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Bioactive prenylated phenyl derivatives derived from marine natural products: novel scaffolds for the design of BACE inhibitors†

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Abnormal accumulation of neurotoxic beta-amyloid peptides (Aβ) is a key factor in the development of Alzheimer's disease (AD) and strategies to reduce Aβ production constitute an active field of research for the development of novel therapeutic agents for the treatment of AD. In particular, β-secretase-1 (BACE-1) has been a prime target for modulating Aβ production although obtaining drug-like BACE-1 inhibitors has proven to be highly challenging. Here we report the isolation and biochemical characterization of a marine natural product, the prenylated hydroxybenzoic acid **1**, with BACE-1 inhibitory activity and ability to decrease Aβ production in cell-based assays. Synthesis and biological activity of a number of new synthetic analogues are also reported, as well as initial structure–activity relationship (SAR) analysis on this chemical family. Hence, these compounds constitute novel scaffolds from which more potent and selective BACE-1 inhibitors could be designed as potential therapeutic agents for the treatment of Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system and the leading cause of dementia in the elderly population. Its clinical symptoms include not only impairment of memory, cognition, temporal and local orientation, judgment and reasoning but also severe emotional disturbances. There are currently no treatments available which can prevent or reverse the disease or its progression. Thus, AD has become a major problem in all societies with high life expectancies and also a significant economic burden for their health systems.

Although the cause of AD remains unknown, a large body of evidence has accumulated suggesting that abnormal accumulation of neurotoxic Aβ peptides is a key factor in the development of AD, and thought to be the likely cause of memory and cognitive loss in this disease condition.[1–4] Neuropathological examination of brains from AD patients reveals that the accumulation of secreted Aβ peptides in extracellular amyloid plaques is involved in neuronal loss in brain regions (hippocampus and cortex) responsible for memory and cognitive functions. Clearly, strategies to reduce Aβ production will facilitate development of therapeutic agents for the treatment of AD.[5]

According to the amyloid hypothesis, the generation of Aβ peptides is a critical event in AD, and inhibiting this process may affect disease progression. Aβ peptides are generated in neuronal secretory vesicles by proteolytic cleavage of the type I transmembrane amyloid precursor protein (APP) by two aspartyl-proteases, called β-secretase and γ-secretase, that sequentially cleave at the N terminus and variant C-termini of Aβ within APP, respectively, resulting in Aβ of 40 or 42 amino acids (Aβ40 and Aβ42, respectively).[5] Both peptides are believed to initiate aggregation and later deposition in the neuritic plaques characteristic of AD.[6]

The importance of the therapeutic potential of inhibiting Aβ deposition has motivated many researchers to isolate and characterize secretase enzymes and to identify potential inhibitors. BACE is a transmembrane aspartyl protease of type I topology with β-secretase activity, in which the N-terminus and catalytic site reside on the luminal or extracellular side of the membrane. In fact, BACE is the major β-secretase responsible for Aβ generation in the brain. BACE catalyses the initial step in the amyloidogenic metabolism of the large transmembrane APP, releasing a soluble APPβ (sAPPβ) ectodomain by specific hydrolysis at the β-site (Met671-Asp672) and simultaneously generating a membrane-bound C-terminal fragment consisting

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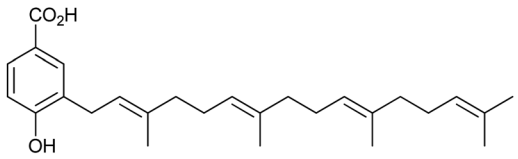
of 99 amino acids (CTF99). The latter is then further processed by the γ-secretase enzyme complex which, in turn, generates the APP intracellular domain and releases the 39–42-amino-acid Aβ-peptide. The identification of the Aβ peptide as the main constituent of the extracellular plaques which characterize AD led to the formulation of the amyloid cascade hypothesis of AD. Interruption of this metabolic cascade at one of several sites could potentially reduce the amyloid burden, and slow or even reverse the devastating consequences of the disease. β-Secretase is particularly attractive in this context as it catalyses the first and rate-limiting step in the pathway. Moreover, the subtype BACE-1 is the major β-secretase responsible for Aβ generation in the brain. Thus, β-secretase inhibition aimed at blocking what is believed to be excessive activity or altered control of BACE-1 in AD is a key target for therapeutic intervention.[7,8]

Therefore, BACE-1 inhibitors have been actively sought by different drug discovery approaches as potential anti-AD drug candidates.[9] The first generation of BACE-1 inhibitors included peptide and peptidomimetic compounds acting as transition-state analogs able to tightly interact with the BACE-1 aspartic acids of the catalytic dyad. Unfortunately, as expected, despite their nanomolar affinity for the enzyme, peptides and peptidomimetics do not have an optimal pharmacokinetic profile.[10] Recently, based on the large amount of structural information, different drug discovery approaches have been employed, and several non-peptidic BACE-1 inhibitors also with nanomolar activity have been identified,[11] including carbinamine,[12] macrocycles,[13] aminoimidazol[14] or hydroxyethylamine moieties.[15] Due to the involvement of β-amyloid in Alzheimer's disease and the need to develop therapeutic agents that effectively reduce β-amyloid deposition, increasing effort has been made in this area of research leading to the development of compounds that have reached phase II clinical trials.[16–18]

Natural products have historically played a critical role in the discovery and development of new drugs. Despite the fact that the marine environment remains largely unexploited, the recognition that marine organisms are a rich source of potential drug candidates in recent years has sparkled the intensive exploration of new substances from marine organisms.[19] Thus, marine organisms represent a diverse source of biologically active compounds as drug leads and inspiration for the synthesis of non-natural molecules with improved drug-like properties. As part of our continuous screening program focused on the identification of novel marine origin compounds with therapeutic potential for neurodegenerative diseases, bioassay guided-fractionation of active extracts from the sponge *Sarcotragus* sp. (Ircinidae family) led to the isolation of bioactive prenylated phenyl derivatives as Aβ peptide inhibitors.

In particular, by using an ELISA cell-based assay we have identified the prenylated hydroxybenzoic acid **1** (Fig. 1), previously isolated from other marine sponges,[20–22] as an inhibitor of Aβ(1−40) peptide production in the micromolar range (10 ± 7.5% (1 μM); 47 ± 19% (10 μM)). Further studies on their mechanism of action showed that this compound also inhibited BACE-1 enzymatic activity (% BACE-1 activity: 45 ± 21% at 1 μM).

In view of the biological activity of this family of marine-derived compounds we considered these prenylated phenyl structures as attractive scaffolds for the design of new analogues. Consequently, we also report here on the synthesis and biological activity of a number of new synthetic analogues by incorporating different structural modifications to initially explore the importance of the substituents of the phenyl ring in the inhibitory activity. Structure–activity relationship studies were also conducted along this work and will be discussed as well.



1

Aβ production Inhib. (%)= 10±7.5 (1μM); 47%±19 (10 μM)

Fig. 1 Chemical structure of the marine prototype **1** and its cell-based assay activity.

Results and discussion

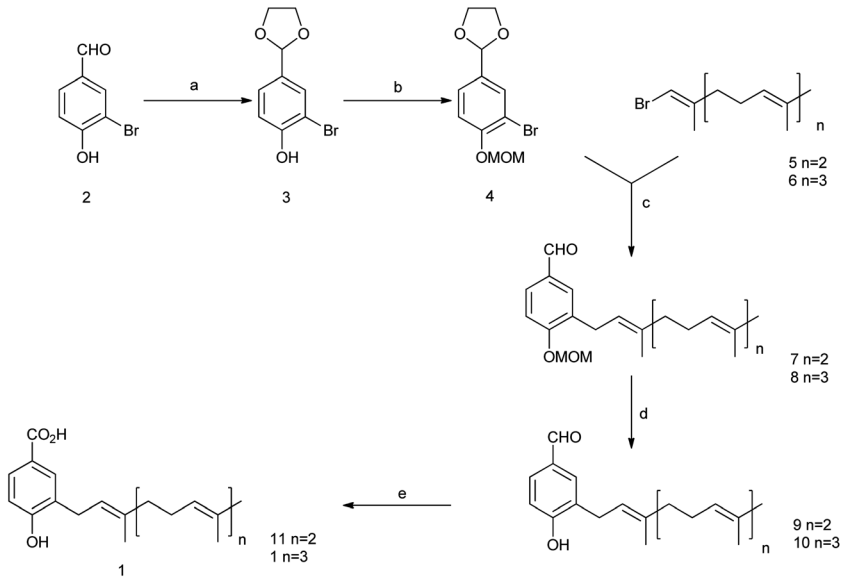
Chemistry

The total synthesis of the marine prototype **1** (Fig. 1) is performed in a similar manner to that previously reported by Lang *et al.*[23] (Scheme 1), aiming to complete its biological characterization and to synthesize derived compounds for further investigation of the influence of both the carboxylic acid and hydroxyl groups present in the molecule in the inhibitory activity.

The key step of the synthesis consisted of the coupling between the phenyl derivative **4** and the required geranylgeranyl bromide **6**. The preparation of the appropriate phenyl derivative **4** is successfully achieved by protection of the aldehyde group of the benzaldehyde **2** through formation of its corresponding acetal **3** followed by protection of the hydroxyl group with methoxymethyl chloride to afford the diprotected derivative **4**. On the other hand, the synthesis of the geranylgeranyl bromide **6** is quantitatively performed, employing the reported methodology (Scheme 1).[24]

Therefore, the coupling between the phenyl derivative **4** and geranylgeranyl bromide **6** is performed employing *n*-butyllithium and copper bromide through formation of its lithium dicuprate derivative, followed by acid mediated MOM-deprotection to obtain the aldehyde **8**. Subsequent deprotection of the aldehyde group with CSA (+/−)-camphor-10-sulfonic acid, followed by oxidation of the aldehyde resulting group with sodium hydrogen phosphate and sodium chlorite, afforded the final synthetic prototype **1**, with moderate yields. The chemical structure of the synthetic prototype **1** is assigned from detailed NMR spectroscopy data and HPLC-MS spectrometry data as well as by comparison with data from the literature,[21] confirming that of the marine prototype.

Regarding the synthesis of compounds derived from prenylated hydroxybenzoic acid **1** the prototype **1** is transformed into the corresponding benzyl esters **12** and **13**, by treatment with



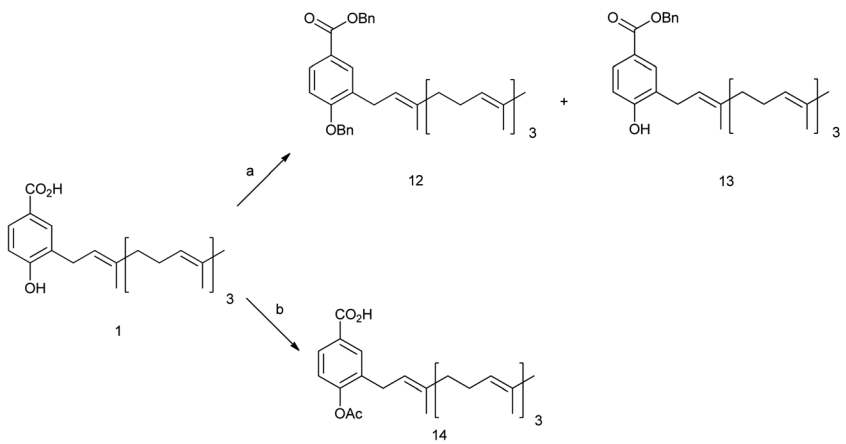
Scheme 1 Synthesis of compounds **1** and **11**. Reagents and conditions: (a) ethylene glycol, pTsOH, toluene; (b) MOMCl; (c) (1) *n*-BuLi, CuBr-DMS; (2) HCl; (d) CSA, MeOH; (e) $NaClO_2$, NaH_2PO_4, THF/H_2O.

benzyl bromide in the presence of a base, and the hydroxyl group is acetylated with acetic anhydride in pyridine to afford the acetylated derivative **14** (Scheme 2).

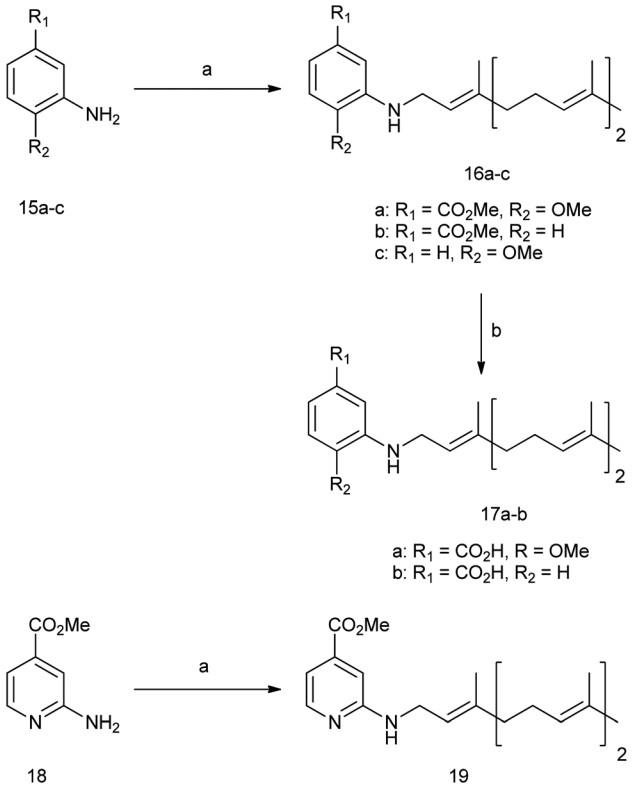
Preparation of synthetic analogues commenced by shortening the prenylated chain present in this molecule in order to evaluate its importance in the inhibitory activity and also aiming to decrease lipophylicity. This new analogue **11** is prepared by coupling the aryl bromide **4** with the corresponding farnesyl bromide **5** following the same methodology as that for the preparation of the prototype **1** (Scheme 1).

As part of the medicinal chemistry strategy, new structural modifications were explored such as incorporating an amino group as linkage between the aryl and the prenylated moieties to see its influence on potency. Furthermore, these analogues were more readily synthesized than those containing a C–C linkage. In general, the preparation of these new analogues, which maintained either two or three substituents in the same relative disposition of the functional groups in the prototype **1**, is based on the alkylation of different anilines with farnesyl bromide (**5**). Thus, alkylation of commercially available anilines **15a–c**, in the presence of K_2CO_3 in DMF or THF (Scheme 3), resulted in the corresponding monoalkylated analogues **16a–c**, in moderate yield. Subsequent hydrolysis of the methyl ester groups in compounds **16a,b** afforded the corresponding carboxylic acid analogues **17a,b** in quantitative yields. The same methodology is employed for the preparation of the pyridine analogue **19** from the protected nicotinic acid **18**.

The replacement of the methoxy group by other alkoxy groups is conducted employing 3-amino-4-hydroxybenzoic acid (**20**) as the starting material as shown in Scheme 4. The carboxylic acid group is protected prior to alkylation, employing H_2SO_4 in MeOH, to give the methyl ester derivative **21**, in quantitative yield. Alkylation of **21** with farnesyl bromide (**5**) in THF, in the absence of a base, produced selectively the alkylated



Scheme 2 Synthesis of compounds **12–14**. Reagents and conditions: (a) BrBn, K_2CO_3, DMF, (b) Ac_2O, pyridine, 0 °C.

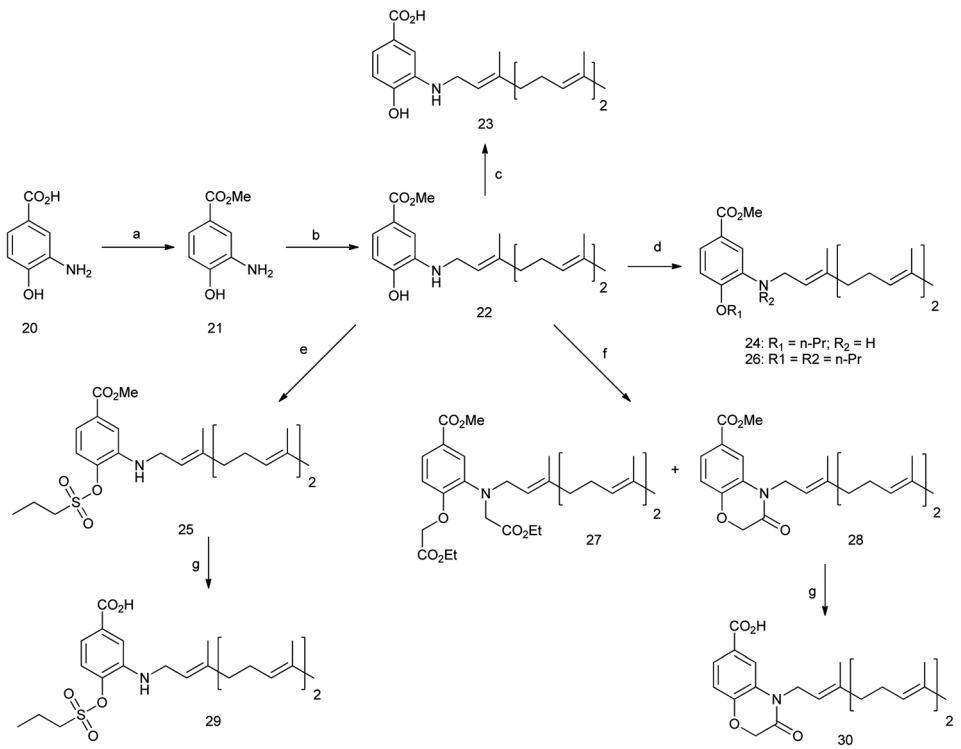


Scheme 3 Synthesis of compounds **16a–c**, **17a–b** and **19**. Reagents and conditions: (a) *trans,trans*-farnesyl bromide, K_2CO_3, THF; (b) $LiOH \cdot H_2O$, H_2O/MeOH (3 : 2).

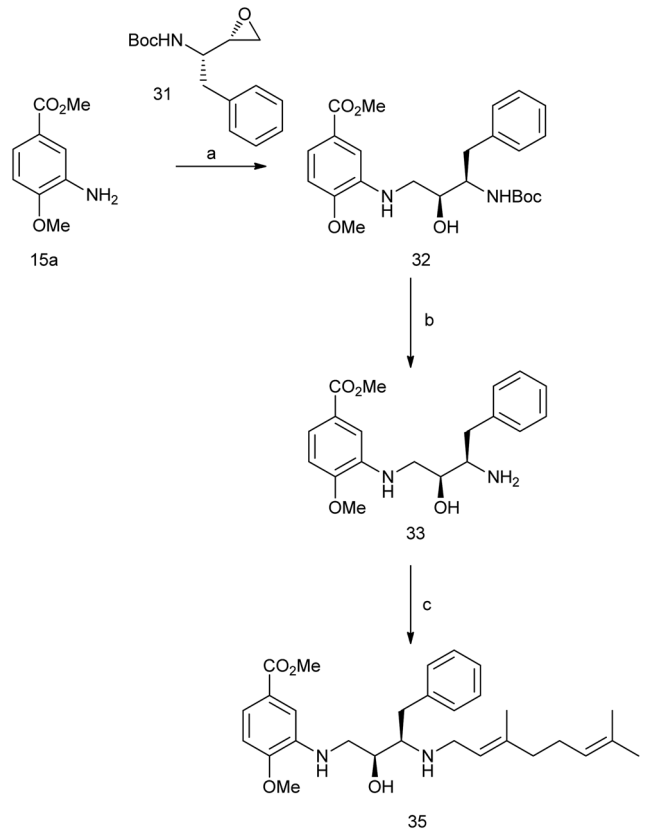
aminophenol derivative **22** in 30% yield, the alkylation of the aromatic hydroxyl group not being detected. Further hydrolysis of its methyl ester group generated the carboxylic acid **23**, in quantitative yields. On the other hand, alkylation of the aminophenol **22** with 1-bromopropyl, employing K_2CO_3 as base in DMF, afforded the corresponding *O*-alkylated compound **24** together with the simultaneously *O*- and *N*-alkylated compound **26.** Similarly, alkylation of the aminophenol **22** with ethyl bromoacetate employing NaH in THF, in the presence of a phase transfer catalyst, produced a mixture of the simultaneously *N*- and *O*-dialkylated analogue **27** together with the monoalkylated intramolecular-cyclized analogue **28** (Scheme 4) in 1 : 7 ratio. Employing the same conditions, alkylation of the aminophenol **22** with 1-propanosulfonyl chloride afforded mainly the *O*-monoalkylated compound **25.**

Subsequent hydrolysis of compound **25** and the cyclized compound **30**, under common basic conditions, yielded the corresponding carboxylic acids **29** and **30.**

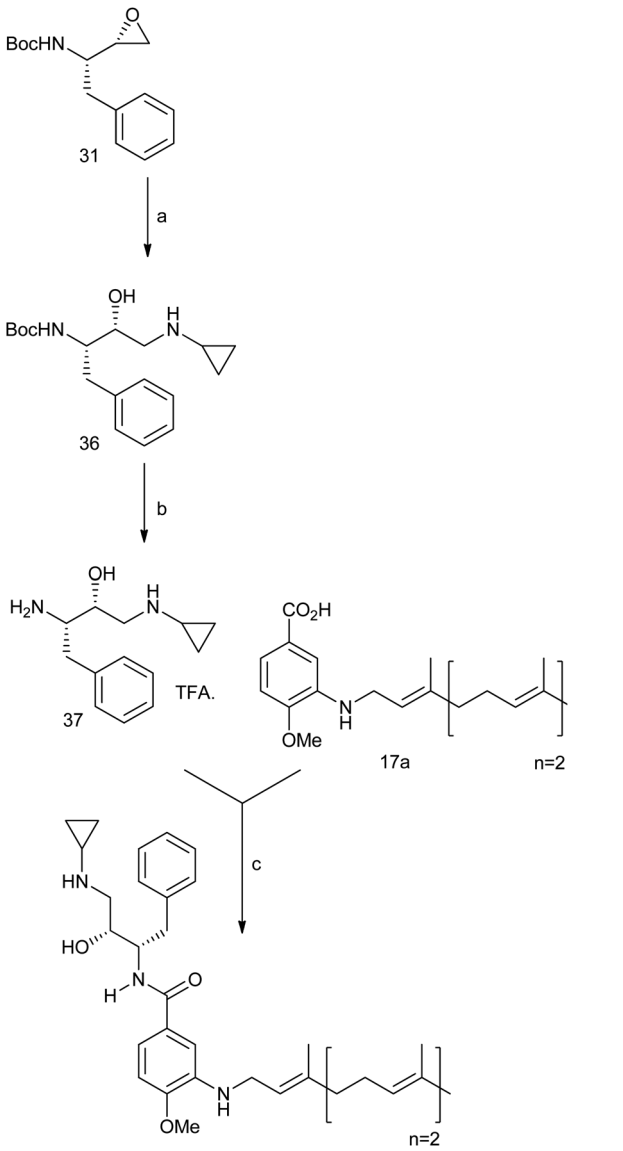
Preparation of analogues, incorporating the ethanolamine moiety by replacing a prenyl unit in the tether chain, is performed employing the epoxide fragment **31** as depicted in Scheme 5. The ethanolamine intermediate **33** is synthesized, in moderate yield, by addition of the 3-amino-4-methoxybenzoic acid methyl ester (**15a**) to the aminoepoxide **31**, in i-PrOH at high temperature, followed by quantitative deprotection of the Boc-amino group with HCl gas at 0 °C. Subsequent alkylation of the aminoalcohol **33** with geranyl bromide, employing $EtB_{3B}N$ in DMF at 50 °C, generated the analogue **35**, in low yields.



Scheme 4 Synthesis of compounds **22–30**. Reagents and conditions: (a) MeOH, H_2SO_4, 70 °C; (b) *trans,trans*-farnesyl bromide (**5**), THF, 30%; (c) $LiOH \cdot H_2O$, H_2O/MeOH (3 : 2); (d) 1-bromopropyl, K_2CO_3, DMF; (e) 1-propanosulfonyl chloride, NaH, THF, 10 min, 0 °C then $INBu_4$ (0.25 eq.), 18-crown-6 (f) ethyl bromoacetate, NaH, THF, 10 min, 0 °C then $INBu_4$ (0.25 eq.), 18-crown-6; (g) $LiOH \cdot H_2O$, THF/H_2O or THF/MeOH/H_2O.



Scheme 5 Synthesis of ethanolamine **35**. Reagents and conditions: (a) *tert*-butyl (1*S*)-1-[(2*S*)-2-oxiranyl]-2-phenylethylcarbamate, ^{i}PrOH, 50 °C; (b) HCl (g), EtOAc/MeOH (4 : 1), 0 °C; (c) geranyl bromide (**34**), Et_3N, DMF/THF (5 : 2), 70 °C.



Scheme 6 Synthesis of ethanolamine **38**. Reagents and conditions: (a) *tert*-butyl (1*S*)-1-[(2*S*)-2-oxiranyl]-2-phenylethylcarbamate (**31**), cyclopropylamine, ^{i}PrOH, 50 °C, 7 h; (b) TFA, DCM; (c) **17a**, **37**, DMF, DIPEA, HOBt and EDC.

The analogue **38**, incorporating the ethanolamino moiety as an amide into the carboxylic acid group, is obtained in 47% yield by coupling the ethanolamine **37** and the carboxylic acid **17a** with HOBt, EDC and DIPEA in DMF (Scheme 6). The required ethanolamine fragment **37** is synthesized following the methodology reported by Stachel *et al.*[25] This methodology consisted of an epoxide ring opening of the aminoepoxide **31** with cyclopropylamine at 50 °C, followed by Boc-amino deprotection employing TFA in DCM.

Biological activity

All compounds synthesized were assayed in both the BACE-1 inhibitory assay and the Aβ peptide cell-based assay (Table 1). Due to intrinsic fluorescence emission of some synthesized analogues several enzymatic assays were attempted. Biological evaluation of the synthetic natural product **1** is shown to be comparable with the natural prototype **1**, in both assays. The analogue program is initiated preparing new semi-synthetic compounds by direct derivatization of the two functional groups present in the phenyl ring of the synthetic prototype **1**, the hydroxyl and the carboxylic acid groups. Among the compounds synthesized **12**, **13** and **14**, only the acetyl derivative **14** maintained the same inhibition pattern of the prototype **1**, in both assays. Considering the high lipophylicity of the prenyl tether chain a new analogue is synthesized shortening the prenyl chain to 3 prenyl units. However, this new analogue **11** results in lower potency than the natural prototype **1**.

The introduction of an amino group as a linkage between the phenyl and the three prenyl unit tether led to new analogues. In an initial investigation of the influence of the substituents of the phenyl ring in the activity, we focused our attention on methylated analogues, readily obtained in the synthetic route. It is evidenced that while simultaneous methylation at the carboxylic acid and the hydroxyl groups in compound **16a** resulted in a decrease of inhibitory activity in comparison to that of the marine prototype **1**, the analogue **17a** methylated only at the hydroxyl group, exhibited equipotent BACE-1 inhibitory activity and Aβ production inhibitory activity compared to the natural prototype **1**. These results suggested

Table 1 Inhibitory activities of natural and synthetic prototype **1** as well as synthesized compounds[e]

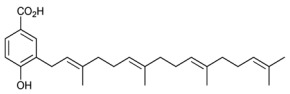
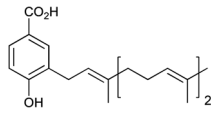
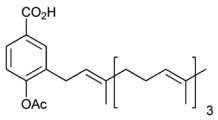
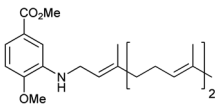
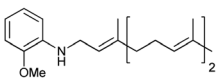
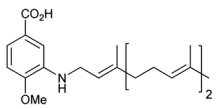
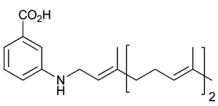
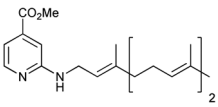
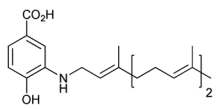
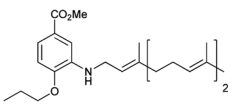
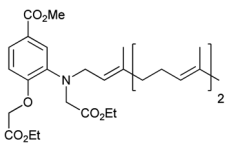
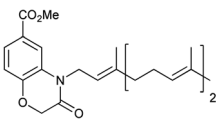
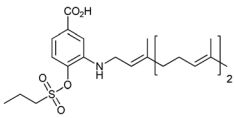
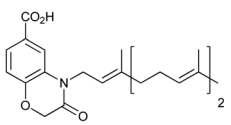
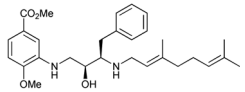
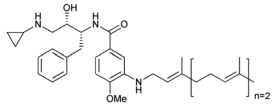
Compound		BACE-1 activity (%)		βA production inhib. (%)	
		1 μM	10 μM	1 μM	10 μM
	Natural 1 Synthetic 1	45 ± 21 42 ± 1	0 0	10 ± 7 8 ± 6	47 ± 19 30 ± 9
	**11**	100	5 ± 9	NA	NA
	**14**[c]	87 ± 14	17 ± 1	24 ± 13	
	**16a**[a]	100	68 ± 11	0	29 ± 13
	**16c**	94 ± 8	64 ± 9	NA	0
	**17a**[a,c]	50 ± 9	0	31 ± 4	
	**17b**[d]	89 ± 9	25 ± 6		
	**19**	100	70 ± 2	NA	0
	**23**	100	36 ± 1	NA	0
	**24**[a]	88 ± 4	27 ± 9	0	0
	**27**[a]	100	0	14 ± 4	36 ± 5
	**28**[a]	59 ± 7	22 ± 11	0	29 ± 6
	**29**	93 ± 2	3 ± 2	14 ± 2	36 ± 4
	**30**	100	17 ± 9	0	0

Table 1 (*Contd.*)

Compound		BACE-1 activity (%)		βA production inhib. (%)	
		1 μM	10 μM	1 μM	10 μM
	**35**[b,c]	100	56 ± 3	3 ± 4	
	**38**[b]	63 ± 6	34 ± 11	10 ± 7	28 ± 1

[a] Autofluorescence employing the FRET assay. Data obtained with kit assay. [b] Autofluorescence employing both the FRET and the kit assay. Data obtained with TRF assay. [c] Toxic in the βA production inhibition assay at 10 μM concentration (*). [d] Toxic in the βA production inhibition assay (*). (*) Toxicity assays were independently conducted with human neuroblastoma cell line SH-SY5Y proving the safety of these compounds in this range of concentration (1.0–10 μM), indicating that the toxicity observed with the CHO-7W cell line is more related to its particular sensitivity. [e] Reference compound OM99-2: BACE-1 activity: IC50 = 7 ± 1 nM; βA production inhib. (%) 80 ± 10 (3 μM).

that the substitution of the hydroxyl group in the phenyl ring lead to an increase of the inhibitory activity in the cell-based Aβ production assay. This result is in accordance with the inhibitory activity pattern of the above acetylated compound **14**, suggesting that the hydroxyl group could be further derivatized to investigate other types of substituents in this position.

In parallel, the influence of the two substituents of the phenyl ring is also studied in analogues **17b** and **16c**, lacking either the methyloxy group **17b** or the carboxilic acid group **16c**. As their inhibition pattern indicates, both of them lose inhibitory activity with respect to their tri-substituted analogue **17a**. Consequently, this suggested that the 2 functional groups are required for activity.

The replacement of the phenyl main core by a bioisosteric ring, such as the pyridine moiety, led to a new analogue **19** which exhibited comparable BACE-1 inhibitory activity to its closest analogue **16a** but devoid of activity in the cell-based assay.

Incorporation of different moieties at the site of the carboxylic acid group is evaluated to further investigate the effect in the inhibitory activity of other moieties rather than the methyl group. The transformation of the carboxylic acid group into a variety of esters and amides did not furnish active compounds.

On the other hand, common hydroxyl-containing inhibitor scaffolds, including hydroxyethylene, hydroxyethylamine core, have been applied successfully to a number of BACE-1 inhibitor series. The hydroxyl groups of the cores engage in hydrogen bonds to the catalytic aspartic acids (Asp32 and Asp128) of the BACE-1 protease. Therefore the incorporation of these kinds of fragments, around different positions of the initial scaffold, is also examined. As result, the new inhibitor containing an hydroxyethylamide scaffold at the site of the carboxylic acid group **38** increased the potency with respect to the methyl ester analogue **16a**, in both enzyme (63.5% at 1 μM) and cell-based assays (10% at 1 μM), exhibiting similar potency to **1**. The hydroxyethylene fragment **35**, also incorporated between the phenyl methyl ester unit and the two prenyl tether units, did not result in any notable improved activity with respect to the prenylated analogue **16a**. When the ester group in **35** is transformed into a carboxylic acid group a loss in the BACE-1 activity is obtained. These data evidenced that further investigation of structural requirements rather than the incorporation of the hydroxyl-containing scaffold fragment should be explored in order to increase potency.

It has so far been proved that while more potent compounds in the BACE-1 assay incorporated the underivatized carboxylic acid group or the hydroxyethylamine fragment in the *meta* position with respect to the amine (**17** and **38** respectively), these analogues contained a methoxy group in the *ortho* position with respect to the prenyl tether chain. As transformation of the hydroxyl group into a methoxy group in this type of analogues containing an amino liker had furnished the most potent compounds, a new series of analogues **24–30** incorporating different alkoxy fragments were synthesized to further explore the influence of these fragments in the inhibitory activity. As shown in Table 1 this position allowed quite a variety of fragments to be introduced, furnishing compounds with BACE-1 inhibitory activities in the micromolar range (**24**, **27**, **28**, **29** and **30**) and what is more encouraging is that some of them were found to be active in the cell-based assay (**27–29**).

Conclusion

The prototype **1** has been successfully synthesized and its biological activity confirmed to be consistent with that of the natural compound isolated from the marine organism. The synthetic prototype **1** was indeed employed for an initial exploration of the structural requirements for inhibitory activity. Since model compounds lacking the prenyl chain proved to be inactive in the enzymatic assay, the chemical modifications were focused on the phenyl core ring. Furthermore, a new family of compounds, more readily synthesized than the prototype, bearing an amino linker between the phenyl ring and the three prenyl unit tether chain furnished a compound **17a** of equal inhibitory profile to the marine

prototype. However, synthesized analogues did not improve the inhibitory potency found in the marine prototype, probably due to the lack of specific interactions between the prenyl unit tether chain and BACE-1 active site cavity.

There is evidence indicating that the carboxylic acid group in the *meta*-position with respect to the tether chain in both the natural product and synthesized analogues is relevant for the inhibitory activity of BACE-1, while the *ortho*-position with respect to the tether chain seems to play an important role in the βA inhibitory activity. Furthermore, compound **38** including the hydroxyethylamine moiety, known to favour the interaction with the BACE active site, displayed activity in both enzymatic and cell-based assays. In conclusion, synthetic efforts have not led to an improvement in the biological activity. Nevertheless, due to synthetic accessibility of the compounds, further investigations on the synthesis of new analogues could be exploited for the preparation of novel chemical entities targeted to other biological profiles.

Experimental section

Chemistry

General methods. Chromatographic purity of all compounds synthesized in this work is determined using HPLC chromatographic methods. To carry out the biological analysis it is strongly required that the purity of the synthesized compounds exceeds 95%. HPLC-MS (electrospray): mass spectra were recorded on a spectrometer Micromass Quatro Micro API ZQ2000. The purity of all compounds was measured using a HPLC analytic Waters Delta, with PDA 2996. Sample preparation is performed as follows: weigh about 1 mg of product in an Eppendorf, dissolve it in a sufficient volume of DMSO with a concentration of 1 mg mL^{-1}, transfer 0.1 mL of this solution to an HPLC vial and dilute with 0.9 mL of mixture of 50% v/v acetonitrile–water. The resulting solution has a concentration of 0.1 mg mL^{-1}. Symmetry column C18, 3.5 μm, 2.1 × 100 mm. Column temperature 30 °C. Mobile phase: phase A: 0.1% formic acid in water, phase B: 0.1% formic acid in acetonitrile. Injection volume: 10 mL. Flow: 0.3 mL min^{-1}, wavelength: 254 nm (normally). Melting points were measured by a DSC technique, using a TA Instruments model Q10. Experimental conditions (general): weight of sample of 1 to 5 mg. Conditions: starting temperature 50 °C. Final temperature: 300 °C, if thermodynamic phenomenon is not observed the final temperature is increased to 400 °C. Temperature gradient: 10 °C min^{-1}. NMR spectra were recorded at room temperature (25 °C) with a Varian spectrometer Mercury Plus 400 MHz using a probe type BBI. Values of chemical shifts (δ) are expressed in ppm, using tetramethylsilane (TMS) as the reference. In the ^{1}H NMR spectra are indicated: the multiplicity of signals (s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet), the number of protons derived by integration and the value of the constants coupling (J) in Hz. To obtain the ^{13}C NMR spectra the same spectrometer at 100 MHz is employed.

2-Bromo-4-[1,3]dioxolan-2-yl-phenol (3).[26] To a solution of 3-bromo 3-bromo-4-hydroxybenzaldehyde (5.0 g, 24.8 mmol) in anhydrous toluene (75 mL), ethylene glycol (1.66 mL, 29.8 mmol) and *p*-toluenesulfonic acid monohydrate (473 mg, 2.49 mmol) are added. The resulting mixture is heated to 135 °C; preferably using a Dean-Stark apparatus, for 5 hours; once this time has elapsed, the mixture is brought to room temperature. Triethylamine (5 mL) is added, and the solvent is eliminated under reduced pressure. A purification process was employed using a silica gel chromatographic column, with a mixture of ethyl acetate–hexane in a ratio of 1 : 2 as an eluent, obtaining 5.4 g of **3** as a white solid (yield: 90%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.54 (d, 1H, $J = 2.0$ Hz), 7.24 (dd, 1H, $J = 2.0$ Hz, 8.3 Hz), 6.88 (m, 1H), 5.63 (s, 1H), 4.02 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm) 156.3, 132.6, 132.0, 128.3, 116.9, 110.5, 104.2, 66.3.

2-(3-Bromo-4-methoxymethoxyphenyl)-[1,3]dioxolane (4).[26] To a solution of dioxolan **3** (5.3 g, 22.0 mmol) in anhydrous THF (75 mL), cooled to 0 °C and under a nitrogen atmosphere, DIPEA (9.4 mL, 54.0 mmol) is slowly added. The resulting mixture is stirred for 15 minutes at 0 °C. Once this time has elapsed, ClMOM (3.5 mL, 43.0 mmol) is added dropwise, and the reaction mixture is stirred for 16 hours at room temperature. The mixture is dried under reduced pressure, and a purification process using a silica gel column is performed, using a mixture of ethyl acetate–hexane as the mobile phase in a ratio of 1 : 10, obtaining 6.0 g of **4** as transparent liquid (yield: 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.66 (s, 1H), 7.33 (dd, 1H, $J = 1.5$ Hz, 8.4 Hz), 7.12 (d, 1H, $J = 8.5$ Hz), 5.72 (s, 1H), 5.20 (m, 2H), 4.04 (m, 4H), 3.48 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 154.3, 132.9, 131.5, 126.8, 115.6, 112.7, 102.7, 95.0, 65.3, 56.3.

General procedure for the synthesis of compounds 7 and 8. To a solution of 2-(3-bromo-4-methoxymethoxy-phenyl)-[1,3] dioxolane (**4**) (3.46 mmol) in anhydrous toluene (6 mL) and anhydrous diethyl ether (10 mL), a small quantity of molecular sieves are added. To this solution, at room temperature and under a nitrogen atmosphere, *n*-BuLi (4.50 mmol, 1.6 M solution in hexane) is added, and the mixture is stirred for 5 minutes. Subsequently, CuBr·DMS (2.07 mmol) is added, and the mixture is stirred for another 30 minutes; once the time has elapsed, the corresponding prenyl bromide (3.80 mmol) is added. After 4 hours, an aqueous saturated solution of ammonium chloride (5 mL) is added. The resulting mixture is extracted with diethyl ether (2 × 50 mL), and the organic phase is acidified with a 1 N solution of hydrochloric acid (2 × 50 mL). The organic phase is dried with sodium sulphate, filtered and dried under reduced pressure. A purification process using a silica gel column is performed, using a mixture of ethyl acetate–hexane in a ratio of 1 : 10 as the mobile phase, obtaining the product as transparent oil.

(2*E*,6*E*)-4-Methoxymethoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl)benzaldehyde (7). Reagents: 2-(3-bromo-4-methoxymethoxyphenyl)-[1,3]dioxolane (**4**) (1.0 g, 3.46 mmol), *n*-BuLi (2.8 mL, 4.50 mmol, 1.6 M in hexanes), *trans*,*trans*-farnesyl bromide (0.9 mL, 3.46 mmol), CuBr·DMS (355 mg, 1.73 mmol), diethyl ether anhydrous (10 mL) and toluene anhydrous (6 mL). Appearance: transparent oil. Yield: 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.87 (s, 1H), 7.70 (m, 2H), 7.17 (d, 1H, $J = 8.7$ Hz), 5.33 (t, 1H, $J = 7.2$ Hz), 5.29 (s, 3H), 5.10 (m, 2H), 3.49 (s, 3H), 3.39 (d, 2H, $J = 7.3$ Hz), 2.05 (m, 8H), 1.73 (s, 3H), 1.67 (s,

3H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 191.2, 159.9, 137.1, 135.1, 131.5, 131.3, 130.8, 130.4, 130.0, 124.3, 124.0, 121.2, 113.2, 94.0, 56.3, 39.8, 39.7, 28.4, 26.7, 26.6, 25.7, 17.7, 16.2, 16.0. RP-HPLC: 13.60 min, purity: 98.9%, ESI-MS$[M]^+$ = 371.08.

(2*E*,6*E*,10*E*)-4-Methoxymethoxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzaldehyde (8). Reagents: 2-(3-bromo-4-methoxymethoxyphenyl)-[1,3]-dioxolane (**4**) (500 mg, 1.73 mmol), *n*-BuLi (1.4 mL, 2.25 mmol, 1.6 M in hexanes), geranyl bromide (611 mg, 1.73 mmol), CuBr·DMS (178 mg, 0.86 mmol), diethyl ether anhydrous (5 mL) and anhydrous toluene (3 mL). Appearance: transparent oil. Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.87 (s, 1H), 7.72–7.65 (m, 2H), 7.17 (d, J = 9.0 Hz, 1H), 5.35–5.30 (m, 1H), 5.29 (s, 2H), 5.16–5.05 (m, 3H), 3.49 (s, 3H), 3.39 (d, J = 7.30 Hz, 2H), 2.21–1.90 (m, 12H), 1.73 (s, 3H), 1.68 (s, 3H), 1.59 (s, 6H), 1.58 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 191.2, 159.9, 137.1, 135.1, 134.9, 131.5, 131.2, 130.8, 130.4, 130.0, 124.4, 124.2, 124.0, 121.2, 113.2, 94.0, 56.2, 39.8, 39.7, 28.4, 26.7, 26.6, 25.7, 17.6, 16.2, 16.0, 16.0. RP-HPLC: 14.10 min, purity: 95%, ESI-MS$[M]^+$ = 438.61.

General procedure for the synthesis of compounds 9 and 10. To a solution of the products **7 and 8** (0.24 mmol), dissolved in methanol (10 mL), (+/−)-camphor-10-sulfonic acid (0.26 mmol) is added. The resulting solution is heated to 70 °C for 4 hours. After 4 hours, an aqueous saturated solution of ammonium chloride (5 mL) is added. The resulting mixture is extracted using diethyl ether (2 × 25 mL), and acidified with water (1 × 25 mL) and brine (1 × 25 mL). The organic phase is dried with sodium sulphate, filtered and dried under reduced pressure. A purification process with a silica gel column is performed, using a mixture of ethyl acetate–hexane in a ratio of 1 : 4 as the mobile phase, obtaining a transparent, oily product.

(2*E*,6*E*)-4-Hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl) benzaldehyde (9). Reagents: (2*E*,6*E*)-4-methoxymethoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl) benzaldehyde (**7**) (100 mg, 0.24 mmol), (+/−)-camphor-10-sulfonic acid (62 mg, 0.26 mmol) and methanol (10 mL). Appearance: transparent oil. Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.85 (s, 1H), 7.67 (m, 2H), 6.91 (d, 1H, J = 8.8 Hz), 5.78 (s, 1H), 5.33 (t, 1H, J = 7.4 Hz), 5.08 (m, 2H), 3.43 (d, 2H, J = 7.1 Hz), 2.07 (m, 8H), 1.79 (s, 3H), 1.67 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 191.0, 160.1, 139.9, 135.7, 131.9, 131.3, 130.4, 130.0, 127.4, 124.3, 123.4, 121.1, 120.4, 116.3, 39.6, 29.6, 26.6, 26.2, 25.6, 17.6, 16.3, 16.0. RP-HPLC: 9.42 min, purity: 98.7%, ESI-MS $[M]^+$ = 327.02.

(2*E*,6*E*,10*E*)-4-Hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzaldehyde (10). Reagents: (2*E*,6*E*,10*E*)-4-methoxymethoxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzaldehyde (**8**) (152 mg, 0.35 mmol), (+/−)-camphor-10-sulfonic acid (88 mg, 0.38 mmol) and methanol (10 mL). Appearance: transparent oil. Yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.84 (s, 1H), 7.67 (m, 2H), 6.92 (d, J = 8.34 Hz, 1H), 5.33 (t, J = 6.59 Hz, 1H), 5.20–5.02 (m, 3H), 3.43 (d, J = 6.70 Hz, 2H), 2.30–1.87 (m, 12H), 1.79 (s, 3H), 1.67 (s, 3H), 1.60 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 191.2, 139.7, 135.7, 134.9, 132.0, 131.2, 130.5, 129.9, 127.6, 124.4, 124.2, 123.5, 121.2, 120.5, 116.2, 39.7, 39.6, 29.5, 26.7, 26.6, 26.3, 26.3, 25.7, 17.7, 16.3, 16.1, 16.0. RP-HPLC: 12.6 min, purity: 94.5%, ESI-MS$[M]^+$ = 395.25.

General procedure for the synthesis of compounds 11 and 1. To a solution of aldehyde **9 and 10** (0.43 mmol) in a mixture of THF–H_2O (5 : 1, 2.5 mL/0.5 mL) and 2-methyl-2-butene (0.1 mL), sodium dihydrogen phosphate (1.01 mmol) and sodium chlorite (1.06 mmol) are added one after the other. The reaction mixture is stirred for 4 hours at room temperature; once the time has elapsed, the mixture is neutralized using a 1 N solution of hydrochloric acid, until slight acidification occurs (pH = 4–5). Water (20 mL) is added, and it is extracted with CH_2Cl_2 (2 × 25 mL); the organic phase is dried with sodium sulphate, filtered and dried under reduced pressure. A purification process using a chromatographic column is performed, using a mixture of dichloromethane with 3% of methanol as an eluent.

(2*E*,6*E*)-4-Hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl) benzoic acid (11). Reagents: (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienyl) benzaldehyde (**9**) (100 mg, 0.24 mmol), NaH_2PO_4 (80 mg, 0.58 mmol), $NaClO_2$ (55 mg, 0.60 mmol), 2-methyl-2-butene (0.1 mL), THF (2.5 mL) and water (0.5 mL). Appearance: transparent oil. Yield: 87%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.95 (dd, 1H, J = 1.2 Hz, J = 8.5 Hz), 7.92 (d, 1H, J = 1.9 Hz), 7.38 (m, 5H), 6.94 (d, 1H, J = 8.5 Hz), 5.34 (t, 1H, J = 7.2 Hz), 5.17 (s, 2H), 5.11 (m, 2H), 3.42 (d, 2H, J = 7.2 Hz), 2.04 (m, 8H), 1.67 (s, 6H), 1.59 (s, 3H), 1.59 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 177.7, 160.8, 136.8, 136.5, 135.0, 131.6, 131.2, 130.6, 130.1, 128.5, 128.0, 127.1, 124.4, 124.1, 121.5, 121.4, 110.8, 70.0, 39.7, 39.6, 28.5, 26.7, 26.6, 25.6, 17.6, 16.1, 16.0. RP-HPLC: 10.62 min, purity: 97.8%, ESI-MS$[M]^+$ = 433.12.

(2*E*,6*E*,10*E*)-4-Hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid (1). Reagents: (2*E*,6*E*,10*E*)-4-hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzaldehyde (**10**) (66 mg, 0.17 mmol), NaH_2PO_4 (48 mg, 0.40 mmol), $NaClO_2$ (38 mg, 0.42 mmol), 2-methyl-2-butane (0.05 mL), THF (2 mL) and water (0.5 mL). Appearance: white solid. Yield: 44%. M.p.: 143 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.88 (m, 2H), 6.83 (d, J = 8.6 Hz, 1H), 5.34 (t, J = 7.0 Hz, 1H), 5.11 (m, 3H), 3.41 (d, J = 7.1 Hz, 2H), 2.61–1.87 (m, 12H), 1.79 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 159.5, 139.6, 135.8, 135.1, 132.8, 131.2, 130.6, 127.1, 124.7, 124.5, 123.8, 122.2, 121.0, 115.9, 39.9, 39.8, 39.7, 29.8, 27.0, 26.9, 26.7, 25.7, 17.7, 16.4, 16.2, 16.1. RP-HPLC: 11.3 min, purity: 91.4%, ESI-MS$[M]^+$ = 411.28.

(2*E*,6*E*,10*E*)-4-Acetoxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid (14). To a solution of (2*E*,6*E*,10*E*)-4-hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid (**1**) (100 mg, 0.25 mmol) in pyridine anhydrous (3 mL) is added acetic anhydride (0.045 mL, 0.50 mmol) at 0 °C. The reaction mixture is stirred for 15 minutes at 0 C and then further stirred for 16 hours at room temperature. The reaction mixture is dried under reduced pressure, obtaining the product as a white solid (113 mg). Yield: 100%. M.p.: 122 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.02 (d, 1H), 7.99 (dd, J = 8.4 Hz, 1H), 7.14 (d, J = 8.34 Hz, 1H), 5.26 (dd, J = 7.2 Hz, 6.1 Hz, 1H), 5.11 (tt, J = 8.3 Hz, 4.2 Hz, 3H), 3.31 (d, J = 7.1 Hz, 2H), 2.33 (s, 3H), 2.20–1.91 (m, 12H), 1.72 (s, 3H), 1.68

(d, $J = 0.8$ Hz, 3H), 1.61 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.7, 153.3, 137.7, 135.2, 134.9, 134.1, 132.3, 131.2, 129.3, 124.4, 124.2, 123.9, 122.5, 120.6, 39.7, 28.7, 26.8, 26.6, 26.6, 25.6, 20.8, 17.6, 16.3, 16.0, 16.0. RP-HPLC: 10.27 min, purity: 95.35%, ESI-MS$[M]^-$ = 451.07.

General procedure for the synthesis of compounds 12 and 13. Benzyl bromide (43 mg, 0.25 mmol) is added portion wise to a suspension of (2*E*,6*E*,10*E*)-4-hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid (**1**) (100 mg, 0.25 mmol) and K_2CO_3 (34 mg, 0.25 mmol) in DMF (1.2 mL) and the mixture is stirred for four hours. After two hours the amber solution with K_2CO_3 in suspension turned to a colorless solution with a white suspension. Water is added and the mixture is extracted with ethyl ether (25 mL). The ether phase is washed eight times with water (10 mL) and once with brine, and the solvent was evaporated. The purification process is performed with radial chromatography 10 : 1 (hexane–ethyl acetate).

(2*E*,6*E*,10*E*)-4-Benzyloxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid benzyl ester (12). Appearance: transparent oil. Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.94–7.90 (m, 2H), 7.47–7.30 (m, 10H), 6.91 (d, $J = 9.2$ Hz, 1H), 5.34 (s, 2H), 5.34–5.30 (m, 1H), 5.15 (s, 2H), 5.14–5.07 (m, 3H), 3.41 (d, $J = 7.2$ Hz, 2H), 2.16–1.92 (m, 12H), 1.69 (d, $J = 1.1$ Hz, 3H), 1.67 (d, $J = 0.9$ Hz, 3H), 1.60 (s, 3H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.4, 160.3, 136.7, 136.6, 136.4, 135.0, 134.9, 131.2, 130.5, 129.4, 128.6, 128.5, 128.0, 128.0, 127.2, 124.4, 124.3, 124.2, 122.4, 121.7, 110.8, 70.0, 66.2, 39.8, 39.7, 28.6, 26.8, 26.7, 26.7, 25.7, 17.7, 16.2, 16.0. RP-HPLC: 11.59 min, purity: 100%, ESI-MS$[M]^-$ = 589.87.

(2*E*,6*E*,10*E*)-4-Hydroxy-3-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl) benzoic acid benzyl ester (13). Appearance: transparent oil. Yield: 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.89–7.85 (m, 2H), 7.46–7.30 (m, 5H), 6.83 (d, $J = 8.9$ Hz, 1H), 5.86 (s, 1H), 5.35 (s, 2H), 5.32 (m, 1H), 5.14–5.07 (m, 3H), 3.40 (d, $J = 7.2$ Hz, 2H), 2.18–1.94 (m, 14H), 1.79 (s, 3H), 1.69 (s, 3H), 1.62–1.59 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.7, 159.2, 139.5, 136.6, 135.8, 135.1, 132.3, 131.4, 130.1, 128.8, 128.3, 128.3, 127.0, 124.6, 124.5, 123.8, 122.7, 121.1, 115.9, 66.6, 39.9, 39.9, 39.9, 29.9, 27.0, 26.8, 26.6, 25.9, 17.9, 16.5, 16.3, 16.2. RP-HPLC: 11.43 min, purity: 99.4%, ESI-MS$[M]^-$ = 499.13.

General procedure for the synthesis of compounds 16a–c. To a solution of 3-amino-4-methoxy-benzoic acid methyl ester (500 mg, 2.8 mmol) in THF anhydrous (25 mL) powder potassium carbonate (1100 mg, 8.3 mmol) is added and the resulting mixture is stirred for 10 minutes. *Trans*,*trans*-farnesyl bromide (0.8 mL, 3.0 mmol) in THF (5 mL) is added and the resulting mixture is stirred for a further 16–18 hours. The solvent is evaporated under reduced pressure and the resulting residue is purified by flash column chromatography.

(2*E*,6*E*)-4-Methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (16a). Reagents: methyl 3-amino-4-methoxybenzoate (500 mg, 2.8 mmol), K_2CO_3 (1.1 g, 8.3 mmol), *trans*,*trans*-farnesyl bromide (0.8 mL, 3.0 mmol), THF (25 + 5 mL). Eluent: AcOEt–hexane 1 : 4. Appearance: transparent oil. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.43 (dd, 1H, $J = 2.1$ Hz, $J = 8.3$ Hz), 7.25 (d, 1H, $J = 2.0$ Hz), 6.75 (d, 1H, $J = 8.4$ Hz), 5.36 (dt, 1H, $J = 1.3$ Hz, $J = 6.7$ Hz), 5.09 (m, 2H), 4.15 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.76 (d, 2H, $J = 6.7$ Hz), 2.06 (m, 8H), 1.74 (s, 3H), 1.68 (s, 3H), 1.60 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.5, 150.5, 139.3, 137.9, 135.2, 131.2, 124.3, 123.8, 122.9, 121.0, 119.2, 110.4, 108.2, 55.5, 51.7, 41.5, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.4, 16.0. RP-HPLC: 13.50 min, purity: 97.9%, ESI-MS$[M]^+$ = 386.7.

(2*E*,6*E*)-3-(3,7,11-Trimethyldodeca-2,6,10-trienylamino)benzoic acid methyl ester (16b). Reagents: methyl 3-aminobenzoate (500 mg, 3.3 mmol), K_2CO_3 (1.1 g, 8.3 mmol), *trans*,*trans*-farnesyl bromide (0.9 mL, 3.6 mmol), THF (25 + 5 mL). Eluent: AcOEt–hexane 1 : 30. Appearance: transparent oil. Yield: 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.36 (td, 1H, $J = 1.2$ Hz, $J = 7.4$ Hz), 7.27 (t, 1H, $J = 2.0$ Hz), 7.22 (t, 1H, $J = 7.9$ Hz), 6.77 (ddd, 1H, $J = 0.9$ Hz, $J = 2.6$ Hz, $J = 8.2$ Hz), 5.32 (dt, 1H, $J = 1.1$ Hz, $J = 6.6$ Hz), 5.09 (m, 2H), 3.89 (s, 3H), 3.74 (d, 2H, $J = 6.7$ Hz), 2.06 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.4, 135.3, 131.3, 131.0, 129.1, 124.2, 123.7, 120.4, 119.1, 119.1, 118.1, 118.0, 114.0, 52.0, 42.3, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.4, 16.0. RP-HPLC: 11.57 min, purity: 99.5%, ESI-MS$[M]^+$ = 356.36.

(2*E*,6*E*)-(2-Methoxyphenyl)-(3,7,11-trimethyldodeca-2,6,10-trienyl)amine (16c). Reagents: 2-methoxyaniline (500 mg, 4.1 mmol), K_2CO_3 (1.3 g, 10.0 mmol), *trans*,*trans*-farnesyl bromide (1.2 mL, 4.5 mmol), THF (25 + 5 mL). Eluent: CH_2Cl_2–hexane 1 : 2. Appearance: transparent oil. Yield: 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.88 (tt, 1H, $J = 1.4$ Hz, $J = 7.6$ Hz), 6.77 (dt, 1H, $J = 1.3$ Hz, $J = 7.9$ Hz), 6.68 (td, 1H, $J = 1.4$ Hz, $J = 2.3$ Hz), 6.62 (dt, 1H, $J = 1.4$ Hz, $J = 7.8$ Hz), 5.38 (dt, 1H, $J = 1.3$ Hz, $J = 6.6$ Hz), 5.11 (m, 2H), 4.14 (s, 1H), 3.84 (s, 3H), 3.73 (d, 2H, $J = 6.6$ Hz), 2.07 (m, 8H), 1.72 (s, 3H), 1.69 (s, 3H), 1.61 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 146.8, 138.7, 138.4, 135.2, 131.2, 124.3, 123.8, 121.7, 121.2, 116.2, 109.9, 109.2, 55.3, 41.6, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.3, 16.0. RP-HPLC: 10.43 min, purity: 99.0%, ESI-MS$[M]^+$ = 327.56.

General procedure for the synthesis of compounds 17a–b. The obtained methyl ester derivative (75 mg, 0.19 mmol) is dissolved in a mixture of THF (2.5 mL), MeOH (1 mL) and water (1.5 mL), and lithium hydroxide monohydrate (82 mg, 1.90 mmol) is added. The reaction mixture is stirred for 48 hours and the reaction mixture is neutralized to pH = 4 with 1 M HCl solution. The resulting mixture is extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (25 mL), saturated NaCl solution (25 mL) and dried with Na_2SO_4 anhydrous. Evaporation of the solvent under reduced pressure gave a residue which is purified by column flash chromatography to give the desired compound.

(2*E*,6*E*)-4-Methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino)benzoic acid (17a). Reagents: (2*E*,6*E*)-4-methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**16a**) (75 mg, 0.19 mmol), $LiOH \cdot H_2O$ (82 mg, 1.9 mmol), THF (2.5 mL), MeOH (1 mL), H_2O (1.5 mL). Appearance: transparent oil. Rto.: 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.52 (td, 1H, $J = 1.8$ Hz, $J = 8.3$ Hz), 7.31 (d, 1H, $J = 1.7$ Hz), 6.79 (d, 1H, $J = 8.4$ Hz), 5.36 (dt, 1H, $J = 1.0$ Hz, $J = 6.7$ Hz), 5.10 (m, 2H), 3.91 (s, 3H), 3.78 (d, 2H, $J = 6.7$ Hz), 2.06 (m, 8H), 1.75 (s, 3H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.1, 151.1, 139.4, 138.0, 135.3, 131.2, 124.3, 123.8,

122.0, 120.9, 120.1, 110.7, 108.3, 55.5, 41.5, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.4, 16.0. RP-HPLC: 12.15 min, purity: 100%, ESI-MS $[M]^+ = 372.03$.

(2*E*,6*E*)-3-(3,7,11-Trimethyldodeca-2,6,10-trienylamino)benzoic acid (17b). Reagents: (2*E*,6*E*)-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**16b**) (576 mg, 1.6 mmol), $LiOH \cdot H_2O$ (680 mg, 16.2 mmol), THF (2.5 mL), MeOH (1 mL), H_2O (1.5 mL). Appearance: white solid. M.p.: 75 °C. Yield: 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.44 (d, 1H, J = 7.6 Hz), 7.32 (s, 1H), 7.25 (t, 1H, J = 7.8 Hz), 6.82 (dd, 1H, J = 2.1 Hz, J = 8.0 Hz), 5.33 (t, 1H, J = 6.9 Hz), 5.10 (q, 2H, J = 5.7 Hz), 3.75 (d, 2H, J = 6.6 Hz), 2.06 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.2, 148.4, 139.6, 135.3, 131.3, 130.1, 129.1, 124.2, 123.7, 120.8, 119.0, 118.1, 113.8, 41.9, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.4, 16.0. RP-HPLC: 4.88 min, purity: 96.4%, ESI-MS$[M]^+ = 342.10$.

(2*E*,6*E*)-2-(3,7,11-Trimethyldodeca-2,6,10-trienylamino)isonicotinic acid methyl ester (19). To a solution of methyl 2-aminoisonicotinate (265 mg, 1.7 mmol) in DMF (5 mL) an aqueous solution of $NaHCO_3$ (365 mg, 4.3 mmol) is added, and the resulting mixture is stirred for 5 minutes. *Trans*,*trans*-farnesyl bromide (0.5 mL, 1.9 mmol) in DMF (1 mL) is added and the resulting mixture is stirred for a further 16 hours at 60 °C. The solvent is evaporated under reduced pressure and the resulting residue is purified by flash column chromatography. Eluent: AcOEt–hexane 1 : 15. Appearance: transparent oil. Yield: 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.18 (d, J = 6.0 Hz, 1H), 7.07 (dd, J = 5.2 Hz, J = 1.4 Hz, 1H), 6.95 (d, J = 0.9 Hz, 1H), 5.32 (t, J = 6.8, J = 6.8 Hz, 1H), 5.10 (d, J = 5.3 Hz, 2H), 4.70 (s, 1H), 3.91 (s, 3H), 3.89 (d, J = 1.9 Hz, 2H), 2.41–1.85 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.5, 159.5, 149.1, 140.1, 138.9, 135.6, 131.5, 124.52, 123.9, 120.7, 111.8, 106.8, 52.6, 40.5, 39.9, 39.8, 26.9, 26.6, 25.9, 17.9, 16.6, 16.2. RP-HPLC: 9.4 min, purity: 99.6%, ESI-MS$[M]^+ =$ 357.38.

3-Amino-4-hydroxybenzoic acid methyl ester (21).[27] To a solution of 3-amino-4-hydroxybenzoic acid (25 g, 16.0 mmol) in MeOH (15 mL), H_2SO_4 conc. (2 mL) is added dropwise at 0 °C. The mixture is stirred for 8 hours at 70 °C, and then the mixture is neutralized with aqueous solution of NaOH 5 N to pH 8–9. The mixture is extracted with AcOEt (2 × 200 mL) and the organic phase dried with Na_2SO_4 anhydrous, filtered and the solvent is evaporated under reduced pressure, to give the desired compound as a white solid. M.p.: 183 °C. Yield: 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 9.90 (s, 1H), 7.25 (d, J = 2.1 Hz, 1H), 7.10 (dd, J = 8.2 Hz, J = 2.1 Hz, 1H), 6.71 (d, J = 8.2 Hz, 1H), 4.77 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 166.5, 148.5, 136.5, 120.6, 118.7, 114.6, 113.6, 51.3. RP-HPLC: 1.46 min, purity: 100%, ESI-MS$[M]^+ = 167.51$.

(2*E*,6*E*)-4-Hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (22). To a solution of 3-amino-4-hydroxybenzoic acid methyl ester (**21**) (12.0 mmol, 2.0 g) in THF anhydrous (40 mL) is added *trans*,*trans*-farnesyl bromide (3.2 mL, 1.8 mmol) and the mixture is stirred for 16 hours at room temperature, after the mixture is neutralized with aqueous solution of NaOH 1 M to pH 6–7 and extracted with CH_2Cl_2 (3 × 25 mL). The organic phase is washed with water (1 × 25 mL), dried with Na_2SO_4 anhydrous, filtered and the solvent is evaporated under reduced pressure. The mixture is purified by flash column chromatography, using AcOEt–hexanes 1 : 10 as an eluent, to give the desired compound as an orange solid. M.p.: 191 °C. Yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.39 (dd, 1H, J = 1.8 Hz, J = 8.1 Hz), 7.36 (d, 1H, J = 1.9 Hz), 6.72 (d, 1H, J = 8.0 Hz), 5.36 (t, 1H, J = 6.7 Hz), 5.10 (m, 2H), 3.87 (s, 3H), 3.75 (d, 2H, J = 6.7 Hz), 2.06 (m, 8H), 1.73 (s, 3H), 1.68 (s, 3H), 1.60 (s, 6H). ^{13}C RMN (100 MHz. $CDCl_3$) δ (ppm) 167.5, 147.9, 139.5, 135.3, 131.3, 124.3, 123.8, 123.3, 120.9, 120.4, 118.0, 113.5, 113.3, 51.8, 42.2, 39.6, 39.5, 26.7, 26.3, 25.6, 17.6, 16.4, 16.0. RP-HPLC: 8.93 min, purity: 99.0%, ESI-MS $[M]^+ = 372.03$.

(2*E*,6*E*)-4-Hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino)benzoic acid (23). A solution of (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**22**) (100 mg, 0.27 mmol) is dissolved in a mixture of THF (2.5 mL), MeOH (1 mL) and water (1.5 mL), and lithium hydroxide monohydrate (114 mg, 2.70 mmol) is added. The reaction mixture is stirred for 24 hours and the reaction mixture is neutralized to pH = 4 with 1 M HCl solution. The resulting mixture is extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (25 mL), saturated NaCl solution (25 mL) and dried with Na_2SO_4 anhydrous. Evaporation of the solvent under reduced pressure gave a residue which is purified by column flash chromatography to give the desired compound as a yellow pale oil. Yield: 36%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.30 (m, 1H), 7.26 (d, 1H, J = 2.0 Hz), 6.71 (d, 1H, J = 8.1 Hz), 5.33 (m, 1H), 5.09 (m, 2H), 3.76 (d, 2H, J = 6.6 Hz), 2.06 (m, 8H), 1.77 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 1.59 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.0, 150.8, 139.8, 138.3, 136.2, 132.0, 125.5, 125.2, 123.3, 123.2, 121.5, 113.6, 113.5, 42.8, 40.8, 40.7, 27.8, 27.4, 25.9, 17.8, 16.5, 16.1. RP-HPLC: 11.5 min, purity: 97.9%, ESI-MS$[M]^+ = 358.16$.

General procedure for the synthesis of compounds 24 and 26. To a solution of (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**22**) (200 mg, 0.54 mmol) in DMF anhydrous (8 mL) under an inert atmosphere is added K_2CO_3 (148 mg, 0.81 mmol) and the mixture is stirred for 40 minutes at room temperature. After 1-iodopropane is added (63 μL, 0.64 mmol) the mixture is stirred for 4 hours. The mixture is concentrated to dryness and the residue is purified by column chromatography, using as an eluent hexane–AcOEt 10 : 1 to obtain the compounds **22** (45 mg, yield 20%) and **24** (10 mg, yield 4%), both as transparent oil.

(2*E*,6*E*)-4-Propoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (24). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.40 (dd, J = 8.3 Hz, J = 2.0 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H), 6.74 (d, J = 8.3 Hz, 1H), 5.35 (dt, J = 6.6 Hz, J = 1.2 Hz, 1H), 5.1–5.0 (m, 2H), 4.18 (s broad, 1H), 4.00 (t, J = 6.6 Hz, 2H), 3.87 (s, 3H), 3.78 (d, J = 6.9 Hz, 2H), 2.24–1.93 (m, 8H), 1.79 (sext, J = 6.6 Hz, 2H), 1.75 (d, J = 1.1 Hz, 3H), 1.68 (d, J = 1.1 Hz, 3H), 1.60 (s, 6H), 1.05 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.6, 149.9, 139.2, 138.0, 135.2, 131.2, 124.3, 123.8, 122.7, 121.2, 119.2, 110.5, 109.1, 69.8, 51.7, 41.6, 39.7, 39.6, 26.7, 26.4, 25.7, 22.7, 17.6, 16.4, 16.0, 10.5. RP-HPLC: 11.02 min, purity: 87.1%, ESI-MS$[M]^+ = 413.6$.

(2*E*,6*E*)-4-Propoxy-3-[propyl-(3,7,11-trimethyldodeca-2,6,10-trienyl)amino] benzoic acid methyl ester (26). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.63 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H), 7.56 (d, J = 1.9 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 5.24 (dt, J = 6.5 Hz, J = 0.9 Hz, 1H), 5.12–5.04 (m, 2H), 4.01 (t, J = 6.6 Hz, 2H), 3.86 (s, 3H), 3.74 (d, J = 6.5 Hz, 2H), 3.10–3.03 (m, 2H), 2.10–1.92 (m, 8H), 1.87 (td, J = 13.9, J = 6.9 Hz, 2H), 1.67 (d, J = 1.1 Hz, 3H), 1.64 (d, J = 0.5 Hz, 3H), 1.59 (d, J = 0.5 Hz, 3H), 1.57 (d, J = 0.7 Hz, 3H), 1.48 (qd, J = 14.9 Hz, J = 7.6 Hz, 2H), 1.07 (t, J = 7.4 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.3, 156.5, 140.0, 137.7, 135.0, 131.2, 124.4, 124.3, 124.0, 122.2, 122.0, 121.7, 111.3, 70.0, 53.1, 51.7, 50.0, 39.7, 39.7, 26.7, 26.5, 25.7, 22.6, 20.3, 17.6, 16.2, 15.9, 11.7, 10.7. RP-HPLC: 12.49 min, purity: 97.9%, ESI-MS$[M]^+$ = 456.

General procedure for the synthesis of compounds 25, 27 and 28. To a solution of (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**22**) (1.0 eq.) in THF anhydrous at 0 °C is added NaH (60% in mineral oil, 1.2 eq.). The mixture is stirred for 10 minutes and then NBu_4I (0.25 eq.), 18-crown-6 (0.01 eq.) and alkylating agent (1.1 eq.) are added. The mixture is stirred for 2 hours at 70 °C, in an inert atmosphere, and then cooled at room temperature and water (25 mL) is added. The mixture is extracted with CH_2Cl_2 (2 × 25 mL) and the organic phase is washed with brine (1 × 25 mL). The combined organic extract is dried with Na_2SO_4 anhydrous, filtered and the solvent is evaporated under reduced pressure. The residue is purified by column flash chromatography to give the desired compounds.

(2*E*,6*E*)-4-(Propane-1-sulfonyloxy)-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (25). Reagent: (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**22**) (0.2 g, 0.54 mmol), NaH (26 mg, 0.64 mmol), NBu_4I (50 mg, 0.13 mmol), 18-crown-6 (1.4 mg, 0.005 mmol), 1-propanesulfonyl chloride (66 μL, 0.59 mmol) and THF anhydrous (1 + 4 mL). Eluent: AcOEt–hexane 1 : 10. Appearance: transparent oil. Yield: 44%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.39 (d, J = 2.0 Hz, 1H), 7.36 (dd, J = 8.3 Hz, J = 2.0 Hz, 1H), 7.21 (d, J = 8.4 Hz, 1H), 5.35 (dt, J = 6.5 Hz, J = 1.1 Hz, 1H), 5.14–5.02 (m, 2H), 4.31 (s, 1H), 4.12–3.98 (m, 2H), 3.92 (s, 3H), 3.79 (d, J = 7.2 Hz, 2H), 3.25 (t, J = 8.8 Hz, 2H), 2.18–1.87 (m, 10H), 1.77 (s, 3H), 1.72 (s, 3H), 1.64 (s, 6H), 1.13 (t, J = 8.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.5, 140.8, 140.5, 140.2, 137.9, 130.9, 130.0, 122.5, 122.3, 121.2, 120.5, 119.2, 112.0, 53.3, 52.5, 42.2, 40.3, 40.3, 27.4, 27.4, 27.0, 18.8, 18.7, 17.5, 17.3, 13.8. RP-HPLC: 12.12 min, purity: 98.8%, ESI-MS$[M]^+$ = 478.1.

(2*E*,6*E*)-4-Ethoxycarbonylmethoxy-3-[ethoxycarbonylmethyl-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)amino]benzoic acid methyl ester (27). Reagent: (2*E*,6*E*)-4-hydroxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**22**) (284 mg, 0.76 mmol), NaH (30 mg, 0.84 mmol), NBu_4I (70.5 mg, 0.19 mmol), 18-crown-6 (2.0 mg, 0.008 mmol), ethyl bromoacetate (93 μL, 0.84 mmol), THF anhydrous (1 + 1 mL). Eluent: AcOEt–hexane 1 : 4. Appearance: transparent oil. Yield: 8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.68 (d, J = 1.9 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.5 Hz, 1H), 5.22 (t, J = 6.6 Hz, 1H), 5.05–4.94 (m, 2H), 4.65 (s, 4H), 4.18 (q, J = 7.1 Hz, 4H), 4.08 (s, 2H), 4.07–4.00 (m, 4H), 3.92 (d, J = 6.1 Hz, 2H), 3.80 (s, 3H), 2.10–1.81 (m, 8H), 1.62 (s, 3H), 1.60 (d, J = 1.1 Hz, 3H), 1.52 (s, 3H), 1.51 (d, J = 0.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.4, 167.0, 153.9, 135.5, 131.5, 124.9, 124.6, 124.0, 120.3, 112.4, 105.0, 65.8, 61.7, 60.7, 52.2, 52.1, 50.4, 40.0, 39.9, 27.0, 26.7, 25.9, 17.9, 16.5, 16.2, 14.4, 14.3. RP-HPLC: 11.6 min, purity: 95.8%, ESI-MS$[M]^+$ = 544.05.

(2*E*,6*E*)-3-Oxo-4-(3,7,11-trimethyldodeca-2,6,10-trienyl)-3,4-dihydro-2*H*-benzo-[1,4]oxazine-6-carboxylic acid methyl ester (28). Reagent: see compound **27**. Eluent: AcOEt–hexane 1 : 4. Appearance: transparent oil. Rto.: 67%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.69 (dd, J = 8.3 Hz, J = 1.9 Hz, 1H), 7.66 (d, J = 1.9 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 5.12 (dt, J = 6.5 Hz, J = 1.1 Hz, 1H), 5.07–4.99 (m, 2H), 4.67 (s, 3H), 4.59 (d, J = 6.6 Hz, 2H), 3.89 (s, 2H), 2.13–1.92 (m, 8H), 1.88 (d, J = 0.7 Hz, 3H), 1.66 (d, J = 1.0 Hz, 3H), 1.56 (s, 3H), 1.55 (d, J = 0.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.4, 167.0, 153.9, 153.2, 135.5, 131.5, 124.9, 124.6, 124.5, 124.0, 122.9, 120.3, 112.4, 105.0, 65.8, 61.7, 60.7, 52.1, 40.0, 27.0, 26.7, 25.9, 17.9, 16.5, 14.3. RP-HPLC: 11.86 min, purity: 95.3%, ESI-MS$[M]^+$ = 412.43.

General procedure for the synthesis of compounds 29 and 30. Example, compound (2*E*,6*E*)-4-(propane-1-sulfonyloxy)-3-(3,7,11-trimethyl-dodeca-2,6,10-trienylamino) benzoic acid (29). A solution of (2*E*,6*E*)-4-(propane-1-sulfonyloxy)-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid methyl ester (**25**) (95 mg, 0.19 mmol) is dissolved in a mixture of THF (2.0 mL) and water (2.0 mL), and lithium hydroxide monohydrate (105 mg, 2.50 mmol) is added. The reaction mixture is stirred for 24 hours and the reaction mixture is neutralized to pH = 4 with 1 M HCl solution. The resulting mixture is extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with water (25 mL), saturated NaCl aqueous solution (25 mL) and dried (Na_2SO_4 anhydrous). Evaporation of the solvent under reduced pressure gave a residue which is purified by column flash chromatography using AcOEt–hexane 1 : 4 as an eluent, to give the desired compound as a transparent oil. Yield: 41%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.40–7.35 (m, 2H), 7.23–7.19 (m, 1H), 5.24 (dt, J = 6.5 Hz, J = 1.0 Hz, 1H), 5.02 (ddd, J = 8.0 Hz, J = 7.0 Hz, J = 4.1 Hz, 2H), 3.72 (d, J = 6.7 Hz, 2H), 3.29–3.16 (m, 2H), 2.13–1.81 (m, 10H), 1.69 (d, J = 0.8 Hz, 3H), 1.60 (d, J = 0.9 Hz, 3H), 1.53 (s, 3H), 1.53 (d, J = 0.5 Hz, 3H), 1.05 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.1, 141.3, 140.7, 140.3, 135.6, 131.5, 128.8, 124.5, 123.9, 122.3, 120.3, 114.2, 105.0, 53.2, 41.7, 39.9, 39.8, 26.9, 26.6, 25.9, 17.9, 17.6, 16.7, 16.2, 13.1. RP-HPLC: 11.25 min, purity: 97.3%, ESI-MS $[M]^-$ = 462.10.

(2*E*,6*E*)-3-Oxo-4-(3,7,11-trimethyldodeca-2,6,10-trienyl)-3,4-dihydro-2H-benzo-[1,4]oxazine-6-carboxylic acid (30). Reagent: (2*E*,6*E*)-3-oxo-4-(3,7,11-trimethyldodeca-2,6,10-trienyl)-3,4-dihydro-2H-benzo-[1,4]oxazine-6-carboxylic acid methyl ester (**28**) (24 mg, 0.052 mmol), $LiOH \cdot H_2O$ (29 mg, 0.68 mmol), THF (1.0 mL), MeOH (0.5 mL), H_2O (1.0 mL). Appearance: transparent oil. Yield: 92%. M.p.: 169 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.78 (dd, J = 8.4 Hz, J = 1.9 Hz, 1H), 7.73 (d, J = 1.9 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 5.14 (dd, J = 6.6 Hz, J = 5.6 Hz, 1H), 5.07–4.99 (m, 2H), 4.71 (s, 2H), 4.62 (d, J = 6.5 Hz, 2H), 2.15–1.91 (m, 8H),

1.90 (s, 3H), 1.65 (d, $J = 0.9$ Hz, 3H), 1.56 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.9, 163.3, 149.8, 140.9, 135.4, 131.2, 128.3, 126.5, 124.3, 123.6, 123.5, 117.8, 117.5, 116.9, 67.5, 39.7, 39.6, 39.5, 26.7, 26.2, 25.6, 17.6, 16.6, 16.0. RP-HPLC: 10.9 min, purity: 94.6%, ESI-MS$[M]^+ = 398.17$, $[M]^+ + Na = 420.16$.

Synthesis of compound 35

(2*S*,3*R*)-3-(3-*tert*-Butoxycarbonylamino-2-hydroxy-4-phenylbutylamino)-4-methoxybenzoic acid methyl ester (32). To a solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (200 mg, 0.76 mmol) in ^{i}PrOH (5 mL) is added methyl 3-amino-4-methoxybenzoate (206 mg, 1.1 mmol). The mixture is stirred at 50 °C, under a nitrogen atmosphere for 16 hours, and then is cooled at room temperature. The reaction mixture is concentrated under reduced pressure and purified by flash column chromatography using AcOEt–hexane 1 : 2 to give the desired compound as a white solid. Yield: 41%. M.p.: 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.46 (dd, $J = 8.3$ Hz, $J = 2.0$ Hz, 1H), 7.36–7.20 (m, 6H), 6.77 (d, $J = 8.4$ Hz, 1H), 4.89–4.36 (m, 2H), 3.96 (dd, $J = 11.2$ Hz, $J = 6.2$ Hz, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 3.49 (d, $J = 4.1$ Hz, 1H), 3.45–3.33 (m, 1H), 3.27–3.14 (m, 1H), 3.00 (dd, $J = 14.1$ Hz, $J = 5.1$ Hz, 1H), 2.95–2.82 (m, 1H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.7, 151.2, 138.2, 137.8, 129.5, 128.8, 126.8, 123.1, 120.2, 120.0, 111.0, 108.8, 80.3, 77.4, 72.7, 55.8, 52.0, 46.5, 36.6, 28.5. RP-HPLC: 9.91 min, purity: 99.4%, ESI-MS$[M]^+ = 444.82$, $[M]^+ + Na = 467.02$.

(2*S*,3*R*)-3-(3-Amino-2-hydroxy-4-phenylbutylamino)-4-methoxybenzoic acid methyl ester (33). To a solution of 3-((2*S*,3*R*)-3-*tert*-butoxycarbonylamino-2-hidroxy-4-fenylbutylamino)-4-metoxybenzoic acid methyl ester (**32**) (100 mg, 0.22 mmol) of AcOEt–methanol 4 : 1 (5 mL) at 0 °C is slowly passed a stream of HCl gas for 5 minutes. The mixture is stirred at 0 °C for 3 hours, and then the reaction mixture is dried under reduced pressure. The solid obtained is washed with Et_2O (10 mL) and is filtered to give the compound as a white solid. Yield: 99%. M.p.: 263 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.02–7.89 (m, 2H), 7.42–7.16 (m, 5H), 7.04 (d, $J = 8.6$ Hz, 1H), 4.27 (d, $J = 8.3$ Hz, 1H), 4.22–4.03 (m, 3H), 3.98 (s, 3H), 3.87 (s, 3H), 3.70 (t, $J = 7.7$ Hz, $J = 7.7$ Hz, 1H), 3.52–3.35 (m, 1H), 3.34–3.20 (m, 2H), 2.98 (d, $J = 7.0$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.1, 155.2, 135.1, 131.2, 131.1, 129.3, 129.2, 127.7, 123.5, 123.2, 111.8, 66.3, 56.7, 55.8, 52.5, 51.4, 33.5. RP-HPLC: 6.75 min, purity: 98.5%, ESI-MS $[M]^+ = 346.14$.

3-[(2*S*,3*R*)-3-[(2*E*,6*E*)-(3,7-Dimethylocta-2,6-dienylamino)]-2-hydroxy-4-phenyl-butylamino]-4-methoxy benzoic acid methyl ester (35). To a solution of (2*S*,3*R*)-3-(3-amino-2-hydroxy-4-phenylbutylamino)-4-methoxy-benzoic acid methyl ester (**33**) (100 mg, 0.29 mmol) in DMF anhydrous (3 mL) is added geranyl bromide (0.8 mL, 0.58 mmol) and Et_3N (0.08 mL, 0.58 mmol). The mixture is stirred for 16 hours at 70 °C under a nitrogen atmosphere, and then the reaction mixture is cooled at room temperature. The reaction mixture is diluted with CH_2Cl_2 (25 mL) and is washed with H_2O (2 × 25 mL) and the organic phase is dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue which is purified by column flash chromatography using CH_2Cl_2/10% MeOH as the eluent to give the desired compound as a pale yellow oil. Yield: 13%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.45 (dd, $J = 8.3$ Hz, $J = 2.0$ Hz, 1H), 7.35–7.16 (m, 6H), 6.77 (d, $J = 8.4$ Hz, 1H), 5.15–4.84 (m, 2H), 3.99 (td, $J = 8.6$ Hz, $J = 3.4$ Hz, 1H), 3.91 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 3.37 (dd, $J = 12.1$ Hz, $J = 3.3$ Hz, 1H), 3.22 (dd, $J = 12.1$ Hz, $J = 8.7$ Hz, 1H), 3.12 (t, $J = 6.2$ Hz, 2H), 3.05–2.99 (m, 1H), 2.88 (dd, $J = 14.0$, $J = 4.9$ Hz, 1H), 2.72 (dd, $J = 13.9$ Hz, $J = 9.6$ Hz, 1H), 2.06–1.97 (m, 2H), 1.97–1.89 (m, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.6, 150.9, 138.3, 138.0, 131.6, 129.2, 129.2, 129.1, 128.8, 128.7, 126.6, 123.9, 122.9, 121.8, 119.6, 110.4, 108.4, 68.8, 61.0, 55.6, 51.8, 45.8, 44.9, 39.5, 35.3, 26.4, 25.7, 17.7, 16.1. RP-HPLC: 8.03 min, purity: 95.1%, ESI-MS$[M]^+ = 481.93$.

Synthesis of compounds 36–38

(1*S*,2*R*)-(1-Benzyl-3-cyclopropylamino-2-hydroxypropyl) carbamic acid *tert*-butyl ester (36).[23] To a solution of (2*S*,3*S*)-1,2-epoxy-3-(Boc-amino)-4-phenylbutane (10 g, 37.0 mmol) in ^{i}PrOH (100 mL) is added cyclopropylamine (22.6 mL, 326.5 mmol) and the mixture is stirred for 7 hours at 50 °C. The reaction mixture is concentrated to dryness to give the desired compound 12.05 g as a white solid. Yield: 99%. M.p. = 233 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.29–7.08 (m, 6H), 6.63 (d, $J = 9.0$ Hz, 1H), 4.73 (d, $J = 5.2$ Hz, 1H), 3.60–3.37 (m, 1H), 2.99 (m, 1H), 2.67 (m, 1H), 2.51 (m, 1H), 2.08 (m, 2H), 1.26 (s, 9H), 1.13 (s, 1H), 0.34 (m, 2H), 0.2 (m, 2H). ESI-MS$[M]^+ = 321.21$.

(2*R*,3*S*)-3-Amino-1-cyclopropylamino-4-phenylbutan-2-ol-ditrifluoroacetate salt (37).[23] To a suspension of (1*S*,2*R*)-(1-benzyl-3-cyclopropylamino-2-hydroxypropyl) carbamic acid *tert*-butyl ester (**36**) (11.8 g, 37.0 mmol) in CH_2Cl_2 (120 mL) is added TFA (11.0 mL, 147.9 mmol) and the reaction mixture is stirred for 24 hours at room temperature. Afterwards, 4 eq. of TFA is added and stirred for an additional 7 hours. The mixture is concentrated to dryness and the oil obtained is washed with CH_2Cl_2 (3 × 20 mL). The compound is purified by precipitation with Et_2O (50 mL) and is washed with Et_2O (2 × 25 mL) to give 12 g of the compound as a white solid. Yield: 72%. M.p. = 181 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.34 (m, 5H), 6.4 (m, 1H), 4.08 (m, 1H), 3.55 (m, 1H), 3.29 (m, 1H), 3.10–2.75 (m, 3H), 2.69 (m, 1H), 0.95–0.59 (m, 4H). ESI-MS$[M]^+ = 221.16$.

N-((1*S*,2*R*)-1-Benzyl-3-cyclopropylamino-2-hydroxypropyl)-(2*E*,6*E*)-4-methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino)benzamide (38). To a solution of (2*R*,3*S*)-3-amino-1-cyclopropylamino-4-phenylbutan-2-ol ditrifluoro-acetate salt (**37**) (310 mg, 0.69 mmol) in DMF anhydrous (2 mL) is sequentially added a solution of (**17a**) (2*E*,6*E*)-4-methoxy-3-(3,7,11-trimethyldodeca-2,6,10-trienylamino) benzoic acid (234 mg, 0.63 mmol) in DMF anhydrous (1 mL), DIPEA (0.7 mL, 3.80 mmol), HOBt (1.0 mL, 1.00 mmol) and EDC (181 mg, 1.00 mmol). The mixture is stirred, under a nitrogen atmosphere, for 16 hours at room temperature, and then HCl 1 N is added until pH 7. Afterwards H_2O (50 mL) is added and the mixture is extracted with AcOEt (3 × 50 mL). The combined organic extracts are dried with $MgSO_4$ anhydrous, filtered and concentrated under reduced pressure to give a residue which is purified by flash column chromatography using CH_2Cl_2/10%

MeOH as an eluent to give the desired compound as a white solid. Yield: 47%. M.p. = 205 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.38–7.12 (m, 5H), 6.89 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H), 6.87 (d, J = 1.7 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 6.39 (d, J = 8.3 Hz, 1H), 5.35 (dt, J = 6.5 Hz, J = 0.8 Hz, 1H), 5.17–5.04 (m, 2H), 4.41–4.20 (m, 1H), 4.14 (s, 1H), 3.89 (t, J = 6.2 Hz, 1H), 3.85 (s, 3H), 3.75–3.63 (m, 4H), 3.10 (d, J = 6.8 Hz, 2H), 3.07–2.81 (m, 3H), 2.28 (d, J = 4.6 Hz, 1H), 2.20–1.91 (m, 8H), 1.72 (s, 3H), 1.68 (d, J = 0.8 Hz, 3H), 1.61 (d, J = 0.6 Hz, 3H), 1.60 (s, 3H), 0.60 (d, J = 10.2 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.0, 149.9, 139.9, 138.6, 137.4, 135.6, 131.5, 129.8, 129.4, 128.9, 127.0, 124.5, 124.0, 120.9, 115.6, 108.6, 108.3, 70.3, 68.8, 55.8, 53.3, 51.9, 41.71 39.9, 39.8, 36.6, 31.0, 27.0, 26.6, 25.9, 17.9, 16.7, 16.3, 6.0, 5.5. RP-HPLC: 9.4 min, purity: 98.3%, ESI-MS$[M]^+$ = 574.3.

Enzyme assay: BACE-1 inhibition

The assay is based on FRET technology, and is performed in a 96 well black microplate. The final concentration of the substrate (Fluorogenic Peptide Substrate IV, R&D Systems, ref.: ES004) was 3.5 μM, and the enzyme concentration (rhBACE-1 β-secretase recombinant human, R&D Systems, ref.: 931-AS) was 0.5 μg mL^{-1}. The final volume of the assay was 100 μL per well and all reagents were diluted in reaction buffer (sodium acetate 50 mM, pH 4.5). The compounds were tested at a concentration of 10^{-5} and 10^{-6} M. The control in the assay was the commercial β-secretase inhibitor OM99-2, from BACHEM, which was tested at 300 nM. All the samples and controls were studied by duplicate. The plate was mixed gently and incubated for one hour in the dark. Changes in the fluorescence were measured using a fluorimeter plate reader (FLUOstar OPTIMA, BMG LABTECH), with 320 nm excitation filter and 405 nm emission filter.

In the cases where abnormal effects in fluorescence were detected, BACE inhibition activity was assayed using BACE-1 (Beta-Secretase) FRET ASSAY KIT (Invitrogen, ref.: P2985), according to the manufacturer's instructions. Fluorescence was measured with a fluorimeter plate reader, with 544 nm excitation filter and 580 nm emission filters.

A third evaluation was performed when samples interfered with the fluorescence signal. This assay was based on time-resolved fluorescence (TRF) technology. In this case, the final concentration of the substrate (TruPoint™ BACE1 substrate, Perkin Elmer, ref.: AD0151R) was 23 nM per well, and the enzyme concentration was 7.44 nM, in reaction buffer (sodium acetate 50 mM, pH 4.5, 1 mM EDTA, 0.2% CHAPS). Changes in fluorescence were measured with a fluorimeter plate reader enabled with TR-capability (POLARstar OPTIMA, BMG LABTECH), using 337 nm as the excitation wavelength and 615 nm as the emission wavelength.

Cell-based assay: inhibition of Aβ(1–40) peptide production

To quantitate Aβ(1–40) levels from cellular supernatants, an ELISA-based method was performed, using a colorimetric commercial kit: Aβ 40 Human ELISA Kit (Invitrogen, ref.: KHB3481). An APP-transfected cell line was employed for the experiments: CHO7W (stably transfected with human APP_{751} cDNA). Cells were grown in a culture medium consisting of DMEM supplemented with 2% foetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine and 200 μg mL^{-1} G418. Cells were seeded in a 96-well culture microplate, at 5000 cells per well and treatment with different compounds, at 10^{-5} and 10^{-6} M concentrations, was performed 24 hours after seeding. 24 hours later culture media were collected and analyzed by the ELISA method. OM99-2, 3 μM, (Bachem, ref.: H-5108) a BACE inhibitor, was used as the Aβ(1–40) secretion reduction positive control.

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