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Article

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Elongation of the hydrophobic chain as a molecular switch: discovery of capsaicin derivatives and endogenous lipids as potent Transient Receptor Potential Vanilloid Channel 2 antagonists

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Abstract

The transient receptor potential vanilloid 2 (TRPV2) is a non-selective Ca^{2+} permeable channel member of the TRPV subfamily, still considered an orphan TRP channel due to the scarcity of available selective and potent pharmacological tools and endogenous modulators. Here we describe the discovery of novel synthetic long-chain capsaicin-derivatives as potent TRPV2 antagonists in comparison to the totally inactive capsaicin, the role of their hydrophobic chain, and how the structure-activity relationships of such derivatives led, through a ligand-based approach, to the identification of endogenous long-chain fatty acid ethanolamides or primary amides acting as TRPV2 antagonists. Both synthetic and endogenous antagonists exhibited differential inhibition against known TRPV2 agonists characterized by distinct kinetic profiles. These findings represent the first example of both synthetic and naturally-occurring TRPV2 modulators with efficacy in the sub/low-micromolar range, which will

be useful to clarify the physio-pathological roles of this receptor, its regulation, and its targeting in pathological conditions.

1. Introduction

TRPV2 belongs to the polymodal transient receptor potential (TRP) superfamily of calcium-permeable non-selective cation channels, activated by a wide variety of physical and chemical stimuli. Due to its mechanosensor property, TRPV2 is considered a stretch-modulated channel and a regulator of calcium homeostasis in different tissues and organs, in particular the heart, where it is 10-fold more abundant than in skeletal muscle[1]. Different lines of evidence suggest for TRPV2 a key role in physiological cardiac function as well as in cardiomyopathies and dystrophic diseases[2–4]. Besides the heart, TRPV2 is also found in the brain, vascular smooth muscle cells, the gastrointestinal tract, macrophages and the urothelial tract[5], and it is involved in a number of physio-pathological processes[6], including cancer[7–9], particularly of the urinary tract[10–13].

Despite its biological and pharmacological relevance, TRPV2 is still considered an orphan TRP channel due to the scarcity of selective drugs and known endogenous ligands. The 2-aminoethoxydiphenyl borate (2APB) is one of the first non-selective activators identified for rat TRPV2 (EC_{50} = 129 μM),[14] although inactive at the human orthologue, suggesting a strong species specificity[15,16]. *Cannabis sativa* derivatives such as Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and Δ^9-tetrahydrocannabivarin (Δ^9-THCV) are TRPV2 activators[17,18], and so is p-(di-n-propylsulfamyl)-benzoic acid (Probenecid)[19]. However, all these agonists are known to modulate other TRP channels. Most TRPV channels are proposed to be modulated also by phosphoinositide lipids[20]. TRPV2-mediated Ca^{2+} influx has been reported following stimulation by endogenous lysophospholipids such as lysophosphatidylcoline (LPC) and lysophosphatidylinositol (LPI)[21], LPC being a relatively potent activator (EC_{50} = 3.4 μM)[22]. To date, the nature of endogenous regulators of TRPV2 activity still remains elusive[23].

Also synthetic inhibitors of TRPV2 are either not specific or endowed with low potency, as exemplified

by: Ruthenium red (IC_{50} = 0.6 μM)[24] a pore blocker that inhibits other twelve ion channels[25]; La^{3+} and Gd^{3+};[26] citral;[27] the alkylated imidazole SKF96365;[16] tetraethylammonium and 4-aminopyridine, two potassium channel blockers; 1-(2-(trifluoromethyl) phenyl) imidazole, an inhibitor of capacitative Ca^{2+} entry;[16] and Tranilast[28], which has been used in several studies[29–34], even though it has never been validated as TRPV2 antagonist.

TRPV2 shares high sequence identity (>50%) with TRPV1 but its threshold of activation by temperature is higher (> 52 °C)[24], and, unlike TRPV1, is not sensitive to capsaicin. The recently solved cryo-EM structures of both TRPV1 and TRPV2[35,36], along with mutagenesis and computational studies, showed that the TRPV1 binding site of capsaicin is not conserved in TRPV2. Furthermore, the replacement of critical residues leads to a mutant (TRPV2-Quad) against which capsaicin behaves as an antagonist, rather than an agonist as in TRPV1[37]. These intriguing results prompted us to investigate a series of capsaicin-derivatives, in which the vanillylamide polar head of capsaicin bears a longer alkyl chain, featuring different length, unsaturation degree and type of polar substituents. The structure-activity relationship (SAR) of these synthetic compounds then suggested the screening of structurally-related endogenous lipids sharing at least one functional group with the capsaicin-derivatives, with the aim of finding new endogenous modulators.

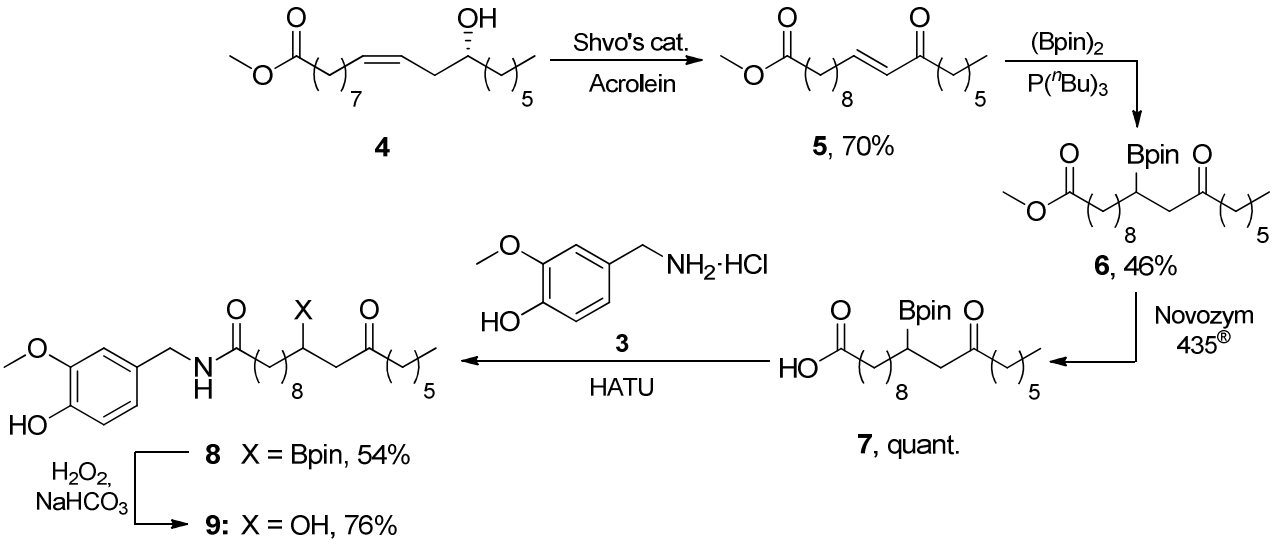
2. Results

2.1 Synthesis

Commercial fatty acids such as ricinoleic acid, oleic acid and palmitic acid were used as starting material to synthesize the **23** compounds tested. **Scheme 1** shows the synthesis of the α,β-unsaturated ketone **5** by the ruthenium-catalyzed oxidation in anhydrous toluene of the homoallylic alcohol of the methyl ricinoleate **4**.[38] Shvo's catalyst and acrolein were used as catalyst and hydrogen scavenger,

respectively.[39] The addition of bis(pinacolato)diboron $(Bpin)_2$ to the enone **5** in presence of tri-*n*-butyl phosphine ($P(^nBu)_3$)[40] yielded the β-boronketone **6** in 46% yield. Enzymatically controlled hydrolysis[41] of the methyl ester **6** with Novozym 435® lipase led to the carboxylic acid **7** quantitatively. This acid **7** was coupled, without any further purification, with 4-hydroxy-3-methoxybenzylamine hydrochloride **3** by HATU[42] and DIPEA in DMFanh. achieving the amide **8**. The oxidative hydrolysis of the boron substituent of the compound **8** led to the β-hydroxyketone **9** in a 76% yield (**Scheme 1**).

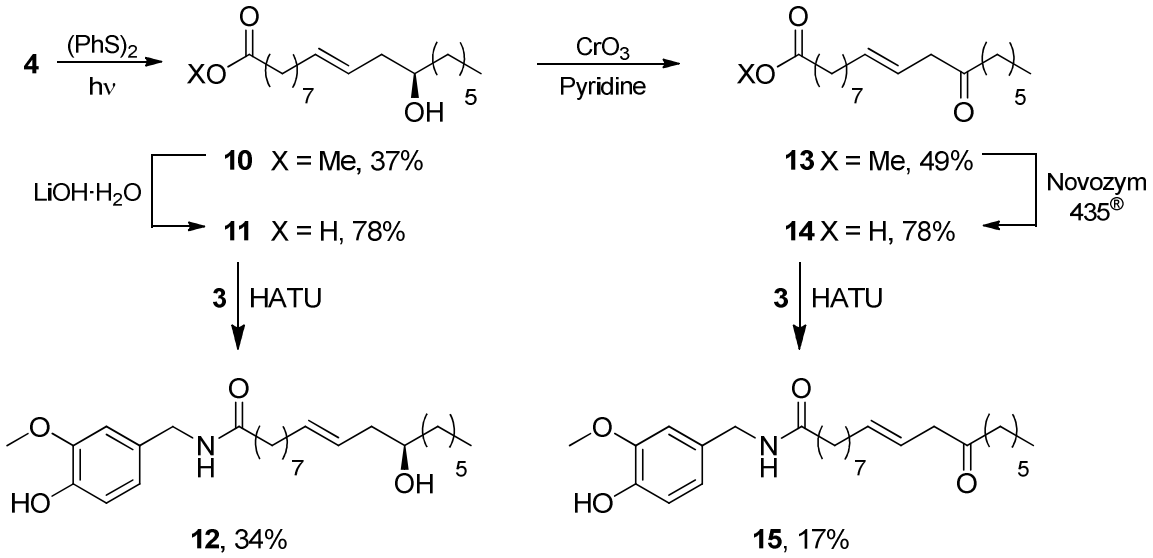
Scheme 1. Synthesis of compound 9.



The irradiation of alcohol **4** with diphenyl sulphide[43] in isooctane in a photochemical reactor for 3 h led to the isomer **10** in 37% yield after several recrystallizations at -30 °C. This compound was used to synthesize two new long-chain *N*-vanillylamides (**12**, **15**). The hydrolysis of the methyl ester of **10** led to the corresponding carboxylic acid **11.** The subsequent coupling of **11** with the 4-hydroxy-3-methoxybenzylamine hydrochloride **3** using the same conditions described above yielded compound **12** in a 34% yield. Compound **10** was also oxidized with CrO_3 in pyridine[44] to prepare the *trans* ketone **13** (49% yield), which was enzymatically hydrolysed to synthesise the corresponding acid **14** in a 78% yield. Subsequently, **14** was coupled with the vanillyl amine **3** to yield the (*E*)-*N*-(4-hydroxy-3-

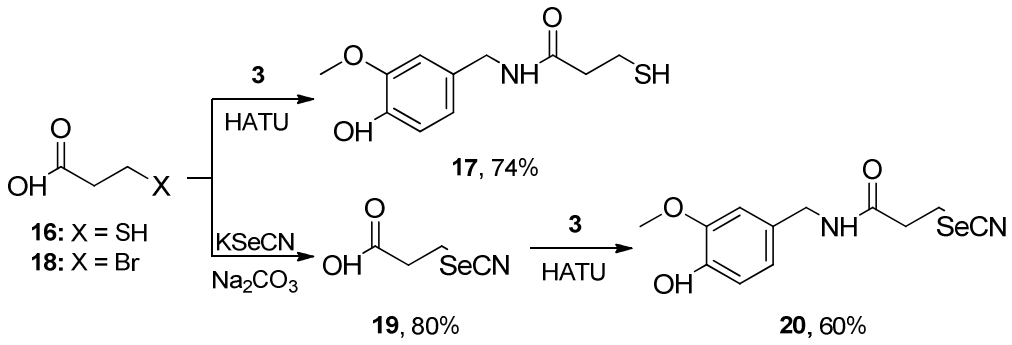
methoxybenzyl)-12-oxooctadec-9-enamide **15** after purification by liquid column chromatography (17% yield) (**Scheme 2**).

Scheme 2. Synthesis of compounds 12 and 15.



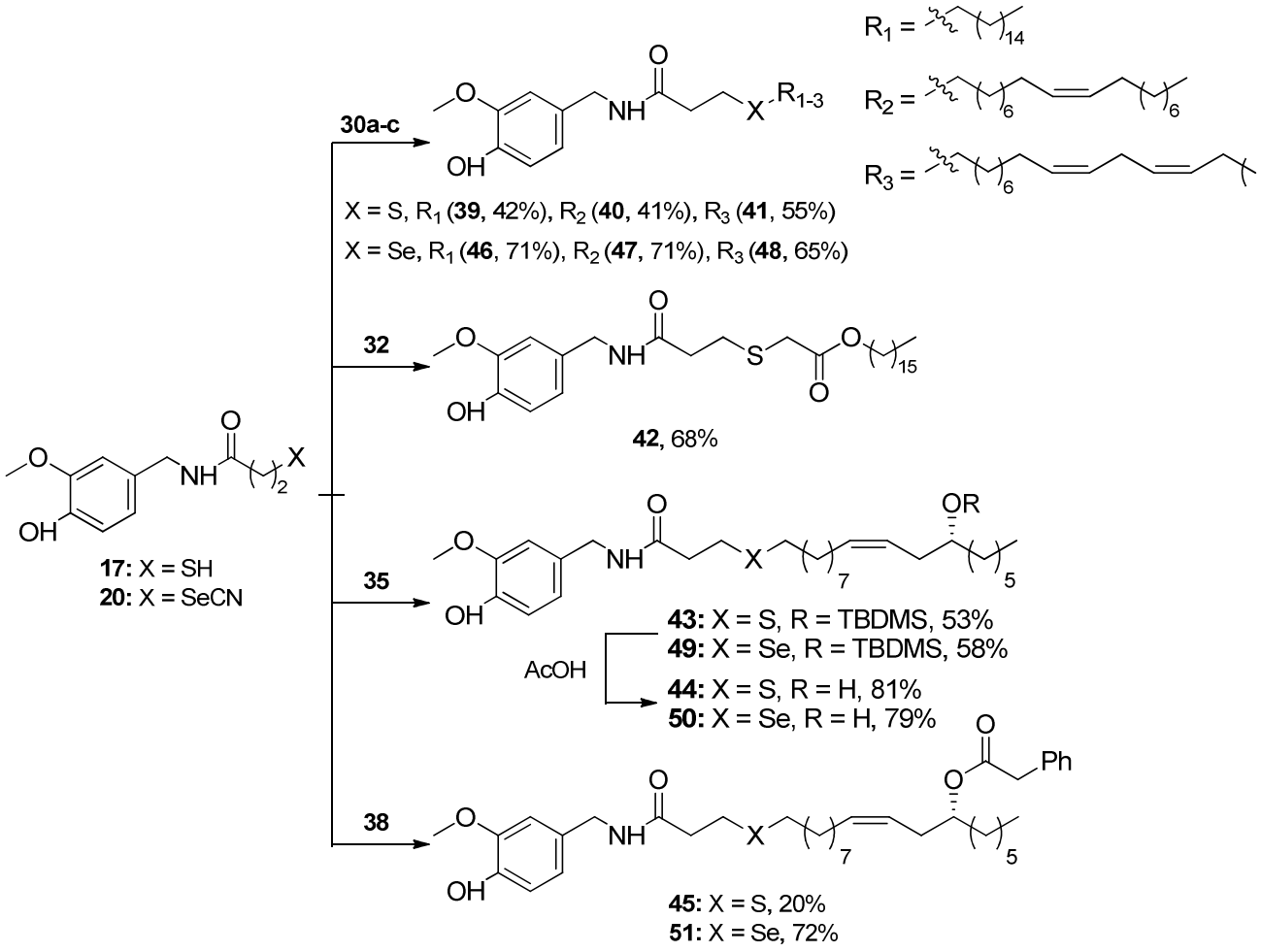
Scheme 3 shows the synthesis of the sulphur- and seleno-derivatives of **3**. Mercaptopropionic acid **16** was coupled with 4-hydroxy-3-methoxybenzylamine hydrochloride **3** using HATU and DIPEA in DMF anh achieving the amide **17** (74% yield). The synthesis of the seleno-derivatives started with bromopropionic acid **18**, which was treated with KSeCN in water: The neutralization with Na_2CO_3, yielded the selenocyanatopropionic acid **19** in 80% without purification. Finally, compound **19** was coupled with the 4-hydroxy-3-methoxybenzylamine hydrochloride **3** to obtain compound **20** after purification by liquid column chromatography (60% yield).

Scheme 3. Synthesis of sulphur- and seleno-intermediates.



Amide **17** was *S*-alkylated with the previously synthesized alkylating derivatives **30a-c**, **32** and **35** (see supporting information) in DMF and triethylamine obtaining the long-chain *N*-vanillylamides **39-43** and **45** in 41-68% yield. *N*-Vanillylamide **44** was successfully achieved after removing the TBDMS protecting group with acetic acid at room temperature (81% yield). New long-chain *N*-vanillylamides were obtained from compound **20**, which was firstly treated with $NaBH_4$ in ethanol at room temperature to remove the cyano protection and regenerate the selenol group.[45] Subsequent *Se*-alkylation was carried out in one-pot with the addition of diverse set of alkylating reagents (**30a-c**, **35** and **38**). *N*-Vanillylamides **46-49** and **51** were synthesized in 71-87% yields. Compound **50** was successfully prepared after removing the TBDMS protecting group with acetic acid at room temperature (79% yield) (**Scheme 4**).

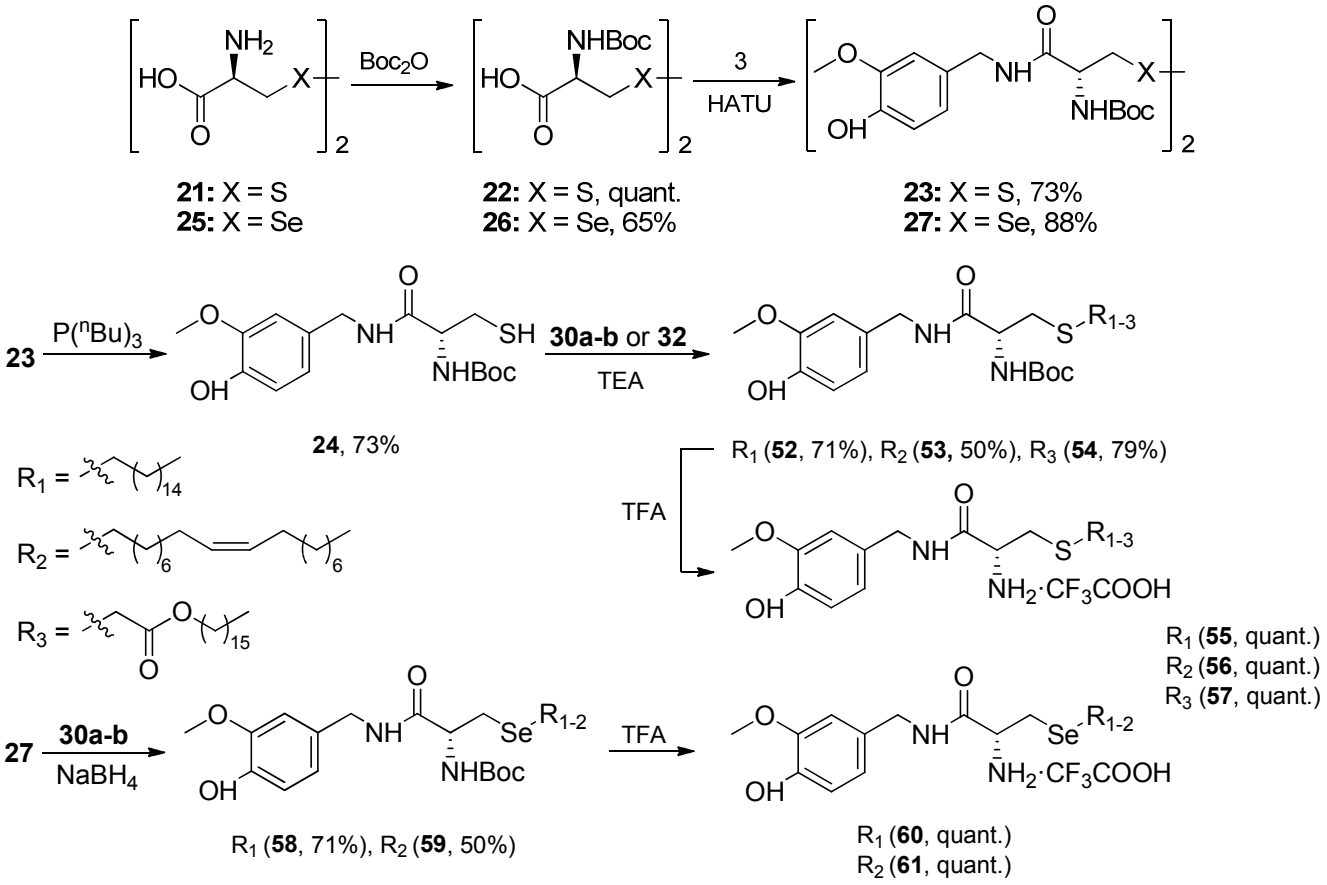
Scheme 4. Synthesis of no-branched sulphur- and seleno-derivatives.



Scheme 5 shows the synthesis of amino-branched analogues. The first step consisted in the treatment of L-cystine **21** or L-selenocystine **25** with Boc_2O in presence of triethylamine to afford the protected derivatives **22**[1] and **26**[2] (quantitative and 65% yield, respectively).[46,47] These compounds were coupled with 4-hydroxy-3-methoxybenzylamine hydrochloride **3** using EDCI, HOBt and triethylamine (TEA) in anhydrous DMF achieving the amides **23** and **27** (74% and 88% yield). The reduction of compound **23** with $P(^nBu)_3$ in wet dichloromethane afforded compound **24** in a 73% yield after purification by liquid column chromatography. New long-chain *N*-vanillylamides were afforded from compound **24**, which was *S*-alkylated with the previously synthesized alkylating derivatives **30a-c** and **32** in presence of triethylamine obtaining the long-chain *N*-vanillylamides **52**, **53** and **54** in moderate yields (50-79%

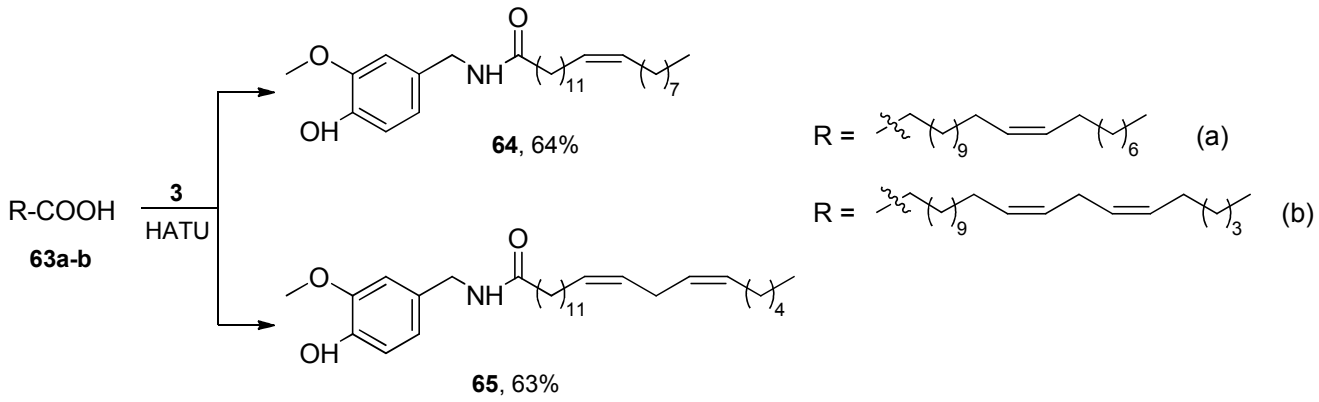
yield). The *N*-Boc deprotection was carried out using trifluoroacetic acid[48] in dichloromethane yielding *N*-vanillylamides **55**, **56** and **57** as trifluoroacetic salts in quantitative yields. Compound **27** was reduced with $NaBH_4$ in ethanol at room temperature to cleave the diselenium bond.[49] The *Se*-alkylation was carried out with the addition of the alkylating derivatives **30a-b** to afford the *N*-vanillylamides **58** and **59** in 74-88% yields. Finally, The *N*-Boc deprotection was carried out using the same conditions described above to afford the *N*-vanillylamides **60** and **61** as trifluoroacetic salts.

Scheme 5. Synthesis of amino-branched analogues.



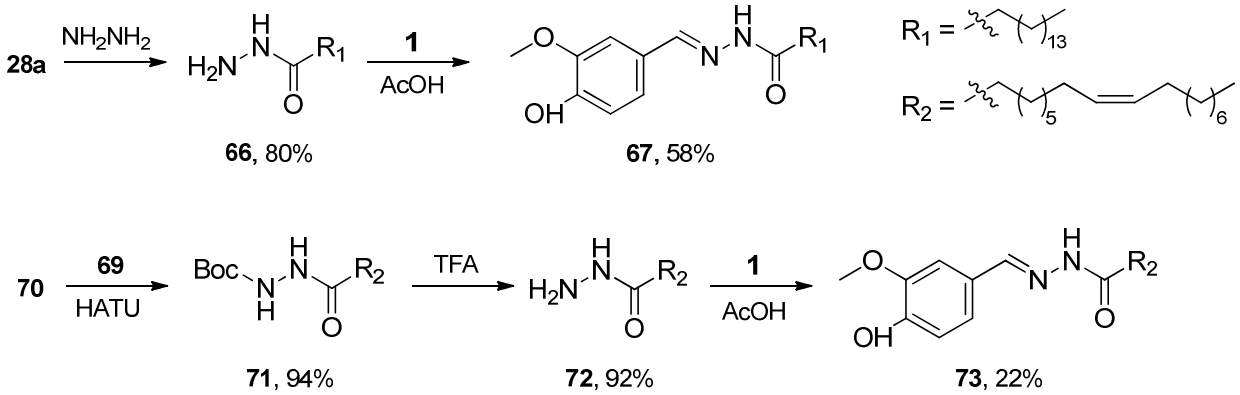
Acids **63a-b**, which were previously obtained from the hydrolysis of their respective methyl esters **62a-b** (see supporting information), were coupled with the 4-hydroxy-3-methoxybenzylamine hydrochloride **3** using HATU and DIPEA in anhydrous DMF achieving the amides **64** and **65** after purification by liquid column chromatography (64 and 63% yield) (**Scheme 6**).

Scheme 6. Synthesis of compounds 64 and 65.



Methyl palmitate **28a** was treated with an excess of hydrazine hydrate in ethanol to synthesize the palmitic acid hydrazide **66** (80% yield). The addition of the aromatic aldehyde vanillin **1** to compound **66** in presence of acetic acid in reflux conditions gave the Schiff's base compound **67** in 58% yield.[50] A similar compound was synthesized starting from oleaic acid **70**, which was coupled to *tert*-butyl hydrazinecarboxylate **69** using HATU and DIPEA in DMF to yield the oleylhydrazide **71** in a 94% yield. The *N*-Boc deprotection of oleylhydrazide **71** with TFA in DCM for 2 h led to oleylhydrazide **72** in 92% yield. Compound **72** refluxed with vanillin **1** in the presence of acetic acid in methanol produced the Schiff base **73** in 22% yield (**Scheme 7**).

Scheme 7. Synthesis of compounds 67 and 73.



2.2 Biological evaluation

2.2.1 Capsaicin-derivatives activate TRPV1 channel

The capsaicin scaffold (Figure **1**) [51] can be ideally divided into three regions: head, neck and tail, formed by the vanillyl moiety, the amidic group and the lipophilic alkyl chain, respectively. Structural variations, including incorporation of sulphur atom, into the head and the neck-regions have been described in the literature[52–55].

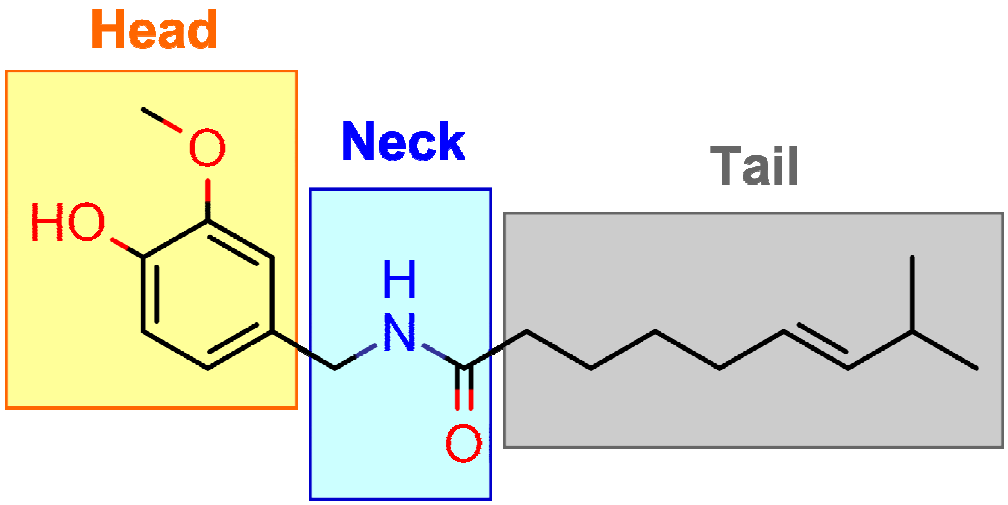


Figure 1. Chemical structure of capsaicin. *The vanillyl head, the amide neck and hydrophobic tail are shaded in yellow, cyan and grey, respectively.*

Instead, the effect of a sulphur atom in the alkyl chain has been less investigated. The recent availability of the 3D structure of TRPV1[56] along with mutagenesis studies[57] allowed the identification of the capsaicin binding site, where the alkyl chain is hosted in a phenylalanine-rich hydrophobic region close to Thr550, a residue involved in H-bond interaction with the ligand amide group. The presence of a sulphur atom near the neck region should in principle lead to an increment of activity due to favourable dipole-dipole and aromatic-sulphur interactions. Since sulphur can be substituted with selenium via isosteric replacement, we also synthetized the corresponding selenium-analogs. Selenium

is an essential trace element whose role in medicine and biology is just starting to be elucidated. Some selenium-containing compounds have provided protection against many degenerative conditions, including cancer. Thus, a series of novel capsaicin-derivatives, i.e. **9**, **12**, **15**, **39**, **46**, **55**, **60**, **42**, **57**, **44**, **56**, **40**, **45**, **65**, **41**, **48**, **64**, **47**, **61**, **51**, **50**, **67**, **73,** whose structures are reported in Tables **1** and **2**, featuring the same "head" and "neck" as capsaicin but differing in length and nature of the hydrocarbon tail, were tested on human TRPV1 heterologously expressed in human embryonic kidney (HEK)-293 cells by fluorometric assay (see **Tables S1** and **S2** in SI). The predicted activities as TRPV1 agonists were confirmed for many compounds within the series, exhibiting EC_{50} values from high- to sub-nanomolar range. A SAR analysis of the results also disclosed the critical role of the region flanking the amide group in modulating the activity. In fact, the insertion of a positive charge next to the amide group was detrimental for activity (compounds **55-57** and **60**), and the introduction of an imido group between the aromatic moiety and the amido group led to totally inactive compounds (compounds **67** and **73**). Conversely, the introduction of a single polar substituent (hydroxyl, ester or ketone) was well-tolerated, and the introduction of a sulphur or selenium atom in the hydrophobic tail even improved the activity. However, on the basis of the antagonist activity exhibited by capsaicin on TRPV2 Quad [37], the new compounds were also tested on TRPV2 to determine if the elongation and the functionalization of the alkyl chain could elicit a functional response at this receptor.

2.2.2 Capsaicin-derivatives inhibit TRPV2 channels activated by LPC

The activity of the synthesized capsaicin-derivatives on TRPV2 was evaluated *in vitro*. The assays were conducted using a fluorometric assay with rat TRPV2 heterologously expressed in HEK-293 cells. The tested compounds did not significantly activate TRPV2-mediated Ca^{2+} elevation in transfected HEK-293 cells. Instead, preincubation (5 min) of TRPV2-HEK-293 cells with different concentrations of the tested compounds, followed by incubation with LPC (3 μM), caused inhibition of

intracellular Ca^{2+} elevation due to TRPV2 response to LPC. The corresponding IC_{50} values are reported in **Table 1**.

The structure-activity relationships (SARs) of these compounds suggested a critical influence on the capability to exert TRPV2 antagonism of the alkyl chain and, in particular, of its hydrophobicity, length and degree of unsaturation. Hydrophobicity is important since, as shown in **Table 1**, the activity dramatically dropped after introduction in the chain of polar substituents such as hydroxyl, keto or ester groups (these latter arising from esterification of the hydroxyl group), or their combinations (**42**, **44**, **50**, **45**, **51**, **9**, **12**, **15**). However, the presence of an amino group next to the amide (**55**, **60**, **56**, **61**), which had marginal effects for already-active compounds, by only slightly increasing their potency (**60** vs **46**), was instead dramatic for those inactive compounds bearing a hydroxyl or an ester moiety in the alkyl chain, whose activity was completely rescued (see **42** vs **57**). The complete recovery of activity after introduction of an amino group next to the amide in derivatives bearing a polar substituent in the alkyl chain suggests that reinforcement of the polar interactions of the "head" avoids the competition with the polar-substituted alkyl chain for interaction with receptor polar residues in a region where the polar head, but not the alkyl chain, should be hosted to elicit a measurable effect. The chain is fairly more tolerant to changes not substantially affecting the hydrophobicity of the alkyl group: replacement of sulphur with selenium in the alkyl chain did not affect significantly ligand activity (**39** vs **46**); its replacement with a carbon atom determined an increase in potency (**64** vs **40**/**47**). While polar functionalization of the alkyl chain caused a dramatic drop of activity, amino or imino groups (**67**, **73**) were well tolerated in the region close to the amide moiety of capsaicin. In particular, the imino derivatives were among the most active compounds within the series (IC_{50} = 0.28 and 0.12 μM; respectively). Also length and unsaturation degree of the alkyl chain significantly affected the activities of the tested compounds. The C16:0 and C18:0 saturated analogs were inactive, whereas the C20:0 derivative showed an IC_{50} =3.1μM. The insertion of a single double bond in C18 chain (Olvanil)

dramatically increased the antagonism, with an IC_{50}= 0.16 μM.

Thus, the screening led to the identification of several very potent TRPV2 antagonists, exhibiting IC_{50} values in the sub- to low-micromolar range. This result is quite remarkable since, despite its close homology to TRPV1, TRPV2 is insensitive to capsaicin, being the residues responsible for capsacin binding and receptor activation in TRPV1 not conserved in TRPV2.[58]

The most striking result from the SAR of capsaicin-derivatives against LPC is that the elongation of the alkyl chain of capsaicin causes a switch of such scaffold from inactivity towards potent antagonism at rat recombinant TRPV2. Intriguingly, the dependence of TRPV2 modulation on the length of the ligand alkyl chain has already been observed for lysophospholipids, which require a carbon chain longer than C12 to stimulate the receptor.[21]

2.2.3 Capsaicin-derivatives inhibit TRPV2 channels activated by CBD

Due to different latency in the activation profile between LPC and cannabidiol (CBD) (see **Figure 2**), we also investigated the effect of a representative panel of capsaicin derivatives against CBD, to ascertain whether the inhibitory activity/potency would vary against agonists exhibiting different kinetics of action. Also in this case, the assays were conducted using a fluorometric assay with recombinant rat TRPV2 heterologously expressed HEK-293 cells. The preincubation (5 min) of TRPV2-HEK-293 cells with different concentrations of the tested compounds, followed by incubation with CBD (2 μM), caused an inhibition of the Ca^{2+} elevation due to the TRPV2 response to CBD. The corresponding IC_{50} values of the tested compounds are reported in **Table 1.** While the trend identified in LPC antagonism for capsaicin derivatives bearing all carbon atoms, selenium or sulphur was substantially conserved, a different behavior was observed with those derivatives featuring polar substituents (i.e. **50**/**51**), since their activity against CBD was not negatively affected by these functional groups, as instead observed against LPC. The imino-derivatives **67** and **73** (see **Table 2**), i.e.

the two most active compounds against LPC (0.28 and 0.12 μM, respectively), were less potent against CBD (IC_{50} = 6.0 and 3.0 μM, respectively). The trend of activity of C16:0, C18:0 and C18:1 derivatives was similar to that observed for LPC, although C18:1 (Olvanil) was less potent as an antagonist (IC_{50} =1.7 μM), whereas, differently from what observed with LPC, C20:0 was totally inactive. These results demonstrate a dependence of the antagonist activity on the type of agonist against which antagonism is tested.

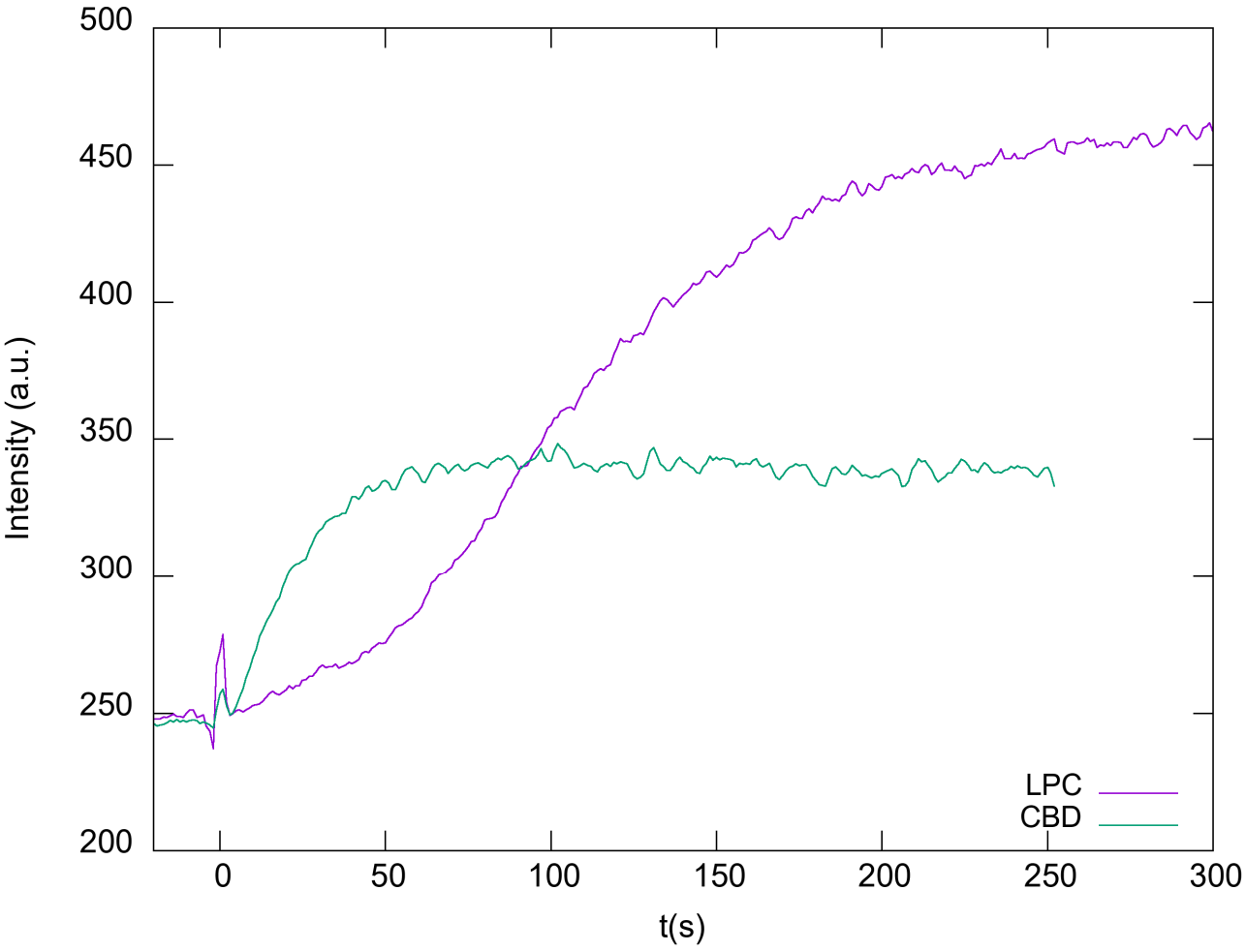
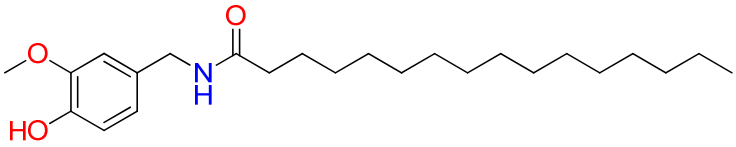
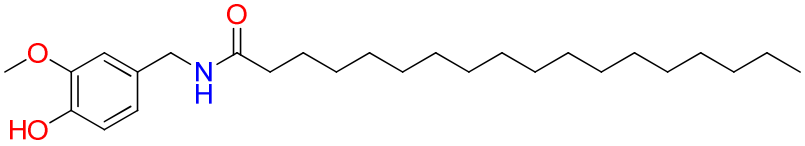
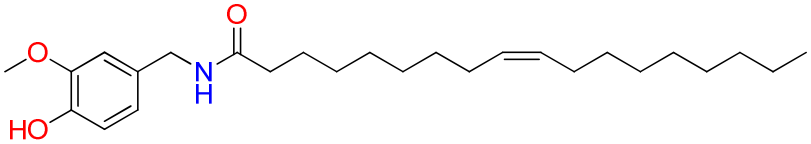
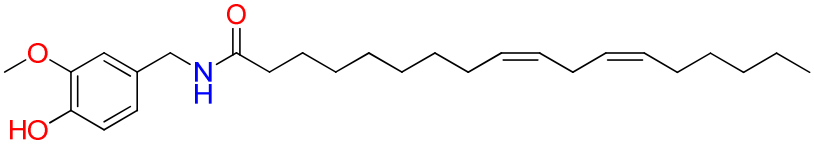
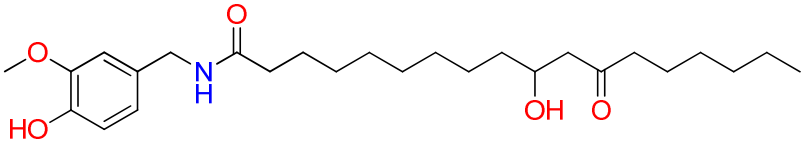
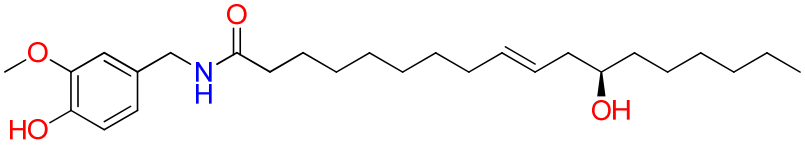
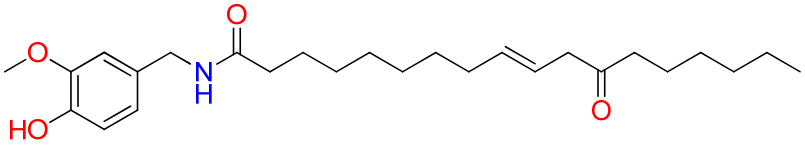
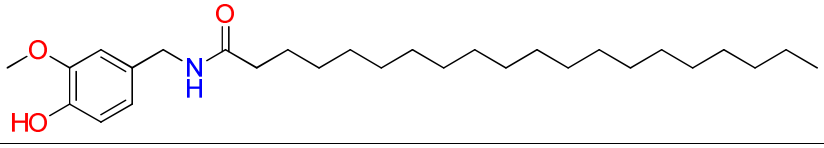
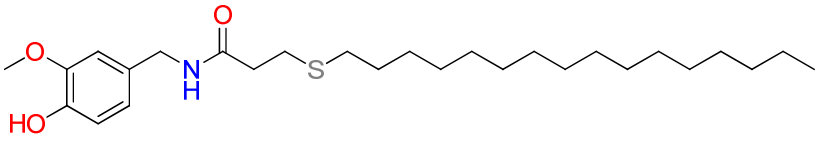
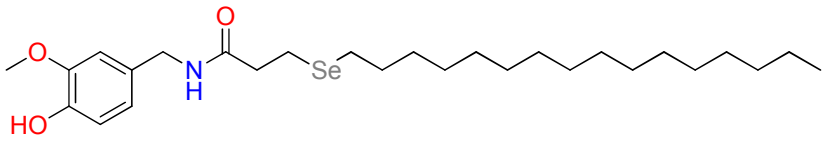
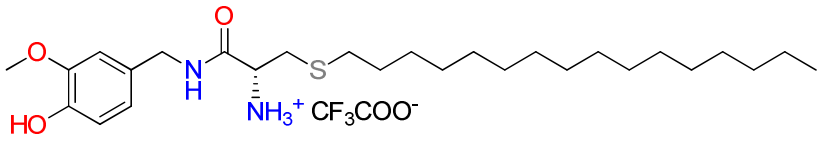
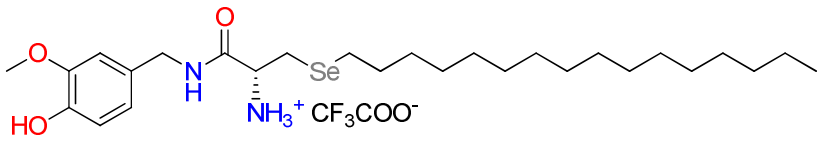
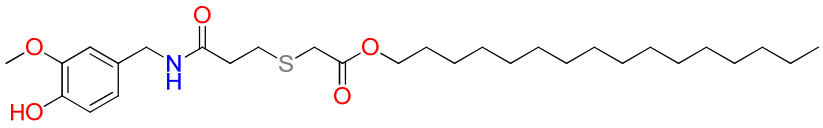
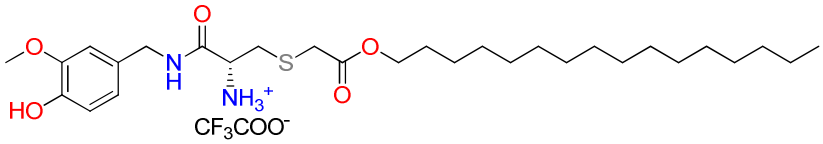
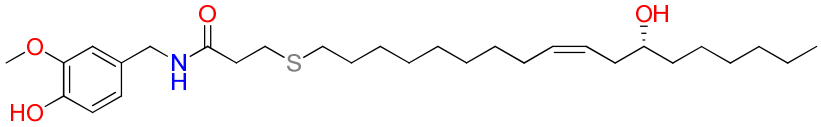
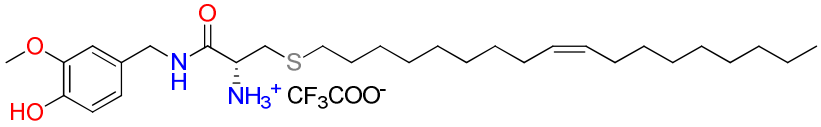
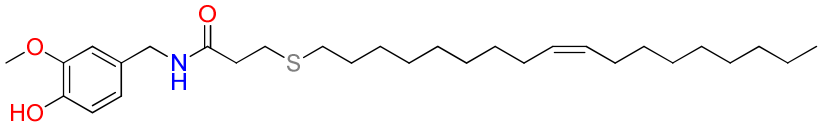
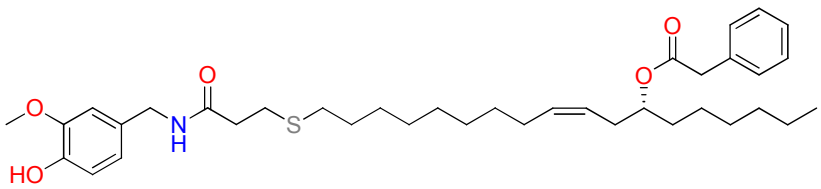
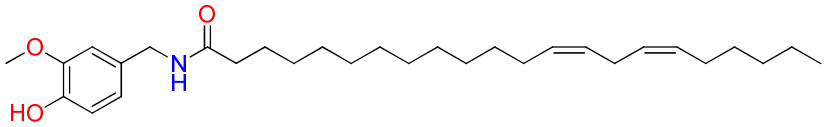
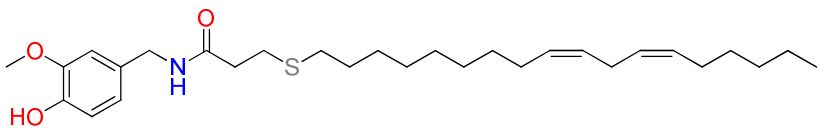
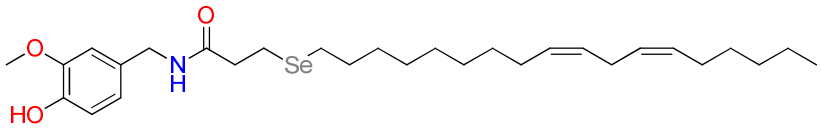
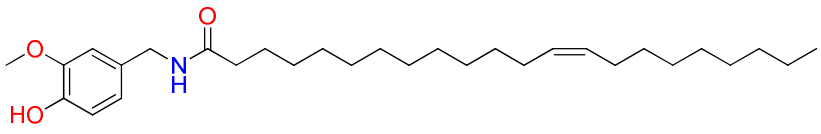
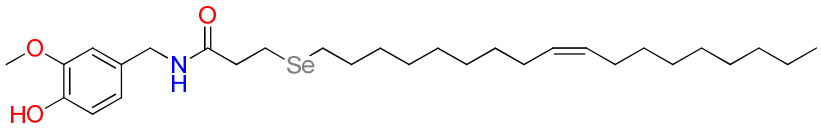
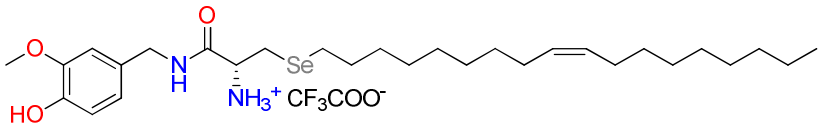
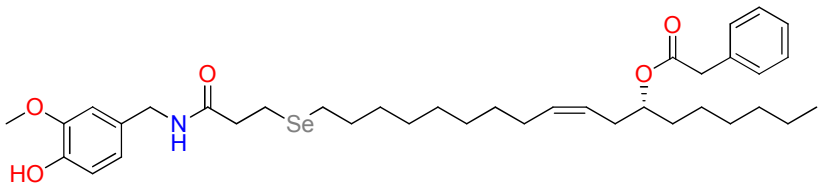
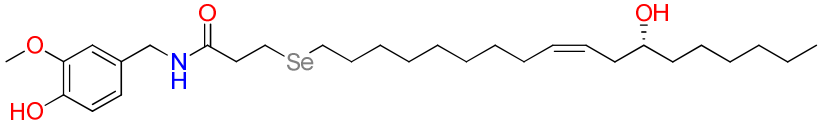


Figure 2. ***TRPV2 is activated by LPC (3 μM) and CBD (2 μM).*** *The graph shows the representative traces of* $[Ca^{2+}]_i$ *increase evoked by the two agonists in HEK293 cells overexpressing TRPV2.*

Table 1. ***Antagonist potency of Capsaicin-like compounds at TRPV2 against LPC (3 μM) and CBD (2 μM), reported as IC_{50} (μM).***

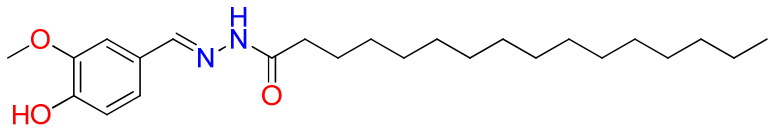
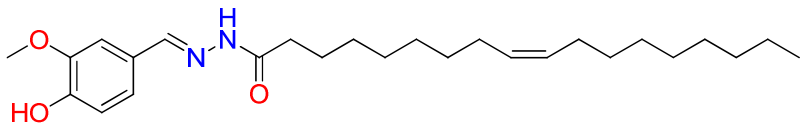
Caps-like	Structure	LPC	CBD
Palvanil (C16:0)[a]		>10	>10
Stevanil (C18:0)		>10	>10
Olvanil (C18:1)		0.16±0.02	1.7±0.1
Livanil (C18:2)		2.6±0.2	2.1±0.1
9 (C18:0)		>10	>10
12 (C18:1)		>10	7.5 ± 1.3
15 (C18:1)		>10	4.4 ± 0.3
Eicosavanillamide (C20:0)		3.1 ± 0.2	>10
39 (C19/S)		3.8 ± 0.8	nd[b]

Caps-like	Structure	LPC	CBD
46 (C19/Se)		4.3 ± 0.9	nd
55 (C19/S)		1.4 ± 0.2	nd
60 (C19/Se)		1.2 ± 0.03	nd
42 (C21/S/O)		>10	nd
57 (C21/S/O)		1.4 ± 0.1	nd
44 (C21/S:1)		>10	nd
56 (C21/S:1)		1.9 ± 0.1	nd
40 (C21/S:1)		2.5 ± 0.1	nd
45 (C21/S:1)		>10	nd
65 (C22:2)		0.82 ± 0.12	1.8 ± 0.3

Caps-like	Structure	LPC	CBD
41 (C22:2)		1.4 ± 0.07	2.8 ± 0.4
48 (C22:2)		1.4 ± 0.06	2.3 ± 0.1
64 (C22:1)		0.49 ± 0.07	1.5 ± 0.2
47 (C21/Se:1)		1.8 ± 0.01	3.2 ± 0.2
61 (C21/Se:1)		1.7 ± 0.01	0.98 ± 0.14
51 (C21/Se:1)		>10	2.3 ± 0.3
50 (C21/Se:1)		>10	1.4 ± 0.1

[a] *In parenthesis, number of C atoms in the alkyl chain: number of unsaturations. When heteroatom X occurs within alkyl chain, it is indicated as "/X";* [b] *nd: Not Determined*

Table 2. ***Antagonist potency of Capsaicin-imino compounds at TRPV2 against LPC (3 μM) and CBD (2 μM), reported as IC_{50} (μM).***

Imino-caps	Structure	LPC	CBD
67 (16:0)		0.28 ± 0.04	6.0 ± 1.0
73 (18:1)		0.12 ± 0.01	3.0 ± 0.4

[a] *In parenthesis, number of C atoms in the alkyl chain: number of unsaturations.*

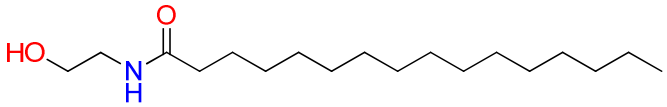
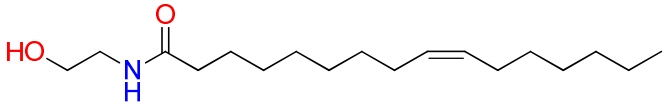
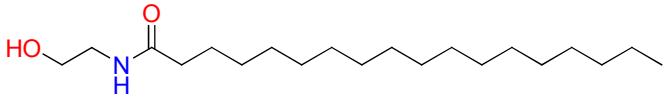
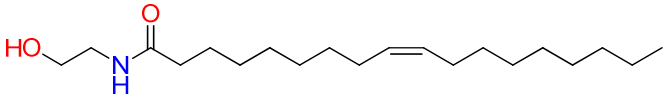
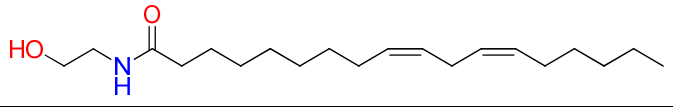
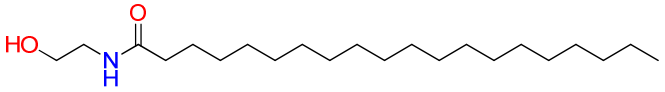
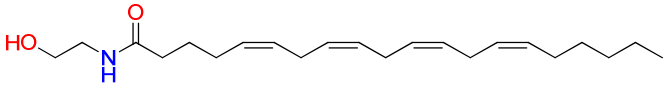
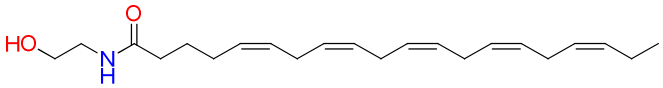
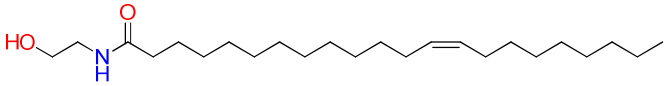
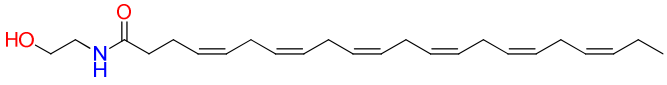
2.2.4 Evaluation of endogenous lipids as potential TRPV2 antagonists

Since the activity of the tested compounds appears to critically depend on the nature of alkyl chain, but is less affected by changes in the polar head, we decided to ascertain the role of the head group of capsaicin, i.e. the vanillyl moiety, by testing a series of naturally-occurring lipids bearing different polar heads and differing in length and unsaturation of the alkyl chain, in order to determine the structural and functional requisites for TRPV2 modulation.

2.2.5 Long-chain ethanolamides exhibit differential inhibition of TRPV2 upon activation by LPC or CBD

To evaluate the contribution of the aromatic moiety to the overall activity, a panel of natural occurring ethanolamides differing in length and unsaturation degree was tested for both agonism and antagonism at TRPV2, using both LPC and CBD as reference activators. Ethanolamides share with the tested capsaicin-derivatives the nature of both the alkyl chain and the hydrophilic groups (amide and hydroxyl moieties) in the polar head. The IC_{50} values (against CBD 2 μM and LPC 3 μM) are reported in **Table 3**. Ethanolamides featuring saturated alkyl chains, regardless of their lengths, were inactive against both agonists, whereas the introduction of a single double bond was sufficient to switch from inactivity to activity against both agonists (see PEA vs POEA, or SEA vs OEA), similarly to what already observed for capsaicin-derivatives. However, while the C20:0 capsaicin-derivative was active against LPC, the homolog ethanolamide was inactive. Moreover, while OEA was less active than the counterpart Olvanil, LEA was more potent than Livanil against both reference agonists. Increasing the number of double bonds increased the potency against CBD, but not LPC.

Table 3. ***Potency of Fatty Ethanolamides as functional antagonists at TRPV2 against LPC (3 μM) and CBD (2 μM), reported as IC_{50} (μM).***

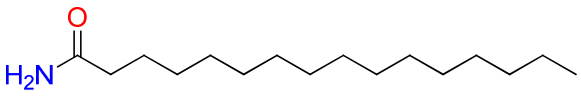
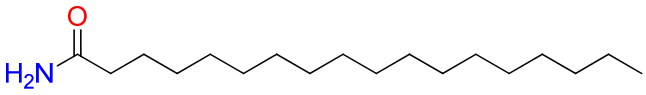
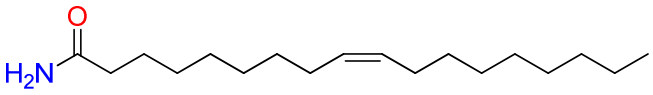
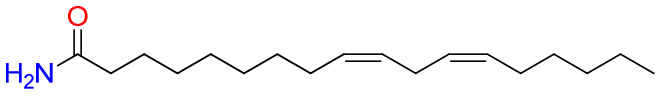
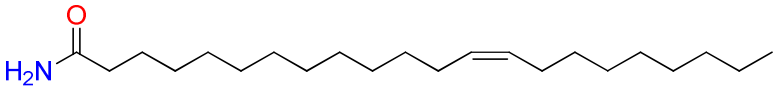
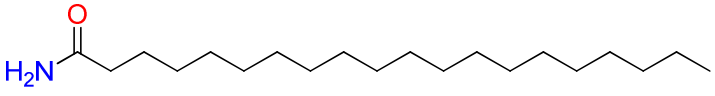
Ethanolamides	Structure	LPC	CBD
PEA[a] (C16:0)[b]		>10	>10
POEA (C16:1)		3.5 ± 0.01	1.7 ± 0.1
SEA (C18:0)		>10	>10
OEA (C18:1)		1.8 ± 0.1	5.4 ± 0.2
LEA (C18:2)		1.4 ± 0.1	0.65 ± 0.07
Arachidoyl-EA (C20:0)		>10	>10
AEA (C20:4)		6.6 ± 0.1	0.96 ± 0.09
EPEA (C20:5)		>10	2.3 ± 0.2
Docosaenoyl-EA (C22:1)		0.74 ± 0.02	>10
DHEA(C22:6)		>10	1.6±0.1

[a]*Abbreviations: Ethanolamide (EA), Palmitoyl Ethanolamide (PEA),Palmitoleoyl Ethanolamide (POEA),Oleoyl Ethanolamide (OEA), Lynoleoyl Ethanolamide (LEA), Arachidonoylethanolamide (AEA), Eicosapentaenoyl Ethanolamide (EPEA), Docosahexaenoyl Ethanolamide (DHEA);*[b] *In parenthesis, number of C atoms in the alkyl chain: number of unsaturations.*

2.2.6 Long-chain primary amides exhibit differential inhibition of TRPV2 channels upon activation by LPC or CBD

To also evaluate the role of the hydroxyl group, we tested a series of amide derivatives. As for capsaicin- and ethanolamine-derivatives, also for the amides the activity strongly depended upon the presence of at least one double bond. In particular, Erucamide is active as TRPV2 antagonist with a potency comparable to that of its capsaicin-derivative (0.67 *vs* 0.49 μM) against LPC, but it is less potent than the capsaicin counterpart against CBD (7.1 *vs* 1.5 μM). As observed with the ethanolamides, also the C20:0 amide-derivative was inactive against both activators (**Table 4**).

Table 4. ***Antagonist potency of Fatty Amides at TRPV2 against LPC (3 μM) and CBD (2 μM), reported as IC_{50} (μM).***

Amides	Structure	LPC	CBD
PA[a] (C16:0)[b]		>10	>10
SA(C18:0)		>10	>10
OA (C18:1)		2.1 ± 0.1	2.1 ± 0.2
LA (C18:2)		2.2 ± 0.1	1.2 ± 0.1
ErA (C22:1)		0.67 ± 0.13	7.1 ± 0.7
Eicosanamide (C20:0)		>10	>10

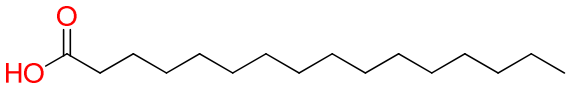
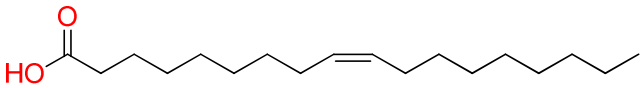
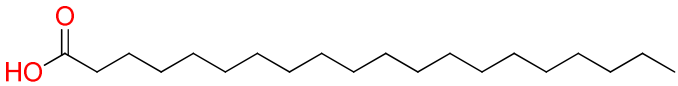
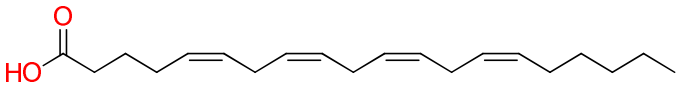
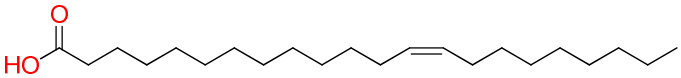
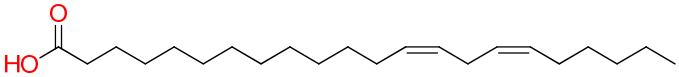
[a] *Abbreviations: Palmitamide (PA), Stearamide (SA), Oleamide (OA), Linoleamide (LA), Erucamide (ErA);*[b] *In parenthesis, number of C atoms in the alkyl chain: number of unsaturations.*

2.2.7 Free fatty acids are poor inhibitors of TRPV2 channels

Finally, to investigate the role of the amide group, we tested against both LPC and CBD a panel of long-chain fatty acids, featuring alkyl chains comparable with those occurring in the already-tested

compounds. The results are reported in **Table 5**. Fatty acids with alkyl chains from C16 up to C22 are by far less potent antagonists against both reference agonists than the other classes of compounds bearing similar alkyl chains, thus suggesting that the amide group is mandatory for potent antagonism.

Table 5. ***Lack of strong antagonist activity of Fatty acids at TRPV2 against LPC (3 μM) and CBD (2 μM), reported as IC_{50} values (μM).***

Acids	Structure	LPC	CBD
Palmitic acid (C16:0)[a]		>10	>10
Oleic acid (C18:1)		>10	>10
Arachidic acid (C20:0)		>10	>10
Arachidonic acid (C20:4)		>10	>10
Erucic acid (C22:1)		>10	>10
Docosadienoic acid (C22:2)		>10	>10

[a] *In parenthesis, number of C atoms in the alkyl chain: number of unsaturations.*

2.2.8 Schild Analysis on selected TRPV2 antagonists

The effect of increasing concentrations of antagonist **61**, Olvanil and Docosaenoyl-EA *vs* LPC and **61**, Olvanil and **50** *vs* CBD were tested against concentration–response curves of LPC and CBD (where the effects of each concentration of LPC and CBD were expressed as percent of their effect of $2x10^{-4}$ M in the absence of the antagonist) to calculate Schild's plots. These compounds have been selected as representative of antagonists active either against both activators (**61**, Olvanil), or selectively towards LPC (Docosaenoyl-EA)/CBD (**50**) alone. In all cases, the plots analyzed by linear regression gave slope values significantly less than unity, as reported in **Table 6**, indicative of a non-competitive behavior. However, this result may be also indicative of a non-equilibrium condition and we do not definitely rule out a competitive behavior.

Table 6. Slope values from linear regression of Schild analysis and t-test statistics

	LPC			CBD		
Compounds	**Slope**[a]	**N**[b]	**P**[c]	**Slope**[a]	**N**[b]	**P**[c]
61	-0.58±0.087	4	<0.0024	-0.74±0.048	4	<0.002
Olvanil	-0.77±0.049	6	<0.001	-0.55±0.068	6	<0.001
Docosaenoyl-EA	-0.54±0.046	6	<0.001	-	-	-
50	-	-	-	-0.63±0.039	5	<0.001

[a] mean value ± standard deviation; [b] number of experiments (each one performed at least in triplicate) used for Schild regression; [c] P values calculated from t-test values for the "slope=1 hypothesis".

3. Discussion

Novel capsaicin-derivatives, initially designed as TRPV1 agonists, behave as potent TRPV2 antagonists. The different types of modifications introduced in this compounds determine different agonist/antagonist profiles and, in particular, opposite behaviors in terms of relative potency/efficacy within a derivative series on the two channels. In fact, the insertion of a positive charge or an imido group close the amido group, detrimental for TRPV1 agonism, is well-tolerated for TRPV2 antagonism, and even leads in some cases to an increment or a rescue of activity. Conversely, the

insertion of a sulfur/selenium atom and/or the presence of a polar group, which increase TRPV1 agonism, leave unaffected, or even decrease, TRPV2 antagonism.

Given the scarcity of known endogenous ligands for TRPV2, the discovery of such long-chain capsaicin-derivatives as potent TRPV2 antagonists prompted us to investigate the following classes of long-chain fatty acid derivatives with at least one functional group in common with capsaicin derivatives as potential TRPV2 modulators: *i*) ethanolamides, *ii*) primary amides and *iii*) free fatty acids, to evaluate the role of the amide group itself. Antagonists were found both in the ethanolamide and primary amide, but not in fatty acid, series.

Activities for both synthetic and endogenous ligands were tested against either LPC or CBD as activators, since, on the basis of their different kinetics of activation, CBD can be defined as a direct TRPV2 agonist, whereas LPC induces TRPV2 activation indirectly, via its G-protein-coupled receptors and PI3,4 Kinase mediated pathways.[21] We found that this different mode of activation is differentially counteracted by the investigated compounds, which can be classified as follows: *a*) compounds endowed with similar antagonist efficacy against both agonists, *b*) compounds selectively active against LPC, *c*) compounds selectively active against CBD. To determine the nature of antagonism, a Schild regression was carried out for the representative members of each class, i.e. Olvanil, Docoesanoyl-EA and compound **50** and in all 3 cases the antagonists behaved as non-competitive ligands, suggesting that these compounds may act as allosteric antagonists. However, we cannot completely rule out a competitive behavior since a Schild plot slope <1 may also suggest non-equilibrium conditions. Moreover, since the hydrophobicity of the alkyl chain of the investigated compounds is a critical requisite for LPC but not for CBD inhibition, it is reasonable to speculate that a different binding site is involved in LPC antagonism, with structural/functional requisites different from those of CBD. This site might be either on TRPV2 or on other targets activated by LPC in its

signaling cascade and would be the target of those compounds selectively antagonizing activation by LPC. A common critical requisite for activity of both ethanolamides and amides as TRPV2 antagonists is the occurrence of at least one double bond in the alkyl chain, since saturated lipids, regardless of the length of their acyl chains, are totally inactive. This suggests that a bent conformation of the alkyl chain is required for a better accommodation into the active site, as previously reported for other TRPV1 agonists[59]. Also C16:0 and C18:0 derivatives of capsaicin result inactive against both CBD and LPC, whereas the C20:0 derivative is selectively active against LPC. Instead, a different behavior is observed with imino-capsaicin derivatives since they are active also when bearing saturated alkyl chain. The aromatic moiety contributes to the overall activity at TRPV2 of the compounds characterized in the present work, since it occurs in the most active antagonists.

4. Conclusions

In summary, the search for structurally-related synthetic or endogenous lipids with structural similarity to capsaicin-derivatives led to identification of Olvanil and **73** as potent TRPV2 antagonists against LPC (0.16 and 0.12 μM, respectively) and of LEA (linoleoyl-ethanolamide) as potent TRPV2 antagonist against CBD (0.65 μM). This finding is both surprising - since all other synthetic and endogenous compounds tested here on TRPV2 behave as antagonists and capsaicin is inactive at this channel - and of great physiological importance, since novel potent endogenous antagonists were been identified following this study.

In conclusion, starting from the testing of a series of synthetic capsaicinoids as modulators of rat TRPV2, we discovered not only new tools for the pharmacological manipulation of the latter, but also that previously described endogenous lipids, i.e. long chain fatty acid ethanolamides and primary amides, behave as negative modulators of this channel. These data are of great potential importance given the increasingly important role assigned to TRPV2 in temperature sensing, pain, insulin

secretion, immune response, muscle and heart function and cancer.[58]

5. Experimental Section

4.1 Compounds

Stevanil, Livanil, ethanolamides, amides and fatty acids when not described in the synthetic section have been purchased from Cayman-Vinci Biochem. Palvanil and PEA are a kind gift from Epitech Group SpA, Saccolongo, Padova, Italy whereas Olvanil is a precious gift from dr. Alberto Minassi, Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Novara, Italy.

5.2 Synthetic Procedures.

Reactions requiring anhydrous conditions were performed in blazed or oven-dried glassware using anhydrous solvents and under inert atmosphere (argon). The solvents and reagents were purchase from Acros Organics, Sigma Aldrich, Fluka, Merk, Panreac, Strem Chemicals or TCI Chemicals. Petroleum ether, EtOAc, DCM and MeOH were used without further purification. In case of anhydrous reactions, solvent and reagents were properly dried. Acrolein was distilled at atmospheric pressure and used immediately. The reactions were monitored until completion by TLC on silica gel 60F-254 precoated plates (Merck). Visualization of the compounds was performed by UV light (254 nm) and stained was performed either by immersion in a 5% solution of concentrated H_2SO_4 in methanol or 5% w/v phosphomolibdic acid in ethanol followed by heating. Flash column chromatography was performed using silica gel (technical grade, 60 Å, 40-63 μm) (Sigma Aldrich) under air pressure. NMR spectra were recorded on a MERCURYplus AS400 MHz Varian spectrometer. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants (*J*) are reported and expressed in hertz (Hz), splitting patterns are designated as: br (broad), s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), dt (double triplet), td (triple doublet), ddd (double double doublet), p (pentuplet) and m (multiplet). All ^{13}C NMR spectra were proton decoupled. High resolution mass spectra (HR-MS) were

recorded on at the Serveis Cientificotècnics of Universitat de Lleida (SCT-UdL) and Servei de Recursos Científics i Tècnics of Universitat Rovira i Virgili (URV) with an Agilent G6510AA Q-TOF MS spectrometer in positive electrospray ionization (ESI^+) and Agilent LC1200 Series coupled to MS6210 TOF spectrometer in electrospray ionization (ESI^+) respectively. Mobile phase was composed of ACN/MeOH 50:50. Flow rate: 0.6 mL/min. Infrared spectra were recorded on Jasco FT-IR 6300 using a diamond ATR crystal cell. Melting points were measured using Gallenkamp capillary apparatus and are uncorrected. Optical rotations were measured at 20 °C with a Perkin Elmer 241 nc polarimeter (λ=589 Na, path length 1 dm). Some recorded values were within the error limit of the polarimeter and therefore were not possible to determine them. It has been indicated as $[\alpha]_D^{20}$<1º. Analytical UPLC-MS was performed on a binary Acquity UPLC with a Acquity PDA UPLC eLambda 800 nm triple quadrupole mass spectrometer (Xevo TQ-S) using a Acquity UPLC® BEH C18 50 x 2.1 mm, 1.7 µm C18 column. UV detection = 210 – 500 nm, mass spectrometry= ESI+ (scan 100-850 m/z). Flow rate was 0.3 mL/min using a solvent gradient of B 100% over 6 min (total runtime with equilibration back to starting conditions = 2 min) where A = MeOH and B = : 85/15/0.2 MeOH/H2O/AcOH. Purities were measured by UV absorption at 254 nm or TIC and are ≥95% unless otherwise stated. Purity of final compounds was assessed by reversed-phase UHPLC with UV diode array detection; all tested compounds were >95% purity.

4.2.1 Procedure I. *Amine bond formation*

To a 0.35 M solution of starting material in anhydrous DMF were added the amine **3** (1.1 eq.), HATU (1.5 eq.) and DIPEA (3 eq.). The mixture was stirred at room temperature for 20 h. To the mixture was added EtOAc and brine, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with 1 M HCl, saturated solution of $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography.

4.2.2 **Procedure II.** *Ester hydrolysis*

To a 0.2 M solution of starting material in THF/H_2O (1:1) $LiOH \cdot H_2O$ (3 eq.) was added. The mixture was stirred at room temperature until completion of the reaction. The reaction mixture was acidified with 1 M HCl until pH 1 and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure to afford the corresponding compound.

4.2.3 **Procedure III**. *Boc protection*

Et_3N (1.5 eq.) was added to a 0.3 M aqueous solution of starting material, cooled in an ice bath. Then Boc_2O (1.5 eq.) was added dropwise and stirred overnight. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with 1 M HCl and brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was thoroughly washed with hexane for several times.

4.2.4 **Procedure IV**. *SS/SeSe bond cleavage*

SS bond cleavage: To a 0.15 M solution of starting material in wet THF was added tri-*n*-butyl phosphine ($P(^nBu)_3$) (1.05 eq.). The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the solvent was removed under reduced pressure to afford the crude product, which was purified by silica gel column chromatography.

SeSe bond cleavage and Se-alkylation: To a 0.13 M solution of starting material in ethanol was added $NaBH_4$ (2.5 eq) at 0 ºC. The reaction mixture was stirred for 20 min, followed by addition of the respective iodinated compound. The reaction mixture was stirred at room temperature for 16 h. Then, the reaction was quenched with 1 M HCl and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography.

4.2.5 **Procedure V**. *Reduction of methyl ester*

To a 0.2 M solution of starting material in anhydrous THF $LiAlH_4$ (2 eq.) was added at 0 ºC. The reaction mixture was stirred at room temperature for 24 h. Then, the reaction was quenched with 1 M HCl, followed by extraction with DCM. The combined organic phases were dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The solid residue was purified by silica gel column chromatography.

4.2.6 **Procedure VI.** *Iodination*

To a 0.25 M solution of starting material in toluene iodine (1.2 eq.), imidazole (3 eq.) and PPh_3 (1.2 eq.) were added. The mixture was stirred at 90 ºC for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with saturated aqueous solution of $KMnO_4$, water and brine, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The solid residue was purified by silica gel column chromatography.

4.2.7 **Procedure VII.** *S-Alkylation*

To a 0.2 M solution of starting material in DMF, TEA (1.5 eq.) and the corresponding iodinated compound (1.12 eq.) were added. The reaction mixture was stirred at 90 ºC overnight. To the mixture was added EtOAc and brine, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with 1 M HCl, saturated solution of $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude residue was purified by silica gel column chromatography.

4.2.8 **Procedure VIII.** *TBDMS deprotection*

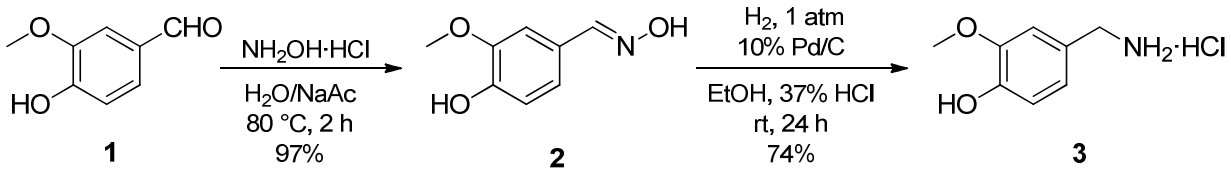
A 0.25 M solution of the starting material in a mixture of AcOH/THF/H_2O was stirred at room temperature until deprotection was complete. The solvent was evaporated under reduced pressure to obtain the reaction crude, which was purified by silica gel column chromatography.

4.2.9 Procedure IX. *Boc deprotection*

To a 0.3 M solution of starting material in DCM TFA (10 eq.) was added. The reaction mixture was stirred for 1 h, followed by removal of the solvent under nitrogen stream and drying *in vacuo* to afford the trifluoroacetate salt of the compound.

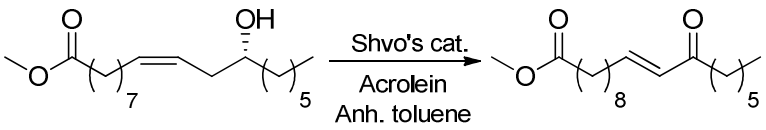
4.2.10 Procedure X. *Base Schiff formation*

To a 0.03 M solution of starting material in MeOH vanillin **1** (1 eq.) was added. The mixture was refluxed for 2 h in presence of small amount of glacial AcOH. After cooling, the reaction mixture was filtered to recover a solid, which was recrystallized from hot MeOH to afford the corresponding compound.



(*E*)-4-Hydroxy-3-methoxybenzaldehyde oxime (2): Hydroxylamine hydrochloride (2.37 g, 34.0 mmol) in H_2O (10 mL) and sodium acetate trihydrate (4.48 g, 32.9 mmol) in H_2O (10 mL) were successively added to a solution of vanillin **1** (5.00 g, 32.9 mmol) in H_2O (30 mL). The reaction mixture was stirred at 80 °C for 2 h. The reaction mixture was extracted with EtOAc, the organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the oxime **2**[1] (5.26 g, 97%) as a white-off solid. mp=118-119 ºC. IR (ATR) ν=3444, 3213, 3008, 2941, 1596, 1513, 1428, 1027, 969 cm^{-1}. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 3.77 (s, 3H, C*H*$_3$O), 6.77 (d, 1H, *J* = 8.1 Hz, H$_{Ar}$), 6.97 (dd, 1H, *J* = 8.1, 2.0 Hz, H$_{Ar}$), 7.16 (d, 1H, *J* = 2.0 Hz, H$_{Ar}$), 7.99 (s, 1H, C*H*=N), 9.33 (s, 1H, O*H*), 10.84 (s, 1H, N-O*H*). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 55.50 (*C*H$_3$O), 109.21 (C$_{Ar}$), 115.49 (C$_{Ar}$), 120.52 (C$_{Ar}$), 124.47 (*C*CHN), 147.85 (*C*OH), 148.01 (*C*CH$_3$O), 148.10 (*C*H=N).

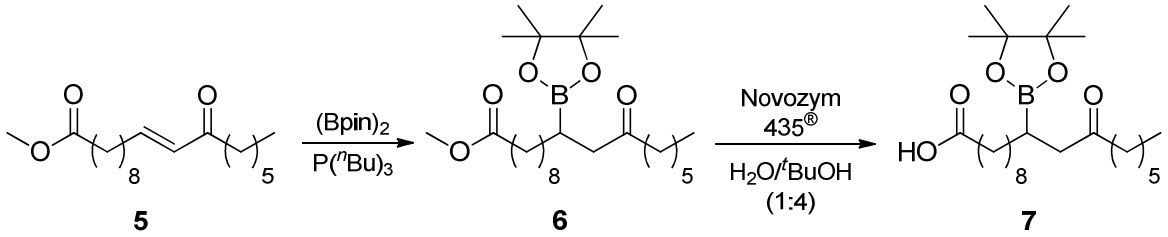
4-Hydroxy-3-methoxybenzylamine hydrochloride (3): A volume of 37% HCl (20 mL, 0.26 mol) and Pd/C (10 wt. % loading) (20% w/w, 1.05 g) were added to a solution of **2** (5.2 g, 0.03 mol) in EtOH (150 mL). The reaction mixture was hydrogenated at 1 atm at room temperature for 24 h. The reaction mixture was filtered over Celite® and the solvent volume was reduced under pressure. The residue was crystallised from EtOAc and filtered to yield the amine hydrochloride salt **3**[2] (4.2 g, 74%) as a white solid. mp=219-222 ºC. IR (ATR) ν=3112, 3024, 2805, 1763, 1377, 1033, 828, 670 cm^{-1}. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 3.77 (s, 3H, CH_3O), 3.83 – 3.90 (m, 2H, CH_2NH_2), 6.79 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 6.85 (dd, 1H, *J* = 8.1, 2.0 Hz, H_{Ar}), 7.18 (d, 1H, *J* = 2.0 Hz, H_{Ar}), 8.40 (br, s, 3H, NH_2, *H*Cl), 9.19 (s, 1H, O*H*). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 42.19 (*C*H_2NH_2), 55.70 (*C*H_3O), 113.45 (C_{Ar}), 115.27 (C_{Ar}), 121.74 (C_{Ar}), 124.64 (*C*CHN), 146.81 (*C*OH), 147.51 (*C*CH_3O).



4 **5**

Methyl 12-oxooctadec-(10*E*)-enoate (5): Shvo's catalyst (9 mg, 8 μmol) and acrolein freshly distilled (390 μL, 4.80 mmol) were added to a solution of methyl ricinoleate **4** (500 mg, 1.60 mmol) in anhydrous toluene (15 mL). The reaction mixture was purged with N_2 and stirred under reflux for 45 min. The solvent was evaporated under reduced pressure and after the purification by silica gel column chromatography (petroleum ether/Et_2O 95:5) the enone **5**[3] (348 mg, 70%) was obtained as a yellowish oil. R_f=0.50 (petroleum ether/Et_2O 9:1). IR (ATR) ν=2927, 2855, 1736, 1709, 1436, 1195, 1169, 1104, 979, 880, 752 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, CH_3), 1.23 – 1.33 (m, 14H, CH_2), 1.38 – 1.48 (m, 2H, CH_2), 1.52 – 1.65 (m, 4H, CH_2), 2.18 (q, 2H, *J* = 6.4 Hz, CH_2), 2.29 (t, 2H, *J* = 6.9 Hz, CH_2), 2.51 (t, 2H, *J* = 6.9 Hz, COCH_2), 3.65 (s, 3H, CH_3O), 6.07 (dt, 1H, *J* = 15.9, 1.5 Hz, CH=C*H*), 6.80 (dt, 1H, *J* = 15.9, 6.9 Hz, C*H*=CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.01 (*C*H_3),

22.48 (CH_2), 24.27 (CH_2), 24.86 (CH_2), 28.04 (CH_2), 28.96 (CH_2), 29.07 (4xCH_2), 31.59 (CH_2), 32.38 (CH_2), 34.02 (CH_2), 40.08 (COCH_2), 51.41 (CH_3O), 130.28 (CH=CH), 147.20 (CH=CH), 174.24 (COO-), 200.99 ($COCH_2$).

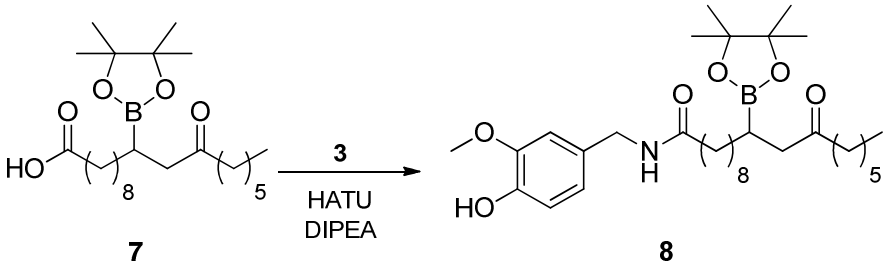


5 **6** **7**

Methyl 12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) octadecanoate (6): Tri-*n*-butylphosphine (26 µL, 0.10 mmol) was added to a solution of anhydrous CuCl (10 mg, 0.10 mmol) in anhydrous DMF (4.5 mL) under argon atmosphere. In another reaction vessel, bis(pinacolato)diboron (283 mg, 1.12 mmol) was added to a solution of methyl 12-oxooctadec-(10*E*)-enoate **5** (290 mg, 0.93 mmol) in anhydrous DMF (4.5 mL) under argon atmosphere. This solution was transferred to the tri-*n*-butylphosphine solution. The reaction mixture was stirred at room temperature for 48 h. The crude was taken up in H_2O and extracted with petroleum ether. The organic solution was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the β-boronketone **6** (190 mg, 46%) as a yellow oil after the purification by silica gel column chromatography (petroleum ether/EtOAc 95:5). R_f=0.49 (petroleum ether/Et_2O 9:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.84 (t, 3H, *J* = 6.9 Hz, CH_3), 1.18 – 1.28 (m, 30H, (CH_3)$_4$, CH_2), 1.34 – 1.39 (m, 1H, C*H*B), 1.49 – 1.60 (m, 4H, CH_2), 2.27 (t, 2H, *J* = 6.9 Hz, CH_2), 2.33 (td, 2H, *J* = 7.4, 3.7 Hz, COCH_2), 2.50 (d, 2H, *J* = 6.8 Hz, CHBCH_2CO), 3.64 (s, 3H, CH_3O).

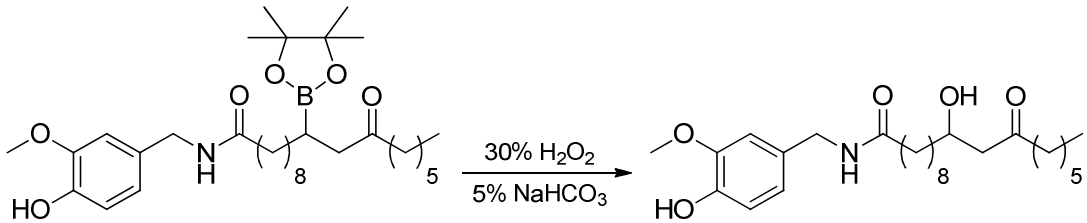
12-Oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) octadecanoic acid (7): Novozym 435® (83 mg, 50% w/w) was added to a solution of the methyl ester **6** (190 mg, 0.43 mmol) in a mixture of H_2O (308 µL) and *tert*-BuOH (922 µL). The reaction mixture was stirred at 45 ºC for 24 h. The mixture was filtered and the solvent was evaporated under reduced pressure to yield the acid **7** (180 mg,

quantitative) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.20 – 1.34 (m, 30H, (C*H*$_3$)$_4$, C*H*$_2$), 1.38 – 1.44 (m, 1H, C*H*B), 1.51 – 1.58 (m, 2H, C*H*$_2$), 1.59 – 1.66 (m, 2H, C*H*$_2$) 2.30 – 2.40 (m, 4H, C*H*$_2$, COC*H*$_2$), 2.53 (d, 2H, *J* = 6.8 Hz, CHBC*H*$_2$CO).



6 **7** **8**

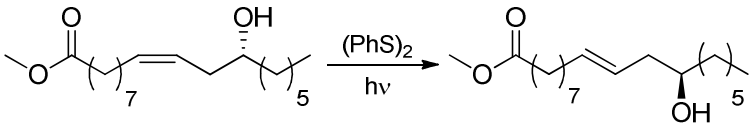
***N*-(4'-Hydroxy-3'-methoxybenzyl)-12-oxo-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) octadecanamide (8):** General procedure I was applied to a solution of the acid **7** (175 mg, 0.41 mmol) dissolved in anhydrous DMF (6 mL), amine hydrochloride salt **3** (69 mg, 0.45 mmol), DIPEA (200 µL, 1.24 mmol), and HATU (235 mg, 0.62 mmol). The amide **8** was obtained (125 mg, 54%) as a brown oil after the purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). R_f=0.55 (petroleum ether/EtOAc 3:7). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.7 Hz, C*H*$_3$), 1.21 – 1.31 (m, 30H, (C*H*$_3$)$_4$, C*H*$_2$), 1.35 – 1.41 (m, 1H, C*H*B), 1.52 – 1.57 (m, 2H, C*H*$_2$), 1.61 – 1.67 (m, 2H, C*H*$_2$), 2.18 (t, 2H, *J* = 6.9 Hz, C*H*$_2$), 2.32 – 2.39 (m, 2H, COC*H*$_2$), 2.52 (d, 2H, *J* = 6.7 Hz, CHBC*H*$_2$CO), 3.88 (s, 3H, C*H*$_3$O), 4.35 (d, 2H, *J* = 5.6 Hz, C*H*$_2$NH), 5.64 – 5.71 (m, 1H, CH$_2$N*H*), 6.82 (ddd, 3H, *J* = 12.5, 9.9, 5.5 Hz, H$_{Ar}$).



7 **8** **9**

***N*-(4'-Hydroxy-3'-methoxybenzyl)-10-hydroxy-12-oxooctadecanamide (9):** A volume of 5% w/v $NaHCO_3$ (2.5 mL, 1.49 mmol) was added to a solution of compound **8** (125 mg, 0.22 mmol) and 2.5

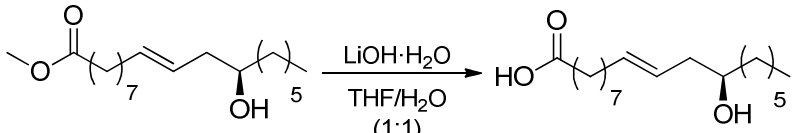
mL of 30% H_2O_2 (0.02 mmol). The reaction mixture was stirred at room temperature for 24 h. Saturated aqueous $Na_2S_2O_4$ (0.25 mL) was added to decompose any remaining peroxide keeping the temperature below 40 °C. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic solution was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to yield the β-hydroxyketone **9** (75 mg, 76%) as a rosaceous solid after the recrystallization from Et_2O. mp=73-75 ºC. IR (ATR) ν=3318, 2912, 2849, 1705, 1638, 1513, 1267, 1240, 1122, 718 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9, Hz, C*H*$_3$), 1.20 – 1.41 (m, 18H, C*H*$_2$), 1.40 – 1.50 (m, 2H, C*H*$_2$), 1.52 – 1.60 (m, 2H, C*H*$_2$), 1.60 – 1.68 (m, 2H, C*H*$_2$), 2.18 (t, 2H, *J* = 6.9 Hz, C*H*$_2$), 2.41 (t, 2H, *J* = 6.9 Hz, COC*H*$_2$), 2.46 – 2.52 (m, 1H, CHC*H*$_{11a}$CO), 2.59 (dd, 1H, *J* = 17.3, 1.8 Hz, CHC*H*$_{11b}$CO), 3.08 (br s, 1H, CHO*H*), 3.87 (s, 3H, C*H*$_3$O), 3.94 – 4.05 (m, 1H, C*H*OH), 4.35 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.69 (br s, 2H, O*H*, CH$_2$N*H*), 6.67 – 6.88 (m, 3H, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (*C*H$_3$), 22.61 (*C*H$_2$), 23.73 (*C*H$_2$), 25.53 (*C*H$_2$), 25.87 (*C*H$_2$), 28.97 (*C*H$_2$), 29.34 (*C*H$_2$), 29.35 (*C*H$_2$), 29.48 (*C*H$_2$), 29.55 (*C*H$_2$), 31.70 (*C*H$_2$), 36.52 (*C*H$_2$), 36.96 (*C*H$_2$), 43.66 (*C*H$_2$NH), 43.84 (CO*C*H$_2$), 49.06 (CH*C*H$_2$CO), 56.08 (*C*H$_3$O), 67.77 (*C*HOH), 110.85 (C$_{Ar}$), 114.53 (C$_{Ar}$), 120.93 (C$_{Ar}$), 130.56 (C$_{Ar}$), 145.25 (C$_{Ar}$), 146.84 (C$_{Ar}$), 172.99 (NH*C*O), 212.84 (*C*OCH$_2$). HR-MS (ESI$^+$): *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{43}NO_5Na$ 472.3033; Found 472.3042.



4 **10**

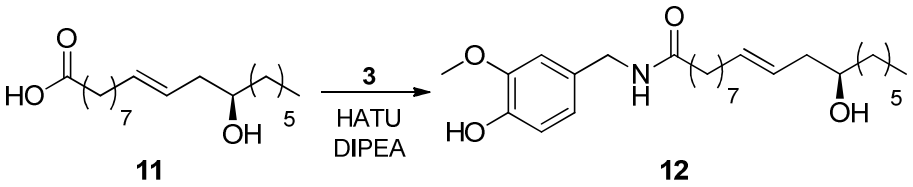
Methyl (12*R*)-hydroxyoctadec-(9*E*)-enoate (10): Diphenyl disulfide (56 mg, 0.26 mmol) was added to a solution of methyl ricinoleate **4** (4 g, 12.8 mmol) in isooctane (120 mL). The reaction mixture was placed in a photochemical reactor and irradiated for 3 h with a Philips HP(L) 400-W medium-pressure mercury lamp. After irradiation the solvent was removed under reduced pressure and the crude reaction mixture was dissolved in hot petroleum ether (185 mL). The filtrate was cooled at -30 ºC and after 48 h

a white solid appeared. This solid was quickly filtered and recovered at -30 ºC to yield the compound **10**[4] (1.49 g, 37%) as a yellowish oil at room temperature. IR (ATR) ν=3431, 2924, 2854, 1740, 1435, 1197, 1171, 969, 860, 724 cm^{-1}. $[\alpha]_D^{20}$=-0.2º (c 2.44, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.23 – 1.39 (m, 16H, C*H*$_2$), 1.39 – 1.48 (m, 3H, C*H*$_2$), 1.56 – 1.71 (m, 2H, C*H*$_2$), 1.97 – 2.09 (m, 3H, C*H*$_2$, H$_{11a}$), 2.18 – 2.26 (m, 1H, H$_{11b}$), 2.29 (t, 2H, *J* = 6.9 Hz, C*H*$_2$), 3.53 – 3.61 (m, 1H, C*H*OH), 3.65 (s, 3H, C*H*$_3$O), 5.47 – 5.56 (m, 1H, CHC*H*), 5.47 – 5.56 (m, 1H, C*H*CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (*C*H$_3$), 22.75 (*C*H$_2$), 25.05 (*C*H$_2$), 25.79 (*C*H$_2$), 29.06 (*C*H$_2$), 29.20 (*C*H$_2$), 29.22 (*C*H$_2$), 29.49 (2x*C*H$_2$), 31.97 (*C*H$_2$), 32.75 (*C*H$_2$), 34.22 (*C*H$_2$), 36.88 (*C*H$_2$), 40.85 (CH*C*H$_2$CHO), 51.57 (*C*H$_3$O), 71.06 (*C*HOH), 126.07 (CH*C*H), 134.69 (*C*HCH), 174.44 (*C*OO-).



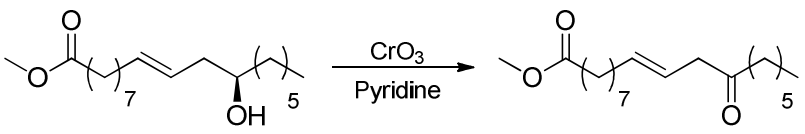
10 **11**

(12*R*)-Hydroxyoctadec-(9*E*)-enoic acid (11): General procedure II was applied to a solution of compound **10** (200 mg, 0.64 mmol) dissolved in THF/H_2O (3 mL, 1:1) and LiOH·H_2O (46 mg, 1.92 mmol) to yield the fatty acid **11**[5] (150 mg, 78%) as a yellowish solid after a recrystallization in hot petroleum ether. mp=49-51 °C. $[\alpha]_D^{20}$=+6.6º (c 1, EtOH). IR (ATR) ν =3321, 3221, 3040, 2955, 2916, 2848, 1690, 1466, 1072, 959, 720, 682 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.22 – 1.40 (m, 16H, C*H*$_2$), 1.40 – 1.50 (m, 4H, C*H*$_2$), 1.58 – 1.68 (m, 2H, C*H*$_2$), 1.97 – 2.11 (m, 3H, C*H*$_2$, H$_{11a}$), 2.18 – 2.28 (m, 1H, H$_{11b}$), 2.33 (t, 2H, *J* = 6.9 Hz, C*H*$_2$), 3.54 – 3.63 (m, 1H, C*H*OH), 5.33 – 5.46 (m, 1H, CHC*H*), 5.45 – 5.58 (m, 1H, *C*HCH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.24 (*C*H$_3$), 22.77 (*C*H$_2$), 24.79 (*C*H$_2$), 25.79 (*C*H$_2$), 29.02 (*C*H$_2$), 29.11 (*C*H$_2$), 29.15 (*C*H$_2$), 29.47 (*C*H$_2$), 29.50 (*C*H$_2$), 31.98 (*C*H$_2$), 32.73 (*C*H$_2$), 34.06 (*C*H$_2$), 36.86 (*C*H$_2$), 40.81 (CH*C*H$_2$CHO), 71.17 (*C*HOH), 126.05 (CH*C*H), 134.74 (*C*HCH), 179.27 (*C*OOH). HR-MS (ESI^+): *m/z*: $[M+Na]^+$ Calcd. for $C_{18}H_{34}O_3Na$ 321.240; Found 321.2411.



11 **12**

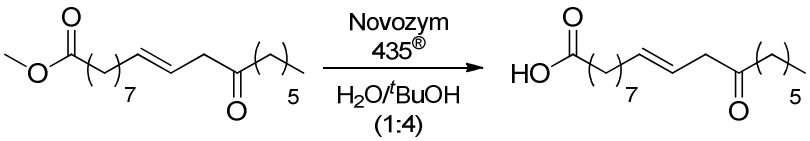
***N*-(4'-Hydroxy-3'-methoxybenzyl)-(12*R*)-hydroxyoctadec-(9*E*)-enamide (12):** General procedure I was applied to a solution of the acid **11** (70 mg, 0.23 mmol) dissolved in anhydrous DMF (3.3 mL), amine hydrochloride salt **3** (53 mg, 0.28 mmol), DIPEA (122 µL, 0.70 mmol), and HATU (133 mg, 0.35 mmol). The compound **12** was afforded (35 mg, 34%) as a white-off solid after the purification by silica gel flash column chromatography (petroleum ether/EtOAc 6:4). $[\alpha]_D^{20}$<+1º (c 0.5, DCM). R_f=0.37 (petroleum ether/EtOAc 6:4). mp=73-75 ºC. IR (ATR) ν=3295, 2920, 2849, 1631, 1515, 1463, 1270, 1030, 959 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.23 – 1.36 (m, 15H, CH_2, H_{13a}), 1.37 – 1.46 (m, 3H, CH_2, H_{13b}), 1.59 – 1.71 (m, 2H, CH_2), 1.96 – 2.09 (m, 3H, CH_2, H_{11a}), 2.14 – 2.27 (m, 3H, CH_2, H_{11b}), 3.53 – 3.61 (m, 1H, C*H*OH), 3.86 (s, 3H, CH_3O), 4.34 (d, *J* = 5.7 Hz, 2H, CH_2NH), 5.35 – 5.44 (m, 1H, CHC*H*), 5.47 – 5.56 (m, 1H, *C*HCH), 5.72 (br s, 2H, CH_2N*H*, O*H*), 6.79 (ddd, 3H, *J* = 16.1, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.23 (*C*H_3), 22.75 (*C*H_2), 25.79 (*C*H_2), 25.86 (*C*H_2), 29.06 (*C*H_2), 29.26 (*C*H_2), 29.35 (*C*H_2), 29.46 (*C*H_2), 29.49 (*C*H_2), 31.97 (*C*H_2), 32.73 (*C*H_2), 36.91 (*C*H_2), 36.96 (*C*H_2), 40.82 (CH*C*H_2CHO), 43.65 (*C*H_2NH), 56.07 (*C*H_3O), 71.07 (*C*HOH), 110.86 (C_{Ar}), 114.53 (C_{Ar}), 120.91 (C_{Ar}), 126.12 (CH*C*H), 130.54 (C_{Ar}), 134.68 (*C*HCH), 145.26 (C_{Ar}), 146.84 (C_{Ar}), 173.01 (NH*C*O). HR-MS (ESI^+): m/z: $[M+H]^+$ Calcd. for $C_{26}H_{44}NO_4$ 434.3265; Found 434.3293.



10 **13**

Methyl 12-oxooctadec-(9*E*)-enoate (13): CrO_3 (960 mg, 9.6 mmol) and pyridine (1.5 mL, 19.2 mmol) were added to a solution of compound **10** (500 mg, 1.6 mmol) in DCM (6 mL). The mixture was

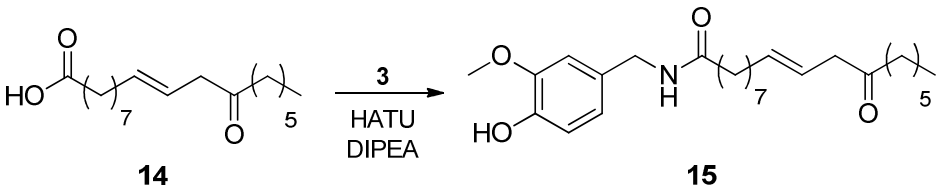
vigorously stirred at room temperature for 2 h. The reaction mixture was filtered over Celite® and washed with 1 M HCl. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to yield the ketone **13**[6] (246 g, 49%) as a yellowish oil after the purification by silica gel column chromatography (petroleum ether/Et_2O 98:2). R_f=0.48 (petroleum ether/Et2O 9:1). IR (ATR) ν=2925, 2854, 1738, 1715, 1435, 1362, 1195, 1170, 968, 725 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.5 Hz, C*H*$_3$), 1.23 – 1.38 (m, 14H, C*H*$_2$), 1.51 – 1.64 (m, 4H, C*H*$_2$), 1.96 – 2.08 (m, 2H, C*H*$_2$), 2.29 (t, *J* = 6.9 Hz, 2H, C*H*$_2$), 2.41 (t, 2H, *J* = 6.9 Hz, COC*H*$_2$), 3.07 (d, 2H, *J* = 5.2 Hz, C*H*$_2$CO), 3.66 (s, 3H, C*H*$_3$O), 5.45 – 5.56 (m, 2H, C*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.16 (*C*H$_3$), 22.63 (*C*H$_2$), 23.84 (*C*H$_2$), 25.06 (*C*H$_2$), 29.03 (*C*H$_2$), 29.06 (*C*H$_2$), 29.21 (2x*C*H$_2$), 29.27 (*C*H$_2$), 31.73 (*C*H$_2$), 32.67 (*C*H$_2$), 34.22(*C*H$_2$), 42.31 (CO*C*H$_2$), 46.95 (*C*H$_2$CO), 51.57 (*C*H$_3$O), 122.13 (CH*C*H), 135.16 (*C*HCH), 174.42 (*C*OO-), 209.95 (*C*OCH$_2$).



13 **14**

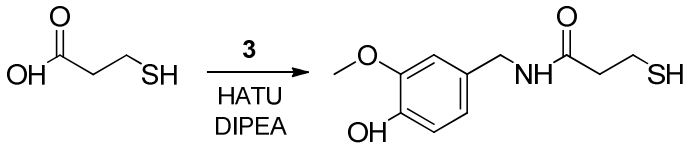
12-Oxooctadec-(9*E*)-enoic acid (14): Novozym 435® (20 mg, 50% w/w) was added to a solution of the methyl ester **13** (20 mg, 0.06 mmol) in a mixture of H_2O (31 µL) and *tert*-BuOH (138 µL). The reaction mixture was stirred at 45 ºC for 24 h. The mixture was filtered and the solvent was evaporated under reduced pressure to yield the acid **14** (17 mg, 89%) as a white solid. mp=71-73 °C. IR (ATR) ν=3121, 2954, 2918, 2848, 1701, 1263, 1082, 962, 720, 689 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.26 – 1.36 (m, 14H, C*H*$_2$), 1.50 – 1.58 (m, 2H, C*H*$_2$), 1.58 – 1.66 (m, 2H, C*H*$_2$), 1.98 – 2.08 (m, 2H, C*H*$_2$), 2.34 (t, 2H, *J* = 6.9 Hz, C*H*$_2$), 2.41 (t, 2H, *J* = 6.9 Hz, COC*H*$_2$), 3.08 (d, 2H, *J* = 5.2 Hz, C*H*$_2$CO), 5.44 – 5.57 (m, 2H, C*HCH*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (*C*H$_3$), 22.63 (*C*H$_2$), 23.85 (*C*H$_2$), 24.79 (*C*H$_2$), 29.03 (2x*C*H$_2$), 29.12 (*C*H$_2$), 29.18 (*C*H$_2$), 29.26 (*C*H$_2$), 31.73 (*C*H$_2$), 32.66 (*C*H$_2$), 34.09 (*C*H$_2$), 42.32 (CO*C*H$_2$), 46.95 (*C*H$_2$CO), 122.13 (CH*C*H), 135.17

(*C*HCH), 179.59 (*C*OOH), 210.13 (CO*C*H_2). HR-MS (ESI$^+$): *m/z*: $[M+Na]^+$ Calcd. for $C_{18}H_{32}O_3Na$ 319.2244; Found 319.2267.



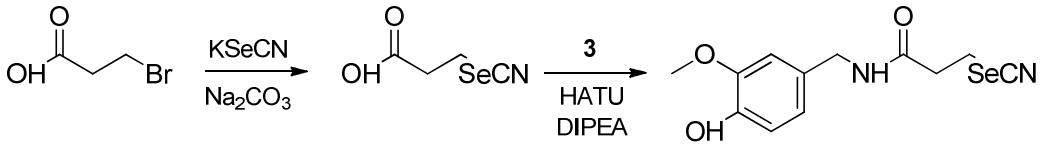
14 **15**

***N*-(4'-Hydroxy-3'-methoxybenzyl)-12-oxooctadec-(9*E*)-enamide (15):** General procedure I was applied to a solution of the acid **14** (210 mg, 0.71 mmol) dissolved in anhydrous DMF (10 mL), amine hydrochloride salt **3** (148 mg, 0.78 mmol), DIPEA (400 µL, 2.1 mmol), and HATU (404 mg, 1.06 mmol). The compound **15** was obtained (52 mg, 17%) as a white-off solid after the purification by silica gel flash column chromatography (petroleum ether/EtOAc 7:3). mp=71-73 ºC. R_f=0.36 (petroleum ether/EtOAc 7:3). IR (ATR) ν=3393, 3312, 2917, 2850, 1703, 1636, 1554, 1509, 1242, 1125, 967, 705 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.22 – 1.38 (m, 14H, C*H*$_2$), 1.50 – 1.58 (m, 2H, C*H*$_2$), 1.59 – 1.69 (m, 2H, C*H*$_2$), 1.97– 2.04 (m, 2H, C*H*$_2$), 2.19 (t, 2H, *J* = 7.4 Hz, C*H*$_2$), 2.40 (t, 2H, *J* = 7.4 Hz, COC*H*$_2$), 3.08 (d, 2H, *J* = 5.2 Hz, C*H*$_2$CO), 3.87 (s, 3H, C*H*$_3$O), 4.35 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.47 – 5.52 (m, 2H, C*HCH*), 5.67 (s, 1H, CH$_2$N*H*), 5.73 (br s, 1H, O*H*), 6.73 – 6.87 (6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.17 (*C*H$_3$), 22.63 (*C*H$_2$), 23.86 (*C*H$_2$), 25.86 (*C*H$_2$), 29.03 (*C*H$_2$), 29.05 (*C*H$_2$), 29.23 (*C*H$_2$), 29.26 (*C*H$_2$), 29.36 (*C*H$_2$), 31.73 (*C*H$_2$), 32.64 (*C*H$_2$), 36.96 (*C*H$_2$), 42.37 (CO*C*H$_2$), 43.66 (*C*H$_2$NH), 46.89 (*C*H$_2$CO), 56.07 (*C*H$_3$O), 110.83 (C$_{Ar}$), 114.50 (C$_{Ar}$), 120.92 (C$_{Ar}$), 122.12 (CH*C*H), 130.56 (C$_{Ar}$), 135.11 (*C*HCH), 145.25 (C$_{Ar}$), 146.82 (C$_{Ar}$), 172.99 (NH*C*O), 210.08 (*C*OCH$_2$). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{26}H_{42}NO_4$ 432.3108; Found 432.3137.



16 **17**

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-mercaptopropanamide (17):** General procedure I was applied to a solution of mercaptopropionic acid (1.2 mL, 12.68 mmol) dissolved in anhydrous DMF (30 mL), amine hydrochloride salt **3** (2.65 g, 13.95 mmol), DIPEA (6.63 mL, 38.04 mmol), and HATU (7.23 g, 19.02 mmol). Compound **17** was obtained after silica gel column chromatography (petroleum ether/EtOAc 5:5) as sticky oil (2.14 g, 74%). R_f=0.60 (petroleum ether/EtOAc 4:6). IR (ATR) ν= 3425, 2922, 2853, 1515, 836 cm^{-1}. ^1H NMR (400 MHz, $(CH_3)_2CO$) δ = 1.86 (t, 1H, *J* = 8.2 Hz, S*H*), 2.54 (t, 2H, *J* = 6.7 Hz, CH_2), 2.70 – 2.82 (m, 2H, CH_2SH), 3.80 (s, 3H, CH_3O), 4.31 (d, 2H, *J* = 5.9 Hz, CH_2NH), 6.74 (d, 2H , *J* = 1.0 Hz, H_{Ar}, OH), 6.92 (s, 1H, H_{Ar}), 7.48 (s, 2H, $H_{Ar,}$ CH_2NH). ^{13}C NMR (101 MHz, $(CH_3)_2CO$) δ = 20.10 (*C*H_2SH), 39.71 (*C*H_2), 42.47 (*C*H_2NH), 55.33 (*C*H_3O), 111.25 (C_{Ar}), 114.66 (C_{Ar}), 120.16 (C_{Ar}), 130.83 (C_{Ar}), 145.61 (C_{Ar}), 147.36 (C_{Ar}), 170.16 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{11}H_{15}NO_3S$: 242.0845; Found 242.0861.

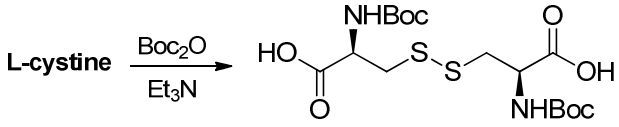


18 **19** **20**

3-Selenocyanatopropanoic acid (19): To a solution of 3-bromopropionic acid **18** (1.5 g, 9.8 mmol) in water (3 mL) was added Na_2CO_3 until pH 7. A volume of 14 mL of 10% KSeCN (1.41 g, 9.8 mmol, 1 eq.) aqueous solution was added. The mixture stirred at room temperature for 2 days. After removing partially the solvent under reduced pressure, the crude was dissolved in Et_2O and washed with 1 M HCl, water and brine. The organic solution was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to yield the 3-selenocyanatopropanoic acid **19**[7]as a yellow oil (1.39 g,

80%) which was used in the next step without further purification. IR (ATR) ν=3024, 2649, 2152, 1703, 1401 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 3.07 (t, 2H, *J* = 6.4 Hz, C*H*$_2$SeCN), 3.24 (dd, 2H, *J* = 6.4 Hz, C*H*$_2$CH$_2$SeCN), 9.52 (br s, 1H, COO*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 22.89 (*C*H$_2$SeCN), 34.90 (*C*H$_2$CH$_2$SeCN), 101.68 (Se*C*N), 176.86 (*C*OOH).

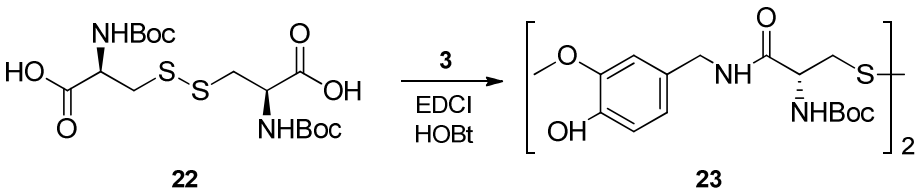
***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-selenocyanatopropanamide (20):** General procedure I was applied to a solution of compound **19** (1.3 g, 7.30 mmol), amine hydrochloride salt **3** (1.52 g, 8.03 mmol), DIPEA (3.82 mL, 21.9 mmol) and HATU (4.16 g, 10.95 mmol) in anhydrous DMF (20 mL). Compound **20** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a white sticky solid (2.14 g, 60%). R_f=0.65 (petroleum ether/EtOAc 4:6). IR (ATR) ν=3315, 2924, 2853, 2148, 1638, 1235 cm^{-1}. ^1H NMR (400 MHz, $(CH_3)_2CO$) δ = 2.94 (t, 2H, *J* = 6.4 Hz, COC*H*$_2$), 3.34 (t, 2H, *J* = 6.4 Hz, C*H*$_2$SeCN), 3.81 (s, 3H, C*H*$_3$), 4.30 (d, 2H, *J* = 5.8 Hz, C*H*$_2$NH), 6.75 (s, 2H, H$_{Ar}$), 6.91 (s, 1H, H$_{Ar}$), 7.48 (s, 1H, O*H*), 7.72 (s, 1H, CH$_2$N*H*). ^{13}C NMR (101 MHz, $(CH_3)_2CO$) δ = 24.79 (*C*H$_2$SeCN), 34.84 (C*H*$_2$CH$_2$SeCN), 42.73 (*C*H$_2$NH), 55.33 (*C*H$_3$O), 104.64 (Se*C*N), 111.35 (C$_{Ar}$), 114.72 (C$_{Ar}$), 120.32 (C$_{Ar}$), 130.19 (C$_{Ar}$), 145.79 (C$_{Ar}$), 147.38 (C$_{Ar}$), 170.92 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{12}H_{15}N_2O_3Se$: 315.0248; Found 315.0242.



21 **22**

***N*,*N*-Di-Boc-L-cystine (22):** General procedure III was applied to L-cystine **21** (10 g, 41.67 mmol), Boc_2O (27.25 g, 124.85 mmol) and Et_3N (17.5 mL, 125.38 mmol) in water (150 mL) to yield compound **22**[8] as a white solid, which was thoroughly washed with petroleum ether for several times (17.56 g, 96%). mp: 145-146 ºC. IR (ATR) ν=3366, 2985, 2936, 1682, 1511, 1163, 1052, 868 cm^{-1}. ^1H NMR (400 MHz, $(CD_3)_2SO$) δ = 1.37 (s, 18H, Boc), 2.87 (dd, 2H, *J* = 13.5, 10.1 Hz, CHC*H*$_2$), 3.09 (dd, 2H, *J* = 13.5, 4.4 Hz, CHC*H*$_2$), 4.16 (td, 2H, *J* = 10.1, 4.4 Hz, C*H*CH$_2$), 7.18 (d, 2H, *J* = 8.4 Hz,

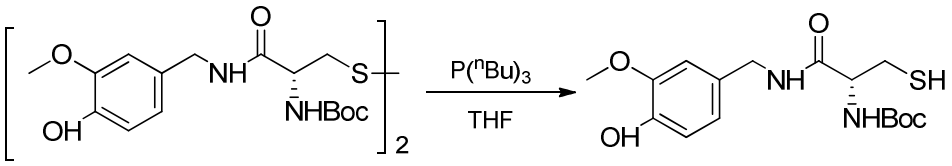
N*H*), 12.79 (s, 2H, COO*H*). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 28.60 (C(CH_3)$_3$), 52.96 (*C*HCH$_2$), 78.70 (*C*(CH$_3$)$_3$), 155.79 (NH*C*O$_2$), 172.82 (*C*OOH).



22 **23**

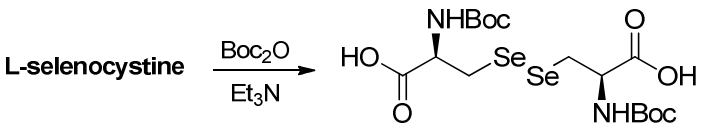
Di-[(2*R*)-*N*-Boc-amino-1-((4'-hydroxy-3'-methoxybenzyl)amino)-1-oxoprop-3-yl]-disulfane (23):

To a solution of compound **22** (5 g, 11.35 mmol) in anhydrous DMF (50 mL) were added HOBt (4.6 g, 34.05 mmol), Et$_3$N (4.74 mL, 34.05 mmol) and the amine hydrochloride salt **3** (5.16 g, 27.24 mmol). The mixture was stirred at 0 ºC during 30 min. EDCI (6.52 g, 34 mmol) was added and the mixture stirred at room temperature during 20 h. To the mixture was added EtOAc and brine, and the aqueous phase was extracted with EtOAc. The combined organic solutions were washed with 1 M HCl, saturated NaHCO$_3$ and brine. The organic solution was dried over anhydrous Na$_2$SO$_4$, filtered and the solvent was evaporated under reduced pressure. Compound **23** was afforded after silica gel column chromatography (PE/EtOAc 1:9) as a white solid (7.58 g, 94%). R_f=0.24 (petroleum ether/EtOAc 1:9). mp: 167-170 ºC. $[\alpha]_D^{20}$= -67.42 (c 0.75, MeOH). IR (ATR) ν = 3330, 2975, 2935, 1658, 1511, 1272, 1033 cm^{-1}. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 1.36 (s, 18H, C(C*H*$_3$)$_3$), 2.86 (dd, 2H, *J* = 13.0, 9.9 Hz, CHC*H*$_2$), 3.07 (dd, 2H, *J* = 13.0, 4.8 Hz, CHC*H*$_2$), 3.72 (s, 6H, C*H*$_3$O), 4.02 – 4.32 (m, 6H, C*H*CH$_2$, C*H*$_2$NH), 6.55 – 6.72 (m, 4H, H$_{Ar}$, N*H*Boc), 6.79 (s, 2H, H$_{Ar}$), 7.06 (d, 2H, *J* = 8.4 Hz, H$_{Ar}$), 8.31 (t, 2H, *J* = 5.4 Hz, CH$_2$N*H*), 8.78 (br s, 2H, O*H*). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 28.59 (C(*C*H$_3$)$_3$), 40.59 (CH*C*H$_2$), 42.40 (*C*H$_2$NH), 54.17 (*C*HCH$_2$), 55.92 (*C*H$_3$O), 78.73 (*C*(CH$_3$)$_3$), 111.82 (C$_{Ar}$), 115.53 (C$_{Ar}$), 119.88 (C$_{Ar}$), 130.37 (C$_{Ar}$), 145.76 (C$_{Ar}$), 147.85 (C$_{Ar}$), 155.70 (NH*C*O$_2$), 170.60 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{32}H_{47}N_4O_{10}S_2$: 711.2734; Found 711.2793.



23 **24**

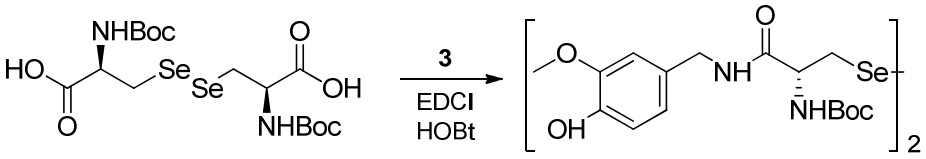
***N*-(4'-Hydroxy-3'-methoxy)benzyl-(2*R*)-(Boc-amino)-3-mercaptopropanamide (24):** General procedure IV (SS bond cleavage) was applied to compound **23** (7 g, 9.86 mmol) dissolved in THF (60 mL), P(^{n}Bu)$_3$ (2.55 mL, 10.35 mmol) in presence of water (1.3 mL). Compound **24** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a white solid (5.11 g, 73%). R_f=0.42 (petroleum ether/EtOAc 4:6). mp: 108-110 ºC. $[\alpha]_D^{20}$= -15.65 (c 1.6, MeOH). IR (ATR) ν = 3456, 3327, 2989, 2934, 2847, 1678, 1513, 1240 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.41 (s, 9H, C(C*H*$_3$)$_3$), 1.54 (t, 1H, *J* = 10.7 Hz, S*H*), 2.74 (ddd, 1H, *J* = 13.8, 10.2, 6.1 Hz, CHC*H*$_2$), 3.09 (ddd, 1H, *J* = 13.6, 7.6, 4.6 Hz, CHC*H*$_2$), 3.84 (s, 3H, C*H*$_3$O), 4.25 – 4.44 (m, 3H, C*H*CH$_2$, C*H*$_2$NH), 5.48 (d, 1H , *J* = 7.8 Hz, CH$_2$N*H*), 5.81 (br s, 1H, OH), 6.67 – 6.89 (m, 4H, H$_{Ar}$, N*H*Boc). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 26.96 (CH*C*H$_2$), 28.23 (C(*C*H$_3$)$_3$), 43.47 (*C*H$_2$NH), 55.67 (*C*HCH$_2$), 55.93 (*C*H$_3$O), 80.69 (*C*(CH$_3$)$_3$), 110.47 (C$_{Ar}$), 114.44 (C$_{Ar}$), 120.58 (C$_{Ar}$), 129.66 (C$_{Ar}$), 145.12 (C$_{Ar}$), 146.74 (C$_{Ar}$), 155.46 (NH*C*O$_2$), 169.88 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{16}H_{24}N_2O_5SNa$: 379.1298; Found 379.1326.



25 **26**

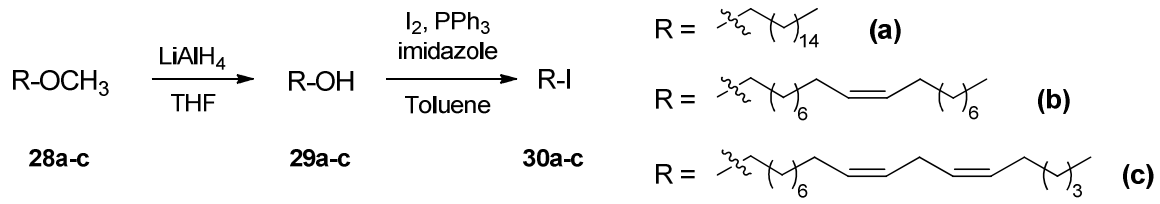
***N*,*N*-Di-Boc-L-selenocystine (26):** General procedure III was applied to L-selenocystine **25** (1.5 g, 4.49 mmol), Boc_2O (3.24 g, 13.48 mmol) and Et_3N (1.88 mL, 13.48 mmol) in water (22 mL) to yield compound **26**[9] as a yellow solid (1.55 g, 65%), which was used in the next step without further purification. mp: 145-147 ºC. $[\alpha]_D^{20}$= -75.63 (c 1.5, DCM). IR (ATR) ν = 3364, 2979, 2557, 1698, 1662, 1506 cm^{-1}. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ = 1.37 (s, 18H, C(C*H*$_3$)$_3$), 3.10 (dd, 2H, *J* = 11.9, 10.2 Hz,

CHCH_2), 3.28 (dd, 2H, J = 11.9, 4.7 Hz, CHCH_2), 4.06 – 4.21 (m, 2H, CHCH$_2$), 7.17 (d, 2H, J = 8.3 Hz, NH), 12.79 (s, 2H, COOH). ^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ = 28.61 (C(CH_3)$_3$), 31.38 (CHCH_2), 54.68 (CHCH$_2$), 78.71 (C(CH$_3$)$_3$), 155.71 (NHCO_2), 172.91 (COOH).



26 **27**

Di-[(2*R*)-*N*-Boc-amino-1-((4'-hydroxy-3'-methoxybenzyl)amino)-1-oxoprop-3-yl]-diseleno (27): To a solution of compound **26** (1.5 g, 2.80 mmol) in anhydrous DMF (14 mL) were added HOBt (1.14 g, 8.4 mmol), Et_3N (1.18 mL, 8.4 mmol),) and the amine hydrochloride salt **3** (1.27 g, 6.72 mmol). The mixture was stirred at 0 ºC during 30 min. EDCI (1.61 g, 8.4 mmol) was added and the mixture stirred at room temperature during 20 h. To the mixture was added EtOAc and brine, and the aqueous phase was extracted. The combined organic layers were washed with 1 M HCl, saturated $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. Compound **27** was afforded after silica gel column chromatography (petroleum ether/EtOAc 1:9) as a white solid (1.98 g, 88%). R_f=0.26 (petroleum ether/EtOAc 5:5). mp: 93-95 ºC. $[\alpha]_D^{20}$= 42.94 (c 0.7, DCM). IR (ATR) ν = 3314, 2975, 2932, 1654, 1513, 1157 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.26 (s, 18H, C(CH_3)$_3$), 3.12 – 3.30 (m, 4H, CHCH_2), 3.83 (s, 6H, CH_3O), 4.25 (dd, 2H, J = 14.7, 5.4 Hz, CH_2NH), 4.48 (dd, 2H, J = 14.7, 6.5 Hz, CH_2NH), 4.75 – 4.94 (m, 2H, CHCH$_2$), 5.58 (d, 2H, J = 9.7 Hz, NHBoc), 5.63 (s, 2H, OH), 6.77 (ddd, 6H, J = 12.5, 9.9, 5.0, H$_{Ar}$), 8.06 (t, 2H, J = 5.6 Hz, CH$_2$NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 28.15 (C(CH_3)$_3$), 37.43 (CHCH_2), 43.28 (CH$_2$NH), 55.24 (CHCH$_2$), 55.86 (CH$_3$O), 78.98 (C(CH$_3$)$_3$), 110.44 (C$_{Ar}$), 114.24 (C$_{Ar}$), 120.77 (C$_{Ar}$), 130.03 (C$_{Ar}$), 145.00 (C$_{Ar}$), 146.58 (C$_{Ar}$), 155.65 (NHCO_2), 170.53 (NHCO). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{32}H_{46}N_4O_{10}Se_2$: 807.1623; Found 807.1621.



1-Hexadecanol (29a): General procedure V was applied to methyl palmitate **28a** (1 g, 3.69 mmol), $LiAlH_4$ (280 mg, 7.38 mmol) in anhydrous THF (20 mL). Compound **29a**[10] was afforded after silica gel column chromatography (petroleum ether/Et_2O 9:1) as a white solid (875 mg, 98%). R_f=0.88 (petroleum ether/Et_2O 9:1). mp: 50-52 ºC. IR (ATR) ν = 3320, 3226, 2915, 2919, 2847, 1462 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, CH_3), 1.15 – 1.41 (m, 24H, CH_2), 1.45 – 1.64 (m, 4H, CH_2, $HOCH_2CH_2$), 3.62 (t, 2H, *J* = 6.9 Hz, $HOCH_2CH_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.08 (CH_3), 22.67 (CH_2), 25.74 (CH_2), 29.35 (CH_2), 29.43 (CH_2), 29.60 (CH_2), 29.61 (CH_2), 29.65 (2xCH_2), 29.67 (CH_2), 29.68 (3xCH_2), 31.91 (CH_2), 32.78 ($HOCH_2CH_2$), 62.99 ($HOCH_2CH_2$).

(9*Z*)-Octadecen-1-ol (29b): General procedure V was applied to methyl oleate **28b** (2.5 g, 8.43 mmol), $LiAlH_4$ (640 mg, 16.86 mmol) in anhydrous THF (50 mL). Compound **29b**[11] was afforded after silica gel column chromatography (petroleum ether/Et_2O 9:1) as a brown oil (2.19 g, 97%). R_f=0.88 (petroleum ether/Et_2O 9:1). IR (ATR) ν = 3320, 2921, 2852, 1463, 1055 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, CH_3), 1.16 – 1.41 (m, 22H, CH_2), 1.47 – 1.62 (m, 2H, $HOCH_2CH_2$), 1.73 (s, 1H, O*H*), 2.00 (q, 4H, *J* = 6.4 Hz, CH_2CH, $CHCH_2$), 3.61 (t, 2H, *J* = 6.9 Hz, $HOCH_2CH_2$), 5.25 – 5.47 (m, 2H, C*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.07 (CH_3), 22.65 (CH_2), 25.73 (CH_2), 27.16 (CH_2CH), 27.18 ($CHCH_2$), 29.22 (CH_2), 29.30 (2xCH_2), 29.40 (CH_2), 29.49 (CH_2), 29.50 (CH_2), 29.72 (CH_2), 29.74 (CH_2), 31.88 (CH_2), 32.75 ($HOCH_2CH_2$), 62.93 ($HOCH_2CH_2$), 129.76 (CH=CH), 129.90 (CH=CH).

(9*Z*,12*Z*)-Octadecadien-1-ol (29c): General procedure V was applied to methyl linoleate **28b** (1 g, 3.39 mmol), $LiAlH_4$ (257 mg, 6.79 mmol) in anhydrous THF (30 mL). Compound **29c**[12] was afforded

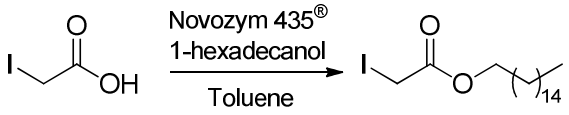
after silica gel column chromatography (petroleum ether/Et_2O 9:1) as a clearless oil (885 mg, 98%). R_f=0.88 (petroleum ether/Et_2O 9:1). IR (ATR) ν = 3373, 2926, 2855, 1719, 1463 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, CH_3), 1.19 – 1.48 (m, 16H, CH_2), 1.51 – 1.61 (m, 2H, HOCH$_2$CH_2), 2.05 (q, 4H, *J* = 6.4 Hz, CH_2CH, CHCH_2), 2.77 (t, 2H, *J* = 6.9 Hz, CHCH_2CH), 3.59 – 3.67 (m, 2H, HOCH_2CH$_2$), 5.14 – 5.52 (m, 4H, 2xC*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.04 (*C*H$_3$), 22.55 (*C*H$_2$), 25.61 (CH*C*H$_2$CH), 25.71 (*C*H$_2$), 27.18 (C*H*$_2$CH), 27.20 (CHC*H*$_2$), 29.22 (*C*H$_2$), 29.33 (*C*H$_2$), 29.38 (*C*H$_2$), 29.48 (*C*H$_2$), 29.63 (*C*H$_2$), 31.51 (*C*H$_2$), 32.78 (HOCH$_2$*C*H$_2$), 63.03 (HO*C*H$_2$CH$_2$), 127.89 (CH=CH), 127.97 (CH=CH), 130.08 (CH=CH), 130.08 (CH=CH).

1-Iodohexadecane (30a): General procedure VI was applied to compound **29a** (1 g, 4.12 mmol), iodine (1.25 g, 4.95 mmol), PPh_3 (1.3 g, 4.95 mmol) and imidazole (0.85 g, 12.36 mmol) in toluene (15 mL). Compound **30a**[13] was afforded after silica gel column chromatography (petroleum ether) as a yellow oil (1.08 g, 75%). R_f=0.1 (petroleum ether). IR (ATR) ν = 2920, 2851, 1464, 1376, 1171, 719 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.26 (s, 24H, CH_2), 1.34 – 1.41 (m, 2H, ICH$_2$CH$_2$CH_2), 1.75 – 1.87 (m, 2H, ICH$_2$CH_2), 3.18 (t, 2H, *J* = 6.9 Hz, ICH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 7.21 (I*C*H$_2$), 14.11 (*C*H$_3$), 22.69 (*C*H$_2$), 28.55 (*C*H$_2$), 29.36 (*C*H$_2$), 29.42 (*C*H$_2$), 29.55 (*C*H$_2$), 29.61 (*C*H$_2$), 29.65 (2x*C*H$_2$), 29.68 (2x*C*H$_2$), 29.69 (*C*H$_2$), 30.51 (*C*H$_2$), 31.92 (*C*H$_2$), 33.58 (ICH$_2$*C*H$_2$).

1-Iodo-(9*Z*)-octadecene (30b): General procedure VI was applied to compound **29b** (2 g, 7.45 mmol), iodine (2.27 g, 8.94 mmol), PPh_3 (2.34 g, 8.94 mmol) and imidazole (1.52 g, 22.35 mmol) in toluene (30 mL). Compound **30b**[14] was afforded after silica gel column chromatography (petroleum ether/Et_2O 9:1) as a yellow oil (2.42 g, 86%). R_f=0.1 (petroleum ether/Et_2O 9:1). IR (ATR) ν = 2921, 2852, 1462, 1181 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.16 – 1.48 (m, 22H, CH_2), 1.72 – 1.91 (m, 2H, ICH$_2$CH_2), 2.01 (q, 4H, *J* = 6.4 Hz, CH_2CH, CHCH_2), 3.18 (t, 2H, *J* = 6.9 Hz, ICH_2), 5.21 – 5.48 (m, 2H, C*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 7.24 (I*C*H$_2$), 14.10 (*C*H$_3$), 22.67

(CH_2), 27.15 (CH_2CH), 27.21 ($CHCH_2$), 28.50 (CH_2), 29.16 (CH_2), 29.29 (CH_2), 29.31 (CH_2), 29.51 (CH_2), 29.68 (CH_2), 29.75 (CH_2), 30.48 (CH_2), 31.89 (CH_2), 33.55 (ICH_2CH_2), 129.73 (CH=CH), 129.98 (CH=CH).

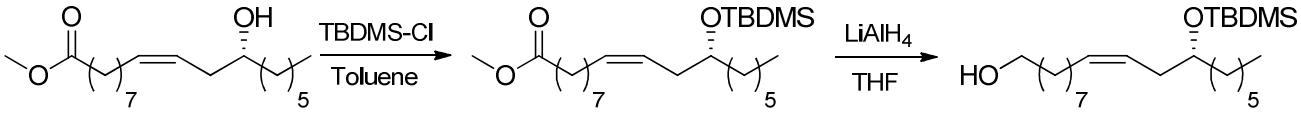
18-Iodo-(6*Z*,9*Z*)-octadecadiene (30c): General procedure VI was applied to compound **29c** (850 mg, 3.18 mmol), iodine (968 mg, 3.81 mmol), PPh_3 (1 g, 3.81 mmol) and imidazole (650 mg, 9.54 mmol) in toluene (15 mL). Compound **30c**[14] was afforded after silica gel column chromatography (petroleum ether) as a yellow oil (1.13 g, 95%). R_f=0.1 (petroleum ether/Et_2O 9:1). IR (ATR) ν = 3439, 2926, 2855, 1707, 1458, 1175 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, CH_3), 1.18 – 1.50 (m, 16H, CH_2), 1.78 – 1.86 (m, 2H, ICH_2CH_2), 2.05 (q, 4H, *J* = 6.4 Hz, CH_2CH, $CHCH_2$), 2.77 (t, 2H, *J* = 6.9 Hz, $CHCH_2CH$), 3.18 (t, 2H, *J* = 6.9 Hz, ICH_2CH_2), 5.25 – 5.50 (m, 2xCH=CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 7.20 (ICH_2), 14.07 (CH_3), 22.57 (CH_2), 25.63 ($CHCH_2CH$), 27.18 (CH_2CH), 27.20 ($CHCH_2$), 28.50 (CH_2), 29.17 (CH_2), 29.30 (CH_2), 29.34 (CH_2), 29.59 (CH_2), 30.48 (CH_2), 31.52 (CH_2), 33.55 (ICH_2CH_2), 127.89 (CH=CH), 128.02 (CH=CH), 130.02 (CH=CH), 130.18 (CH=CH).



31 **32**

Hexadecyl 2-iodoacetate (32): To a solution of iodoacetic acid **31** (500 mg, 2.69 mmol) in toluene (5 mL) were added 1-hexadecanol (978 mg, 4.03 mmol, 1.5 eq.) and Novozym 435® (150 mg). The reaction mixture was stirred at 50 ºC for 2 days. The mixture was filtered off, EtOAc was added and the organic phase was washed with saturated solution of $NaHCO_3$, water and brine. The organic solution was then dried over Na_2SO_4 and the solvent was removed under reduced pressure. Compound **32**[15] was afforded after silica gel column chromatography (petroleum ether/ Et_2O 9:1) as a yellow oil (562 mg, 51%). R_f=0.36 (petroleum ether/Et_2O 9:1). IR (ATR) ν = 2920, 2851, 1733, 1259, 1089 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, CH_3), 1.14 – 1.41 (m, 26H, CH_2), 1.54 – 1.74 (m, 2H,

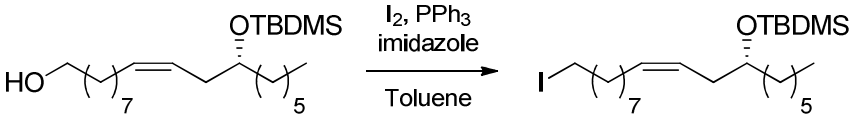
$COOCH_2CH_2$), 3.68 (s, 2H, ICH_2), 4.13 (t, 2H, J = 6.9 Hz, $COOCH_2CH_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 5.19 (ICH_2), 14.27 (CH_3), 22.84 (CH_2), 25.90 (CH_2), 28.50 (CH_2), 29.33 (CH_2), 29.51 (CH_2), 29.63 (CH_2), 29.70 (CH_2), 29.78 (CH_2), 29.80 (CH_2), 29.82 (CH_2), 29.84 (3xCH_2), 32.07 (CH_2), 66.41 ($COOCH_2$), 169.00 ($COOCH_2$).



4 **33** **34**

Methyl (12*R*)-[(*tert*-butyldimethylsilyl)oxy]octadec-(9*Z*)-enoate (33): To a solution of methyl ricinoleate **4** (2 g, 6.4 mmol) in DCM (40 mL) was added DMAP (31 mg, 0.25 mmol) and Et_3N (2.23 mL, 16 mmol). TBDMS-Cl was slowly added (1.5 g, 9.92 mmol). The mixture stirred at room temperature for 2 days. Then, the organic phase was washed with 1 M HCl, water and brine, dried over anhydrous $NaSO_4$ and the solvent was removed under reduced pressure. Compound **33**[16] was afforded after silica gel column chromatography (petroleum ether/Et_2O 9:1) as a colourless oil (2.37 g, 87%). R_f=0.1 (petroleum ether/Et_2O 9:1). $[\alpha]_D^{20}$= 9.98 (c 2.8, DCM). IR (ATR) ν = 2927, 2855, 1742, 1461, 1251 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.04 (s, 6H, $Si(CH_3)_2$), 0.78 – 0.95 (m, 12H, $SiC(CH_3)_3$, CH_3), 1.16 – 1.46 (m, 18H, CH_2), 1.51 – 1.68 (m, 2H, $COCH_2CH_2$), 2.01 (q, 2H, J = 6.4 Hz, CH_2CH), 2.17 (t, 2H, J = 6.9 Hz, $CHCH_2$), 2.29 (t, 2H, J = 6.9 Hz, $COCH_2CH_2$), 3.59 – 3.73 (m, 4H, CH_3O, CH_2CHO), 5.29 – 5.51 (m, 2H, CH=CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.59 ($SiCH_3$), -4.38 ($SiCH_3$), 14.06 (CH_3), 18.11 ($SiC(CH_3)_3$), 22.61 (CH_2), 24.92 ($COCH_2CH_2$), 25.38 (CH_2), 25.89 ($SiC(CH_3)_3$), 27.40 (CH_2CH), 29.10 (CH_2), 29.12 (CH_2), 29.14 (CH_2), 29.45 (CH_2), 29.58 (CH_2), 31.87 (CH_2), 34.06 ($COCH_2CH_2$), 35.23 ($CHCH_2$), 36.84 (CH_2), 51.38 (CH_3O), 72.37 (CH_2CHO), 125.95 (CH=CH), 131.28 (CH=CH), 174.23 (COOH).

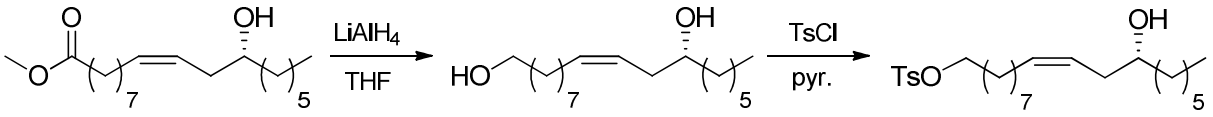
(12*R*)-[(*tert*-Butyldimethylsilyl)oxy]octadec-(9*Z*)-en-1-ol (34): General procedure V was applied to compound **33** (2.20 g, 5.15 mmol) with anhydrous $LiAlH_4$ (390 mg, 10.30 mmol) in dry THF (50 mL). Compound **34**[17] was afforded after silica gel column chromatography (petroleum ether/ Et_2O 9:1) as a brown oil (1.91 g, 93%). R_f=0.86 (petroleum ether/Et_2O 9:1). $[\alpha]_D^{20}$= 13.21 (c 2.6, DCM). IR (ATR) ν = 3330, 2926, 2854, 1461, 1253, 1054 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.04 (s, 6H, $Si(CH_3)_2$), 0.78 – 0.93 (m, 12H, $SiC(CH_3)_3$, CH_3), 1.14 – 1.50 (m, 20H, CH_2), 1.51 – 1.62 (m, 2H, $HOCH_2CH_2$), 2.04 (q, 2H, *J* = 6.4 Hz, CH_2CH), 2.18 (t, 2H, *J* = 6.9 Hz, $CHCH_2$), 3.54 – 3.74 (m, 3H, $HOCH_2CH_2$, CH_2CHO), 5.30 – 5.50 (m, 2H, CH=CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.58 ($SiCH_3$), -4.37 ($SiCH_3$), 14.07 (CH_3), 18.12 ($SiC(CH_3)_3$), 22.61 (CH_2), 25.39 (CH_2), 25.72 (CH_2), 25.90 ($SiC(CH_3)_3$), 27.43 (CH_2CH), 29.26 (CH_2), 29.38 (CH_2), 29.46 (CH_2), 29.49 (CH_2), 29.64 (CH_2), 31.87 (CH_2), 32.77 ($HOCH_2CH_2$) 35.24 ($CHCH_2$), 36.84 (CH_2), 63.00 ($HOCH_2CH_2$), 72.40 (CH_2CHO), 125.91 (CH=CH), 131.36 (CH=CH).



34 **35**

(12*R*)-[(*tert*-Butyldimethylsilyl)oxy]-1-iodo-octadec-(9*Z*)-ene (35): General procedure VI was applied to compound **34** (1.8 g, 4.51 mmol), iodine (1.37 g, 5.42 mmol), PPh_3 (1.42 g, 5.42 mmol) and imidazole (921 mg, 13.53 mmol) in toluene (20 mL). Compound **35** was afforded after silica gel column chromatography (petroleum ether) as a colourless oil (1.86 g, 81%). R_f=0.1 (petroleum ether/Et_2O 9:1). $[\alpha]_D^{20}$= 7.12 (c 0.6, DCM). IR (ATR) ν = 2925, 2854, 1461, 1252, 1063 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.05 (s, 6H, $Si(CH_3)_2$), 0.80 – 0.97 (m, 12H, $SiC(CH_3)_3$, CH_3), 1.15 – 1.49 (m, 20H, CH_2), 1.71 – 1.92 (m, 2H, ICH_2CH_2), 2.02 (q, 2H, *J* = 6.4 Hz, CH_2CH), 2.18 (t, 2H, *J* = 6.9 Hz, $CHCH_2$), 3.18 (t, 2H, *J* = 7.1 Hz, ICH_2CH_2), 3.57 – 3.75 (m, 1H, CH_2CHO), 5.29 – 5.52 (m, 2H, CH=CH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.56 ($SiCH_3$), -4.35 ($SiCH_3$), 7.19 (ICH_2), 14.09 (CH_3),

18.13 (Si*C*(CH$_3$)$_3$), 22.63 (*C*H$_2$), 25.40 (*C*H$_2$), 25.91 (SiC(*C*H$_3$)$_3$), 27.42 (*C*H$_2$CH), 28.50 (*C*H$_2$), 29.21 (*C*H$_2$), 29.31 (*C*H$_2$), 29.47 (*C*H$_2$), 29.61 (*C*H$_2$), 30.48 (*C*H$_2$), 31.89 (*C*H$_2$), 33.55 (ICH$_2$*C*H$_2$), 35.25 (CH*C*H$_2$), 36.86 (*C*H$_2$), 72.38 (CH$_2$*C*HO), 125.97 (CH=*C*H), 131.30 (*C*H=CH).

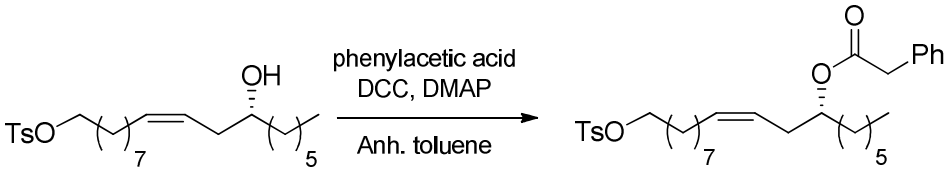


4 **36** **37**

Octadec-(9Z)-ene-1,(12*R*)-diol (36): General procedure V was applied to methyl ricinoleate **4** (2.50 g, 8 mmol) with LiAlH$_4$ (607 mg, 16 mmol) in anhydrous THF (40 mL). Compound **36**[18] was afforded after silica gel column chromatography (petroleum ether/Et$_2$O 9:1) as a colourless oil (1.95 g, 86%). R_f=0.82 (petroleum ether/Et$_2$O 9:1). IR (ATR) ν = 3329, 2923, 2853, 1458, 1053 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.19 – 1.39 (m, 18H, C*H*$_2$), 1.40 – 1.49 (m, 2H, C*H*$_2$), 1.51 – 1.58 (m, 2H, HOCH$_2$C*H*$_2$), 1.59 (br s, 2H, O*H*), 2.04 (q, 2H, *J* = 6.4 Hz, C*H*$_2$CH), 2.20 (t, 2H, *J* = 6.9 Hz, CHC*H*$_2$), 3.62 (m, 3H, HOC*H*$_2$CH$_2$, CH$_2$C*H*O), 5.29 – 5.47 (m, 1H, C*H*=C*H*), 5.47 – 5.66 (m, 1H, C*H*=C*H*). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.06 (*C*H$_3$), 22.59 (*C*H$_2$), 25.68 (*C*H$_2$), 25.69 (*C*H$_2$), 27.36 (*C*H$_2$CH), 29.17 (*C*H$_2$), 29.31 (*C*H$_2$), 29.33 (*C*H$_2$), 29.40 (*C*H$_2$), 29.59 (*C*H$_2$), 31.81 (*C*H$_2$), 32.73 (HOCH$_2$*C*H$_2$), 35.32 (CH*C*H$_2$), 36.81 (*C*H$_2$), 62.96 (HO*C*H$_2$CH$_2$), 71.49 (CH$_2$*C*HO), 125.14 (CH=*C*H), 133.39 (*C*H=CH).

(12'*R*)-Hydroxyoctadec-(9'*Z*)-en-1-yl-4-methylbenzenesulfonate (37): To a solution of compound **36** (1.6 g, 5.62 mmol) in a mixture of DCM and pyridine (6 mL, 5:5) was added TsCl (1.07 g, 5.62 mmol, 1 eq.) in portions and DMAP (27 mg, 0.22 mmol). The mixture was stirred at room temperature for 20 h. The mixture was washed with 1 M HCl and extracted with EtOAc. The organic phase was dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure. Compound **37**[19] was afforded after silica gel column chromatography (petroleum ether/Et$_2$O 7:3) as a yellow oil (1.11 g, 45%). R_f=0.84 (petroleum ether/Et$_2$O 7:3). $[\alpha]_D^{20}$= 4.40 (c 1.4, DCM). IR (ATR) ν = 2924, 2854, 1458, 1358 cm^{-1}. ^{1}H

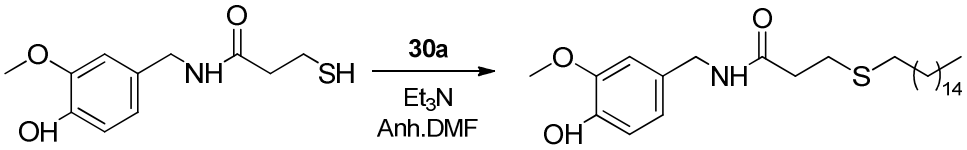
NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.11 – 1.39 (m, 18H, CH_2), 1.39 – 1.54 (m, 2H, CH_2), 1.53 – 1.70 (m, 2H, OCH_2CH_2) 2.03 (q, 2H, *J* = 6.4 Hz, CH_2CH), 2.20 (t, 2H, *J* = 6.9 Hz, CHCH_2), 2.44 (s, 3H, CH_3C) 3.54 – 3.71 (m, 1H, CH_2CHO), 4.01 (t, 2H, *J* = 6.9 Hz, OCH_2CH_2), 5.31 – 5.47(m, 1H, CH=CH), 5.48 – 5.68 (m, 1H, CH=CH), 7.33 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 7.78 (d, 2H, *J* = 7.9 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.06 (CH_3), 21.60 (CH_3C), 22.59 (CH_2), 25.28 (CH_2), 25.69 (CH_2), 27.35 (CH_2CH), 28.78 (OCH_2CH_2), 28.84 (CH_2), 29.10 (CH_2), 29.22 (CH_2), 29.32 (CH_2), 29.56 (CH_2), 31.81 (CH_2), 35.34 (CHCH_2), 36.83 (C-CH_2), 70.64 (OCH_2CH_2), 71.45 (CH_2CHO), 125.23 (CH=CH), 127.84 (2xC_{Ar}), 129.76 (2xC_{Ar}), 133.22 (C_{Ar}), 133.27 (CH=CH), 144.58 (C_{Ar}).



37 **38**

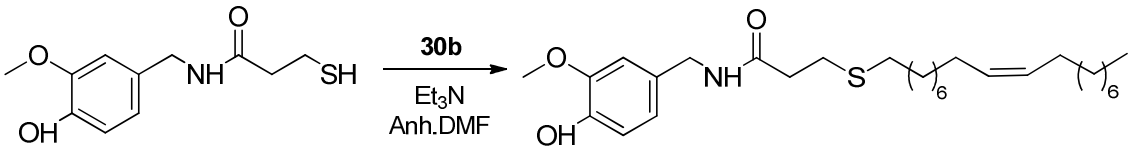
1''-Hexyl-12''-(Tosyloxy)dodec-(3''*Z*)-en-(1''*R*)-yl-2-phenylacetate (38): To a solution of compound **37** (900 mg, 2.05 mmol) in anhydrous toluene (10 mL), phenylacetic acid (307 mg, 2.25 mmol, 1.1 eq.), DCC (1.02 g, 5.13 mmol, 2.5 eq.) and DMAP (500 mg, 4.1 mmol, 2 eq.) were added. The mixture left stirred at room temperature overnight and then filtered off to remove DCU. The solvent was partially evaporated; the crude was dissolved in EtOAc and washed with 1 M HCl, water and brine. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Compound **38** was afforded after silica gel column chromatography (petroleum ether/EtOAc 8:2) as a colourless oil (935 mg, 82%). R_f=0.53 (petroleum ether/EtOAc 8:2). $[\alpha]_D^{20}$= 16.91 (c 5, DCM). IR (ATR) ν = 2925, 2855, 1730, 1361, 1187 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, CH_3), 1.11 – 1.39 (m, 18H, CH_2), 1.42 – 1.56 (m, 2H, CH_2), 1.58 – 1.67 (m, 2H, OCH_2CH_2), 1.97 (q, 2H, *J* = 6.4, CH_2CH), 2.13 – 2.38 (m, 2H, CHCH_2), 2.44 (s, 3H, CH_3C) 3.58 (s, 2H, COCH_2), 4.01 (t, 2H, *J* = 6.9 Hz, OCH_2CH_2), 4.87 (p, 1H, *J* = 6.1 Hz, CH_2CHO), 5.19 – 5.37 (m, 1H, CH=CH), 5.37 –

5.55 (m, 1H, C*H*=CH), 7.19 – 7.43 (m, 7H, H_{Ar}), 7.79 (d, 2H, *J* = 8.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.04 (*C*H_3), 21.61 (*C*H_3C), 22.50 (*C*H_2), 25.17 (*C*H_2), 25.31 (*C*H_2), 27.27 (*C*H_2CH), 28.80 (OCH$_2$*C*H$_2$), 28.88 (*C*H_2), 29.04 (*C*H_2), 29.13 (*C*H_2), 29.27 (*C*H_2), 29.49 (*C*H_2), 31.66 (*C*H_2), 31.89 (CH*C*H$_2$), 33.53 (C*H*$_2$), 41.74 (CO*C*H$_2$), 70.64 (O*C*H$_2$CH$_2$), 74.44 (CH$_2$*C*HO), 124.15 (CH=*C*H), 126.92 (C_{Ar}), 127.85 (2xC_{Ar}), 128.44 (2xC_{Ar}), 129.20 (2xC_{Ar}), 129.76 (2xC_{Ar}), 132.57 (*C*H=CH), 133.25 (C_{Ar}), 134.31 (C_{Ar}), 144.57 (C_{Ar}), 171.27 (O*C*OCH$_2$). HR-MS (ESI$^+$): *m/z*: $[M+NH_4]^+$ Calcd. for $C_{33}H_{52}NO_5S$: 574.3561; Found 573.3563.



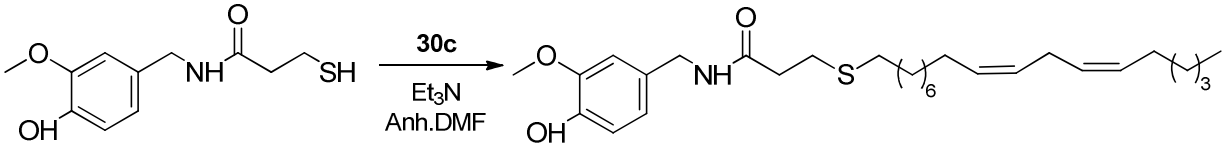
17 **39**

3-(Hexadecylthio)-*N*-(4'-hydroxy-3'-methoxybenzyl)propanamide (39): General procedure VII was applied to **32** (150 mg, 0.62 mmol), compound **30a** (245 mg, 0.70 mmol) and Et_3N (175 µL, 1.24 mmol) dissolved in anhydrous DMF (4 mL). Compound **39** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a white solid (136 mg, 42%). mp=72-73 ºC. R_f=0.48 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 2925, 2855, 1730, 1361, 1187 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.23 – 1.32 (m, 24H, C*H*$_2$), 1.56 – 1.60 (m, 4H, SCH$_2$C*H*$_2$), 2.40 – 2.58 (m, 4H, COC*H*$_2$S, SC*H*$_2$CH$_2$), 2.84 (t, 2H, *J* = 6.9 Hz, C*H*$_2$S), 3.88 (s, 3H, C*H*$_3$O), 4.37 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.59 (s, 1H, CH$_2$N*H*), 5.90 (br s, 1H, O*H*), 6.81 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.28 (*C*H_3), 22.85 (*C*H_2), 28.04 (*C*H_2S), 29.05 (*C*H_2), 29.40 (*C*H_2), 29.52 (*C*H_2), 29.69 (*C*H_2), 29.77 (*C*H_2), 29.81 (3x*C*H_2), 29.85 (4x*C*H_2), 32.08 (*C*H_2), 32.63 (CO*C*H$_2$), 37.07 (S*C*H$_2$CH$_2$), 43.80 (*C*H$_2$NH), 56.13 (*C*H$_3$O), 110.80 (C_{Ar}), 114.49 (C_{Ar}), 120.97 (C_{Ar}), 130.24 (C_{Ar}), 145.28 (C_{Ar}), 146.84 (C_{Ar}), 171.12 (NH*C*O). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{27}H_{48}NO_3S$: 466.3355; Found 466.3378.



17 40

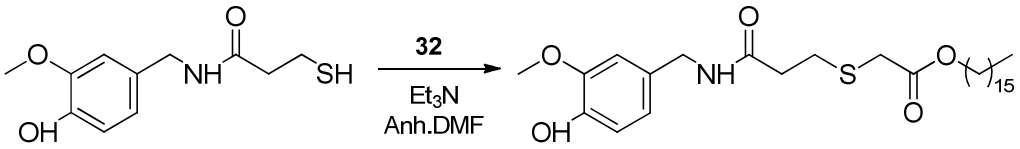
***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-(octadec-(9''*Z*)-en-1-ylthio)propanamide (40):** General procedure VII was applied to compound **17** (100 mg, 0.41 mmol), compound **30b** (174 mg, 0.46 mmol) and Et_3N (115 µL, 0.82 mmol) dissolved in anhydrous DMF (2 mL). Compound **40** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a white sticky solid (83 mg, 41%). R_f=0.73 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 3505, 3323, 2919, 2851, 1640, 1519 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.23 – 1.37 (m, 22H, C*H*$_2$), 1.51 – 1.61 (m, 2H, SCH$_2$C*H*$_2$), 2.01 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.44 – 2.55 (m, 4H, COC*H*$_2$, SC*H*$_2$CH$_2$), 2.83 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.88 (s, 3H, C*H*$_3$O), 4.37 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.28 – 5.40 (m, 2H, C*H*=C*H*), 5.64 (s, 1H, O*H*), 5.94 (br s, 1H, CH$_2$N*H*), 6.81 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.26 (*C*H$_3$), 22.82 (*C*H$_2$), 27.33 (*C*H$_2$CH), 27.36 (CH*C*H$_2$), 28.03 (*C*H$_2$S), 29.03 (*C*H$_2$), 29.35 (*C*H$_2$), 29.39 (*C*H$_2$), 29.46 (2x*C*H$_2$), 29.57 (*C*H$_2$), 29.66 (*C*H$_2$), 29.76 (*C*H$_2$), 29.88 (*C*H$_2$), 29.91 (*C*H$_2$), 32.04 (*C*H$_2$), 32.61 (CO*C*H$_2$), 37.08 (S*C*H$_2$CH$_2$), 43.77 (*C*H$_2$NH), 56.11 (*C*H$_3$O), 110.80 (C$_{Ar}$), 114.49 (C$_{Ar}$), 120.93 (C$_{Ar}$), 129.93 (*C*H=CH), 130.11 (CH=*C*H), 130.21 (C$_{Ar}$), 145.27 (C$_{Ar}$), 146.83 (C$_{Ar}$), 171.13 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{50}NO_3S$: 492.3511; Found 492.3502.



17 41

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-(octadeca-(9''*Z*,12''*Z*)-dien-1-ylthio)propanamide (41):** General procedure VII was applied to compound **17** (100 mg, 0.41 mmol), compound **30c** (173 mg,

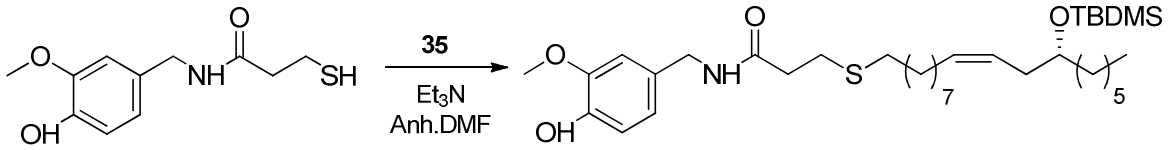
0.46 mmol) and Et_3N (115 µL, 0.82 mmol) dissolved in anhydrous DMF (2 mL). Compound **41** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellow oil (110 mg, 55%). R_f=0.66 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 2923, 2854, 1643, 1515, 1273 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.25 – 1.39 (m, 16H, C*H*$_2$), 1.51 – 1.62 (m, 2H, SCH$_2$C*H*$_2$), 2.04 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.42 – 2.59 (m, 4H, , SC*H*$_2$CH$_2$), 2.69 – 2.90 (m, 4H, COCH$_2$C*H*$_2$, CHC*H*$_2$CH), 3.87 (s, 3H, C*H*$_3$O), 4.36 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.26 – 5.43 (m, 4H, 2xC*H*=C*H*), 5.66 (s, 1H, O*H*), 5.96 (s, 1H, CH$_2$N*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (*C*H$_3$), 22.71 (*C*H$_2$), 25.77 (CH*C*H$_2$CH), 27.34 (*C*H$_2$CH), 27.35 (CH*C*H$_2$), 28.02 (*C*H$_2$S), 29.02 (*C*H$_2$), 29.34 (*C*H$_2$), 29.38 (*C*H$_2$), 29.48 (*C*H$_2$), 29.56 (*C*H$_2$), 29.78 (*C*H$_2$), 29.66 (*C*H$_2$), 31.59 (*C*H$_2$), 32.59 (CO*C*H$_2$), 37.03 (S*C*H$_2$CH$_2$), 43.77 (*C*H$_2$NH), 56.10 (*C*H$_3$O), 110.80 (C$_{Ar}$), 114.49 (C$_{Ar}$), 120.92 (C$_{Ar}$), 128.04 (*C*H=CH), 128.14 (CH=*C*H), 130.19 (C$_{Ar}$), 130.22 (*C*H=CH), 130.34 (CH=*C*H), 145.27 (C$_{Ar}$), 146.83 (C$_{Ar}$), 171.14 (NH*C*O). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{48}NO_3S$: 490.3355; Found 490.3351.



17 **42**

Hexadecyl 2-[(3'-((4''-hydroxy-3''-methoxybenzyl)amino)-3'-oxopropyl)thio]acetate (42): General procedure VII was applied to compound **17** (50 mg, 0.21 mmol), compound **32** (95 mg, 0.23 mmol) and Et_3N (60 µL, 0.42 mmol) dissolved in anhydrous DMF (2 mL). Compound **42** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a white solid (75 mg, 68%). mp: 59-60 ºC. R_f=0.61 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 3370, 3278, 2955, 2917, 2849, 1726, 1269 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, *J* = 6.9 Hz, 3H, C*H*$_3$), 1.24 – 1.33 (m, 26H, C*H*$_2$), 1.57 – 1.65 (m, 2H, COOCH$_2$C*H*$_2$), 2.53 (t, 2H, *J* = 6.9 Hz, COC*H*$_2$), 2.97 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.24 (s, 2H, SC*H*$_2$), 3.88 (s, 3H, C*H*$_3$OH), 4.06 (t, 2H, *J* = 6.9 Hz, COOC*H*$_2$CH$_2$), 4.37 (d, 2H, *J* = 5.7

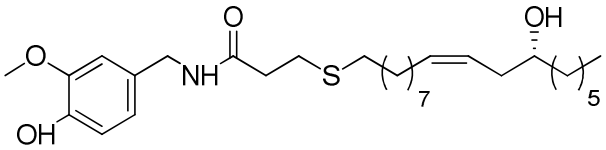
Hz, C*H*$_2$NH), 5.63 (br s, 1H, O*H*), 6.09 (br s, 1H, CH$_2$N*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.26 (*C*H$_3$), 22.83 (*C*H$_2$), 25.96 (*C*H$_2$), 28.65 (*C*H$_2$), 29.26 (*C*H$_2$S), 29.36 (*C*H$_2$), 29.50 (*C*H$_2$), 29.65 (*C*H$_2$), 29.72 (*C*H$_2$), 29.79 (*C*H$_2$), 29.79 (*C*H$_2$), 29.82 (*C*H$_2$), 29.83 (3x*C*H$_2$), 32.06 (*C*H$_2$), 34.40 (S*C*H$_2$), 36.55 (CO*C*H$_2$), 43.76 (*C*H$_2$NH), 56.12 (*C*H$_3$O), 65.91 (COO*C*H$_2$), 110.77 (C$_{Ar}$), 114.44 (C$_{Ar}$), 120.91 (C$_{Ar}$), 130.22 (C$_{Ar}$), 145.23 (C$_{Ar}$), 146.83 (C$_{Ar}$), 170.75 (NH*C*O), 170.80 (*C*OOCH$_2$). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{29}H_{50}NO_5S$: 524.3404; Found 524.3437.



17 **43**

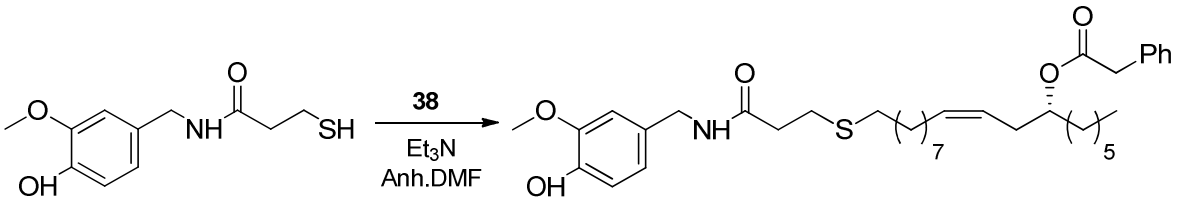
***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-[(((12''*R*)-*tert*-butyldimethylsilyl)oxy)-octadec-(9''*Z*)-en-1-ylthio]propanamide (43):** General procedure VII was applied to compound **17** (100 mg, 0.41 mmol), compound **35** (236 mg, 0.46 mmol) and Et$_3$N (120 µL, 0.82 mmol) dissolved in DMF (2 mL). Compound **43** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a yellow oil (135 mg, 53%). R_f=0.45 (petroleum ether/EtOAc 5:5). $[\alpha]_D^{20}$= -4.71 (c 0.45, DCM). IR (ATR) ν = 3370, 3278, 2955, 2917, 2849, 1726, 1269 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$) δ = 0.03 (s, 6H, Si(C*H*$_3$)$_2$), 0.73 – 0.94 (m, 12H, SiC(C*H*$_3$)$_3$, C*H*$_3$), 1.14– 1.42 (m, 20H, C*H*$_2$), 1.47 – 1.67 (m, 2H, SCH$_2$C*H*$_2$), 2.00 (q, 2H, *J* = 6.4 Hz, C*H*$_2$CH), 2.11 – 2.26 (m, 2H, CHC*H*$_2$), 2.41 – 2.57 (m, 4H, COC*H*$_2$, SC*H*$_2$CH$_2$), 2.83 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.55 – 3.74 (m, 1H, CH$_2$C*H*O), 3.86 (s, 3H, C*H*$_3$O), 4.34 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.27 – 5.51 (m, 2H, C*H*=C*H*), 5.76 (s, 1H, O*H*), 6.03 (s, 1H, CH$_2$N*H*), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 4.57 (Si*C*H$_3$), -4.36 (Si*C*H$_3$), 14.09 (*C*H$_3$), 18.13 (Si*C*(CH$_3$)$_3$), 22.62 (*C*H$_2$), 25.38 (*C*H$_2$), 25.91 (SiC(*C*H$_3$)$_3$), 27.44 (*C*H$_2$CH), 27.87 (*C*H$_2$S), 28.87 (*C*H$_2$), 29.20 (*C*H$_2$), 29.28 (*C*H$_2$), 29.44 (*C*H$_2$), 29.46 (*C*H$_2$), 29.60

(CH_2), 29.65 (CH_2), 31.87 (CH_2), 32.43 (CH_2), 35.24 (CHCH_2), 36.84 (COCH_2, SCH_2CH_2), 43.59 (CH_2NH), 55.93 (CH_3O), 72.38 (CH_2CHO), 110.66 (C_{Ar}), 114.36 (C_{Ar}), 120.74 (C_{Ar}), 125.93 (CH=CH), 130.02 (C_{Ar}), 131.34 (CH=CH), 145.12 (C_{Ar}), 146.71 (C_{Ar}), 171.04 (NHCO). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{35}H_{64}NO_4SSi$: 622.4307; Found 622.4307.

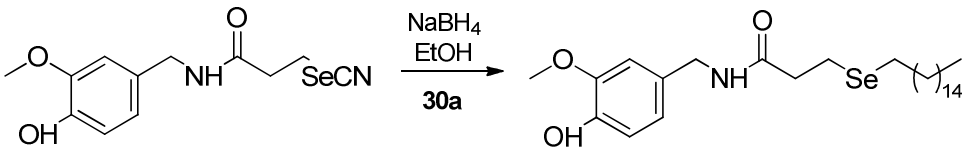


44

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-[((12''*R*)-hydroxy)-octadec-(9''*Z*)-en-1-ylthio]propanamide (44):** General procedure VIII was applied to compound **43** (100 mg, 0.16 mmol) in AcOH/THF/H_2O (1 mL, 6:2:2). Compound **44** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a colourless oil (66 mg, 81%). R_f=0.62 (petroleum ether/EtOAc 5:5). $[\alpha]_D^{20}$= -1.37 (c 0.4, DCM). IR (ATR) ν = 3290, 2923, 2852, 1645, 1514, 1273 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.21– 1.38 (m, 18H, CH_2), 1.41 – 1.49 (m, 4H, CH_2), 1.51 – 1.60 (m, 2H, SCH_2CH_2), 2.04 (q, 2H, *J* = 6.4 Hz, CH_2CH), 2.22 (t, 2H, *J* = 6.9 Hz, CHCH_2), 2.43 – 2.55 (m, 4H, COCH_2, SCH_2CH_2), 2.83 (t, 2H, *J* = 6.9 Hz, COCH_2CH_2), 3.56 – 3.65 (m, 1H, CH_2CHO), 3.88 (s, 3H, CH_3O), 4.37 (d, 2H, *J* = 5.7 Hz, CH_2NH), 5.34 – 5.46 (m,1H, CH=CH), 5.50 – 5.60 (m, 1H, CH=CH), 6.00 (s, 1H, CH_2NH), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ =14.23 (CH_3), 22.76 (CH_2), 25.86 (CH_2), 27.53 (CH_2CH), 28.04 (CH_2S), 28.95 (CH_2), 29.28 (CH_2), 29.35 (CH_2), 29.49 (2xCH_2), 29.71 (CH_2), 29.76 (CH_2), 31.98 (CH_2), 32.59 (SCH_2CH_2), 35.49 (CHCH_2), 36.98 (COCH_2), 36.99 (SCH_2CH_2), 43.81 (CH_2NH), 56.12 (CH_3O), 71.67 (CH_2CHO), 110.83 (C_{Ar}), 114.52 (C_{Ar}), 120.94 (C_{Ar}), 125.31 (CH=CH), 130.13 (C_{Ar}), 133.59 (CH=CH), 145.30 (C_{Ar}), 146.86 (C_{Ar}), 171.25 (NHCO). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{50}NO_4Si$: 508.3461; Found 508.3451.

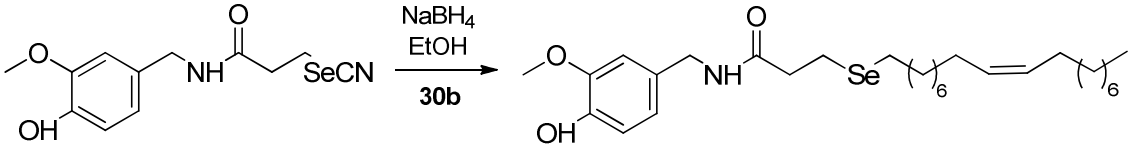


1''-Hexyl-12''-[(3'''-((4''''-hydroxy-3''''-methoxybenzyl)amino)-3'''-oxopropyl)thio]dodec-(3''*Z*)-en-(1''*R*)-yl 2-phenylacetate (45): General procedure VII was applied to compound **17** (100 mg, 0.41 mmol), compound **38** (255 mg, 0.46 mmol) and Et_3N (115 µL, 0.82 mmol) dissolved in anhydrous DMF (2 mL). Compound **45** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a yellow oil (51 mg, 20%). R_f=0.78 (petroleum ether/EtOAc 6:4). $[\alpha]_D^{20}$= 7.90 (c 0.4, DCM). IR (ATR) ν = 3290, 2924, 2853, 1729, 1646, 1514 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.06 – 1.40 (m, 18H, C*H*$_2$), 1.46 – 1.60 (m, 4H, C*H*$_2$, SCH$_2$C*H*$_2$), 1.99 (q, 2H, *J* = 6.4 Hz ,C*H*$_2$CH), 2.19 – 2.35 (m, 2H, CHC*H*$_2$), 2.44 – 2.56 (m, 4H, COC*H*$_2$, SC*H*$_2$CH$_2$), 2.83 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.58 (s, 2H, OCOC*H*$_2$), 3.87 (s, 3H, C*H*$_3$O), 4.36 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 4.86 (p, 1H, *J* = 6.2 Hz, CH$_2$C*H*O), 5.22 – 5.32 (m, 1H, C*H*=CH), 5.39 – 5.48 (m, 1H, CH=C*H*), 6.04 (br s, 1H, CH$_2$N*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$), 7.21 – 7.34 (m, 5H, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.20 (*C*H$_3$), 22.66 (*C*H$_2$), 25.33 (*C*H$_2$), 27.45 (*C*H$_2$CH), 27.01 (*C*H$_2$S), 28.99 (*C*H$_2$), 29.20 (*C*H$_2$), 29.32 (*C*H$_2$), 29.37 (*C*H$_2$), 29.55 (*C*H$_2$), 29.69 (*C*H$_2$), 29.73 (*C*H$_2$), 31.82 (*C*H$_2$), 32.04 (CH*C*H$_2$), 32.57 (CO*C*H$_2$), 33.69 (*C*H$_2$), 36.91 (S*C*H$_2$CH$_2$), 41.90 (OCO*C*H$_2$), 43.84 (*C*H$_2$NH), 56.11 (*C*H$_3$O), 74.65 (CH$_2$*C*HO), 110.81 (C$_{Ar}$), 114.50 (C$_{Ar}$), 120.94 (C$_{Ar}$), 124.25 (CH=*C*H), 127.09 (C$_{Ar}$), 128.60 (2xC$_{Ar}$), 129.36 (2xC$_{Ar}$), 130.06 (C$_{Ar}$), 132.80 (*C*H=CH), 134.46 (C$_{Ar}$), 145.30 (C$_{Ar}$), 146.84 (C$_{Ar}$), 171.37 (NH*C*O), 171.48 (O*C*OCH$_2$). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{37}H_{56}NO_5S$: 626.3879; Found 626.3870.



20 **46**

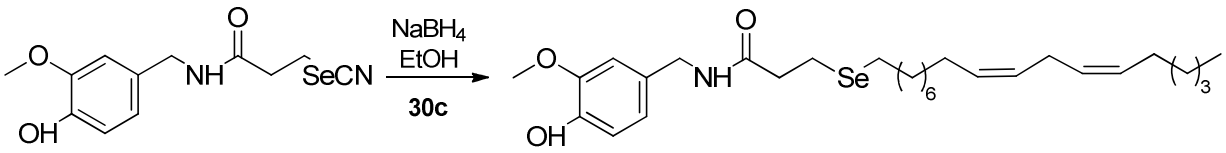
3-(Hexadecylseleno)-*N*-(4'-hydroxy-3'-methoxybenzyl)propanamide (46): General procedure IV was applied to compound **20** (100 mg, 0.32 mmol), $NaBH_4$ (30 mg, 0.8 mmol) and compound **30a** (126 mg, 0.36 mmol) dissolved in EtOH (2 mL). Compound **46** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellow sticky solid (166 mg, 71%). R_f=0.55 (petroleum ether/EtOAc 7:3). IR (ATR) ν = 3504, 3317, 2917, 2848, 1645, 1519 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.22 – 1.36 (m, 26H, C*H*$_2$), 1.59 – 1.68 (m, 2H, SeCH$_2$C*H*$_2$), 2.53 – 2.62 (m, 4H, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.83 (t, 2H, *J* = 6.9 Hz, C*H*$_2$Se), 3.88 (s, 3H, C*H*$_3$O), 4.36 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.66 (s, 1H, CH$_2$N*H*), 5.88 (br s, 1H, O*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.26 (*C*H$_3$), 18.69 (*C*H$_2$Se), 22.83 (*C*H$_2$), 24.84 (Se*C*H$_2$CH$_2$), 29.31 (*C*H$_2$), 29.49 (*C*H$_2$), 29.68 (*C*H$_2$), 29.75 (*C*H$_2$), 29.79 (2x*C*H$_2$), 29.83 (4x*C*H$_2$), 30.08 (*C*H$_2$), 30.74 (*C*H$_2$), 32.06 (*C*H$_2$), 38.03 (CO*C*H$_2$), 43.78 (*C*H$_2$NH), 56.12 (*C*H$_3$O), 110.83 (C$_{Ar}$), 114.49 (C$_{Ar}$), 120.96 (C$_{Ar}$), 130.20 (C$_{Ar}$), 145.28 (C$_{Ar}$), 146.84 (C$_{Ar}$), 171.41 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{27}H_{48}NO_3Se$: 514.2799; Found 514.2795.



20 **47**

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-(octadec-(9''*Z*)-en-1-yl-seleno)propanamide (47):** General procedure IV was applied to compound **20** (200 mg, 0.64 mmol), $NaBH_4$ (59 mg, 1.6 mmol) and compound **30b** (271 mg, 0.72 mmol) dissolved in EtOH (2 mL). Compound **47** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellow sticky solid (244 mg, 71%).

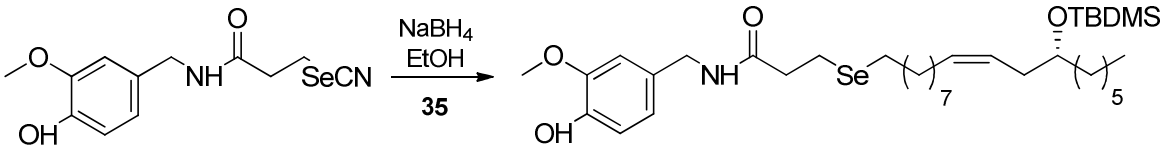
R_f=0.71 (petroleum ether/EtOAc 7:3). IR (ATR) ν = 3509, 3321, 2919, 2850, 1646, 1519 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.24 – 1.37 (m, 22H, C*H*$_2$), 1.60 – 1.68 (m, 2H, SeCH$_2$C*H*$_2$), 2.01 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.54 – 2.61 (m, 4H, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.84 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.88 (s, 3H, C*H*$_3$O), 4.37 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.29 – 5.40 (m, 2H, C*H*=C*H*), 5.61 (s, 1H, O*H*), 5.83 (br s, 1H, CH$_2$N*H*), 6.82 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.27 (*C*H$_3$), 18.70 (*C*H$_2$Se), 22.83 (*C*H$_2$), 24.84 (Se*C*H$_2$CH$_2$), 27.35 (*C*H$_2$CH), 27.37 (CH*C*H$_2$), 29.29 (*C*H$_2$), 29.40 (*C*H$_2$), 29.47 (2x*C*H$_2$), 29.58, (*C*H$_2$) 29.67 (*C*H$_2$), 29.89 (*C*H$_2$), 29.92 (*C*H$_2$), 30.08 (*C*H$_2$), 30.74 (*C*H$_2$), 32.05 (*C*H$_2$), 38.06 (CO*C*H$_2$), 43.80 (*C*H$_2$NH), 56.14 (*C*H$_3$O), 110.83 (C_{Ar}), 114.48 (C_{Ar}), 120.99 (C_{Ar}), 129.94 (*C*H=CH), 130.11 (*C*H=CH), 130.22 (C_{Ar}), 145.29 (C_{Ar}), 146.84 (C_{Ar}), 171.37 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{50}NO_3Se$: 540.2956; Found 540.2957.



20 **48**

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-(octadeca-(9''*Z*,12''*Z*)-dien-1-ylseleno)propanamide (48):** General procedure IV was applied to compound **20** (100 mg, 0.32 mmol), $NaBH_4$ (30 mg, 0.80 mmol) and compound **30c** (135 mg, 0.36 mmol) dissolved in EtOH (2 mL). Compound **48** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellowish oil (111 mg, 65%). R_f=0.7 (petroleum ether/EtOAc 7:3). IR (ATR) ν = 3288, 3008, 2923, 2852, 1644, 1514 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.25 – 1.38 (m, 16H, C*H*$_2$), 1.59 – 1.68 (m, 2H, SeCH$_2$C*H*$_2$), 2.04 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.54 – 2.61 (m, 4H, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.77 (t, 2H, *J* = 6.9 Hz, 2H, CHC*H*$_2$CH), 2.83 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.88 (s, 3H, C*H*$_3$O), 4.36 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.28 – 5.42 (m, 4H, 2xC*H*=C*H*), 5.66 (s, 1H, O*H*), 5.88 (br s, 1H, CH$_2$N*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.21 (*C*H$_3$), 18.69 (*C*H$_2$),

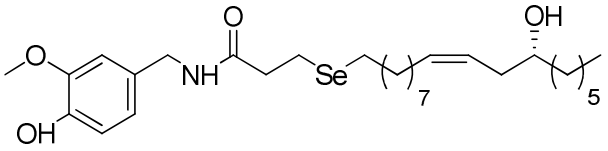
22.70 (*C*H$_2$), 24.81 (Se*C*H$_2$CH$_2$), 25.77 (CH*C*H$_2$CH), 27.33 (*C*H$_2$CH), 27.35 (CH*C*H$_2$), 29.26 (*C*H$_2$), 29.38 (*C*H$_2$), 29.48 (*C*H$_2$), 29.56 (*C*H$_2$), 29.77 (*C*H$_2$), 30.06 (*C*H$_2$), 30.72 (*C*H$_2$), 31.66 (*C*H$_2$), 38.02 (CO*C*H$_2$), 43.78 (CH$_2$NH), 56.12 (CH$_3$O), 110.82 (C$_{Ar}$), 114.48 (C$_{Ar}$), 120.95 (C$_{Ar}$), 128.04 (*C*H=CH), 128.14 (CH=*C*H), 130.19 (C$_{Ar}$), 130.22 (*C*H=CH), 130.34 (CH=*C*H), 145.28 (C$_{Ar}$), 146.83 (C$_{Ar}$), 171.39 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{29}H_{48}NO_3Se$: 538.2799; Found 538.2761.



20 **49**

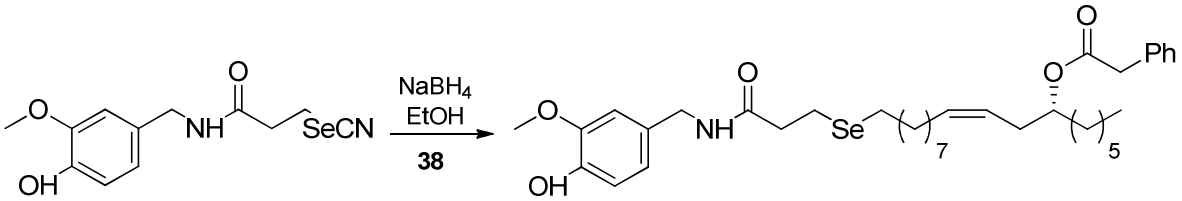
***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-[(((12''*R*)-*tert*-butyldimethylsilyl)oxy)-octadec-(9''*Z*)-en-1-ylseleno]propanamide (49):** General procedure IV was applied to compound **20** (100 mg, 0.32 mmol), $NaBH_4$ (30 mg, 0.80 mmol) and compound **35** (233 mg, 0.46 mmol) dissolved in EtOH (2 mL). Compound **49** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellow oil (124 mg, 58%). R_f: 0.54 (petroleum ether/EtOAc 7:3). $[\alpha]_D^{20}$= -2.21 (c 0.7, DCM). IR (ATR) ν = 3288, 2924, 2853, 1645, 1514. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.04 (s, 6H, Si(C*H*$_3$)$_2$), 0.80 – 0.97 (m, 12H, SiC(C*H*$_3$)$_3$, C*H*$_3$), 1.15– 1.32 (m, 20H, C*H*$_2$), 1.52 – 1.71 (m, 2H, SeCH$_2$C*H*$_2$), 2.01 (q, 2H, *J* = 6.4 Hz, C*H*$_2$CH), 2.18 (t, 2H, *J* = 6.9 Hz, CHC*H*$_2$), 2.58 (t, 4H, *J* = 6.9 Hz, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.84 (s, 2H, COCH$_2$C*H*$_2$), 3.58 – 3.70 (m, 1H, CH$_2$C*H*O), 3.89 (s, 3H, C*H*$_3$O), 4.37 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.32 – 5.49 (m, 2H, C*H*=C*H*), 5.58 (s, 1H, O*H*), 5.80 (s, 1H, CH$_2$N*H*), 6.81 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = -4.56 (Si*C*H$_3$), -4.36 (Si*C*H$_3$), 14.09 (*C*H$_3$), 18.14 (Si*C*(CH$_3$)$_3$), 18.55 (*C*H$_2$Se), 22.62 (*C*H$_2$), 24.71 (Se*C*H$_2$CH$_2$), 25.39 (*C*H$_2$), 25.91 (SiC(*C*H$_3$)$_3$), 27.45 (*C*H$_2$CH), 29.13 (*C*H$_2$), 29.29 (*C*H$_2$), 29.44 (*C*H$_2$), 29.46 (*C*H$_2$), 29.65 (*C*H$_2$), 29.68 (*C*H$_2$), 29.93 (*C*H$_2$), 31.88 (*C*H$_2$), 35.25 (CH*C*H$_2$), 36.85 (*C*H$_2$), 37.90 (CO*C*H$_2$), 43.64 (*C*H$_2$NH), 55.97 (*C*H$_3$O), 72.39 (CH$_2$*C*HO), 110.65 (C$_{Ar}$), 114.31 (C$_{Ar}$), 120.83 (C$_{Ar}$), 125.93 (CH=*C*H), 130.05 (C$_{Ar}$), 131.35

(*C*H=CH), 145.12 (C_{Ar}), 146.66 (C_{Ar}), 171.19 (NH*C*O).



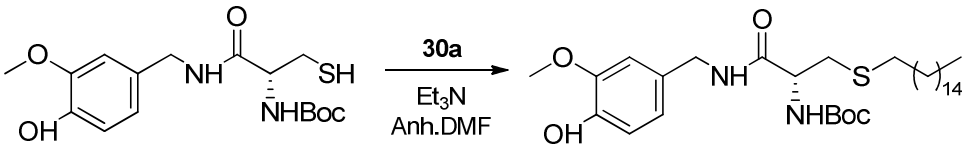
50

***N*-(4'-Hydroxy-3'-methoxybenzyl)-3-[((12''*R*)-hydroxy)-octadec-(9''*Z*)-en-1-ylseleno] propanamide (50):** General procedure VIII was applied to compound **49** (100 mg, 0.18 mmol) in AcOH/THF/H_2O (1 mL, 6:2:2). Compound **50** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a pale yellow oil (79 mg, 79%). R_f=0.77 (petroleum ether/EtOAc 7:3). $[\alpha]_D^{20}$= -7.88 (c 0.3, DCM). IR (ATR) ν = 3288, 2923, 2852, 1646, 1514 1273 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.21 – 1.39 (m, 18H, C*H*$_2$), 1.42 – 1.48 (m, 2H, COHC*H*$_2$), 1.58 – 1.67 (m, 2H, SeCH$_2$C*H*$_2$), 2.04 (q, 2H, *J* = 6.4 Hz, C*H*$_2$CH), 2.20 (t, 2H, *J* = 6.9 Hz, CHC*H*$_2$), 2.53 – 2.61 (m, 4H, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.83 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.57 – 3.65 (m, 1H, CH$_2$C*H*O), 3.87 (s, 3H, C*H*$_3$O), 4.36 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.34 – 5.45 (m, 1H, C*H*=CH), 5.49 – 5.60 (m, 1H, CH=C*H*), 5.73 (br s, 1H, O*H*), 5.93 (br s, 1H, CH$_2$N*H*), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.23 (*C*H$_3$), 18.71 (*C*H$_2$Se), 22.76 (*C*H$_2$), 24.81 (Se*C*H$_2$CH$_2$), 25.86 (*C*H$_2$), 27.53 (*C*H$_2$), 29.21 (*C*H$_2$), 29.35 (*C*H$_2$), 29.49 (2x*C*H$_2$), 29.75 (*C*H$_2$), 30.00 (*C*H$_2$), 30.69 (SeCH$_2$*C*H$_2$), 31.98 (*C*H$_2$), 35.50 (CH*C*H$_2$), 36.98 (*C*H$_2$), 38.04 (CO*C*H$_2$), 43.79 (*C*H$_2$NH), 56.13 (*C*H$_3$O), 71.65 (CH$_2$*C*HO), 110.85 (C_{Ar}), 114.51 (C_{Ar}), 120.97 (C_{Ar}), 125.31 (CH=*C*H), 130.21 (C_{Ar}), 133.58 (*C*H=CH), 145.29 (C_{Ar}), 146.85 (C_{Ar}), 171.39 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{29}H_{50}NO_4Se$: 556.2905; Found 556.2901.



20 **51**

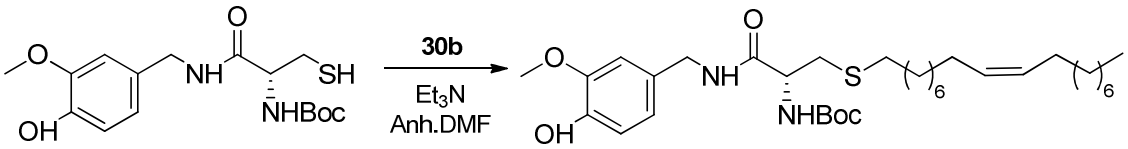
1''-Hexyl-12''-[(3'''-((4''''-hydroxy-3''''-methoxybenzyl)amino)-3'''-oxopropyl)seleno]dodec-(3''*Z*)-en-(1''*R*)-yl 2-phenylacetate (51): General procedure IV was applied to compound **20** (100 mg, 0.32 mmol), $NaBH_4$ (30 mg, 0.80 mmol) and compound **38** (200 mg, 0.36 mmol) dissolved in EtOH (2 mL). Compound **51** was afforded after silica gel column chromatography (petroleum ether/EtOAc 5:5) as a yellow oil (155 mg, 72%). R_f=0.58 (petroleum ether/EtOAc 5:5). $[\alpha]_D^{20}$= 14.78 (c 1.8, DCM). IR (ATR) ν = 3291, 2924, 2853, 1729, 1645, 1514 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.20 – 1.40 (m, 18H, C*H*$_2$), 1.50 – 1.58 (m, 2H, SeCH$_2$C*H*$_2$), 1.61 – 1.71 (m, 2H, COHC*H*$_2$), 2.01 (q, 2H, *J* = 6.4 Hz, C*H*$_2$CH), 2.23 – 2.37 (m, 2H, CHC*H*$_2$), 2.60 (t, 4H, *J* = 6.9 Hz, COC*H*$_2$, SeC*H*$_2$CH$_2$), 2.86 (t, 2H, *J* = 6.9 Hz, COCH$_2$C*H*$_2$), 3.61 (s, 2H, OCOC*H*$_2$), 3.89 (s, 3H, C*H*$_3$O), 4.38 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 4.90 (p, 1H, *J* = 6.3 Hz, CH$_2$C*H*O), 5.26 – 5.35 (m, 1H, C*H*=CH), 5.42 – 5.51 (m, 1H, CH=C*H*), 5.75 (s, 1H, O*H*), 5.98 (br s, 1H, CH$_2$N*H*), 6.83 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$), 7.16 – 7.42 (m, 5H, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.18 (*C*H$_3$), 18.68 (*C*H$_2$), 22.63 (*C*H$_2$), 24.76 (Se*C*H$_2$CH$_2$), 25.30 (*C*H$_2$), 27.43 (*C*H$_2$CH), 29.18 (*C*H$_2$), 29.23 (*C*H$_2$), 29.35 (*C*H$_2$), 29.53 (*C*H$_2$), 29.66 (*C*H$_2$), 30.02 (*C*H$_2$), 30.68 (*C*H$_2$), 31.79 (*C*H$_2$), 32.01 (CH*C*H$_2$), 33.66 (*C*H$_2$), 37.97 (CO*C*H$_2$), 41.87 (OCO*C*H$_2$), 43.74 (*C*H$_2$NH), 56.09 (*C*H$_3$O), 74.62 (CH$_2$*C*HO), 110.82 (C$_{Ar}$), 114.48 (C$_{Ar}$), 120.91 (C$_{Ar}$), 124.22 (CH=*C*H), 127.06 (C$_{Ar}$), 128.57 (2xC$_{Ar}$), 129.33 (2xC$_{Ar}$), 130.17 (C$_{Ar}$), 132.78 (*C*H=CH), 134.42 (C$_{Ar}$), 145.26 (C$_{Ar}$), 146.83 (C$_{Ar}$), 171.41 (NH*C*O), 171.46 (O*C*OCH$_2$). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{37}H_{56}NO_5Se$: 674.3324; Found 674.3315.



24 **52**

(2*R*)-Boc-amino-3-(hexadecylthio)-*N*-(4'-hydroxy-3'-methoxybenzyl)-propanamide (52): General procedure VII was applied to compound **24** (200 mg, 0.56 mmol), compound **30a** (220 mg, 0.63 mmol) and Et_3N (0.16 mL, 1.12 mmol) in anhydrous DMF (5 mL). Compound **52** was afforded after silica gel

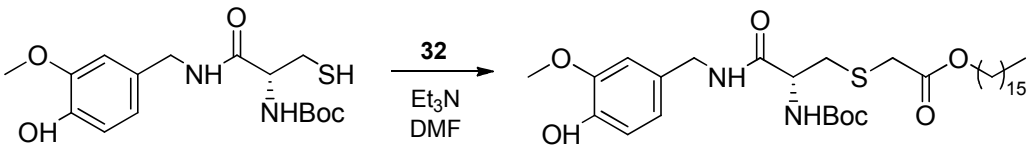
column chromatography (petroleum ether/EtOAc 6:4) as a white solid (230 mg, 71%). R_f=0.29 (petroleum ether/EtOAc 5:5). mp: 76-77 ºC. $[\alpha]_D^{20}$= -2.28 (c 0.6, DCM). IR (ATR) ν = 3449, 3336, 2918, 2850, 1681, 1659, 1513 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.15 – 1.35 (m, 26H, C*H*$_2$), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.47 – 1.60 (m, 2H, SCH$_2$C*H*$_2$), 2.52 (td, 2H, *J* = 6.9, 1.7 Hz, SC*H*$_2$CH$_2$), 2.84 (dd, 1H, *J* = 13.7, 6.9 Hz, CHC*H*$_2$S), 2.98 (dd, 1H *J* = 13.7, 5.5 Hz, CHC*H*$_2$S), 3.86 (s, 3H, C*H*$_3$O), 4.25 (d, 1H, *J* = 5.7 Hz, C*H*$_2$NH), 4.29 – 4.45 (m, 2H, C*H*CH$_2$S), 5.39 (d, 1H, *J* = 5.7 Hz, CH$_2$N*H*), 5.70 (s, 1H, O*H*), 6.67 (t, *J* = 5.5 Hz, 1H, N*H*Boc), 6.78 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.25 (*C*H$_3$), 22.82 (*C*H$_2$), 28.39 (C(*C*H$_3$)$_3$), 28.92 (*C*H$_2$), 29.36 (*C*H$_2$), 29.49 (*C*H$_2$), 29.65 (*C*H$_2$), 29.74 (*C*H$_2$), 29.78 (2x*C*H$_2$), 29.81 (*C*H$_2$), 29.82 (4x*C*H$_2$), 32.05 (*C*H$_2$), 32.82 (S*C*H$_2$CH$_2$), 34.61 (CH*C*H$_2$S), 43.68 (*C*H$_2$NH), 54.25 (*C*HCH$_2$S), 56.08 (*C*H$_3$O), 80.59 (*C*(CH$_3$)$_3$), 110.63 (C$_{Ar}$), 114.50 (C$_{Ar}$), 120.76 (C$_{Ar}$), 129.81 (C$_{Ar}$), 145.24 (C$_{Ar}$), 146.83 (C$_{Ar}$), 155.51 (NH*C*O$_2$), 170.58 (NH*C*O). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{32}H_{57}N_2O_5S$: 581.3988; Found 581.3978.



24 **53**

(2*R*)-Boc-amino-*N*-(4'-hydroxy-3'-methoxybenzyl)-3-(octadec-(9''*Z*)-en-1-ylthio)propanamide (53): General procedure VII was applied to compound **24** (100 mg, 0.42 mmol), compound **30b** (179 mg, 0.47 mmol) and Et_3N (117 µL mL, 0.84 mmol) dissolved in DMF (2 mL). Compound **53** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a white solid (127 mg, 50%). mp: 43-44 ºC. R_f=0.58 (petroleum ether /EtOAc 7:3). $[\alpha]_D^{20}$= 0.26 (c 1.2, DCM). IR (ATR) ν = 3450, 3333, 2918, 2850, 1514, 1240 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.18 – 1.38 (m, 22H, C*H*$_2$), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.48 –1.61 (m, 2H, SCH$_2$C*H*$_2$), 2.01 (q, 4H, *J*

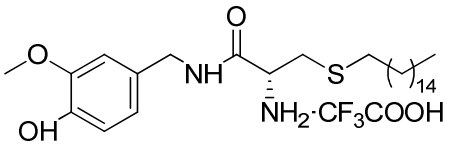
= 6.4 Hz, CH_2CH, CHCH_2), 2.45 –2.58 (m, 2H, SCH_2CH$_2$), 2.84 (dd, 1H, *J* = 13.7, 6.9 Hz, CHCH_2S), 3.00 (dd, 1H, *J* = 13.7, 5.5 Hz, CHCH_2S), 3.88 (s, 3H, CH_3O), 4.24 (dd, 1H, *J* = 12.5, 6.1 Hz CH_2NH), 4.30 – 4.48 (m, 2H, C*H*CH$_2$S), 5.22 – 5.44 (m, 3H, C*H*=C*H*, CH$_2$N*H*), 5.59 (s, 1H, O*H*), 6.61 (t, 1H, *J* = 5.5 Hz, N*H*Boc), 6.80 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.10 (*C*H$_3$), 22.66 (*C*H$_2$), 27.18 (*C*H$_2$CH), 27.20 (CH*C*H$_2$), 28.24 (C(*C*H$_3$)$_3$), 28.76 (*C*H$_2$), 29.18 (*C*H$_2$), 29.23 (*C*H$_2$), 29.29 (*C*H$_2$), 29.30 (*C*H$_2$), 29.40 (SCH$_2$*C*H$_2$), 29.50 (*C*H$_2$), 29.59 (*C*H$_2$), 29.68 (*C*H$_2$), 29.73 (*C*H$_2$), 29.75 (*C*H$_2$), 31.88 (*C*H$_2$), 32.66 (S*C*H$_2$CH$_2$), 34.44 (CH*C*H$_2$S), 43.55 (*C*H$_2$NH), 54.12 (*C*HCH$_2$S), 55.94 (*C*H$_3$O), 80.57 (*C*(CH$_3$)$_3$), 110.45 (C$_{Ar}$), 114.31 (C$_{Ar}$), 120.64 (C$_{Ar}$), 129.68 (C$_{Ar}$), 129.76 (CH=*C*H), 129.95 (CH=*C*H), 145.10 (C$_{Ar}$), 146.65 (C$_{Ar}$), 155.55 (NH*C*O$_2$), 170.37 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for C$_{34}$H$_{59}$N$_2$O$_5$S: 607.4145; Found 607.4138.



24 **54**

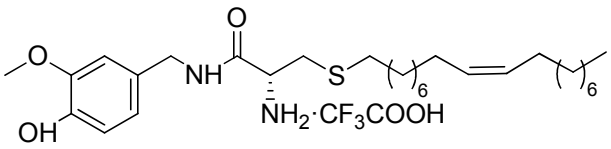
Hexadecyl 2-[((2'*R*)-Boc-amino-3'-((4''-hydroxy-3''-methoxybenzyl)amino)-3'-oxopropyl) thio]acetate (54): General procedure VII was applied to compound **24** (200 mg, 0.56 mmol), compound **35** (258 mg, 0.63 mmol) and Et$_3$N (160 µL, 1.12 mmol) dissolved in anhydrous DMF (2 mL). Compound **54** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a white solid (282 mg, 79%). Mp: 74-75 ºC. *R$_f$*=0.75 (petroleum ether/EtOAc 7:3). $[\alpha]_D^{20}$= -8.04 (c 1, MeOH). IR (ATR) ν = 3493, 3326, 2917, 2849, 1655, 1518 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$) δ = 0.88 (t, *J* = 6.9 Hz, 3H, C*H*$_3$), 1.17 – 1.35 (m, 26H, C*H*$_2$), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.55 – 1.65 (m, 2H, COOCH$_2$C*H*$_2$), 2.88 (dd, 1H, *J* = 13.7, 6.9 Hz, CHC*H*$_2$S), 3.07 (dd, 1H, *J* = 13.7, 6.9 Hz, CHC*H*$_2$S), 3.35 (s, 2H, SC*H*$_2$), 3.87 (s, 3H, C*H*$_3$OH), 4.07 (t, 2H, *J* = 6.9 Hz, COOC*H*$_2$CH$_2$), 4.25 – 4.49 (m, 3H, COC*H*CH$_2$, C*H*$_2$NH), 5.47 – 5.69 (m, 2H, CH$_2$N*H,* O*H*), 6.73 – 6.87 (m, 3H, H$_{Ar}$), 7.04 (t, 1H, *J* = 5.0 Hz, N*H*Boc). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.09 (*C*H$_3$), 22.66 (*C*H$_2$), 25.78 (*C*H$_2$), 28.26

(C(CH_3)$_3$), 28.44 (CH_2), 29.20 (CH_2), 29.33 (CH_2), 29.48 (CH_2), 29.55 (CH_2), 29.62 (CH_2), 29.63 (CH_2), 29.65 (CH_2), 29.67 (3xCH_2), 31.90 (CH_2), 34.70 (S$CH_2$$CH_2$), 35.89 (CH$CH_2$S), 43.50 ($CH_2$NH), 53.59 ($CH$$CH_2$S), 55.93 ($CH_3$O), 66.07 (COO$CH_2$), 80.35 ($C$($CH_3$)$_3$), 110.42 ($C_{Ar}$), 114.28 ($C_{Ar}$), 120.61 ($C_{Ar}$), 129.70 ($C_{Ar}$), 145.00 ($C_{Ar}$), 146.62 ($C_{Ar}$), 155.46 (NH$CO_2$), 170.00 (NH$CO$), 171.34 ($COOCH_2$). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{34}H_{59}N_2O_7S$: 639.4043; Found 639.4040.



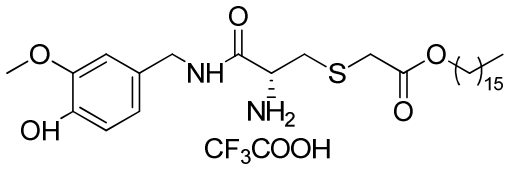
55

2-(Hexadecylthio)-1-[*N*-(4'-hydroxy-3'-methoxybenzyl)carbamoyl]-(1*R*)-ethylammonium trifluoroacetate (55): General procedure IX was applied to compound **52** (200 mg, 0.34 mmol), TFA (0.26 mL, 3.4 mmol) in DCM (1 mL). Compound **55** was afforded after flushing nitrogen and drying *in vacuo* as a yellow oil (195 mg, quantitative). $[\alpha]_D^{20}$= -6.67 (c 0.6, DCM). IR (ATR) ν = 3093, 2921, 2852, 1779, 1667, 1153 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.21 – 1.31 (m, 26H, CH_2), 1.45 – 1.54 (m, 2H, SCH$_2$CH_2), 2.48 (t, 2H, *J* = 6.9 Hz, SCH_2CH$_2$), 2.85 – 3.03 (m, 2H, CHCH_2S), 3.83 (s, CH_3O), 4.22 – 4.38 (m, 3H, CHCH$_2$S, CH_2NH), 6.52 (br s, 2H, NH_2), 6.68 – 6.85 (m, 4H, OH, H_{Ar}), 7.55 (t, 1H, *J* = 5.0 Hz, CH$_2$NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.26 (CH_3), 22.85 (CH_2), 28.82 (CH_2), 29.30 (CH_2), 29.32 (CH_2), 29.52 (CH_2), 29.65 (CH_2), 29.74 (2xCH_2), 29.84 (CH_2), 29.86 (4xCH_2), 32.08 (CH_2), 32.50 (S$CH_2$$CH_2$), 33.06 (CH$CH_2$S), 44.38 ($CH_2$NH), 52.72 ($CHCH_2$S), 56.01 ($CH_3$O), 110.67 ($C_{Ar}$), 114.71 ($C_{Ar}$), 116.86 ($CF_3$COOH), 120.92 ($C_{Ar}$), 128.31 ($C_{Ar}$), 145.52 ($C_{Ar}$), 146.95 ($C_{Ar}$), 161.37 (CF$_3$$C$OOH), 167.54 (NH$CO$). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{27}H_{49}N_2O_3S$: 481.3458; Found 481.3497.



56

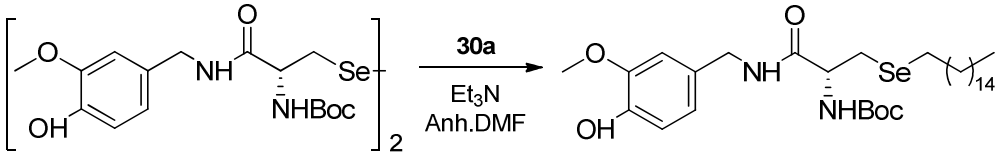
1-[*N*-(4'-Hydroxy-3'-methoxybenzyl)carbamoyl]-2-(octadec-(9''*Z*)-en-1-yl-thio)-(1*R*)-ethylammonium trifluoroacetate (56): General procedure IX was applied to compound **53** (100 mg, 0.16 mmol), TFA (120 µL, 1.64 mmol) in DCM (1 mL). Compound **56** was afforded after flushing nitrogen and drying *in vacuo* as a yellow oil (98 mg, quantitative). $[\alpha]_D^{20}$= 0.62 (c 2.2, DCM). IR (ATR) ν = 2922, 2853, 1662, 1199, 1133 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.21 – 1.35 (m, 22H, C*H*$_2$), 1.43 – 1.51 (m, 2H, SCH$_2$C*H*$_2$), 2.00 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.45 (t, 2H, *J* = 6.9 Hz, SC*H*$_2$CH$_2$), 2.94 (d, 2H, *J* = 6.0 Hz, CHC*H*$_2$S), 3.78 (s, 3H, C*H*$_3$O), 4.13 – 4.34 (m, 3H, C*H*CH$_2$S, C*H*$_2$NH), 5.26 – 5.43 (m, 2H, C*H*=C*H*), 6.70 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$), 7.87 (t, 1H, *J* = 5.0 Hz, CH$_2$N*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.25 (*C*H$_3$), 22.83 (*C*H$_2$), 27.37 (*C*H$_2$CH, CH*C*H$_2$), 28.86 (*C*H$_2$), 29.34 (*C*H$_2$), 29.41 (*C*H$_2$), 29.44 (*C*H$_2$), 29.46 (*C*H$_2$), 29.47 (*C*H$_2$), 29.61 (*C*H$_2$), 29.68 (*C*H$_2$), 29.82 (*C*H$_2$), 29.85 (*C*H$_2$), 29.92 (*C*H$_2$), 32.05 (*C*H$_2$), 32.66 (S*C*H$_2$CH$_2$), 32.96 (CH*C*H$_2$S), 44.0 (*C*H$_2$NH), 52.77 (*C*HCH$_2$S), 55.96 (*C*H$_3$O), 110.71 (C$_{Ar}$), 114.67 (C$_{Ar}$), 120.74 (C$_{Ar}$), 128.82 (C$_{Ar}$), 129.90 (*C*H=CH), 130.11 (CH=*C*H), 145.27 (C$_{Ar}$), 146.93 (C$_{Ar}$), 167.76 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{31}H_{51}N_2O_3S$: 507.3615; Found 507.3616.



57

2'-Hexadecyloxy-1-[*N*-(4''-hydroxy-3''-methoxybenzyl)]carbamoyl-2-[(oxoethyl)thio]ethan-(1*R*)-ammonium trifluoroacetate (57): General procedure IX was applied to compound **54** (200 mg, 0.31 mmol), TFA (240 µL, 3.1 mmol) in DCM (1 mL). Compound **57** was afforded after flushing nitrogen and drying *in vacuo* as a yellow oil (201 mg, quantitative). $[\alpha]_D^{20}$= -7.53 (c 0.4, MeOH). IR (ATR) ν = 2917, 2850, 1662, 1176, 1131 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, *J* = 6.9 Hz, 3H, C*H*$_3$), 1.18 – 1.34 (m, 26H, C*H*$_2$), 1.53 – 1.64 (m, 2H, COOCH$_2$C*H*$_2$), 2.98 – 3.14 (m, 2H, C*H*CH$_2$S), 3.37 (s, 2H,

SCH_2), 3.82 (s, 3H, CH_3OH), 3.99 – 4.11 (m, 2H, COOCH_2CH$_2$), 4.22 – 4.43 (m, 3H, COCHCH$_2$, H_2NH), 6.67 – 6.83 (m, 3H, H$_{Ar}$), 7.94 (t, 1H, J = 5.0 Hz, CH$_2$NH). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.26 (*C*H$_3$), 22.84 (*C*H$_2$), 25.87 (*C*H$_2$), 28.43 (*C*H$_2$), 29.35 (*C*H$_2$), 29.51 (2x*C*H$_2$), 29.64 (*C*H$_2$), 29.73 (*C*H$_2$), 29.81 (*C*H$_2$), 29.83 (*C*H$_2$), 29.85 (3x*C*H$_2$), 32.08 (*C*H$_2$), 34.65 (*C*H$_2$), 34.95 (*C*H$_2$), 44.24 (*C*H$_2$NH), 53.08 (*C*HCH$_2$S), 55.99 (*C*H$_3$O), 67.26 (COO*C*H$_2$), 110.62 (C$_{Ar}$), 114.64 (C$_{Ar}$), 120.80 (C$_{Ar}$), 128.61 (C$_{Ar}$), 145.35 (C$_{Ar}$), 146.91 (C$_{Ar}$), 167.33 (NH*C*O), 172.72 (*C*OOCH$_2$). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{29}H_{51}N_2O_5S$: 539.3513; Found 539.3557.

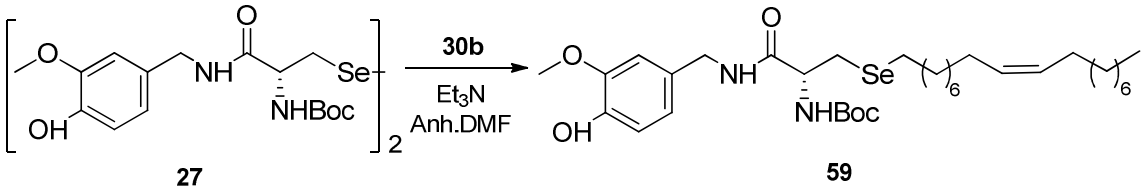


27 **58**

(2*R*)-Boc-amino-3-(hexadecylseleno)-*N*-(4'-hydroxy-3'-methoxybenzyl)-propanamide (58):

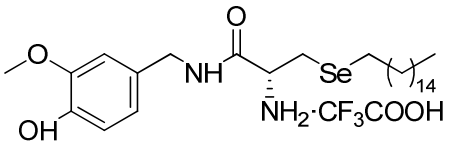
General procedure III was applied to compound **27** (200 mg, 0.25 mmol), $NaBH_4$ (24 mg, 0.62 mmol) and compound **30a** (197 mg, 0.56 mmol) dissolved in EtOH (2 mL). Compound **58** was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a white solid (231 mg, 74%). R_f=0.37 (petroleum ether/EtOAc 6:4). mp: 75-76 ºC. $[\alpha]_D^{20}$= -5.24 (c 1.3, DCM). IR (ATR) ν = 3281, 3008, 2924, 2854, 1666, 1516 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$) δ = 0.88 (t, 1H, J = 6.9 Hz, CH_3), 1.17 – 1.38 (m, 26H, CH_2), 1.42 (s, 9H, J = 4.9 Hz, C(CH_3)$_3$), 1.58 – 1.69 (m, 2H, SeCH$_2$CH_2), 2.46 – 2.67 (m, 2H, SeCH_2CH$_2$), 2.83 (dd, 1H, J = 12.8, 6.9 Hz, CHCH_2Se), 3.05 (dd, 1H, J = 12.8, 5.2 Hz, CHCH_2Se), 3.88 (s, 3H, CH_3O), 4.22 – 4.36 (m, 1H, CHCH$_2$Se), 4.37 (d, 2H, J = 5.7 Hz, CH_2NH), 5.33 (s, 1H, CH$_2$NH), 5.58 (s, 1H, OH), 6.55 (t, 1H, J = 5.5 Hz, NHBoc), 6.80 (ddd, 3H, J = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, CDCl$_3$) δ = 14.10 (*C*H$_3$), 22.67 (*C*H$_2$), 25.37 (Se*C*H$_2$CH$_2$), 25.88 (CH*C*H$_2$Se), 28.24 (C(*C*H$_3$)$_3$), 29.13 (*C*H$_2$), 29.34 (*C*H$_2$), 29.51 (*C*H$_2$), 29.59 (*C*H$_2$), 29.63 (3x*C*H$_2$), 29.66 (*C*H$_2$), 29.67 (2x*C*H$_2$), 29.81 (*C*H$_2$), 30.51 (*C*H$_2$), 31.90 (*C*H$_2$), 43.54 (*C*H$_2$NH), 54.63

(*C*HCH$_2$Se), 55.95 (*C*H$_3$O), 80.37 (*C*(CH$_3$)$_3$), 110.49 (C$_{Ar}$), 114.32 (C$_{Ar}$), 120.65 (C$_{Ar}$), 129.68 (C$_{Ar}$), 145.10 (C$_{Ar}$), 146.67 (C$_{Ar}$), 155.30 (NH*C*O$_2$), 170.46 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{32}H_{57}N_2O_5Se$: 629.3433; Found 629.3431.



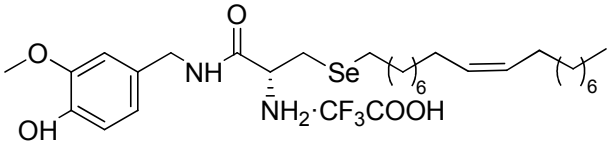
27 59

(2*R*)-Boc-amino-*N*-(4'-hydroxy-3'-methoxybenzyl)-3-(octadec-(9''*Z*)-en-1-ylseleno)propanamide (59): General procedure III was applied to compound **27** (200 mg, 0.25 mmol), $NaBH_4$ (24 mg, 0.62 mmol) and compound **30b** (212 mg, 0.56 mmol) dissolved in EtOH (2 mL). Compound **59** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a yellow oil (287 mg, 88%). R_f=0.66 (petroleum ether/EtOAc 7:3). $[\alpha]_D^{20}$= -4.90 (c 1.4, DCM). IR (ATR) ν = 3444, 3337, 2919, 2850, 1676, 1511 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.16 – 1.39 (m, 22H, C*H*$_2$), 1.42 (s, 9H, C(C*H*$_3$)$_3$), 1.57 – 1.68 (m, 2H, SeCH$_2$C*H*$_2$), 2.01 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.44 – 2.70 (m, 2H, SeC*H*$_2$CH$_2$), 2.83 (dd, 1H, *J* = 12.8, 6.9 Hz, CHC*H*$_2$Se), 3.05 (dd, 1H, *J* = 12.8, 5.2 Hz, CHC*H*$_2$Se), 3.88 (s, 3H, C*H*$_3$O), 4.26 – 4.35 (m, C*H*CH$_2$Se), 4.37 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.23 – 5.43 (m, 3H, CH=CH, CH$_2$N*H*), 5.60 (s, 1H, O*H*), 6.56 (t, 1H, *J* = 5.5 Hz, N*H*Boc), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.10 (*C*H$_3$), 22.66 (*C*H$_2$), 25.36 (Se*C*H$_2$CH$_2$), 25.90 (CH*C*H$_2$Se), 27.18 (*C*H$_2$CH), 27.20 (CH*C*H$_2$), 28.24 (C(*C*H$_3$)$_3$), 29.11 (*C*H$_2$), 29.23 (*C*H$_2$), 29.30 (2x*C*H$_2$), 29.41 (*C*H$_2$), 29.50 (*C*H$_2$), 29.72 (*C*H$_2$), 29.75 (*C*H$_2$), 29.80 (*C*H$_2$), 30.50 (*C*H$_2$), 31.88 (*C*H$_2$), 43.55 (C*H*$_2$NH), 54.42 (*C*HCH$_2$Se), 55.95 (*C*H$_3$O), 80.57 (*C*(CH$_3$)$_3$), 110.48 (C$_{Ar}$), 114.31 (C$_{Ar}$), 120.66 (C$_{Ar}$), 129.68 (C$_{Ar}$), 129.76 (CH=*C*H), 129.95 (CH=*C*H), 145.10 (C$_{Ar}$), 146.65 (C$_{Ar}$), 155.54 (NH*C*O$_2$), 170.43 (NH*C*O). HR-MS (ESI$^+$): *m/z*: [M+H]$^+$ Calcd. for $C_{34}H_{59}N_2O_5Se$: 655.3589; Found 655.3583.



60

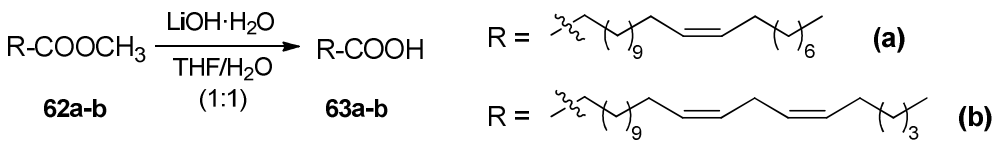
2-(Hexadecylseleno)-1-[*N*-(4'-hydroxy-3'-methoxybenzyl)carbamoyl]-(1*R*)-ethylammonium trifluoroacetate (60): General procedure IX was applied to compound **58** (200 mg, 0.32 mmol), TFA (240 µL, 3.2 mmol) in DCM (1 mL). Compound **60** was afforded after flushing nitrogen and drying *in vacuo* as a yellow oil (201 mg, quantitative). $[\alpha]_D^{20}$= 0.65 (c 1.4, MeOH). IR (ATR) ν = 3425, 3316, 2916, 2849, 1658, 1187 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.20 – 1.34 (m, 26H, C*H*$_2$), 1.53 – 1.61 (m, 2H, SeCH$_2$C*H*$_2$), 2.55 (t, 2H, *J* = 6.9 Hz, SeC*H*$_2$CH$_2$), 2.85 – 3.01 (m, 2H, CHC*H*$_2$Se), 3.82 (s, 3H, C*H*$_3$O), 4.21 – 4.37 (m, 3H, C*H*CH$_2$Se, C*H*$_2$NH), 6.73 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$), 7.53 (t, 1H, *J* = 5.0 Hz, CH$_2$N*H*), 7.98 (br s, 1H, OH), 9.42 (br s, 2H, N*H*$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.25 (*C*H$_3$), 22.84 (*C*H$_2$), 23.51 (CH*C*H$_2$Se), 25.89 (*C*H$_2$), 27.72 (*C*H$_2$), 29.22 (*C*H$_2$), 29.51 (*C*H$_2$), 29.64 (*C*H$_2$), 29.73 (*C*H$_2$), 29.80 (*C*H$_2$), 29.81 (*C*H$_2$), 29.83 (*C*H$_2$), 29.85 (3x*C*H$_2$), 30.19 (*C*H$_2$), 32.08 (*C*H$_2$), 44.50 (*C*H$_2$NH), 53.54 (*C*HCH$_2$Se), 55.94 (*C*H$_3$O), 110.72 (C$_{Ar}$), 114.77 (C$_{Ar}$), 116.78 (*C*F$_3$COOH), 120.96 (C$_{Ar}$), 128.09 (C$_{Ar}$), 145.43 (C$_{Ar}$), 146.96 (C$_{Ar}$), 160.81 – 162.0 (CF$_3$*C*OOH), 167.72 (NHCO). HR-MS (ESI$^+$): *m/z*: $[M+H]^+$ Calcd. for $C_{27}H_{49}N_2O_3Se$: 529.2903; Found 529.2905.



61

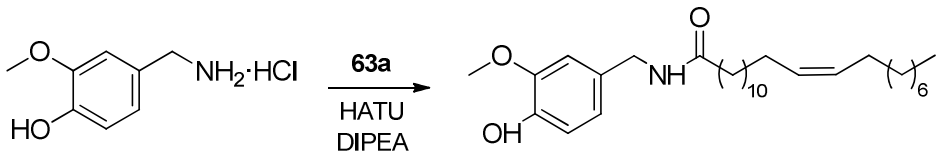
1-[*N*-(4'-Hydroxy-3'-methoxybenzyl)carbamoyl]-2-(octadec-(9''*Z*)-en-1-ylseleno)-(1*R*)-ethylammonium trifluoroacetate (61): General procedure IX was applied to compound **59** (200 mg, 0.30 mmol), TFA (230 µL, 3 mmol) in DCM (1 mL). Compound **61** was afforded after flushing

nitrogen and drying *in vacuo* as a yellow oil (199 mg, quantitative). $[\alpha]_D^{20}$= -2.58 (c 0.3, DCM). IR (ATR) ν = 2922, 2853, 1666, 1199 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.22 – 1.34 (m, 22H, C*H*$_2$), 1.51 – 1.61 (m, 2H, SeCH$_2$C*H*$_2$), 2.00 (q, 4H, *J* = 6.4 Hz,, C*H*$_2$CH, CHC*H*$_2$), 2.54 (t, 2H, *J* = 6.9 Hz, SeC*H*$_2$CH$_2$), 2.93 (d, 2H, *J* = 6.4 Hz, CHC*H*$_2$Se), 3.81 (s, 3H, C*H*$_3$O), 4.17 – 4.34 (m, 3H, C*H*CH$_2$Se, C*H*$_2$NH), 5.28 – 5.42 (m, 2H, C*H*=C*H*), 6.72 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$), 7.64 (t, 1H, *J* = 5.5 Hz, CH$_2$N*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.26 (*C*H$_3$), 22.83 (*C*H$_2$), 23.56 (CH*C*H$_2$Se), 25.89 (Se*C*H$_2$CH$_2$), 27.37 (*C*H$_2$CH, CH*C*H$_2$), 29.24 (*C*H$_2$), 29.42 (*C*H$_2$), 29.47 (*C*H$_2$), 29.47 (*C*H$_2$), 29.59 (*C*H$_2$), 29.68 (*C*H$_2$), 29.87 (*C*H$_2$), 29.91 (*C*H$_2$), 29.92 (*C*H$_2$), 30.26 (*C*H$_2$), 32.06 (*C*H$_2$), 44.17 (*C*H$_2$NH), 53.40 (*C*HCH$_2$Se), 56.00 (*C*H$_3$O), 110.73 (C$_{Ar}$), 114.68 (C$_{Ar}$), 120.87 (C$_{Ar}$), 128.61 (C$_{Ar}$), 129.90 (*C*H=CH), 130.12 (CH=*C*H), 145.38 (C$_{Ar}$), 146.93 (C$_{Ar}$), 167.76 (NH*C*O). HR-MS (ESI^+): *m/z*: $[M+H]^+$ Calcd. for $C_{29}H_{51}N_2O_3Se$: 555.3059; Found 555.3067.



(13*Z*)-Docosenoic acid (63a): General procedure II was applied to a solution of methyl (13*Z*)-docosenoate **62a** (500 µL, 1.23 mmol) dissolved in THF/H_2O (6 mL, 1:1) and LiOH·H_2O (155 mg, 3.70 mmol) to yield compound **63a** as a white solid (360 mg, 86 %). mp: 30-32 ºC. IR (ATR) ν = 2916, 2849, 1691, 1471 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.17 – 1.39 (m, 28H, C*H*$_2$), 1.58 – 1.70 (m, 2H, OHCOCH$_2$C*H*$_2$), 2.02 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.34 (t, 2H, *J* = 6.9 Hz, OHCOC*H*$_2$CH$_2$), 5.24 – 5.42 (m, 2H, C*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.09 (*C*H$_3$), 22.67 (*C*H$_2$), 24.67 (OHCOCH$_2$*C*H$_2$), 27.20 (*C*H$_2$CH, CH*C*H$_2$), 29.05 (*C*H$_2$), 29.23 (*C*H$_2$), 29.30 (*C*H$_2$), 29.31 (2x*C*H$_2$), 29.42 (*C*H$_2$), 29.51 (*C*H$_2$), 29.53 (*C*H$_2$), 29.57 (*C*H$_2$), 29.59 (*C*H$_2$), 29.76 (2x*C*H$_2$), 31.90 (*C*H$_2$), 34.01 (OHCO*C*H$_2$CH$_2$), 129.86 (CH=*C*H), 129.89 (*C*H=CH), 179.89 (OH*C*OCH$_2$CH$_2$).

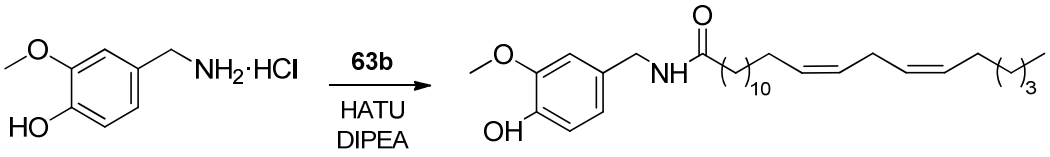
(13*Z*,16*Z*)-Docosadienoic acid (63b): General procedure II was applied to a solution of methyl (13*Z*,16*Z*)-docosadienoate **62b** (25 µL, 0.07 mmol) in THF/H_2O (1 mL, 1:1) and LiOH·H_2O (9 mg, 0.21 mmol) to yield compound **63b**[20] as a sticky solid (23 mg, quantitative). IR (ATR) ν = 2922, 2853, 1708, 1458 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, CH_3), 1.17 – 1.45 (m, 22H, CH_2), 1.53 – 1.72 (m, 2H, $COCH_2CH_2$), 2.05 (q, 4H, *J* = 6.4 Hz, CH_2CH, $CHCH_2$), 2.34 (t, 2H, *J* = 6.9 Hz, $COCH_2CH_2$), 2.77 (t, 2H, *J* = 6.9 Hz, $CHCH_2CH$), 5.24 – 5.44 (m, 4H, 2xC*H*=C*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.07 (CH_3), 22.58 (CH_2), 24.68 ($OHCOCH_2CH_2$), 25.63 ($CHCH_2CH$), 27.20 (CH_2CH), 27.24 ($CHCH_2$), 29.07 (CH_2), 29.24 (CH_2), 29.32 (CH_2), 29.36 (CH_2), 29.43 (CH_2), 29.54 (CH_2), 29.58 (CH_2), 29.60 (CH_2), 29.68 (CH_2), 31.53 (CH_2), 34.05 ($OHCOCH_2CH_2$), 127.94 (2xCH=CH), 130.17 (2xCH=CH), 179.96 ($OHCOCH_2CH_2$).



3 **64**

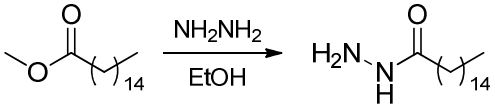
***N*-(4'-Hydroxy-3'-methoxybenzyl)docosa-(13*Z*)-enamide (64):** General procedure I was applied to a solution of compound **63a** (200 mg, 0.59 mmol) in anhydrous DMF (5 mL), amine hydrochloride salt **3** (123 mg, 0.65 mmol), DIPEA (309 µL, 1.77 mmol) and HATU (337 mg, 0.88 mmol). Compound **64** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a sticky solid (179 mg, 64%). R_f=0.42 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 3489, 3315, 3304, 2918, 2849, 1648, 1465 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, CH_3), 1.23 – 1.36 (m, 28H, CH_2), 1.59 – 1.69 (m, 2H, $COCH_2CH_2$), 2.01 (q, 4H, *J* = 6.4 Hz, CH_2CH, $CHCH_2$), 2.19 (t, 2H, *J* = 6.9 Hz, $COCH_2CH_2$), 3.87 (s, 3H, CH_3O), 4.34 (d, 2H, *J* = 5.7 Hz, CH_2NH), 5.29 – 5.39 (m, 2H, C*H*=C*H*), 5.69 (s, 2H, OH, CH_2NH), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.25 (CH_3), 22.82 (CH_2), 25.94 ($COCH_2CH_2$), 27.35 (CH_2CH, $CHCH_2$), 29.46 (3xCH_2), 29.50 (CH_2), 29.66 (2xCH_2), 29.69 (CH_2), 29.75 (2xCH_2), 29.83 (CH_2), 29.91 (CH_2), 29.92 (CH_2), 32.04 (CH_2),

37.00 (CO*C*H$_2$CH$_2$), 43.66 (C*H*$_2$NH), 56.05 (C*H*$_3$O), 110.82 (C$_{Ar}$), 114.50 (C$_{Ar}$), 120.92 (C$_{Ar}$), 130.00 (CH=*C*H), 130.04 (*C*H=CH), 130.51 (C$_{Ar}$), 145.26 (C$_{Ar}$), 146.83 (C$_{Ar}$), 173.04 (*C*OCH$_2$CH$_2$). HR-MS (ESI$^+$): *m/z*: [M+Na]$^+$ Calcd. for $C_{30}H_{51}NO_3Na$: 496.3767; Found 496.3756.



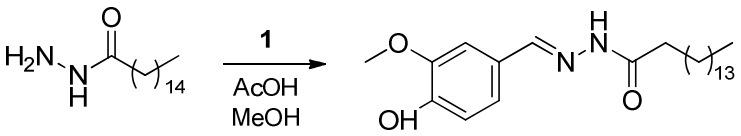
3 **65**

***N*-(4'-Hydroxy-3'-methoxybenzyl) docosa-(13*Z*,16*Z*)-dienamide (65):** General procedure I was applied to a solution of compound **63b** (23 mg, 0.07 mmol) dissolved in DMF (1 mL), amine hydrochloride salt **3** (15 mg, 0.08 mmol), DIPEA (38 µL, 0.21 mmol), and HATU (39 mg, 0.10 mmol). Compound **65** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a sticky oil (21 mg, 63%). R_f=0.40 (petroleum ether/EtOAc 5:5). IR (ATR) ν = 3489, 3316, 3302, 2919, 2849, 1639, 1518 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.89 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.24 – 1.38 (m, 22H, C*H*$_2$), 1.59 – 1.70 (m, 2H, COCH$_2$C*H*$_2$), 2.05 (q, 4H, *J* = 6.4 Hz, C*H*$_2$CH, CHC*H*$_2$), 2.19 (t, 2H, *J* = 6.9 Hz, COC*H*$_2$CH$_2$), 2.77 (t, 2H, *J* = 6.9 Hz, CHC*H*$_2$CH), 3.87 (s, 3H, C*H*$_3$O), 4.35 (d, 2H, *J* = 5.7 Hz, C*H*$_2$NH), 5.28 – 5.43 (m, 4H, 2xC*H*=C*H*), 5.59 – 5.72 (m, 2H, O*H*, CH$_2$N*H*), 6.79 (ddd, 3H, *J* = 12.5, 9.9, 5.0 Hz, H$_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.22 (*C*H$_3$), 22.72 (*C*H$_2$), 25.78 (CH*C*H$_2$CH), 25.94 (COCH$_2$*C*H$_2$), 27.35 (*C*H$_2$CH), 27.39 (CH*C*H$_2$), 29.48 (2x*C*H$_2$), 29.50 (2x*C*H$_2$), 29.65 (*C*H$_2$), 29.70 (*C*H$_2$), 29.75 (2x*C*H$_2$), 29.83 (*C*H$_2$), 31.68 (*C*H$_2$), 37.03 (CO*C*H$_2$CH$_2$), 43.68 (*C*H$_2$NH), 56.08 (*C*H$_3$O), 110.82 (C$_{Ar}$), 114.49 (C$_{Ar}$), 120.95 (C$_{Ar}$), 128.09 (2x*C*H=CH), 130.31 (CH=*C*H), 130.34 (CH=*C*H), 130.53 (C$_{Ar}$), 145.26 (C$_{Ar}$), 146.82 (C$_{Ar}$), 173.05 (*C*OCH$_2$CH$_2$). HR-MS (ESI$^+$): *m/z*: [M+Na]$^+$ Calcd. for $C_{30}H_{49}NO_3Na$: 494.3610; Found 494.3606.



28a **66**

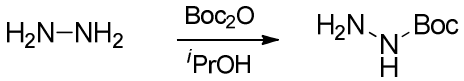
Hexadecanohydrazide (66): To a suspension of methyl palmitate **28a** (1 g, 3.69 mmol) in ethanol (20 mL), hydrazyne hydrate (64%, 370 µL, 7.38 mmol, 2 eq.) was added. Then, the mixture was heated at 150 ºC for 3 h. The mixture was cooled and the solid precipitated was recovered by filtration to yield compound **66**[21] as a white solid (800 mg, 80%). mp: 110-111 ºC. IR (ATR) ν = 3315, 3288, 3199, 2956, 2917, 2848, 1627, 1535 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.06 – 1.42 (m, 24H, C*H*$_2$), 1.55 – 1.74 (m 2H, NHCOCH$_2$C*H*$_2$), 2.08 – 2.23 (m, 2H, NHCOC*H*$_2$CH$_2$), 3.89 (br s, 2H, N*H*$_2$NH), 6.66 (s, 1H, NH$_2$N*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.10 (*C*H$_3$), 22.67 (CH$_2$), 25.48 (NHCOCH$_2$*C*H$_2$), 29.25 (*C*H$_2$), 29.27 (*C*H$_2$), 29.34 (*C*H$_2$), 29.44 (*C*H$_2$), 29.57 (*C*H$_2$), 29.62 (*C*H$_2$), 29.63 (*C*H$_2$), 29.64 (*C*H$_2$), 29.66 (*C*H$_2$), 29.67 (*C*H$_2$), 31.90 (*C*H$_2$), 34.59 (NHCO*C*H$_2$CH$_2$), 173.97 (NH*C*OCH$_2$).



66 **67**

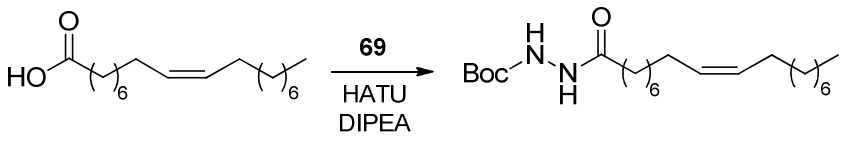
***N*'-(4'-Hydroxy-3'-methoxybenzylidene)hexadecanohydrazide (67):** General procedure X was applied to compound **66** (280 mg, 1.03 mmol), vanillin **1** (157 mg, 1.03 mmol), AcOH (60 µL, 1.03 mmol) in MeOH (30 mL). Compound **67** was afforded as a white solid (242 mg, 58%) after recrystallization from hot MeOH. The ^1H NMR analysis confirmed the presence of the *cis* isomer of the imine as the minor product. mp: 109-110 ºC. IR (ATR) ν = 3202, 3054, 2917, 2849, 1659, 1510 cm^{-1}. *Trans isomer*: ^1H NMR (400 MHz, $CDCl_3$) δ = 0.88 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.23 – 1.42 (m, 24H, C*H*$_2$), 1.69 – 1.78 (m, 2H, NHCOCH$_2$C*H*$_2$), 2.74 (t, 2H, *J* = 6.9 Hz, NHCOC*H*$_2$CH$_2$), 3.95 (s, 3H, C*H*$_3$O), 5.86 (s, 1H, O*H*), 6.93 (d, 1H, *J* = 8.2 Hz, H$_{Ar}$), 7.09 (dd, 1H, *J* = 8.2, 1.8 Hz, H$_{Ar}$), 7.25 (d, 1H, *J* = 1.8 Hz, H$_{Ar}$), 7.65 (s, 1H, *H*C=NNH), 9.02 (s, 1H, N*H*CO). *Cis isomer*: ^1H NMR (400 MHz, $CDCl_3$) δ = 2.28 (t, 2H, *J* = 6.9 Hz, NHCOC*H*$_2$CH$_2$), 3.94 (s, 1H, CH$_3$OH), 5.91 (br s, 1H, O*H*), 6.89 (d, 1H, *J* = 8.2 Hz, H$_{Ar}$), 6.98 (dd, 1H, *J* = 8.2, 1.8 Hz, H$_{Ar}$), 7.49 (br s, 1H, H$_{Ar}$), 8.00 (s, 1H,

*H*C=NNH), 8.46 (s, 1H, N*H*CO). The rest of signals are common to *trans* isomer. *Trans isomer*: ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.27 (*C*H_3), 22.85 (NHCOCH$_2$*C*H$_2$), 24.97 (*C*H$_2$), 29.51 (*C*H$_2$), 29.59 (*C*H$_2$), 29.64 (*C*H$_2$), 29.72 (*C*H$_2$), 29.81 (2x*C*H$_2$), 29.85 (4x*C*H$_2$), 32.08 (*C*H$_2$), 32.96 (NHCO*C*H$_2$CH$_2$), 56.09 (*C*H$_3$O), 107.97 (C$_{Ar}$), 114.61 (C$_{Ar}$), 122.37 (C$_{Ar}$), 126.49 (C$_{Ar}$), 143.20 (H*C*=NNH), 147.07 (C$_{Ar}$), 147.90 (C$_{Ar}$), 176.00 (NH*C*O). *Cis isomer*: ^{13}C NMR (101 MHz, $CDCl_3$) δ = 56.38 (*C*H$_3$O), 107.86 (C$_{Ar}$), 114.13 (C$_{Ar}$), 123.80 (C$_{Ar}$), 126.20 (C$_{Ar}$). The rest of signals are common to *trans* isomer. HR-MS (ESI$^+$): *m/z*: [M+Na]$^+$ Calcd. for $C_{48}H_{80}N_4O_6Na$: 831.5976; Found 831.5968.



68 **69**

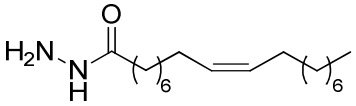
***tert*-Butyl hydrazinecarboxylate (69):** Hydrazyne hydrate **68** (64%, 1.52 mL, 31.2 mmol) was mixed with isopropanol (3 mL) at 0 ºC. Then, a solution of Boc_2O (6.8 g, 31.2 mmol, 1 eq.) in isopropanol (6 mL) was added dropwise. The reaction mixture turned cloudy upon addition and was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in DCM, washed with 1M HCl and brine. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was recrystallized from hexane to yield compound **69**[22] as a white solid (1.94 g , 47%). mp: 38-40 ºC. IR (ATR) ν = 3374, 3324, 2981, 1692, 1627, 1502 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.44 (s, 9H, C(C*H*$_3$)$_3$), 3.57 (s, 2H, N*H*$_2$) 6.00 (s, 1H, N*H*CO). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 28.28 (C(*C*H$_3$)$_3$), 80.42 (*C*(CH$_3$)$_3$), 158.22 (*C*OO).



70 **71**

***N'-(tert*-Butyloxycarbonyl)-octadec-(9*Z*)-enohydrazide (70):** General procedure I was applied to a solution of oleic acid **70** (1 g, 3.54 mmol) dissolved in DMF (30 mL), compound **69** (524 mg, 3.96

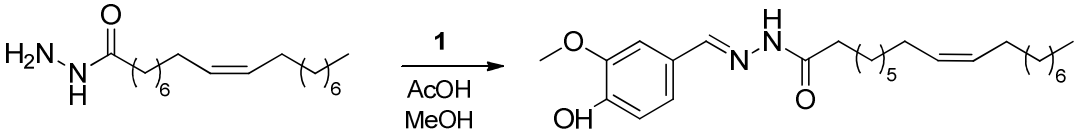
mmol), DIPEA (1.85 mL, 10.62 mmol) and HATU (2.02 g, 5.31 mmol). Compound **71**[23] was afforded after silica gel column chromatography (petroleum ether/EtOAc 7:3) as a yellow oil (1.32 g, 94%). R_f=0.47 (petroleum ether/EtOAc 6:4). IR (ATR) ν = 3280, 2924, 2854, 1729, 1673, 1242 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.86 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.16 – 1.40 (m, 20H, C*H*$_2$) 1.44 (s, 9H, C(C*H*$_3$)$_3$), 1.57 – 1.74 (m, 2H, NHCOCH$_2$C*H*$_2$), 1.90 – 2.07 (m, 4H, C*H*$_2$CH, CHC*H*$_2$), 2.11 – 2.28 (m, 2H, NHCOC*H*$_2$CH$_2$), 5.22 – 5.43 (m, 2H, C*H*=C*H*), 6.85 (s, 1H, N*H*NH), 8.06 (s, 1H, NHN*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.07 (*C*H$_3$), 22.64 (*C*H$_2$), 25.25 (NHCOCH$_2$*C*H$_2$), 27.14 (*C*H$_2$CH), 27.18 (CH*C*H$_2$), 28.11 (C(*C*H$_3$)$_3$), 29.08 (*C*H$_2$), 29.17 (*C*H$_2$), 29.19 (*C*H$_2$), 29.27 (*C*H$_2$), 29.29 (*C*H$_2$), 29.48 (*C*H$_2$), 29.67 (*C*H$_2$), 29.72 (*C*H$_2$), 31.86 (*C*H$_2$), 33.97 (NHCO*C*H$_2$CH$_2$), 81.66 (*C*(CH$_3$)$_3$), 129.68 (CH=*C*H), 129.93 (*C*H=CH), 155.85 (*C*OC(CH$_3$)$_3$), 172.80 (NH*C*OCH$_2$).



72

Oleylhydrazine (72): To a solution of compound **71** (1 g, 2.52 mmol) in DCM (3 mL), TFA (1.93 mL, 25.2 mmol, 10 eq.) was added. The mixture stirred for 2 h at room temperature. Then, the solvent was partially evaporated. Water was added and the pH was adjusted to 7 with saturated solution of $NaHCO_3$. The aqueous phase was extracted with DCM and the organic solution was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to yield the compound **72** as a yellow solid (687 mg, 92%). mp: 109-110 ºC. IR (ATR) ν = 3316, 3214, 2919, 2849, 1628, 1596 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.12 – 1.42 (m, 20H, C*H*$_2$) 1.53 – 1.74 (m, 2H, NHCOCH$_2$C*H*$_2$), 1.88 – 2.05 (m, 4H, C*H*$_2$CH, CHC*H*$_2$), 2.08 – 2.24 (m, 2H, NHCOC*H*$_2$CH$_2$), 3.97 (s, 2H, *H*$_2$N), 5.20 – 5.43 (m, 2H, C*H*=C*H*), 6.84 (s, 1H, N*H*). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.08 (*C*H$_3$), 22.65 (*C*H$_2$), 25.46 (NHCOCH$_2$*C*H$_2$), 27.13 (*C*H$_2$CH), 27.19 (CH*C*H$_2$), 29.07 (*C*H$_2$), 29.18 (*C*H$_2$), 29.22 (*C*H$_2$), 29.29 (2x*C*H$_2$), 29.49 (*C*H$_2$), 29.66 (*C*H$_2$), 29.73 (*C*H$_2$), 31.87 (*C*H$_2$), 34.55

(NHCO*C*H$_2$CH$_2$), 129.67 (CH=*C*H), 129.99 (*C*H=CH), 173.98 (NH*C*OCH$_2$).



72 **73**

***N*'-(4'-Hydroxy-3'-methoxybenzylidene)-octadec-(9*Z*)-enohydrazide (73):** General procedure X was applied to compound **72** (300 mg, 1.01 mmol), vanillin **1** (153 mg, 1.01 mmol), AcOH (60 µL, 1.01 mmol) in MeOH (30 mL). Compound **73** was afforded after silica gel column chromatography (petroleum ether/EtOAc 6:4) as a colourless oil (1.32 g, 94%). The ^{1}H NMR analysis confirmed the presence of the *cis* isomer of the imine as a minor product. IR (ATR) ν = 3452, 3194, 2921, 2852, 1650, 1211 cm^{-1}. *Trans isomer*: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.87 (t, 3H, *J* = 6.9 Hz, C*H*$_3$), 1.22 – 1.43 (m, 20H, C*H*$_2$), 1.69 – 1.78 (m, 2H, NHCOCH$_2$C*H*$_2$), 1.94 – 2.07 (m, 4H, C*H*$_2$CH, CHC*H*$_2$), 2.74 (t, 2H, *J* = 6.9 Hz, NHCOC*H*$_2$CH$_2$), 3.95 (s, 3H, C*H*$_3$O), 5.31 – 5.36 (m, 2H, C*H*=C*H*), 5.93 (br s, 1H, O*H*), 6.93 (d, 1H, *J* = 8.2 Hz, H$_{Ar}$), 7.10 (dd, 1H, *J* = 8.2, 1.8 Hz, H$_{Ar}$), 7.25 (d, 1H, *J* = 1.8 Hz, H$_{Ar}$), 7.69 (s, 1H, *H*C=NNH), 9.43 (s, 1H, N*H*CO). *Cis isomer*: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.28 (t, 2H, *J* = 6.9 Hz, NHCOC*H*$_2$CH$_2$), 3.93 (s, 1H, CH_3OH), 5.36 – 5.39 (m, 2H, C*H*=C*H*), 5.97 (br s, 1H, O*H*), 6.89 (d, 1H, *J* = 8.2 Hz, H$_{Ar}$), 6.97 (dd, 1H, *J* = 8.2, 1.8 Hz, H$_{Ar}$), 7.49 (d, 1H, *J* = 1.8 Hz, H$_{Ar}$), 8.00 (s, 1H, *H*C=NNH), 8.62 (s, 1H, N*H*CO). The rest of signals are common to *trans* isomer. *Trans isomer:* ^{13}C NMR (101 MHz, $CDCl_3$) δ = 14.26 (*C*H$_3$), 22.82 (NHCOCH$_2$*C*H$_2$), 25.00 (*C*H$_2$), 27.34 (*C*H$_2$CH), 27.36 (CH*C*H$_2$), 29.35 (*C*H$_2$), 29.46 (*C*H$_2$), 29.46 (*C*H$_2$), 29.49 (*C*H$_2$), 29.61 (*C*H$_2$), 29.66 (*C*H$_2$), 29.84 (*C*H$_2$), 29.91 (*C*H$_2$), 32.04 (*C*H$_2$), 32.94 (NHCO*C*H$_2$CH$_2$), 56.08 (*C*H$_3$O), 108.06 (C$_{Ar}$), 114.63 (C$_{Ar}$), 122.32 (C$_{Ar}$), 126.54 (C$_{Ar}$), 129.88 (CH=*C*H), 130.13 (*C*H=CH), 143.54 (H*C*=NNH), 147.06 (C$_{Ar}$), 147.89 (C$_{Ar}$), 176.30 (NH*C*O). *Cis isomer*: ^{13}C NMR (101 MHz, $CDCl_3$) δ = 56.35 (*C*H$_3$O), 107.87 (C$_{Ar}$), 114.11 (C$_{Ar}$), 123.79 (C$_{Ar}$), 126.16 (C$_{Ar}$), 147.24 (C$_{Ar}$), 147.73 (C$_{Ar}$). The rest of signals are common to *trans* isomer. HR-MS (ESI$^+$): *m/z*: [M+Na]$^+$ Calcd. for $C_{52}H_{84}N_4O_6Na$: 883.6289; Found

883.6286.

4.3 TRP channels assays.

Assays of TRP-mediated elevation of $[Ca^{2+}]_i$ were performed as previously described.[60] HEK-293 (human embryonic kidney) cells wild-type or stably over-expressing recombinant human TRPV1 or rat TRPV2 were grown on 100 mm diameter Petri dishes as mono layers in Eagle's Minimum Essential Medium (EMEM) supplemented with 1% non-essential amino acids, 10% foetal bovine serum (FBS), 50 U/mL penicillin plus 50 µg/mL streptomycin and 2 mM glutamine, maintained under 5% CO_2 at 37°C and only for the over-expressing cells selected by G-418 (Geneticin, 600 mg mL^{-1}; Thermo-Fisher Scientific). On the day of the experiment, the cells were loaded for 1 h at 25 °C with the Ca^{2+} indicator Fluo-4-AM (Thermo-Fisher Scientific) 4 µM in DMSO containing 0.02% Pluronic F-127 (Thermo-Fisher Scientific) in EMEM without FBS. After loading, cells were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose and 10 mM HEPES, pH 7.4) resuspended in the same buffer, and transferred, about 100,000 cells for each determination, to the quartz cuvette of the spectrofluorimeter (λ_{ex} = 488 nm; λ_{em} = 516 nm) Perkin-Elmer LS50B equipped with PTP-1 Fluorescence Peltier System (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring at 25 °C. Experiments were carried by measuring cell fluorescence before and after the addition of test compounds at various concentrations. The values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e. not transfected with any TRP construct) HEK-293 cells were taken as baselines. Potency (EC_{50} values) was determined as the concentration of test compounds exerting a half-maximal agonist effect (i.e. half-maximal increases in $[Ca^{2+}]_i$). The efficacy of the agonists was determined by comparing their effect to the maximal effect on $[Ca^{2+}]_i$ observed with 4 µM ionomycin. Antagonist/desensitizing behaviour was evaluated against the agonist capsaicin 0.1 µM (Sigma-Aldrich) for TRPV1 and the agonists lysophosphatidylcholine (LPC) (Sigma-Aldrich) 3 µM and cannabidiol (CBD) 2 µM (a kind gift by GW Pharmaceuticals) for TRPV2 by adding the test

compounds in the quartz cuvette 5 min before stimulation of cells with the agonist. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]_i$ elevation (IC_{50}). Concentration–response curves were fitted by a sigmoidal regression with variable slope. Curve fitting and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by Bonferroni's test.

Ancillary Information

Supporting Information: Tables S1 and S2 of TRPV1 activity; copies of ^{1}H, ^{13}C NMR spectra; Molecular Formula Strings.

Author Contributions: A.S.M., S.L.C. and O.N.F. contributed equally to the work.

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Abbreviations Used: Transient Receptor Potential Vanilloid 2 (TRPV2); Transient Receptor Potential Vanilloid 1 (TRPV1); Ethanolamide (EA); Lysophosphatidylcoline (LPC); Cannabidiol (CBD); Palmitoyl Ethanolamide (PEA); Palmitoleoyl Ethanolamide (POEA); Oleoyl Ethanolamide (OEA);

Lynoleoyl Ethanolamide (LEA); Arachidonoylethanolamide (AEA); Eicosapentaenoyl Ethanolamide (EPEA); Docosahexaenoyl Ethanolamide (DHEA); Palmitamide (PA); Stearamide (SA); Oleamide (OA); Linoleamide (LA); Erucamide (ErA);

References

(1) Iwata, Y.; Katanosaka, Y.; Arai, Y.; Komamura, K.; Miyatake, K.; Shigekawa, M. A Novel Mechanism of Myocyte Degeneration Involving the Ca(2+)-Permeable Growth Factor–regulated Channel. *J. Cell Biol.* **2003**, *161* (5), 957–967.

(2) Iwata, Y.; Katanosaka, Y.; Arai, Y.; Shigekawa, M.; Wakabayashi, S. Dominant-Negative Inhibition of Ca2+ Influx via TRPV2 Ameliorates Muscular Dystrophy in Animal Models. *Hum. Mol. Genet.* **2009**, *18* (5), 824–834.

(3) Nadège, Z.; Yuko, I.; Olivier, S.; Jean, L.; Shigeo, W.; Philippe, G. Essential Role of TRPV2 Ion Channel in the Sensitivity of Dystrophic Muscle to Eccentric Contractions. *FEBS Lett.* **2009**, *583* (22), 3600–3604.

(4) Aguettaz, E.; Bois, P.; Cognard, C.; Sebille, S. Stretch-Activated TRPV2 Channels: Role in Mediating Cardiopathies. *Prog. Biophys. Mol. Biol.* **2017**, *130*, 273–280.

(5) Wouter, E.; Thomas, G.; Bernd, N.; Dirk, D. R. On the Origin of Bladder Sensing: Tr(i)Ps in Urology. *Neurourol. Urodyn.* **2008**, *27* (4), 264–273.

(6) Nilius, B.; Owsianik, G.; Voets, T.; Peters, J. A. Transient Receptor Potential Cation Channels in Disease. *Physiol. Rev.* **2007**, *87* (1), 165–217.

(7) Prevarskaya, N.; Zhang, L.; Barritt, G. TRP Channels in Cancer. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2007**, *1772* (8), 937–946.

(8) Lehen′kyi, V.; Prevarskaya, N. Study of TRP Channels in Cancer Cells. In *TRP Channels*; Zhu, M. X., Ed.; CRC Press/Taylor & Francis: Boca Raton (FL), 2011; pp 375–394.

(9) Santoni, G.; Farfariello, V. TRP Channels and Cancer: New Targets for Diagnosis and Chemotherapy. *Endocrine, Metab. Immune Disord. - Drug Targets* **2011**, *11* (1), 54–67.

(10) Caprodossi, S.; Lucciarini, R.; Amantini, C.; Nabissi, M.; Canesin, G.; Ballarini, P.; Di Spilimbergo, A.; Cardarelli, M. A.; Servi, L.; Mammana, G.; et al. Transient Receptor Potential Vanilloid Type 2 (TRPV2) Expression in Normal Urothelium and in Urothelial Carcinoma of Human Bladder: Correlation with the Pathologic Stage. *Eur. Urol.* **2008**, *54* (3), 612–620.

(11) Monet, M.; Lehen'kyi, V.; Gackiere, F.; Firlej, V.; Vandenberghe, M.; Roudbaraki, M.; Gkika, D.; Pourtier, A.; Bidaux, G.; Slomianny, C.; et al. Role of Cationic Channel TRPV2 in Promoting Prostate Cancer Migration and Progression to Androgen Resistance. *Cancer Res.* **2010**, *70* (3), 1225–1235.

(12) Gkika, D.; Prevarskaya, N. TRP Channels in Prostate Cancer: The Good, the Bad and the Ugly? *Asian J. Androl.* **2011**, *13* (5), 673–676.

(13) Ziglioli, F.; Frattini, A.; Maestroni, U.; Dinale, F. Vanilloid-Mediated Apoptosis in Prostate Cancer Cells through ATRPV-1 Dependent and a TRPV-1-Independent Mechanism. *Acta Bio Medica Atenei Parm.* **2009**, *80* (1), 13–20.

(14) Wang, C.; Hu, H.-Z.; Colton, C. K.; Wood, J. D.; Zhu, M. X. An Alternative Splicing Product of the Murine Trpv1 Gene Dominant Negatively Modulates the Activity of TRPV1 Channels. *J. Biol. Chem.* **2004**, *279* (36), 37423–37430.

(15) Neeper, M. P.; Liu, Y.; Hutchinson, T. L.; Wang, Y.; Flores, C. M.; Qin, N. Activation Properties of Heterologously Expressed Mammalian TRPV2. *J. Biol. Chem.* **2007**, *282* (21), 15894–15902.

(16) Juvin, V.; Penna, A.; Chemin, J.; Lin, Y.-L.; Rassendren, F.-A. Pharmacological Characterization and Molecular Determinants of the Activation of Transient Receptor Potential V2 Channel

Orthologs by 2-Aminoethoxydiphenyl Borate. *Mol. Pharmacol.* **2007**, *72* (5), 1258 LP-1268.

(17) Qin, N.; Neeper, M. P.; Liu, Y.; Hutchinson, T. L.; Lubin, M. Lou; Flores, C. M. TRPV2 Is Activated by Cannabidiol and Mediates CGRP Release in Cultured Rat Dorsal Root Ganglion Neurons. *J. Neurosci.* **2008**, *28* (24), 6231 LP-6238.

(18) De Petrocellis, L.; Ligresti, A.; Moriello, A. S.; Allarà, M.; Bisogno, T.; Petrosino, S.; Stott, C. G.; Di Marzo, V. Effects of Cannabinoids and Cannabinoid-Enriched Cannabis Extracts on TRP Channels and Endocannabinoid Metabolic Enzymes. *Br. J. Pharmacol.* **2011**, *163* (7), 1479–1494.

(19) Bang, S.; Kim, K. Y.; Yoo, S.; Lee, S.-H.; Hwang, S. W. Transient Receptor Potential V2 Expressed in Sensory Neurons Is Activated by Probenecid. *Neurosci. Lett.* **2007**, *425* (2), 120–125.

(20) Rohacs, T. Phosphoinositide Regulation of TRP Channels. In *Mammalian Transient Receptor Potential (TRP) Cation Channels*; Springer: Cham (CH), 2014; pp 1143–1176.

(21) Monet, M.; Gkika, D.; Lehen'kyi, V.; Pourtier, A.; Abeele, F. Vanden; Bidaux, G.; Juvin, V.; Rassendren, F.; Humez, S.; Prevarsakaya, N. Lysophospholipids Stimulate Prostate Cancer Cell Migration via TRPV2 Channel Activation. *Biochim. Biophys. Acta - Mol. Cell Res.* **2009**, *1793* (3), 528–539.

(22) De Petrocellis, L.; Ligresti, A.; Moriello, A. S.; Allarà, M.; Bisogno, T.; Petrosino, S.; Stott, C. G.; Di Marzo, V. Effects of Cannabinoids and Cannabinoid-Enriched Cannabis Extracts on TRP Channels and Endocannabinoid Metabolic Enzymes. *Br. J. Pharmacol.* **2011**, *163* (7), 1479–1494.

(23) Alex, P.; Pau, D.; Rachelle, G. What Do We Know about the Transient Receptor Potential Vanilloid 2 (TRPV2) Ion Channel? *FEBS J.* **2013**, *280* (21), 5471–5487.

(24) Caterina, M. J.; Rosen, T. A.; Tominaga, M.; Brake, A. J.; Julius, D. A Capsaicin-Receptor

Homologue with a High Threshold for Noxious Heat. *Nature* **1999**, *398*, 436.

(25) Alexander, S.; Mathie, A.; Peters, J. Guide to Receptors and Channels (GRAC), 5th Edition. *Br. J. Pharmacol.* **2011**, *164* (s1), S1–S2.

(26) Andreas, L.; Madalina, L. R.; Carla, N.; Peter, R.; Alexandru, B. A High□threshold Heat□activated Channel in Cultured Rat Dorsal Root Ganglion Neurons Resembles TRPV2 and Is Blocked by Gadolinium. *Eur. J. Neurosci.* **2007**, *26* (1), 12–22.

(27) Stotz, S. C.; Vriens, J.; Martyn, D.; Clardy, J.; Clapham, D. E. Citral Sensing by TRANSient Receptor Potential Channels in Dorsal Root Ganglion Neurons. *PLoS One* **2008**, *3* (5), e2082.

(28) Nie, L.; Oishi, Y.; Doi, I.; Shibata, H.; Kojima, I. Inhibition of Proliferation of MCF-7 Breast Cancer Cells by a Blocker of Ca2+-Permeable Channel. *Cell Calcium* **1997**, *22* (2), 75–82.

(29) Hisanaga, E.; Nagasawa, M.; Ueki, K.; Kulkarni, R. N.; Mori, M.; Kojima, I. Regulation of Calcium-Permeable TRPV2 Channel by Insulin in Pancreatic -Cells. *Diabetes* **2009**, *58* (1), 174–184.

(30) Aoyagi, K.; Ohara-Imaizumi, M.; Nishiwaki, C.; Nakamichi, Y.; Nagamatsu, S. Insulin/Phosphoinositide 3-Kinase Pathway Accelerates the Glucose-Induced First-Phase Insulin Secretion through TrpV2 Recruitment in Pancreatic β-Cells. *Biochem. J.* **2010**, *432* (2), 375–386.

(31) Mihara, H.; Boudaka, A.; Shibasaki, K.; Yamanaka, A.; Sugiyama, T.; Tominaga, M. Involvement of TRPV2 Activation in Intestinal Movement through Nitric Oxide Production in Mice. *J. Neurosci.* **2010**, *30* (49), 16536–16544.

(32) Lin, Y.; Sun, Z. Antiaging Gene Klotho Enhances Glucose-Induced Insulin Secretion by Up-Regulating Plasma Membrane Levels of TRPV2 in MIN6 β-Cells. *Endocrinology* **2012**, *153* (7), 3029–3039.

(33) Iwata, Y.; Ohtake, H.; Suzuki, O.; Matsuda, J.; Komamura, K.; Wakabayashi, S. Blockade of

Sarcolemmal TRPV2 Accumulation Inhibits Progression of Dilated Cardiomyopathy. *Cardiovasc. Res.* **2013**, *99* (4), 760–768.

(34) Nadège, Z.; Ludivine, M.; Clarisse, F.; François, S.; Inès, D.; Marie, C.; Olivier, S.; Nicolas, T.; Yuko, I.; Shigeo, W.; et al. Osmosensation in TRPV2 Dominant Negative Expressing Skeletal Muscle Fibres. *J. Physiol.* **2015**, *593* (17), 3849–3863.

(35) Zubcevic, L.; Herzik Jr, M. A.; Chung, B. C.; Liu, Z.; Lander, G. C.; Lee, S.-Y. Cryo-Electron Microscopy Structure of the TRPV2 Ion Channel. *Nat. Struct. &Amp; Mol. Biol.* **2016**, *23*, 180.

(36) Huynh, K. W.; Cohen, M. R.; Jiang, J.; Samanta, A.; Lodowski, D. T.; Zhou, Z. H.; Moiseenkova-Bell, V. Y. Structure of the Full-Length TRPV2 Channel by Cryo-EM. *Nat. Commun.* **2016**, *7*, 11130.

(37) Yang, F.; Vu, S.; Yarov-Yarovoy, V.; Zheng, J. Rational Design and Validation of a Vanilloid-Sensitive TRPV2 Ion Channel. *Proc. Natl. Acad. Sci.* **2016**, *113* (26), E3657–E3666.

(38) Miao, X.; Malacea, R.; Fischmeister, C.; Bruneau, C.; Dixneuf, P. H. Ruthenium-Alkylidene Catalysed Cross-Metathesis of Fatty Acid Derivatives with Acrylonitrile and Methyl Acrylate: A Key Step toward Long-Chain Bifunctional and Amino Acid Compounds. *Green Chem.* **2011**, *13* (10), 2911–2919.

(39) Maletz, R.; Schäfer, H. J.; Quermann, R. Conversion of Unsaturated Fatty Acids — Nucleophilic Additions to Methyl (E)-12-Oxo-10-Octadecenoate [1]. *Lipid/Fett* **1996**, *98* (11), 370–379.

(40) Stewart, I. C.; Bergman, R. G.; Toste, F. D. Phosphine-Catalyzed Hydration and Hydroalkoxylation of Activated Olefins: Use of a Strong Nucleophile to Generate a Strong Base. *J. Am. Chem. Soc.* **2003**, *125* (29), 8696–8697.

(41) Straathof, A. J. J.; Adlercreutz, P. *Applied Biocatalysis*; CRC Press: London, 2000.

(42) Carpino, L. A.; El-Faham, A.; Albericio, F. Racemization Studies during Solid-Phase Peptide Synthesis Using Azabenzotriazole-Based Coupling Reagents. *Tetrahedron Lett.* **1994**, *35* (15),

2279–2282.

(43) Thalmann, A.; Oertle, K.; Gerlach, H. Ricinelaidic Acid Lactone. In *Organic Syntheses*; Major Reference Works; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2003; pp 192–192.

(44) Collins, J. C.; Hess, W. W.; Frank, F. J. Dipyridine-Chromium(VI) Oxide Oxidation of Alcohols in Dichloromethane. *Tetrahedron Lett.* **1968**, *9* (30), 3363–3366.

(45) Müller, J.; Terfort, A. Synthesis of Pure Aromatic, Aliphatic, and Araliphatic Diselenides. *Inorganica Chim. Acta* **2006**, *359* (15), 4821–4827.

(46) Salunke, D. B.; Connelly, S. W.; Shukla, N. M.; Hermanson, A. R.; Fox, L. M.; David, S. A. Design and Development of Stable, Water-Soluble, Human Toll-like Receptor 2 Specific Monoacyl Lipopeptides as Candidate Vaccine Adjuvants. *J. Med. Chem.* **2013**, *56* (14), 5885–5900.

(47) Pedzisa, L.; Li, X.; Rader, C.; Roush, W. R. Assessment of Reagents for Selenocysteine Conjugation and the Stability of Selenocysteine Adducts. *Org. Biomol. Chem.* **2016**, *14* (22), 5141–5147.

(48) López, S. E.; Salazar, J. Trifluoroacetic Acid: Uses and Recent Applications in Organic Synthesis. *J. Fluor. Chem.* **2013**, *156*, 73–100.

(49) Agnihotri, G.; Crall, B. M.; Lewis, T. C.; Day, T. P.; Balakrishna, R.; Warshakoon, H. J.; Malladi, S. S.; David, S. A. Structure–Activity Relationships in Toll-Like Receptor 2-Agonists Leading to Simplified Monoacyl Lipopeptides. *J. Med. Chem.* **2011**, *54* (23), 8148–8160.

(50) Kumar, V.; Sharma, A.; Chander Sharma, P. Synthesis of Some Novel 2,5-Disubstituted Thiazolidinones from a Long Chain Fatty Acid as Possible Anti-Inflammatory, Analgesic and Hydrogen Peroxide Scavenging Agents. *J. Enzyme Inhib. Med. Chem.* **2011**, *26* (2), 198–203.

(51) Yang, F.; Zheng, J. Understand Spiciness: Mechanism of TRPV1 Channel Activation by Capsaicin. *Protein Cell* **2017**, *8* (3), 169–177.

(52) Gao, M.; Li, J.; Nie, C.; Song, B.; Yan, L.; Qian, H. Design, Synthesis and Biological Evaluation of Novel Hydrogen Sulfide Releasing Capsaicin Derivatives. *Bioorg. Med. Chem.* **2018**, *26* (9), 2632–2639.

(53) Walpole, C. S.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure-Activity Studies. 2. The Amide Bond "B-Region". *J. Med. Chem.* **1993**, *36* (16), 2373–2380.

(54) Janusz, J. M.; Buckwalter, B. L.; Young, P. A.; LaHann, T. R.; Farmer, R. W.; Kasting, G. B.; Loomans, M. E.; Kerckaert, G. A.; Maddin, C. S.; Berman, E. F. Vanilloids. 1. Analogs of Capsaicin with Antinociceptive and Antiinflammatory Activity. *J. Med. Chem.* **1993**, *36* (18), 2595–2604.

(55) Lee, J.; Kang, S.-U.; Choi, H.-K.; Lee, J.; Lim, J.-O.; Kil, M.-J.; Jin, M.-K.; Kim, K.-P.; Sung, J.-H.; Chung, S.-J.; et al. Analysis of Structure–activity Relationships for the 'B-Region' of N -(3-Acyloxy-2-Benzylpropyl)- N ′ -[4-(Methylsulfonylamino)Benzyl]Thiourea Analogues as Vanilloid Receptor Antagonists: Discovery of an N -Hydroxythiourea Analogue with Potent Analgesic A. *Bioorg. Med. Chem. Lett.* **2004**, *14* (9), 2291–2297.

(56) Cao, E.; Liao, M.; Cheng, Y.; Julius, D. TRPV1 Structures in Distinct Conformations Reveal Activation Mechanisms. *Nature* **2013**, *504* (7478), 113–118.

(57) Gavva, N. R.; Klionsky, L.; Qu, Y.; Shi, L.; Tamir, R.; Edenson, S.; Zhang, T. J.; Viswanadhan, V. N.; Toth, A.; Pearce, L. V.; et al. Molecular Determinants of Vanilloid Sensitivity in TRPV1. *J. Biol. Chem.* **2004**, *279* (19), 20283–20295.

(58) Shibasaki, K. Physiological Significance of TRPV2 as a Mechanosensor, Thermosensor and Lipid Sensor. *J. Physiol. Sci.* **2016**, *66* (5), 359–365.

(59) Appendino, G.; Minassi, A.; Morello, A. S.; De Petrocellis, L.; Di Marzo, V. N -

Acylvanillamides: Development of an Expeditious Synthesis and Discovery of New Acyl Templates for Powerful Activation of the Vanilloid Receptor. *J. Med. Chem.* **2002**, *45* (17), 3739–3745.

(60) Moriello, A. S.; De Petrocellis, L. Assay of TRPV1 Receptor Signaling. In *Endocannabinoid Signaling: Methods and Protocols*; Press, H., Ed.; Springer: New York, NY, 2016; pp 65–76.

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