, 12H), 2.73 (t, 2H, $J$ = 7.5 Hz), 3.46 (t, 1H, $J$ = 7.0 Hz), 4.18 (q, 4H, $J$ = 7.5 Hz), 5.50 (d, 1H, $J$ = 18.0 Hz), 6.52 and 6.57 (td, 1H, $J$ = 18.0 Hz and $J'$ = 7.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.2, 24.7, 34.6, 51.0, 61.6, 83.2, 121.0, 148.8, 168.8. IR (neat) 1731, 1361 $cm^{-1}$. MS ($ESI^+$) *m/z* 349 $[M+Na]^+$. HRMS ($ESI^+$) calcd for $C_{16}H_{27}O_6BNa$ $[M+Na]^+$: 349.1798, found 349.1814.

### 5.1.33. (*E*)-Diethyl 2-(3-(5-((*tert*-butyldimethylsilyloxy)methyl)-1-methyl-1*H*-imidazol-2-yl)allylmalonate (28a)

Compound **30** (1.202 g, 3.08 mmol), sodium bicarbonate (0.358 g, 3.38 mmol) and tetrakis(triphenylphosphine) palladium (0.178 g, 0.154 mmol) were added to a solution of **29** (1 g, 3.08 mmol) in DMF (6 mL). The reaction mixture was warmed to 130 °C and stirred for 4 h. The reaction was allowed to cool to room temperature and was extracted with ethyl acetate and $H_2O$. The crude product was purified by column chromatography (gradient from heptane/AcOEt 7:3 to heptane/AcOEt 2:8) to afford pure compound **28a** as a yellow oil (1.05 g, 80%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.08 (s, 6H), 0.91 (s, 9H), 1.29 (t, 6H, $J$ = 7.0 Hz), 2.86 (t, 2H, $J$ = 7.0 Hz), 3.53 (t, 1H, $J$ = 7.0 Hz), 3.61 (s, 3H), 4.20 (m, 4H), 4.69 (s, 2H), 6.38 (d, 1H, $J$ = 15.5 Hz), 6.65 (m, 1H), 6.89 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ −5.0, 14.4, 26.1, 30.5, 32.6, 51.9, 55.7, 61.9, 119.1, 127.7, 130.8, 131.8, 146.3, 169.0. IR (neat) 1730, 1050 $cm^{-1}$.

### 5.1.34. Diethyl 2-(3-(5-((*tert*-butyldimethylsilyloxy)methyl)-1-methyl-1*H*-imidazol-2-yl)propyl) malonate (28b)

Palladium on activated carbon Pd 10% (0.300 g, 88% w/w) was added to **28a** (0.340 g, 0.802 mmol) in ethyl acetate (8 mL). The

reaction mixture was stirred under hydrogen atmosphere at room temperature for 18 h. The mixture was filtered over silica gel and washed with ethyl acetate, methanol and dichloromethane. The solvent was removed under reduced pressure to afford pure compound **28b** as a colorless oil (341 mg, 100%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.00 (s, 6H), 0.84 (s, 9H), 1.22 (t, 6H, $J$ = 7.0 Hz), 1.78 (m, 2H), 1.95 (m, 2H), 2.65 (t, 2H, $J$ = 7.0 Hz), 3.34 (t, 1H, $J$ = 7.0 Hz), 3.50 (s, 3H), 4.14 (dd, 4H, $J$ = 7.0 Hz, $J'$ = 2.0 Hz), 4.57 (s, 2H), 6.76 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ −5.3, 14.1, 25.3, 25.8, 26.7, 28.4, 30.2, 51.8, 55.5, 61.4, 125.8, 129.0, 146.3, 169.3. MS (ESI$^+$) $m/z$ 427 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{39}N_2O_5Si$ [M+H]$^+$: 427.2628, found 427.2607.

### 5.1.35. 2-((6*E*,10*E*)-4,4-Bis(ethoxycarbonyl)-7,11,15-trimethylhexadeca-6,10,14-trienyl)-5-(hydroxymethyl)-1-methyl-3-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)-1*H*-imidazol-3-ium (31)

Prepared according to general procedure E on **28b** (0.267 g, 0.96 mmol) with NaH (0.046 g, 1.15 mmol) and **6** (0.26 mL, 0.96 mmol) in 1 mL solvent for 3 h. After work up, the crude product was purified by column chromatography (gradient $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 15 min then $CH_2Cl_2$/MeOH 9:1 for 10 min) to afford compound **33b** as a colorless oil (0.345 g, 50%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.26 (t, 6H, $J$ = 7.5 Hz), 1.61 (m, 17H), 1.69 (m, 6H), 1.82 (s, 3H), 1.90 (m, 2H), 1.96–2.13 (m, 16H), 2.61 (d, 2H, $J$ = 8.0 Hz), 3.03 (t, 2H, $J$ = 8.0 Hz), 3.90 (s, 3H), 4.18 (q, 4H, $J$ = 7.5 Hz), 4.69 (m, 4H), 4.95 (t, 1H, $J$ = 8.0 Hz), 5.09 (m, 4H), 5.28 (t, 1H, $J$ = 8.0 Hz), 7.17 (s, 1H), 7,72 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 16.0, 16.4,16.9, 17.7, 22.5, 24.5, 25.7, 26.1, 26.6, 26.7, 30.9, 32.4, 32.6, 33.1, 39.4, 39.7, 40.0, 46.4, 52.7, 57.3, 61.5, 115.2, 117.0, 119.2, 123.0, 123.7, 124.1, 124.2, 131.4, 131.5, 134.5, 135.4, 136.1, 139.8, 145.8, 145.9, 171.0. MS (ESI$^+$) $m/z$ 721 [M]$^+$. HRMS (ESI$^+$) calcd for $C_{45}H_{73}N_2O_5$ [M]$^+$: 721.5519, found 721.5542.

### 5.1.36. (*E*)-Diethyl 2-(3-(5-formyl-1-methyl-1*H*-imidazol-2-yl)-allyl)malonate (32a)

Prepared according to general procedure D on **28a** (0.300 g, 0.707 mmol) in 4 mL solvent with TBAF (1.41 mL, 1.41 mmol) for 2 h. After work up, the crude product was purified by column chromatography (gradient $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 15 min) to afford pure alcohol as a yellow oil (115 mg, 52%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.28 (t, 6H, $J$ = 8.0 Hz), 2.86 (t, 2H, $J$ = 7.0 Hz), 3.53 (t, 1H, $J$ = 8.0 Hz), 3.65 (s, 3H), 4.21 (m, 4H), 4.61 (s, 2H), 6.38 (d, 1H, $J$ = 15.0 Hz), 6.63 (m, 1H), 6.90 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 30.2, 32.2, 51.5, 54.6, 61.6, 118.6, 127.9, 131.2, 131.4, 146.7, 168.7. MS (ESI$^+$) $m/z$ 333 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{15}H_{22}N_2O_5Na$ [M+Na]$^+$: 333.1426, found 333.1435. Activated manganese dioxide(IV) (0.208 g, 2.39 mmol) was then added to the yellow oil (0.124 g, 0.399 mmol) in 3 mL solvent to afford **32a** (104 mg, 85%) as a colorless oil. $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 1.28 (t, 6H, $J$ = 8.0 Hz), 2.91 (t, 2H, $J$ = 7.0 Hz), 3.54 (t, 1H, $J$ = 8.0 Hz), 3.93 (s, 3H), 4.22 (m, 4H), 6.44 (d, 1H, $J$ = 15.0 Hz), 6.92 (m, 1H), 7.72 (s, 1H), 9.68 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 31.9, 32.2, 51.1, 61.7, 117.1, 131.8, 137.0, 143.9, 151.3, 168.4, 178.8. IR (neat) 1726, 1660 cm$^{-1}$. MS (ESI$^+$) $m/z$ 333 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{15}H_{22}N_2O_5Na$ [M+Na]$^+$: 333.1426, found 333.1435.

### 5.1.37. Diethyl 2-(3-(5-formyl-1-méthyl-1*H*-imidazol-2-yl)propyl)-malonate (32b)

Prepared according to general procedure D on **28b** (0.342 g, 0.803 mmol) in 4 mL solvent with TBAF (1.60 mL, 1.60 mmol) for 3 h. After work up, the crude product was purified by column chromatography (gradient $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 15 min) to afford pure compound **32b** as a colorless oil (70.5 mg, 29%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.27 (t, 6H, $J$ = 7.0 Hz), 1.78 (qi, 2H, $J$ = 7.5 Hz), 1.95 (q, 2H, $J$ = 7.5 Hz), 2.70 (t, 2H, $J$ = 7.5 Hz), 3.34 (t, 1H, $J$ = 7.5 Hz), 3.59 (s, 3H), 4.14 (m, 4H), 4.57 (s, 2H), 6.77 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.0, 25.2, 26.6, 28.3, 30.2, 51.7, 54.4, 61.4, 125.7, 131.3, 148.8, 169.3. MS (ESI$^+$) $m/z$ 313 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{15}H_{25}N_2O_5$ [M+H]$^+$: 313.1363, found 313.1753. Activated manganese dioxide(IV) (0.272 g, 3.12 mmol) was then added to the colorless oil (0.150 g, 0.48 mmol) in 5 mL solvent to afford **32b** (95 mg, 64%) as a colorless oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.29 (t, 6H, $J$ = 9.0 Hz), 1.79 (qi, 2H, $J$ = 7.0 Hz), 2.01 (q, 2H, $J$ = 7.0 Hz), 2.80 (t, 2H, $J$ = 7.0 Hz), 3.41 (t, 1H, $J$ = 7.0 Hz), 3.88 (s, 3H), 4,20 (m, 4H), 7.71 (s, 1H), 9.69 (s, 1H).

### 5.1.38. Diethyl 2-((*E*)-3-(5-formyl-1-methyl-1*H*-imidazol-2-yl)-allyl)-2-((2*E*,6*E*)-3,7,11-trimethyl dodeca-2,6,10-trienyl)malonate (33a)

Prepared according to general procedure E on **32a** (0.073 g, 0.237 mmol) with NaH (0.057 g, 0.237 mmol) and **6** (0.064 mL, 0.237 mmol) in 2 mL solvent. Compound **33a** was obtained after work up as gray oil (0.123 g, 100%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.26 (t, 6H, $J$ = 8.0 Hz), 1.62 (s, 6H), 1.64 (s, 3H), 1.70 (s, 3H), 1.99–2.09 (m, 8H), 2.69 (d, 2H, $J$ = 6.0 Hz), 2.89 (t, 2H, $J$ = 6.0 Hz), 3.92 (s, 3H), 4.21 (q, 4H, $J$ = 7.0 Hz), 5.03 (t, 1H, $J$ = 8.0 Hz), 5.11 (t, 2H, $J$ = 7.0 Hz), 6.35 (d, 1H, $J$ = 16.0 Hz), 6.86 (m, 1H), 7.75 (s, 1H), 9.69 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.2, 16.0, 16.5, 17.7, 25.7, 26.5, 26.7, 30.9, 31.3, 36.4, 39.7, 40.0, 57.7, 61.4, 117.3, 117.9, 123.8, 124.3, 131.9, 132.4, 135.3, 136.4, 139.6, 144.0, 151.3, 170.7, 178.9. IR (neat) 1727, 1669 cm$^{-1}$. MS (ESI$^+$) $m/z$ 535 [M+Na]$^+$. HRMS (ESI$^+$) calcd for $C_{30}H_{44}N_2O_5Na$ [M+Na]$^+$: 535.3148, found 535.3132.

### 5.1.39. Dimethyl 2-(3-(5-formyl-1-methyl-1*H*-imidazol-2-ylpropyl)-2-((2*E*,6*E*)-3,7,11-trimethyl dodeca-2,6,10-trienyl)malonate (33b)

Prepared according to general procedure E on **32b** (0.095 g, 0.306 mmol) with NaH (0.008 g, 0.337 mmol) and **6** (0.083 mL, 0.306 mmol) in 5 mL solvent. Compound **33b** was obtained after work up as a colorless oil (121 mg, 77%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.25 (t, 6H, $J$ = 7.0 Hz), 1.61 (m, 9H), 1.72 (s, 3H), 1.72–1.77 (m, 2H), 1.97–2.10 (m, 12H), 2.66 (d, 2H, $J$ = 8.0 Hz), 2.76 (t, 2H, $J$ = 8.0 Hz), 3.89 (s, 3H), 4,18 (q, 4H, $J$ = 7.0 Hz), 4.98 (t, 1H, $J$ = 8.0 Hz), 5.11 (m, 2H), 7.71 (s, 1H), 9.68 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 16.0, 16.4, 17.7, 22.2, 25.7, 26.6, 26.8, 30.0, 31.9, 32.3, 39.7, 57.4, 61.3, 117.5, 123.9, 124.5, 131.7, 135.4, 136.3, 139.8, 150.5, 158.2, 171.6, 179.1. IR (neat) 1727, 1669 cm$^{-1}$. MS (ESI$^+$) $m/z$ 515 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{30}H_{47}N_2O_5$ [M+H]$^+$: 515.3485, found 515.3478.

### 5.1.40. Methyl *N*-((2-((1*E*,6*E*,10*E*)-4,4-bis(ethoxycarbonyl)-7,11,15-trimethylhexadeca-1,6,10,14-tetraen-1-yl)-1-methyl-1*H*-imidazol-5-yl)methyl)-L-valyl-L-phenylalanyl-L-methioninate (34a)

According to general procedure F on **33a** (0.112 g, 0.22 mmol) with tripeptide (0.135 g, 0.26 mmol) deprotected with TFA (2.7 mL, 75%), triethylamine (0.030 mL, 0.22 mmol) and $NaBH_3CN$ (0.016 g, 0.26 mmol). The crude product was purified by column chromatography (gradient from $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 15 min) to afford pure compound **34a** as a colorless oil (67 mg, 34%). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.74 and 0.82 (d, 6H, $J$ = 7.0 Hz), 1.27 (t, 6H, $J$ = 7.0 Hz), 1.61 (s, 9H), 1.66 (s, 3H), 1.69 (s, 3H), 1.76–1.92 (m, 3H), 1.94- 2.14 (m, 11H), 2.43 (t, 2H, $J$ = 7.0 Hz), 2.69 (t, 2H, $J$ = 7.0 Hz), 2.87 (m, 3H), 3.14 (m, 2H), 3.49 (s, 3H), 3.72 (s, 3H), 3.84 (s, 2H), 4.19 (m, 4H), 4.64 (q, 1H, $J$ = 7.0 Hz), 4.75 (q, 1H, $J$ = 7.0 Hz), 5.06 (m, 1H), 5.20 (m, 2H), 6.35 (d, 1H, $J$ = 15.0 Hz), 6.70 (m, 1H), 7.22–7.33 (m, 6H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 15.4, 15.5, 16.0, 17.9, 17.9, 19.4, 25.7, 26.6, 26.7, 29.7, 30.2, 31.0, 36.2, 38.0, 39.7, 40.0, 42.8, 51.6, 52.5, 54.0, 57.9, 61.3, 67.9, 117.5, 119.9, 123.9, 124.3, 126.9,

127.1, 128.6, 129.2, 136.2, 131.3, 135.2, 136.4, 139.3, 146.0, 170.9, 171.8, 173.8. IR (neat) 3295, 1730, 1644, 1442 $cm^{-1}$. MS ($ESI^+$) *m/z* 906 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{50}H_{76}N_5O_8S$ $[M+H]^+$: 906.5415, found 906.5396.

#### 5.1.41. Diethyl 2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)-malonate-1-methyl-1*H*-imidazol-5-yl)methyl)-L-valyl-L-phenylalanyl-L-methioninate (34b)

According to general procedure F on **33b** (0.066 g, 0.128 mmol) with tripeptide (0.052 g, 0.128 mmol) deprotected with TFA (1.6 mL, 75%), triethylamine (0.018 mL, 0.128 mmol) and $NaBH_3CN$ (0.010 g, 0.154 mmol). The crude product was purified by column chromatography (gradient from $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 15 min) to afford pure product **34b** as a colorless oil (67 mg, 34%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.82 and 0.85 (d, 6H, $J$ = 7.0 Hz), 1.26 (t, 6H, $J$ = 7.0 Hz), 1.60, 1.62 and 1.63 (s, 9H), 1.69 (s, 3H), 1.87–2.14 (m, 17H), 2.46 (t, 2H, $J$ = 7.0 Hz), 2.64 (d, 2H, $J$ = 8 Hz), 2.79 (m, 2H), 2.83 (d, 2H, $J$ = 7.0 Hz), 3.04–3.20 (m, 2H), 3.45–3.60 (m, 2H), 3.57 (s, 3H), 3.74 (s, 3H), 4.18 (q, 4H, $J$ = 7.0 Hz), 4.64 (t, 1H, $J$ = 8.0 Hz), 4.79 (q, 1H, $J$ = 8.0 Hz), 4.99 (t, 1H, $J$ = 8.0 Hz), 5.08 (m, 2H), 6.73 (s, 1H), 6.80 (d, 1H, $J$ = 7.0 Hz), 7.20–7.32 (m, 5H), 7.41 (d, 1H, $J$ = 8.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 15.4, 16.0, 16.4, 17.7, 18.2, 19.4, 25.7, 26.7, 29.7, 30.8, 31.6, 39.7, 40.0, 46.3, 51.6, 52.5, 54.1, 57.5, 61.4, 67.7, 117.4, 123.9, 124.3, 127.1, 128.7, 129.3, 135.2, 136.6, 139.1, 148.2, 171.0, 171.4, 171.8, 173.8. IR (neat) 3295, 1730, 1644, 1442 $cm^{-1}$. MS ($ESI^+$) *m/z* 908 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{50}H_{78}N_5O_8S$ $[M+H]^+$: 908.5571, found 908.5546.

#### 5.1.42. *N*-((2-((1*E*,6*E*,10*E*)-4-Carboxy-4-(ethoxycarbonyl)-7,11,15-trimethylhexadeca-1,6,10,14-tetraen-1-yl)-1-methyl-1*H*-imidazol-5-yl)methyl)-L-valyl-L-phenylalanyl-L-methionine (35a)

Prepared according to general procedure G on **34a** (0.046 g, 0.051 mmol) with LiOH (0.021 g, 0.51 mmol) in 2 mL solvent for seven days. After work up, compound **35a** was obtained as a colorless oil (42 mg, 100%). $^1H$ NMR (500 MHz, $CD_3OD$) $\delta$ 1.00 and 1.09 (d, 6H, $J$ = 7.0 Hz), 1.29 (t, 3H, $J$ = 6.0 Hz), 1.63 (s, 6H), 1.70 (s, 6H), 2.00–2.20 (m, 14H), 2.56 (m, 2H), 2.62 (m, 2H), 2.95 (m, 3H), 3.27–3.41 (m, 2H), 3.60 (m, 1H), 3.68 (s, 3H), 4.10 (m, 1H), 4.24 (m, 2H), 4.60 (m, 1H), 4.91 (m, 1H), 5.11 (m, 3H), 6.75 (d, 1H, $J$ = 16.0 Hz), 6.94 (m, 1H), 7.17 (t, 1H, $J$ = 7.0 Hz), 7.32 (t, 2H, $J$ = 7.0 Hz), 7.40 (d, 2H, $J$ = 8.0 Hz), 7.58 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 4.4, 15.2, 16.2, 16.7, 17.8, 18.6, 19.3, 25.9, 27.5, 27.8, 29.8, 31.2, 31.8, 32.2, 33.1, 38.1, 39.2, 40.2, 40.9, 52.6, 55.8, 59.1, 62.7, 67.8, 115.0, 118.7, 122.7, 125.1, 125.4, 128.0, 129.7, 130.7, 138.7, 132.2, 136.4, 143.4, 145.4, 145.8, 172.2, 172.9, 173.5, 174.7. MS ($ESI^+$) *m/z* 864 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{47}H_{70}N_5O_8S$ $[M+H]^+$: 864.4945 found 864.4958.

#### 5.1.43. *N*-((2-((4*E*,8*E*)-4-Carboxy-4-(ethoxycarbonyl)-7,11,15-trimethylhexadeca-1,6,10,14-tetraen-1-yl)-1-methyl-1*H*-imidazol-5-yl)methyl)-L-valyl-L-phenylalanyl-L-methionine (35b)

Prepared according to general procedure G on **34b** (0.025 g, 0.027 mmol) with LiOH (0.023 g, 0.55 mmol) in 2 mL solvent for eight days. After work up, compound **35b** was obtained as a colorless oil (6 mg, 27%). $^1H$ NMR (500 MHz, $CD_3OD$) $\delta$ 1.02 and 1.11 (d, 6H, $J$ = 7.0 Hz), 1.29 (m, 3H), 1.63 (s, 6H), 1.70 (s, 6H), 1.79 (m, 2H), 1.89 (m, 2H), 2.00–2.11 (m, 16H), 2.53 (m, 2H), 2.62 (m, 2H), 2.52–2.74 (m, 4H), 2.95 (m, 1H), 3.05–3.08 (m, 2H), 3.28 (m, 2H), 3.63 (s, 3H), 3.74 (m, 3H), 4.14–4.24 (m, 2H), 4.61 (m, 1H), 4.98 (m, 1H), 5.12 (m, 3H), 7.15 (t, 1H, $J$ = 7.0 Hz), 7.32 (t, 2H, $J$ = 7.0 Hz), 7.41 (d, 2H, $J$ = 8.0 Hz), 7.62 (s, 1H), 8.55 (d, 1H, $J$ = 7.0 Hz), 8.85 (d, 1H, $J$ = 7.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.4, 15.2, 16.2, 16.6, 17.8, 18.6, 19.3, 23.1, 25.9, 26.2, 27.1, 27.6, 27.8, 31.2, 31.8, 32.2, 32.7, 32.8, 33.1, 39.2, 40.4, 40.9, 41.1, 48.4, 48.6, 48.9, 49.0, 49.6, 49.8, 52.6, 55.8, 58.6, 62.5, 67.9, 119.0, 125.2, 125.4, 128.0, 129.7, 130.7, 132.2, 136.3, 138.8, 140.5, 150.0, 172.9, 173.0, 174.2, 174.7. IR (neat) 3300, 1730, 1650 $cm^{-1}$. MS ($ESI^+$) *m/z* 866 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{47}H_{72}N_5O_8S$ $[M+H]^+$: 866.5102 found 866.5071.

#### 5.1.44. (*E*)-Diethyl-2-(3-(1-methyl-1*H*-imidazol-2-yl)allyl)malonate (37)

2-Iodo-1-(1*H*)-methyl-imidazole (0.435 g, 2.09 mmol), sodium bicarbonate (0.244 g, 2.30 mmol) and tetrakis(triphenylphosphine)palladium (0.121 g, 0.10 mmol) were added to a solution of **30** (3.818 g, 2.51 mmol) in DMF (6 mL). The reaction mixture was warmed to 130 °C and stirred for 3 h. The reaction was stopped by filtration on silica gel and extracted with ethyl acetate and $H_2O$. The organic layer was dried over $Na_2SO_4$. The crude product was purified by chromatography on silica gel ($CH_2Cl_2$/MeOH 98:2) to afford pure compound **37** as a brown oil (497.4 mg, 85%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.27 (m, 6H), 2.86 (td, 2H, $J$ = 7.5 Hz, $J'$ = 2.0 Hz), 3.53 (t, 1H, $J$ = 7.5 Hz), 3.63 (s, 3H), 4.22 (m, 4H), 6.38 (d, 1H, $J$ = 15.5 Hz), 6.59 (m, 1H), 6.82 (s, 1H), 7.00 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 32.2, 32.7, 51.6, 61.6, 118.4, 121.1, 128.3, 130.6, 144.9, 168.7. MS ($ESI^+$) *m/z* 281 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{14}H_{21}N_2O_4$ $[M+H]^+$: 281.1501, found 281.1507.

#### 5.1.45. Diethyl 2-(3-(1-methyl-1*H*-imidazol-2-yl)propyl)-2-((1*E*,5*E*)-2,6,10-trimethyldodeca-1,5,9-trienyl)malonate (38)

Palladium on activated carbon Pd 10% (0.140 g, 14% w/w) was added to **37** (0.100 g, 0.357 mmol) in ethyl acetate (4 mL). The reaction mixture was stirred under hydrogen atmosphere at room temperature for 4 h at room temperature. The mixture was filtered over silica gel and washed with ethyl acetate, methanol and dichloromethane. The solvent was removed under reduced pressure to afford pure compound **38** as an orange oil (94.1 mg, 93%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.27 (m, 6H), 1.84 (q, 2H, $J$ = 7.0 Hz), 2.01 (q, 2H, $J$ = 7.0 Hz), 2.73 (t, 2H, $J$ = 8.0 Hz), 3.39 (t, 1H, $J$ = 8.0 Hz), 3.60 (s, 3H), 4.21 (m, 4H), 6.81 (s, 1H), 6.95 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.5, 25.8, 26.6, 28.7, 37.7, 52.0, 61.8, 120.9, 127.2, 147.5, 169.7. MS ($ESI^+$) *m/z* 283 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{14}H_{23}N_2O_4$ $[M+H]^+$: 283.1658, found 283.1652. The orange oil (0.0764 g, 0.271 mmol) was added to a suspension of NaH, 60% in oil (0.011 g, 0.271 mmol) in anhydrous THF (2 mL) at 0 °C. The reaction mixture was stirred 1 h at 0 °C then *trans–trans*-farnesyl bromide **6** (0.073 mL, 0. 271 mmol) was added. After being stirred 1 h 30 min at 0 °C, the reaction mixture was extracted with ethyl acetate and washed with $H_2O$. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (gradient from $CH_2Cl_2$ to $CH_2Cl_2$/MeOH 9:1 in 30 min) to afford pure product **38** as a colorless oil (85.1 mg, 64%). $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 1.25 (t, 6H, $J$ = 7.0 Hz), 1.60 and 1.62 (s, 9H), 1.69 (m, 5H), 1.97–2.08 (m, 10H), 2.64 (d, 2H, $J$ = 7.0 Hz), 2.75 (t, 2H, $J$ = 8.0 Hz), 3.61 (s, 3H), 4.17 (qi, 4H, $J$ = 8.0 Hz), 4.98 (t, 1H, $J$ = 8.0 Hz), 5.11 (m, 2H), 6.81 (s, 1H), 6.99 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 14.1, 16.0, 16.3, 17.7, 22.8, 24.9, 25.7, 26.6, 31.0, 32.0, 32.8, 39.7, 40.0, 57.5, 61.2, 117.6, 120.5, 123.9, 124.3, 128.6, 131.3, 135.2, 139.1, 147.7, 171.4. IR (neat) 1727 $cm^{-1}$. MS ($ESI^+$) *m/z* 487 $[M+H]^+$. HRMS ($ESI^+$) calcd for $C_{29}H_{47}N_2O_4$ $[M+H]^+$: 487.3536, found 487.3519.

#### 5.1.46. 2-(3-(1-Methyl-1*H*-imidazol-2-yl)propyl)-2-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)malonic acid (36)

Lithium hydroxide (0.147 g, 3.5 mmol) was added to a solution of **38** (0.085 g, 0.175 mmol) in THF/$H_2O$ 1:1 (1 mL). The reaction mixture was stirred at room temperature for eight days then stopped by neutralization with a solution of HCl 10% and the reaction mixture was extracted with dichloromethane. The organic layer was dried over $Na_2SO_4$ and the solvent was removed in

reduced pressure to afford pure compound **36** as colorless oil (21 mg, 27%). $^1$H NMR ($CDCl_3$) $\delta$ 1.60, 1.61 and 1.62 (s, 9H), 1.68 (m, 5H), 1.90–2.08 (m, 10H), 2.53 (d, 2H, $J$ = 8 Hz), 2.91 (t, 2H, $J$ = 8 Hz), 3.78 (s, 3H), 5.10 (m, 3H), 7.28 (s, 1H), 7.33 (s, 1H). $^{13}$C NMR ($CDCl_3$) $\delta$ 16.8, 17.2, 18.5, 25.0, 26.0, 25.5, 28.3, 35.0, 38.5, 39.7, 41.5, 41.8, 58.9, 120.8, 121.4, 124.7, 126.0, 126.2, 132.7, 136.7, 140.3, 147.7, 166.3. MS ($ESI^+$) $m/z$ 453 $[M+Na]^+$. HRMS ($ESI^+$) calcd for $C_{25}H_{38}N_2O_4Na$ $[M+Na]^+$: 453.2729, found 453.2721.

### 5.2. Biological assays

#### 5.2.1. Yeast FTase assay

Assays were realized on 96-well plates, prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read on Wallac Victor fluorimeter from Perkin–Elmer. Per well 20 μL of farnesyl pyrophosphate (10 μM) was added to 180 μL of a solution containing 2 μL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 μL of a solution composed by 0.1 mL of partially purified recombinant yeast FTase (2.2 mg/mL) and 7.0 mL of Dansyl-GCVLS peptide (in the following buffer: 5.8 mM DTT, 6 mM $MgCl_2$, 12 μM $ZnCl_2$ and 0.09% (w/v) CHAPS, 53 mM Tris/HCl, pH 7.5). Then the fluorescence development was recorded for 15 min (0.7 s per well, 20 repeats) at 30 °C with an excitation filter at 340 nm and an emission filter at 486 nm. Each measurement was realized twice as duplicate or triplicate.

#### 5.2.2. Human FTase assay

Assays were realized on 96-well plates, as described for yeast FTase but octyl-D-glucopyranoside (0.18%) was used instead of CHAPS and the solution contains 5 μL of partially purified human FTase (1.5 mg/mL)[24] in 1 mL peptide solution.

The kinetic experiments were realized under the same conditions either with FPP as varied substrate with constant concentration of Dns-GCVLS of 2.5 μM or with Dns-GCVLS as varied substrate with constant concentration of FPP of 10 μM. Non-linear regressions were made by KALEIDAGRAPH 4.03 software. $K_m^{app}$ and $V_{max}^{app}$ were obtained from Michaelis–Menten fits of fluorescent data and $K_i$ and $K_i'$ were deduced from the linear regression of $K_m^{app}/V_{max}^{app}$ and $1/V_{max}^{app}$ as a function of the inhibitor concentration, respectively.

#### 5.2.3. Assay for in vitro inhibition of *P. falciparum* growth

The chloroquine-resistant strain FcB1/Colombia of *P. falciparum* was maintained in vitro on human erythrocytes in RPMI 1640 medium supplemented by 8% (v/v) heat-inactivated human serum, at 37 °C, under an atmosphere of 3% $CO_2$, 6% $O_2$, 91% $N_2$. In vitro drug susceptibility was measured by [$^3$H]-hypoxanthine incorporation as described.[51] Drugs were prepared in DMSO at a 10 mM concentration. Compounds were serially diluted twofold with 100 μL culture medium in 96-well plates. Asynchronous parasite cultures (100 μL, 1% parasitemia and 1% final hematocrit) were then added to each well and incubated for 24 h at 37 °C prior to the addition of 0.5 μCi of [$^3$H]-hypoxanthine (GE Healthcare, France, 1–5 Ci mmol/mL) per well. After a further incubation of 24 h, plates were frozen and thawed. Cell lysates were then collected onto glass-fiber filters and counted in a liquid scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% growth inhibition ($IC_{50}$) was obtained from the drug concentration–response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments.

#### 5.2.4. Assay for in vitro inhibition of *T. brucei* growth

Procyclic forms of the *T. brucei brucei* strain WT 2913 were maintained in semi-defined medium 79 containing 10% (v/v) heat-inactivated fetal calf serum (FCS) at 27 °C.[52] Bloodstream forms of *T. brucei brucei* strain 93 were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% $CO_2$.[53] In all experiments, log-phage cell cultures were harvested by centrifugation at 3000*g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells[54] and were performed according to the manufacturer recommendations (Alamar-Blue®Assay, Invitrogen Corporation). Drug stock solutions were prepared in pure DMSO. *T. brucei* procyclic ($1 \times 10^6$ cells/mL) or bloodstream forms ($3 \times 10^4$ cells/mL) were cultured as described above in 24-well plates (1 mL per well) either in the absence or in the presence of different concentrations of inhibitors (0, 0.1, 1, 10 and 100 μM) with a final DMSO concentration of 1%. After a 72-h incubation, AlamarBlue® solution (100 μL) was added in each well and fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after a further 4-h incubation. Each inhibitor concentration was tested in triplicate and the experiment repeated twice. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that of in the absence of drug.

#### 5.2.5. Cytotoxicity[55]

Human mouth epidermal carcinoma (KB) or human diploid embryonic lung (MRC-5) cells were seeded into 96-well microplates at 2000 cells per well. The cell lines were incubated for 72 h with different concentrations of drugs. The final volume in each experiment was made up with the media containing 1% DMSO final volume. Docetaxel was used as positive control. The experiments were performed in triplicate. Cell growth inhibition was determined by the MTS assay according to the recommendations of the manufacturer [Promega]. The optical density was measured at 490 nm. The number of viable cells was proportional to the extent of formazan production. The percent cytotoxicity index [(OD490 treated/OD490 control) × 100] was calculated from three experiments. For $IC_{50}$ determinations, the cytotoxicity index was plotted against the drug concentration ranged over 10–0.5 nM and the value resulting in 50% cytotoxicity was determined.

### Acknowledgments

The authors thank J.-B. Créchet for the generous gift of the *E. coli* strain expressing the yeast FTase, Dr. J. Ouazzani and P. Lopez for the production and purification of recombinant yeast and human FTases, G. Aubert for cytotoxicity assays and Research Minister and CNRS for financial support.

### Supplementary data

Supplementary data (Lineweaver–Burk plots of the fluorescent data and $K_i$ determination for compounds **16**, **35a** and **35b**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.12.017.

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