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Structure–activity relationships of natural and non-natural amino acid-based amide and 2-oxoamide inhibitors of human phospholipase A_2 enzymes

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ABSTRACT

A variety of 2-oxoamides and related amides based on natural and non-natural amino acids were synthesized. Their activity on two human intracellular phospholipases (GIVA cPLA$_2$ and GVIA iPLA$_2$) and one human secretory phospholipase (GV sPLA$_2$) was evaluated. We show that an amide based on (*R*)-γ-norleucine is a highly selective inhibitor of GV sPLA$_2$.

1. Introduction

The phospholipase A_2 (PLA$_2$) superfamily consists of a broad range of structurally distinct enzymes that catalyze the hydrolysis of the *sn*-2 ester bond of phospholipids. The PLA$_2$ superfamily has been systematically grouped based on the source (i.e. organism) and primary sequence.[1–3] In mammals, some of these enzymes are particularly interesting anti-inflammatory drug targets, in part due to their ability to liberate arachidonic acid for subsequent eicosanoid biosynthesis.[1–4] Specifically in human macrophages, three of these groups are known to play significant roles in immune response: cytosolic Ca^{2+}-dependent PLA$_2$ (GIVA cPLA$_2$), cytosolic Ca^{2+}-independent PLA$_2$ (GVIA iPLA$_2$) and Ca^{2+}-dependent secretory PLA$_2$ (GV sPLA$_2$).

GIVA cPLA$_2$ is an attractive target for drug development since it is the rate-limiting provider of arachidonic acid and lysophospholipids that are subsequently converted into prostaglandins, leukotrienes and PAF, respectively.[5] Various studies on transgenic mice lacking GIVA cPLA$_2$ showed a 90% reduction in the production of prostaglandins and leukotrienes.[6,7] Recently, Kalyvas and David demonstrated that GIVA cPLA$_2$ plays an important role in the pathogenesis of experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis,[8] while it was reported that cytosolic phospholipase A_2-deficient mice were resistant to experimental autoimmune encephalomyelitis.[9] While the exact physiological roles remain in question, multiple studies confirm the primacy of GIVA cPLA$_2$ in lipid mediator production. Consequently, much effort has been focused on the design, synthesis and characterization of GIVA cPLA$_2$ inhibitors as potential agents for experimental exploration as well as treatment of various inflammatory conditions.

The role of the GVIA iPLA$_2$ in the inflammatory process is still unclear, and it has not been a target for the development of novel medicines up to now.[10] This enzyme appears to be the primary PLA$_2$ for basal metabolic functions within the cell.

It has been shown that in macrophages and other cells, GIVA cPLA$_2$ and secretory phospholipase A_2 work together to release arachidonic acid.[11–15] Group GV sPLA$_2$ was identified at the DNA-level in humans,[16] and subsequently expressed and charactized.[17] Several experiments suggested that GV sPLA$_2$ has a role in amplifying the action of GIVA cPLA$_2$ in supplying arachidonic acid for eicosanoid production.[18] In addition, GV sPLA$_2$ has cellular functions, independent of its ability to provide arachidonic acid, that include regulation of phagocytosis and foam cell formation.[19] Thus, this enzyme was recently proposed to have a unique role in atherosclerosis.[19b]

Kokotos and Magrioti recently reviewed many synthetic inhibitors of GIVA cPLA$_2$ and GVIA iPLA$_2$.[20] Since then, new *N*-benzydryl indole inhibitors[21–23] as well as 1-indol-1-yl-propan-2-ones[24] have

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been reported. Our recent efforts are focused on the development of a novel class of GIVA $cPLA_2$ inhibitors, initially designed to contain 2-oxoamide functionality and a free carboxyl group.[25–32] 2-Oxoamides based on γ-aminobutyric acid (**1a**, Fig. 1) and the non-natural amino acids γ-norleucine or δ-norleucine (**1b** and **1c**, Fig. 1) are potent inhibitors of GIVA $cPLA_2$ presenting in-vivo anti-inflammatory and analgesic activity.[26,30] We have also demonstrated that ethyl 4-[(2-oxohexadecanoyl)amino]butanoate (**1d**, Fig. 1) inhibits both GIVA $cPLA_2$ and GVIA $iPLA_2$ and is the first systemically bioavailable compound with a significant affinity for GIVA $cPLA_2$, which produces potent hyperalgesia.[28] In-vivo, intrathecal and systemic administration of inhibitor **1d** blocked carrageenan hyperalgesia.[28] Recently, a large number of lipophilic 2-oxoamides have been tested for inhibition of GV $sPLA_2$.[30] Only one compound (**2**, Fig. 1) significantly inhibited GV $sPLA_2$ with an $X_I(50)$ of 0.035 mole fraction. However, compound **2** inhibited both GIVA $cPLA_2$ and GVIA $iPLA_2$, in addition to GV $sPLA_2$, with no statistical preference.

Known inhibitors of secreted PLA_2 enzyme groups have been reviewed.[33] Among them, non-phospholipid amide inhibitors based on non-natural amino acids, such as compounds **3**[34] and **4**[35] (Fig. 1), presented interesting inhibition of pancreatic and non-pancreatic GI and GII $sPLA_2$. Inhibitor **4** has been reported to protect rat small intestine from ischaemia and reperfusion injury[36] and trinitrobenzene sulfonic acid-induced colitis.[37]

The selective inhibition of the each PLA_2 class is very important to understand enzyme-specific roles in cells and in-vivo. Therefore, we synthesized a variety of 2-oxoamide esters and related amides based on natural and non-natural amino acids and we evaluated their activity on GIVA $cPLA_2$, GV s PLA_2 and GVIA $iPLA_2$. While most of these compounds exhibited inhibitory properties for all three PLA_2 classes, we demonstrate that an amide based on (*R*)-γ-norleucine is a highly selective inhibitor of GV $sPLA_2$.

2. Results

2.1. Design and synthesis of inhibitors

Our previous work has shown that 2-oxoamides containing a free carboxyl group are selective inhibitors of GIVA $cPLA_2$, not affecting GVIA $iPLA_2$ and GV $sPLA_2$.[26,29,30] On the contrary, methyl or ethyl esters of 2-oxoamides may inhibit both GIVA $cPLA_2$ and GVIA $iPLA_2$.[29] In addition, the 2-oxoamide that contains a methyl ester group (**2**) inhibits all the three forms: GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$.[30] In this study, we decided to systematically evaluate: (a) the distance between the ester group and the 2-oxoamide functionality, and (b) the nature of the ester group. For structure-activity relationship (SAR) studies, we evaluated a variety of amino acid and ester substitutions such as glycine, β-alanine, γ-aminobutyric acid, δ-aminovaleric acid, and *p*-aminobenzoic acid as well as methyl, ethyl, and *tert*-butyl esters. The esters of amino acids **5a–l** were coupled with 2-hydroxy-hexadecanoic acid (**6**) using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide as a condensing agent in the presence of 1-hydroxybenzotriazole (Scheme 1). The 2-hydroxyamides **7a–l** were oxidized to target compounds **8a–l** by treatment with Dess–Martin reagent.[38]

Three 2-oxoamide esters based on γ-leucine, γ-norleucine and γ-alanine were prepared starting from the corresponding amino alcohols **9a–c** (Scheme 2) according to procedures we described previously.[26,30] Oxidation by the NaOCl/AcNH-TEMPO method[39,40] followed by Wittig reaction with methyl or *tert*-butyl (triphenylphosphoranylidene)acetate led to α,β-unsaturated esters **10a–c**. Oxidation of compounds **11a–c** by the Dess–Martin method produced the target compounds **12a–c**. To prepare 2-oxoamide esters based on ε-norleucine, Boc-L-norleucinol (**9b**) was oxidized to aldehyde and directly reacted with triethyl phosphonocrotonate[41] (Scheme 3). Catalytic hydrogenation of compound **13**, removal of the Boc group by HCl/Et_2O and coupling with 2-hydroxy-hexadecanoic acid produced compound **16**. The unsaturated 2-hydroxyamide **15** was prepared by similar reaction. Both compounds **15** and **16** were oxidized to the corresponding 2-oxoamides **17** and **18** by the Dess–Martin method. 2-Oxoamide **19** containing a free carboxyl group was also prepared.

Ketone and ether analogues of the in-vivo active inhibitor **1d** were prepared as described in Scheme 4. Treatment of Boc-GABA with *n*-BuLi led to ketone **22** in moderate yield. Etherification of compound **21** under phase-transfer conditions produced ether **23**. Both compounds **22** and **23** were deprotected, coupled and oxidized to obtain the target derivatives **26** and **27**.

The known inhibitors **3** and **4** (Fig. 1) of GI and GII $sPLA_2$ are amides consisting of a 7-phenylheptanoyl acyl residue and a non-natural γ-amino acid bearing a side chain with two aromatic groups. We decided to synthesize 2-oxoamides containing the 7-phenylheptanoyl residue instead of the long aliphatic chain. The synthesis procedure of 2-oxoamides **37a,b** and amides **32a,b**, based on methyl ester of glycine and *tert*-butyl ester of β-alanine, is depicted in Scheme 5.

In addition, we synthesized a γ-norleucine-based amide containing a 7-phenylheptanoyl chain. Boc-D-norleucinol (**38**), prepared according to the literature,[42,43] was oxidized to aldehyde

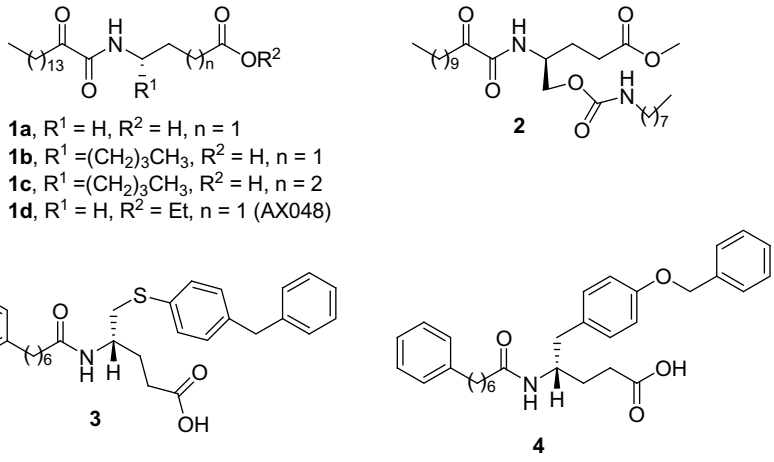
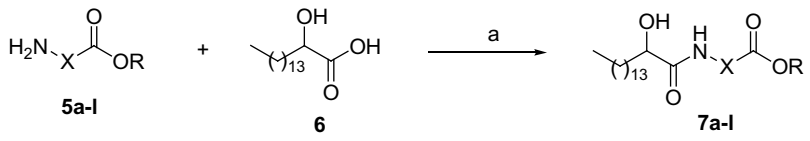
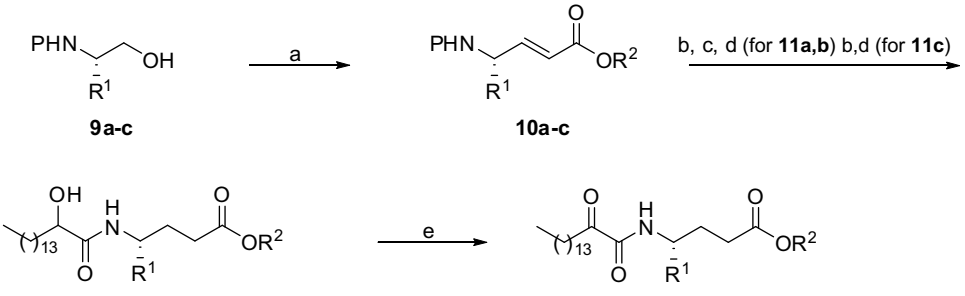


Fig. 1. Amino acid-based inhibitors of phospholipase A_2 enzymes.



5,7,8	X	R
a	CH_2	Me
b	CH_2	Et
c	CH_2	Bu^t
d	$(CH_2)_2$	Me
e	$(CH_2)_2$	Et
f	$(CH_2)_2$	Bu^t
g	$(CH_2)_3$	Bn
h	$(CH_2)_3$	All
i	$(CH_2)_4$	Me
j	$(CH_2)_4$	Et
k	$(CH_2)_4$	Bu^t
l	C_6H_4	Et

Scheme 1. Reagents: (a) WSCI, HOBt, Et_3N, CH_2Cl_2; (b) Dess–Martin periodinane, CH_2Cl_2.



9-12	R^1	R^2	P
a	i-Bu	Me	Boc
b	n-Bu	Me	Boc
c	Me	Bu^t	Z

Scheme 2. Reagents and conditions: (a) i—NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C; ii—Ph_3P=$CHCOOR^2$, THF, reflux; (b) H_2, 10% Pd/C, EtOH; (c) 4 N HCl/Et_2O; (d) $CH_3(CH_2)_{13}CH(OH)COOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (e) Dess–Martin periodinane, CH_2Cl_2.

and treated with methyl or ethyl (phosphoranylidene)acetate to produce compounds **39a,b** (Scheme 6). After hydrogenation and removal of Boc group, coupling with 7-phenylheptanoic acid[35] gave amides **41a,b**. The target compound **42** was obtained after saponification of either **41a** or **41b**. Its enantiomer **43** was synthesized following similar reactions.

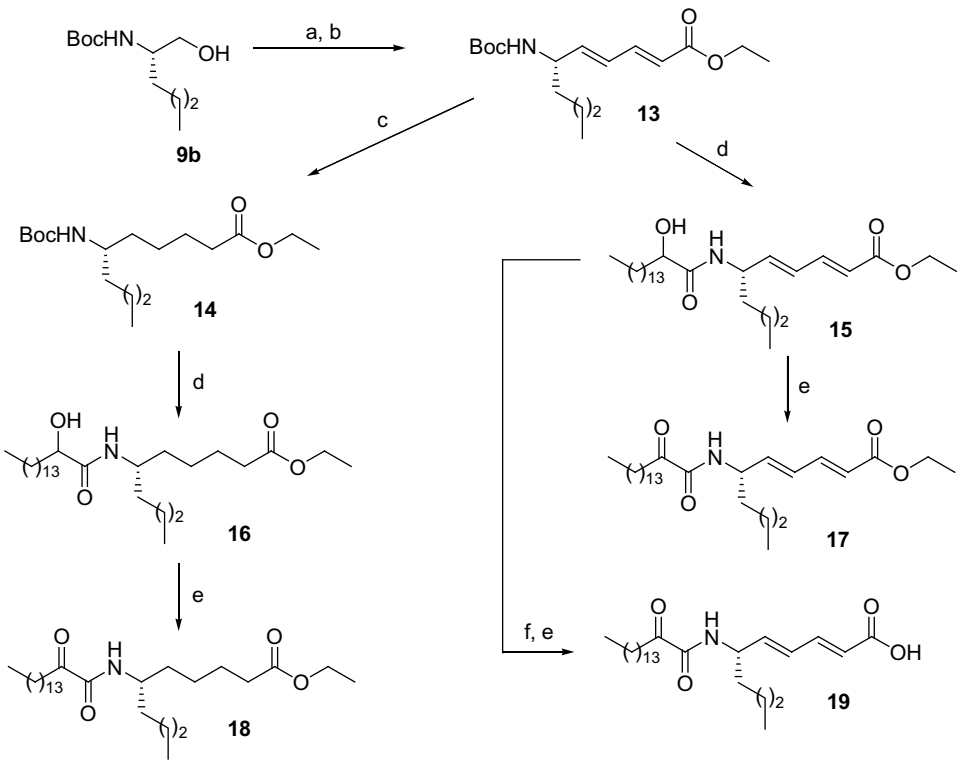
2.2. In-vitro inhibition of GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$

Twenty-six new derivatives including 2-oxoamides and amides were tested for inhibition of human GIVA $cPLA_2$, GVIA $iPLA_2$ and GV $sPLA_2$ using previously described mixed micelle-based assays.[26,29,30] The relative degrees of inhibition are presented in Tables 1–3 as either percent inhibition or $X_I(50)$ values. Initially, the percent of inhibition for each PLA_2 enzyme at 0.091 mole fraction of each inhibitor was determined, and $X_I(50)$ values were estimated for compounds that displayed greater than 90% inhibition. Each $X_I(50)$ value is derived from the mole fraction of the inhibitor in the total substrate interface required to inhibit the enzyme by 50%.

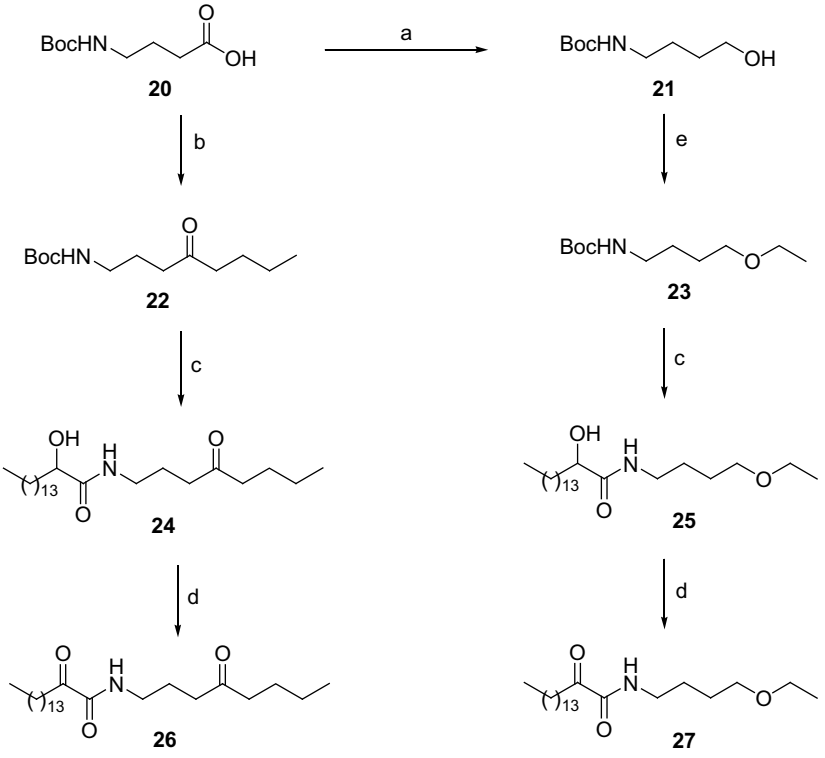
None of the methyl, ethyl or *tert*-butyl esters of 2-oxoamides based on glycine (**8a**–**c**), β-alanine (**8d**–**f**), and δ-aminovaleric acid (**8i**–**k**) (Table 1) display potent inhibition of GIVA $cPLA_2$ or GVIA $iPLA_2$. *tert*-Butyl esters (**8c**, **8f**, and **8k**) are better inhibitors than the corresponding methyl and ethyl esters. Benzyl ester (**8g**) and allyl ester (**8h**) analogues of inhibitor **1d** were found to be weak inhibitors of GIVA $cPLA_2$ and GVIA $iPLA_2$, indicating that the ethyl ester of GABA leads to better in-vitro results.[28]

The esters of the 2-oxoamides based on γ-leucine (**12a**), γ-norleucine (**12b**) and γ-alanine (**12c**) are good inhibitors of GIVA $cPLA_2$ (Table 2). They also weakly inhibit GVIA $iPLA_2$. Derivative **8l** (Table 2) based on the aromatic amino acid seems to selectively inhibit GIVA $cPLA_2$, without affecting GVIA $iPLA_2$ activity. 2-Oxoamide ethyl esters based on ε-norleucine (saturated **18** or unsaturated **17**, Table 2) are weak inhibitors of both enzymes. It should be noticed that compound **19** (Table 2) containing a free carboxyl group is a selective inhibitor of GIVA $cPLA_2$, without affecting the activity of GVIA $iPLA_2$. This observation is in full agreement with our previous report that 2-oxoamides based on γ-amino acids are selective inhibitors of GIVA $cPLA_2$.[29,30]

Among the 2-oxoamides based on esters of γ- and ε-amino acids, two derivatives (**12b** and **12c**, Table 2) based on esters of γ-norleucine and γ-alanine displayed inhibition of GV $sPLA_2$. However, compounds **12b** and **12c** also inhibited GIVA $cPLA_2$ and GVIA



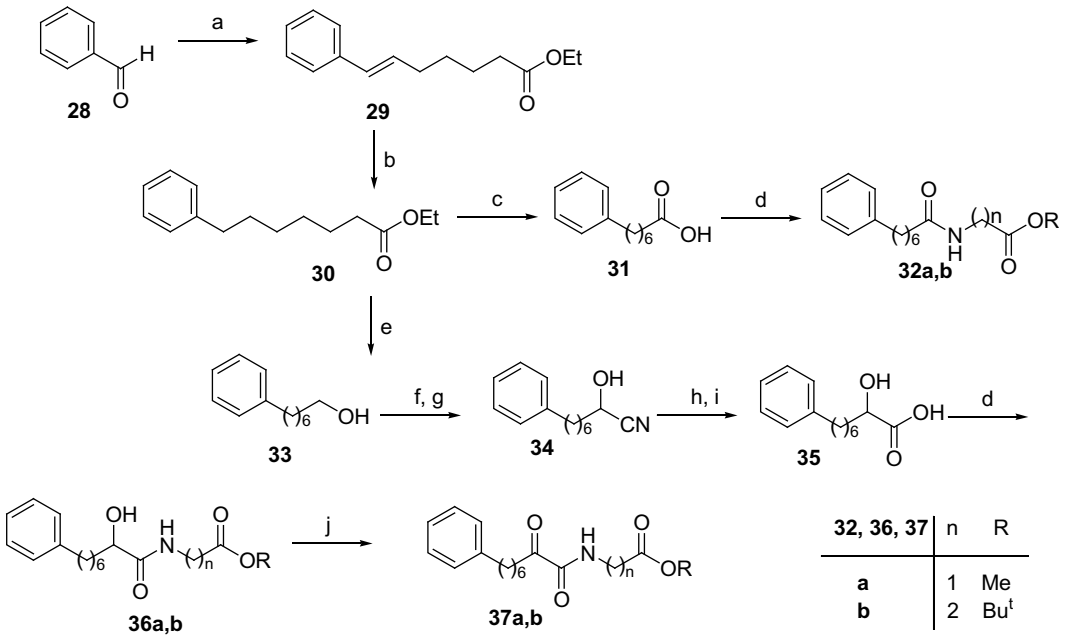
Scheme 3. Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C; (b) $(EtO)_2P(=O)CH_2CH=CHCOOEt$, LiOH, $PhCH_3$, rt; (c) H_2, 10% Pd/C, EtOH; (d) i—4 N HCl/Et_2O; ii—$CH_3(CH_2)_{13}CH(OH)COOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (e) Dess–Martin periodinane, CH_2Cl_2; (f) 1 N NaOH, CH_3OH.



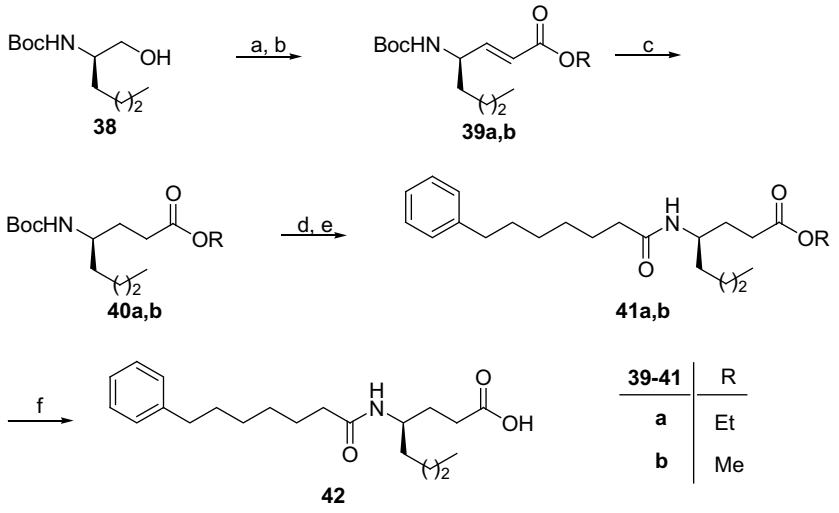
Scheme 4. Reagents: (a) i—NMM, $ClCO_2Et$, THF; ii—$NaBH_4$, MeOH; (b) *n*-BuLi/hexane, THF; (c) i—4 N HCl/Et_2O; ii—$CH_3(CH_2)_{13}CH(OH)COOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (d) Dess–Martin periodinane, CH_2Cl_2; (e) EtBr, Bu_4NHSO_4, 50% NaOH, C_6H_6.

$iPLA_2$, and therefore demonstrated no statistical preference for the inhibition of the three enzymes.

To characterize the mode of interaction between the ethyl ester derivative **1d** and the active site of GIVA $cPLA_2$, we prepared two structurally related compounds, a ketone derivative **26** and an ether derivative **27**. In compound **26**, the carbonyl group is at the same position as in the ester derivative **1d**, although the alkoxy group was replaced by an alkyl group. In compound **27**, the ethoxy



Scheme 5. Reagents and conditions: (a) $Br^{-}Ph_3P^{+}(CH_2)_5COOEt$, NaH, THF; (b) H_2, 10% Pd/C, EtOH; (c) 1 N NaOH, dioxane; (d) $H_2N(CH_2)_nCOOR$, WSCI, HOBt, Et_3N, CH_2Cl_2; (e) DIBALH, dry Et_2O; (f) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C; (g) $NaHSO_3$, KCN, H_2O, CH_2Cl_2; (h) concd HCl; (i) KOH, EtOH/H_2O; (j) Dess–Martin periodinane, CH_2Cl_2.



Scheme 6. Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O 3:3:0.5, −5 °C; (b) $Ph_3P{=}CHCOOR$, THF, reflux; (c) H_2, 10% Pd/C, EtOH or MeOH; (d) 4 N HCl/Et_2O, (e) $Ph(CH_2)_6COOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (f) 1 N NaOH, dioxane.

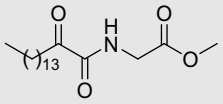
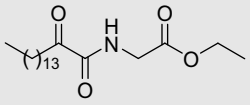
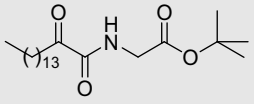
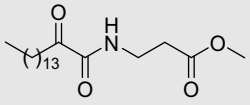
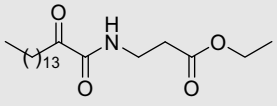
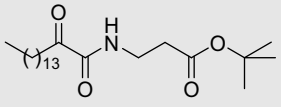
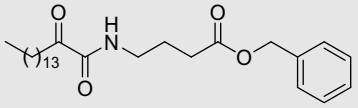
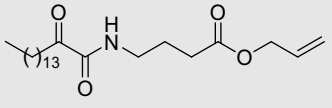
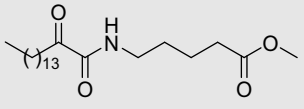
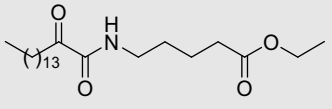
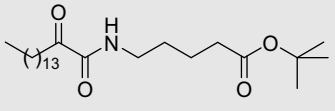
group of the ester derivative was kept constant, while the carbonyl group is absent. For synthetic reasons, we prepared a ketone derivative containing one additional carbon atom in the right chain of the ketone chain. However, this modification was not expected to play an essential role in inhibition. Neither compounds **26** or **27** presented interesting inhibitory properties, thereby confirming the essential inhibitory role of the ester group of compound **1d.**

Table 3 summarizes the in-vitro inhibition activities caused by 2-oxoamides and amides containing the 7-phenylheptanoyl chain instead of the long aliphatic chain. Comparing **37a** with **8a**, it seems that such a replacement of the long chain led to decreased activity for all the enzymes, as observed in the case of glycine methyl ester. However, similar activities were observed for **37b** and **8b**. The corresponding amides **32a** and **32b** were either totally inactive or very weak inhibitors. Interestingly, amide **42** based on (*R*)-γ-norleucine is a potent and selective GV $sPLA_2$ inhibitor, not affecting at all the activities of GIVA $cPLA_2$ and GVIA $iPLA_2$. The dose-response curve for the inhibition of GV $sPLA_2$ by amide **42** is shown in Fig. 2, and a $X_I(50)$ value 0.003 ± 0.0004 was calculated. The configuration of the substituted amino acid is very important, since the amide **43** based on (*S*)-γ-norleucine was inactive for all the three PLA_2 enzymes.

3. Discussion

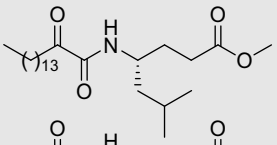
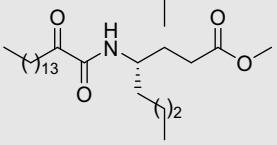
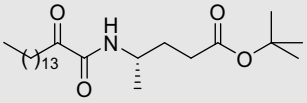
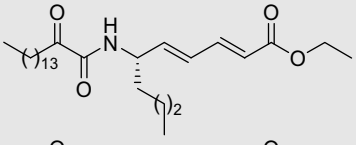
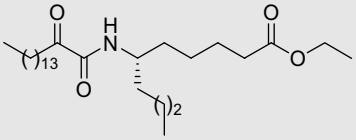
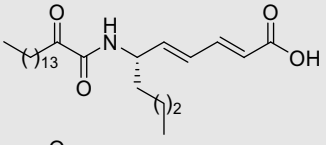
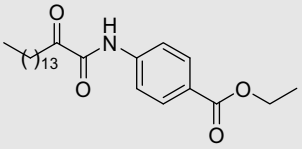
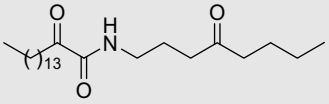
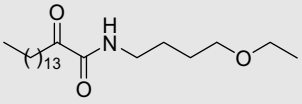
We previously demonstrated that 2-oxoamide **1d**, which is based on the ethyl ester of γ-aminobutyric acid, inhibits the GIVA $cPLA_2$ and GVIA $iPLA_2$ and exhibits very interesting antihyperalgesic activity.[28] The *tert*-butyl ester variant of compound **1d** (AX057) also inhibits both enzymes.[28] In addition, 2-oxoamides based on

Table 1
Inhibition of PLA_2 by 2-oxoamide esters

Entry	Structure	GIVA $cPLA_2$	GVIA $iPLA_2$	GV $sPLA_2$
8a		62 ± 1	45 ± 13	52 ± 4
8b		74 ± 1	62 ± 13	62 ± 5
8c		80 ± 2	53 ± 13	82 ± 8
8d		N.D.	N.D.	48 ± 9
8e		44 ± 2	51 ± 13	59 ± 8
8f		79 ± 2	54 ± 13	72 ± 7
8g		35 ± 1	N.D.	58 ± 12
8h		53 ± 2	63 ± 5	38 ± 8
8i		N.D.	N.D.	42 ± 8
8j		31 ± 2	35 ± 12	47 ± 6
8k		74 ± 2	62 ± 13	72 ± 10

Average percent inhibition and standard error ($n = 3$) reported for each compound at 0.091 mole fraction. N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

Table 2
Inhibition of PLA2 by 2-oxoamides based on esters of γ- and ε-amino acids and related compounds

Entry	Structure	GIVA $cPLA_2$	GVIA $iPLA_2$	GV $sPLA_2$
12a		83 ± 4	65 ± 9	65 ± 9
12b		87 ± 13	78 ± 9	86 ± 6
12c		85 ± 5	72 ± 2	87 ± 4
17		64 ± 4	74 ± 5	52 ± 7
18		62 ± 12	66 ± 5	N.D.
19		85 ± 4	N.D.	N.D.
8l		87 ± 1	N.D.	78 ± 8
26		32 ± 5	N.D.	N.D.
27		62 ± 14	51 ± 2	64 ± 12

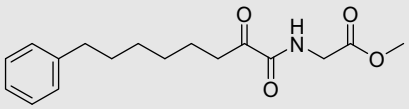
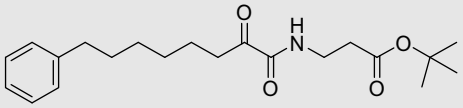
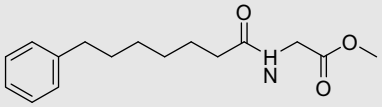
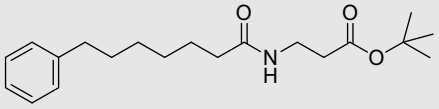
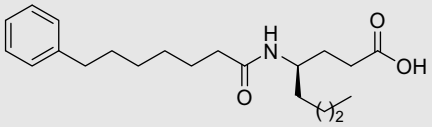
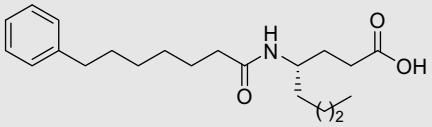
Average percent inhibition and standard error ($n = 3$) reported for each compound at 0.091 mole fraction. N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

methyl esters of γ-aminobutyric acid or α,β-unsaturated γ-norleucine are able to inhibit the same enzymes.[29] From the results of Table 1, we show that 2-oxoamide esters with varying distance between the 2-oxoamide functionality and the ester group (one to four carbon atoms) do not present any significant inhibition of GIVA $cPLA_2$ or GVIA $iPLA_2$. Interestingly, the introduction of a side-chain to the non-natural amino acids (Table 2) increases the potency of methyl or *tert*-butyl esters of γ-leucine, γ-norleucine, and γ-alanine (**12a**–**c**) for GIVA $cPLA_2$; but display rather weak inhibition of GVIA $iPLA_2$. Even so, increasing the spacer length to five carbon atoms, as seen in the ethyl ester of an ε-amino acid variant (**17**), leads to inhibition of both GIVA $cPLA_2$ and GVIA $iPLA_2$.

The 2-oxoamide compounds are inhibitors of various lipolytic enzymes (pancreatic and gastric lipases,[44–48] GIVA $cPLA_2$,[25,26,29,30] GVIA $iPLA_2$[29]) that utilize a serine residue as the nucleophile in their catalytic mechanism. In the cases of such serine esterases, it has been proposed that the serine hydroxyl group interacts with the activated carbonyl group of the 2-oxoamide functionality. The small, secreted human GV $sPLA_2$, as well as other $sPLA_2$ enzymes, utilize a histidine residue in the catalytic site to coordinate a water molecule for hydrolysis at the *sn*-2 ester bond of phospholipids. It is likely that the long chain 2-oxoamides able to inhibit GV $sPLA_2$ resemble the transition state, explaining its high-affinity for the active site of GV $sPLA_2$.

We demonstrate that an amide based on a non-natural amino acid with a short linear aliphatic side chain (**42**) is a potent and

Table 3
Inhibition of PLA_2 by 2-oxoamide and amide containing 7-phenylheptanoyl chain

Entry	Structure	GIVA $cPLA_2$	GVIA $iPLA_2$	GV $sPLA_2$
37a		54 ± 4	31 ± 10	30 ± 2
37b		82 ± 9	81 ± 10	69 ± 9
32a		N.D.	N.D.	N.D.
32b		36 ± 3	57 ± 12	66 ± 9
42		N.D.	N.D.	95 $X_i(50)$ 0.003 ± 0.0004
43		N.D.	N.D.	N.D.

Average percent inhibition and standard error ($n = 3$) reported for each compound at 0.091 mole fraction. N.D. signifies compounds with less than 25% inhibition (or no detectable inhibition).

selective inhibitor of GV $sPLA_2$. Comparing the present results with our previous studies, we conclude that a 2-oxoamide based on (*S*)-γ-norleucine, containing a long aliphatic chain, is a potent and selective inhibitor of GIVA $cPLA_2$,[26] while a simple amide based on (*R*)-γ-norleucine, containing a 7-phenylheptanoyl chain, is a potent and selective inhibitor of GV $sPLA_2$.

4. Conclusions

In conclusion, we synthesized a variety of 2-oxoamides and amides based on various amino acids and studied their activity on two human intracellular phospholipases (GIVA $cPLA_2$ and GVIA $iPLA_2$) and one human secretory phospholipase (GV $sPLA_2$). In summary, compounds with (*S*) configuration containing the 2-oxoamide functionality and a long fatty acyl chain provide selective inhibition of GIVA $cPLA_2$, while amide functionality in combination with the 7-phenylheptanoyl chain and the (*R*) configuration lead to selective inhibition of GV $sPLA_2$. Since both of these PLA_2s have been shown to contribute to the production of arachidonic acid, selective 2-oxoamide and amide inhibitors will be valuable tools for studies in cells and in-vivo.

5. Experimental

5.1. General procedures

Melting points were determined on a Buchi 530 apparatus and are uncorrected. Specific rotations were measured at 25 °C on a Perkin-Elmer 343 polarimeter using a 10 cm cell. NMR spectra were recorded on a Varian Mercury (200 Mz) spectrometer. Fast atom bombardment (FAB) mass spectra were recorded using a VG analytical ZAB-SE instrument. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan, Surveyor MSQ Plus spectrometer. All amino acid derivatives were purchased from Fluka Chemical Co. TLC plates (silica gel 60 F_{254}) and silica gel 60 (70–230 or 230–400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain. THF, toluene, and Et_2O were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were reagent grade and used without further purification.

5.2. Synthesis of 2-oxoamide Inhibitors

5.2.1. Coupling of 2-hydroxy-acids with amino components

To a stirred solution of 2-hydroxy-acid (1.0 mmol) and the amino component (1.0 mmol) in CH_2Cl_2 (10 mL), Et_3N (3.1 mL, 2.2 mmol) and subsequently 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCI) (0.21 g, 1.1 mmol) and 1-hydroxybenzotriazole (HOBt) (0.14 g, 1.0 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at rt. The solvent was evaporated under reduced pressure and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1 N HCl, brine, 5% $NaHCO_3$, and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column-chromatography using $CHCl_3$/MeOH as eluent.

5.2.1.1. Methyl 2-(2-hydroxyhexadecanamido)acetate (7a). Yield 82%; White solid; mp 116–118 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.02 (t, $J = 4.8$ Hz, 1H), 4.20–4.14 (m, 1H), 4.10–4.06 (m, 2H), 3.77 (s, 3H), 2.78 (br, 1H), 1.94–1.56 (m, 2H), 1.26 (br s, 24H), 0.89 (t,

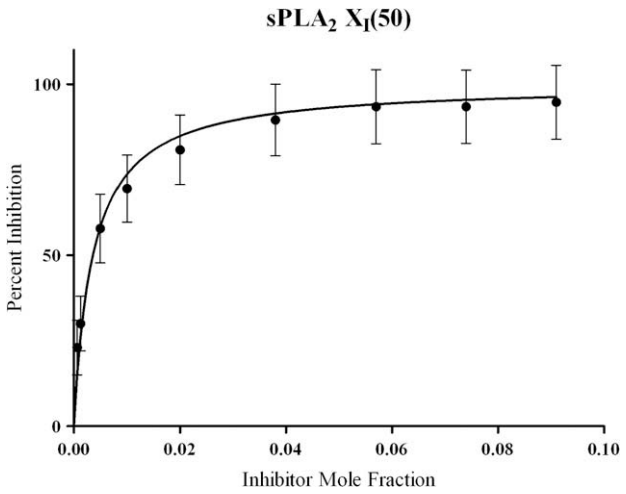


Fig. 2. Inhibition curve for amide **42** in a mixed-micelle assay with human GV $sPLA_2$. Non-linear regression (hyperbolic) estimated a $X_I(50)$ value of 0.003 ± 0.0004. Compound **42** did not inhibit GIVA $cPLA_2$ and GVIA $iPLA_2$ at 0.091 mole fraction.

J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.2, 170.3, 72.2, 52.4, 40.8, 34.8, 31.9, 29.6, 29.5, 29.4, 29.3, 24.9, 22.7, 14.1. Anal. Calcd for $C_{19}H_{37}NO_4$: C, 66.43; H, 10.86; N, 4.08. Found: C, 66.65; H, 10.71; N, 4.13.

5.2.1.2. Ethyl 2-(2-hydroxyhexadecanamido)acetate (7b). Yield 75%; White solid; mp 115–117 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (m, 1H), 4.29–4.07 (m, 5H), 1.93–1.53 (m, 2H), 1.26 (br s, 27H), 0.89 (t, J = 5.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.2, 169.9, 72.2, 61.6, 40.9, 34.8, 31.9, 29.6, 29.5, 29.4, 29.3, 24.9, 22.7, 14.1. Anal. Calcd for $C_{20}H_{39}NO_4$: C, 67.19; H, 10.99; N, 3.92. Found: C, 67.33; H, 10.83; N, 3.99.

5.2.1.3. *tert*-Butyl 2-(2-hydroxyhexadecanamido)acetate (7c). Yield 77%; White solid; mp 53–56 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (t, J = 5.2 Hz, 1H), 4.18–4.12 (m, 1H), 3.99–3.93 (m, 2H), 3.15 (br, 1H), 1.89–1.50 (m, 2H), 1.47 (s, 9H), 1.25 (br s, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.3, 169.1, 82.4, 72.2, 41.5, 34.7, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.0, 25.0, 22.7, 14.1. Anal. Calcd for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.45; H, 11.37; N, 3.71.

5.2.1.4. Methyl 3-(2-hydroxyhexadecanamido)propanoate (7d). Yield 79%; White solid; mp 111–114 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00 (br, 1H), 4.12–4.06 (m, 1H), 3.71 (s, 3H), 3.61–3.51 (m, 2H), 2.86 (br, 1H), 2.57 (t, J = 6 Hz, 2H), 1.86–1.55 (m, 2H), 1.25 (br s, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0, 172.9, 72.0, 51.9, 34.9, 34.5, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 24.9, 22.7, 14.1. Anal. Calcd for $C_{20}H_{39}NO_4$: C, 67.19; H, 10.99; N, 3.92. Found: C, 67.03; H, 11.21; N, 3.86.

5.2.1.5. Ethyl 3-(2-hydroxyhexadecanamido)propanoate (7e). Yield 68%; White solid; mp 102–104 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.07 (t, J = 6 Hz, 1H), 4.15 (q, J = 7.2 Hz, 2H), 4.09–4.04 (m, 1H), 3.59–3.49 (m, 2H), 3.20 (br, 1H), 2.54 (t, J = 6.2 Hz, 2H), 1.86–1.50 (m, 2H), 1.24 (br s, 27H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 172.4, 72.0, 60.8, 34.8, 34.5, 34.0, 31.9, 29.6, 29.5, 29.4, 29.3, 24.9, 22.6, 14.1, 14.0. Anal. Calcd for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.64; H, 11.34; N, 3.71.

5.2.1.6. *tert*-Butyl 3-(2-hydroxyhexadecanamido)propanoate (7f). Yield 63%; White solid; mp 95–97 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.97 (t, J = 5.8 Hz, 1H), 4.11–4.05 (m, 1H), 3.56–3.46 (m, 2H), 2.83 (br, 1H), 2.44 (t, J = 5.8 Hz, 2H), 1.88–1.51 (m, 2H), 1.44 (s, 9H), 1.23 (br s, 24H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.8, 171.7, 81.2, 72.0, 35.1, 35.0, 34.7, 31.9, 29.6, 29.5, 29.4, 29.3, 28.1, 24.8, 22.7, 14.1. Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 69.01; H, 11.48; N, 3.44.

5.2.1.7. Benzyl 4-(2-hydroxyhexadecanamido)butanoate (7g). Yield 64%; White solid; mp 89–91 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.36 (m, 5H), 6.72 (t, J = 5.8 Hz, 1H), 5.13 (s, 2H), 4.09–4.03 (m, 1H), 3.37–3.28 (m, 2H), 2.85 (br, 1H), 2.42 (t, J = 7 Hz, 2H), 1.95–1.50 (m, 4H), 1.26 (br s, 24H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 173.1, 135.7, 128.6, 128.3, 128.2, 72.1, 66.4, 38.4, 34.9, 31.9, 31.6, 29.7, 29.62, 29.58, 29.5, 29.4, 29.3, 25.0, 24.6, 22.7, 14.1. Anal. Calcd for $C_{27}H_{45}NO_4$: C, 72.44; H, 10.13; N, 3.13. Found: C, 72.61; H, 9.95; N, 3.23.

5.2.1.8. Allyl 4-(2-hydroxyhexadecanamido)butanoate (7h). Yield 71%; White solid; mp 91–93 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.71 (t, J = 5.4 Hz, 1H), 6.02–5.82 (m, 1H), 5.36–5.22 (m, 2H), 4.61–4.57 (m, 2H), 4.12–4.06 (m, 1H), 3.39–3.29 (m, 2H), 2.59 (br, 1H), 2.40 (t, J = 7.2 Hz, 2H), 1.95–1.52 (m, 4H), 1.25 (br s, 24H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0, 173.0, 132.0, 118.4, 72.1, 65.3, 38.5, 34.9, 31.9, 31.6, 29.7, 29.6, 29.5, 29.4, 29.3, 25.0, 24.6, 22.7, 14.1. Anal. Calcd for $C_{23}H_{43}NO_4$: C, 69.48; H, 10.90; N, 3.52. Found: C, 69.62; H, 10.75; N, 3.57.

5.2.1.9. Methyl 5-(2-hydroxyhexadecanamido)pentanoate (7i). Yield 66%; White solid; mp 105–107 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.73 (t, J = 5.8 Hz, 1H), 4.10–4.04 (m, 1H), 3.66 (s, 3H), 3.32–3.22 (m, 2H), 3.17 (br, 1H), 2.34 (t, J = 7 Hz, 2H), 1.79–1.51 (m, 6H), 1.24 (br s, 24H), 0.87 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.2, 174.0, 72.1, 51.6, 38.5, 34.9, 33.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 25.0, 22.6, 22.0, 14.1. Anal. Calcd for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.77; H, 11.13; N, 3.58.

5.2.1.10. Ethyl 5-(2-hydroxyhexadecanamido)pentanoate (7j). Yield 68%; White solid; mp 95–97 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.69 (t, J = 5.8 Hz, 1H), 4.17–4.06 (m, 3H), 3.33–3.23 (m, 2H), 3.15 (br, 1H), 2.33 (t, J = 7 Hz, 2H), 1.79–1.58 (m, 6H), 1.24 (br s, 27H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0, 173.5, 72.1, 60.4, 38.5, 34.9, 33.7, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 25.0, 22.7, 22.0, 14.1. Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 68.97; H, 11.59; N, 3.43.

5.2.1.11. *tert*-Butyl 5-(2-hydroxyhexadecanamido)pentanoate (7k). Yield 61%; White solid; mp 60–63 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.71 (t, J = 5.6 Hz, 1H), 4.11–4.06 (m, 1H), 3.32–3.23 (m, 2H), 2.24 (t, J = 7 Hz, 2H), 1.81–1.46 (m, 6H), 1.43 (s, 9H), 1.23 (br s, 24H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 173.0, 80.4, 72.1, 38.6, 34.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.9, 28.0, 25.0, 22.7, 22.1, 14.1. Anal. Calcd for $C_{25}H_{49}NO_4$: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.02; H, 11.68; N, 3.32.

5.2.1.12. Ethyl 4-(2-hydroxyhexadecanamido)benzoate (7l). Yield 43%; Oily solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.06 (s, 1H), 7.95–7.33 (m, 3H), 4.37 (q, J = 7 Hz, 2H), 4.28–4.22 (m, 1H), 3.35 (br, 1H), 1.90–1.61 (m, 2H), 1.25 (br s, 27H), 0.88 (t, J = 5.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.7, 166.3, 137.5, 131.1, 129.1, 125.4, 124.1, 120.6, 72.6, 61.2, 34.7, 31.9, 29.7, 29.5, 29.4, 29.3, 25.0, 22.6, 14.2, 14.1. Anal. Calcd for $C_{25}H_{41}NO_4$: C, 71.56; H, 9.85; N, 3.34. Found: C, 71.33; H, 9.97; N, 3.42.

5.2.1.13. (4*R*)-Methyl 4-(2-hydroxyhexadecanamido)-6-methylheptanoate (11a). Yield 57%; White solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ *6.40–6.20* (m, 1H), 4.20–3.90 (m, 2H), 3.66 (s, 3H), 3.10–

2.90 (m, 1H), 2.35 (t, J = 7.8 Hz, 2H), 1.95- 1.47 (m, 5H), 1.40–1.18 (m, 26H), 0.95-0.81 (m, 9H). Anal. Calcd for $C_{25}H_{49}NO_4$: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.34; H, 11.46; N, 3.22.

5.2.1.14. (4*S*)-Methyl 4-(2-hydroxyhexadecanamido)octanoate (11b). Yield 62%; White solid; 1H NMR (200 MHz, $CDCl_3$) δ *6.35–6.15* (m, 1H), 4.15–4.00 (m, 1H), 4.00–3.80 (m, 1H), 3.67 (s, 3H), 2.80–2.70 (m, 1H), 2.35 (t, J = 7.8 Hz, 2H), 1.97–1.42 (m, 6H), 1.38–1.15 (m, 28H), 0.98–0.81 (m, 6H). Anal. Calcd for $C_{25}H_{49}NO_4$: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.04; H, 11.62; N, 3.36.

5.2.1.15. (4*S*)-*tert*-Butyl 4-(2-hydroxyhexadecanamido)pentanoate (11c). Yield 58%; White solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ *6.60–6.40* (m, 1H), 4.10–3.90 (m, 2H), 3.15–2.95 (m, 1H), 2.27 (t, J = 7.8 Hz, 2H), 1.98–1.52 (m, 4H), 1.44 (s, 9H), 1.40–1.21 (m, 24H), 1.16 (d, J = 6.6 Hz, 3H), 0.88 (t, J = 6.6 Hz, 3H). Anal. Calcd for $C_{25}H_{49}NO_4$: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.05; H, 11.74; N, 3.21.

5.2.1.16. (2*E*,4*E*,6*S*)-Ethyl 6-(2-hydroxyhexadecanamido)deca-2,4-dienoate (15). Yield 58%; Waxy solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 (dd, J_1 = 15.6 Hz, J_2 = 10.8 Hz, 1H), 6.73–6.67 (m, 1H), 6.26 (dd, J_1 = 15.2 Hz, J_2 = 11 Hz, 1H), 6.12(dd, J_1 = 15 Hz, J_2 = 5.8 Hz, 1H), 5.95 (d, J = 15.4 Hz, 1H), 4.73–4.57 (m, 1H), 4.35–4.24 (m, 3H), 3.11 (d, J = 4.8 Hz, $^2/_3$H), 3.03 (d, J = 4.8 Hz, $^1/_3$H), 1.92–1.58 (m, 4H), 1.29 (br s, 31H), 0.89 (t, J = 6.6 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.2, 167.0, 143.7, 142.5, 128.0, 121.5, 72.2, 60.4, 50.1, 34.9, 34.5, 31.9, 29.7, 29.6, 29.4, 29.3, 27.8, 24.9, 22.7, 22.4, 14.2, 14.1, 13.9. Anal. Calcd for $C_{28}H_{51}NO_4$: C, 72.21; H, 11.04; N, 3.01. Found: C, 72.34; H, 10.97; N, 2.97.

5.2.1.17. (6*S*)-Ethyl 6-(2-hydroxyhexadecanamido)decanoate (16). Yield 55%; White solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ *6.28–6.18* (m, 1H), 4.16–4.06 (m, 3H), 3.92–3.89 (m, 1H), 3.11 (d, J = 4.4 Hz, $^2/_3$H), 2.94 (d, J = 5 Hz, $^1/_3$H), 2.28 (t, J = 7.4 Hz, 2H), 1.93–1.21 (m, 41H), 0.88 (t, J = 6.6 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.9, 173.5, 72.2 (72.0), 60.3, 48.6 (48.8), 35.1, 34.9, 34.8, 34.7, 34.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.0, 25.4, 25.3, 24.9, 24.8, 24.7, 22.7 (22.6), 14.2, 14.1, 14.0. Anal. Calcd for $C_{28}H_{55}NO_4$: C, 71.59; H, 11.80; N, 2.98. Found: C, 71.28; H, 11.95; N, 2.94.

5.2.1.18. 2-Hydroxy-*N*-(4-oxooctyl)hexadecanamide (24). Yield 70%; White solid; mp 122–124 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.73 (t, J = 5.8 Hz, 1H), 4.22–4.06 (m, 1H), 3.34–3.28 (m, 2H), 2.56–2.40 (m, 4H), 1.97–1.73 (m, 3H), 1.73–1.20 (m, 30H), 0.88 (t, J = 6.6 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 211.2, 174.0, 72.1, 42.7, 40.0, 38.7, 34.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 25.9, 25.0, 23.3, 22.7, 22.3, 14.1, 13.8. Anal. Calcd for $C_{24}H_{47}NO_3$: C, 72.49; H, 11.91; N, 3.52. Found: C, 72.38; H, 11.99; N, 3.56.

5.2.1.19. *N*-(4-Ethoxybutyl)-2-hydroxyhexadecanamide (25). Yield 67%; White solid; mp 92–95 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.75 (t, J = 5.8 Hz, 1H), 4.10–4.02 (m, 1H), 3.52–3.26 (m, 7H), 1.95–1.50 (m, 6H), 1.25 (br s, 27H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.1, 72.0, 70.1, 66.2, 38.8, 35.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 27.1, 26.4, 25.0, 22.6, 15.1, 14.1. Anal. Calcd for $C_{22}H_{45}NO_3$: C, 71.11; H, 12.21; N, 3.77. Found: C, 71.24; H, 12.15; N, 3.72.

5.2.1.20. Methyl 2-(7-phenylheptanamido)acetate (32a). Yield 45%; White solid; mp 48–50 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ *7.40–7.21* (m, 5H), 6.30 (b, 1H), 4.11 (d, J = 5.6 Hz, 2H), 3.83 (s, 3H), 2.69 (t, J = 7.2 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 1.81–1.62 (m, 4H), 1.55–1.38 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.3, 170.4, 142.5, 128.2, 128.1, 125.5, 52.1, 41.0, 36.1, 35.7, 31.1, 28.9, 28.8, 25.3; MS (ESI) m/z (%): 278 (100) $[M+H]^+$; Anal. Calcd for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05. Found: C, 68.92; H, 8.47; N, 5.11.

5.2.1.21. *tert*-Butyl 3-(7-phenylheptanamido)propanoate (32b). Yield 45%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ *7.40–7.23* (m, 5H), 6.18 (br s, 1H), 3.56 (q, 2H), 2.68 (t, J = 7.4 Hz, 2H), 2.53 (t, J = 6.2 Hz, 2H), 2.23 (t, J = 7.0 Hz, 2H), 1.80–1.62 (m, 4H), 1.54 (s, 9H), 1.51–1.38 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.0, 172.2, 142.6, 128.3, 128.2, 125.5, 81.0, 36.7, 35.8, 35.0, 34.8, 31.2, 29.0, 28.9, 28.0, 25.6; MS (ESI) m/z (%): 356 (100) $[M+Na]^+$; Anal. Calcd for $C_{20}H_{31}NO_3$: C, 72.04; H, 9.37; N, 4.20. Found: C, 71.76; H, 9.59; N, 4.36.

5.2.1.22. Methyl 2-(2-hydroxy-8-phenyloctanamido)acetate (36a). Yield 41%; White solid; mp 65–67 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39–7.25 (m, 6H), 4.27–4.08 (m, 3H), 3.83 (s, 3H), 3.32 (br s, 1H), 2.68 (t, J = 7.4 Hz, 2H), 1.98–1.62 (m, 4H), 1.58–1.35 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.7, 170.4, 142.7, 128.3, 128.2, 125.5, 72.1, 52.3, 40.7, 35.8, 34.6, 31.3, 29.2, 29.1, 24.8; Anal. Calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56. Found: C, 66.29; H, 8.11; N, 4.61.

5.2.1.23. *tert*-Butyl 3-(2-hydroxy-8-phenyloctanamido)propanoate (36b). Yield 32%; White solid; mp 62–64 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–7.23 (m, 5H), 7.13 (t, J = 5.8 Hz, 1H), 4.16 (dd, J_1 = 3.6 Hz, J_2 = 7.2 Hz, 1H), 3.59 (q, J = 6.4 Hz, 2H), 3.10 (br s, 1H), 2.68 (t, J = 7.4 Hz, 2H), 2.54 (t, J = 6.2 Hz, 2H), 1.95–1.78 (m, 1H), 1.76–1.61 (m, 3H), 1.54 (s, 9H), 1.42–1.35 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.0, 171.7, 142.7, 128.3, 128.2, 125.5, 81.2, 71.9, 35.9, 35.2, 34.8, 34.7, 31.4, 29.2, 29.1, 28.0, 24.8; Anal. Calcd for $C_{21}H_{33}NO_4$: C, 69.39; H, 9.15; N, 3.85. Found: C, 69.28; H, 9.21; N, 3.94.

5.2.1.24. (*R*)-Ethyl 4-(7-phenylheptanamido)octanoate (41a). Yield 89%; White solid; mp 54–56 °C; $[\alpha]_D$ +8.2 (c 1 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39–7.23 (m, 5H), 5.88 (d, J = 9.0 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 4.03 (br s, 1H), 2.69 (t, J = 7.4 Hz, 2H), 2.43 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 6.8 Hz, 2H), 1.99–1.87 (m, 2H), 1.83–1.60 (m, 6H), 1.56–1.36 (m, 8H), 1.32 (t, J = 7.4 Hz, 3H), 0.98 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.5, 172.6, 142.4, 128.1, 128.0, 125.4, 60.2, 48.5, 36.6, 35.6, 34.8, 31.1, 30.9, 29.8, 28.9, 28.8, 27.8, 25.6, 22.3, 14.0, 13.9; MS (ESI) m/z (%): 398 (100) $[M+Na]^+$; Anal. Calcd for $C_{23}H_{37}NO_3$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.22; H, 10.18; N, 3.79.

5.2.1.25. (*R*)-Methyl 4-(7-phenylheptanamido)octanoate (41b). Yield 70%; White solid; mp 62–64 °C; $[\alpha]_D$ +9.3 (c 1 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–7.23 (m, 5H), 5.45 (d, J = 8.2 Hz, 1H), 4.12–3.95 (m, 1H), 3.74 (s, 3H), 2.69 (t, J = 7.0 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 2.23 (t, J = 7.4 Hz, 2H), 2.05–1.85 (m, 1H), 1.79–1.60 (m, 5H), 1.55–1.36 (m, 10H), 0.97 (t, J = 6.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.2, 172.7, 142.6, 128.3, 128.2, 125.5, 51.6, 48.8, 36.8, 35.8, 31.2, 30.8, 30.0, 29.1, 28.9, 28.0, 25.7, 22.5, 13.9; Anal. Calcd for $C_{22}H_{35}NO_3$: C, 73.09; H, 9.76; N, 3.87. Found: C, 72.77; H, 9.97; N, 3.95.

5.2.2. Oxidation of 2-hydroxy-amides

To a solution of 2-hydroxy-amide (1 mmol) in dry CH_2Cl_2 (10 mL) Dess–Martin periodinane was added (0.64 g, 1.5 mmol) and the mixture was stirred for 1 h at rt. The organic solution was washed with 10% aqueous $NaHCO_3$, dried over Na_2SO_4 and the organic solvent was evaporated under reduced pressure. The residue was purified by column-chromatography using $CHCl_3$ as eluent.

5.2.2.1. Methyl 2-(2-oxohexadecanamido)acetate (8a). Yield 91%; White solid; mp 79–81 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42–7.36 (m, 1H), 4.09 (d, J = 5.8 Hz, 2H), 3.79 (s, 3H), 2.91 (t, J = 7.8 Hz, 2H), 1.68–1.52 (m, 2H), 1.25 (br s, 22H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.2, 169.3, 160.2, 52.5, 40.9, 36.7, 31.9, 29.62, 29.56, 29.4, 29.33, 29.30, 29.0, 23.1, 22.7, 14.1; MS (ESI): m/z (%): 364 (51) $[M+Na]^+$; Anal. Calcd for $C_{19}H_{35}NO_4$: C, 66.83; H, 10.33; N, 4.10. Found: C, 66.92; H, 10.25; N, 4.04.

5.2.2.2. Ethyl 2-(2-oxohexadecanamido)acetate (8b). Yield 84%; White solid; mp 66–68 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.10–6.91 (m, 1H), 4.22 (q, J = 7 Hz, 2H), 4.07 (d, J = 5.4 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H), 1.89–1.56 (m, 4H), 1.53–1.08 (br s, 23H), 0.86 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.2, 168.8, 160.2, 61.7, 41.0, 36.7, 31.9, 29.6, 29.5, 29.4, 29.30, 29.26, 29.0, 23.1, 22.6, 14.1; MS (ESI): m/z (%): 378 (100) $[M+Na]^+$, 356 (5) $[M+H]^+$; Anal. Calcd for $C_{20}H_{37}NO_4$: C, 67.57; H, 10.49; N, 3.94. Found: C, 67.71; H, 10.42; N, 3.89.

5.2.2.3. *tert*-Butyl 2-(2-oxohexadecanamido)acetate (8c). Yield 81%; White solid; mp 54–56 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43–7.32 (m, 1H), 3.97 (d, J = 5.6 Hz, 2H), 2.91 (t, J = 7.4 Hz, 2H), 1.65–1.52 (m, 2H), 1.48 (s, 9 H), 1.25 (br s, 22H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.3, 167.9, 160.1, 82.7, 41.7, 36.7, 31.9, 29.63, 29.57, 29.4, 29.34, 29.30, 29.0, 28.0, 23.1, 22.7, 14.1; MS (ESI): m/z (%): 406 (78) $[M+Na]^+$; Anal. Calcd for $C_{22}H_{41}NO_4$: C, 68.89; H, 10.77; N, 3.65. Found: C, 68.95; H, 10.69; N, 3.72.

5.2.2.4. Methyl 3-(2-oxohexadecanamido)propanoate (8d). Yield 74%; White solid; mp 83–86 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45–7.33 (m, 1H), 3.71 (s, 3H), 3.62–3.53 (m, 2H), 2.89 (t, J = 6.6 Hz, 2H), 2.58 (t, J = 6.2 Hz, 2H), 1.62–1.52 (m, 2H), 1.25 (br s, 22H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.9, 172.2, 160.2, 51.9, 36.7, 34.7, 33.5, 31.9, 29.61, 29.56, 29.4, 29.30, 29.32, 29.0, 23.1, 22.7, 14.1; MS (ESI): m/z (%): 378 (100) $[M+Na]^+$, 356 (9) $[M+H]^+$; Anal. Calcd for $C_{20}H_{37}NO_4$: C, 67.57; H, 10.49; N, 3.94. Found: C, 67.46; H, 10.53; N, 3.98.

5.2.2.5. Ethyl 3-(2-oxohexadecanamido)propanoate (8e). Yield 83%; White solid; mp 60–62 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42 (t, J = 6.2 Hz, 1H), 4.16 (q, J = 7.2 Hz, 2H), 3.61–3.72 (m, 2H), 2.89 (t, J = 7.4 Hz, 2H), 2.56 (t, J = 6.2 Hz, 2H), 1.78–1.18 (m, 27H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.9, 171.8, 160.1, 60.9, 36.7, 34.7, 33.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.1, 22.7, 14.1; MS (FAB): m/z (%): 370 (100) $[M+H]^+$; Anal. Calcd for $C_{21}H_{39}NO_4$: C, 68.25; H, 10.64; N, 3.79. Found: C, 68.17; H, 10.61; N, 3.84.

5.2.2.6. *tert*-Butyl 3-(2-oxohexadecanamido)propanoate (8f). Yield 75%; White solid; mp 44–45 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43 (t, J = 6.2 Hz, 1H), 3.57–3.48 (m, 2H), 2.90 (t, J = 7.4 Hz, 2H), 2.47 (t, J = 5.8 Hz, 2H), 1.62–1.55 (m, 2H), 1.45 (s, 9H), 1.24 (br s, 22H), 0.87 (t, J = 7 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.0, 171.0, 160.0, 81.4, 36.7, 34.8, 31.9, 29.61, 29.55, 29.4, 29.3, 29.0, 28.0, 23.1, 22.7, 14.1; MS (ESI): m/z (%): 420 (100) $[M+Na]^+$; Anal. Calcd for $C_{23}H_{43}NO_4$: C, 69.48; H, 10.90; N, 3.52. Found: C, 69.41; H, 10.84; N, 3.62.

5.2.2.7. Benzyl 4-(2-oxohexadecanamido)butanoate (8g). Yield 91%; White solid; mp 68–70 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H), 7.11 (t, J = 5.8 Hz, 1H), 5.13 (s, 2H), 3.40–3.30 (m, 2H), 2.90 (t, J = 7.4 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.98–1.84 (m, 2H), 1.68–1.53 (m, 2H), 1.26 (br s, 22H), 0.89 (t, J = 6.2 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.2, 172.7, 160.3, 135.7, 128.6, 128.31, 128.25, 66.5, 38.6, 36.7, 31.9, 31.5, 29.6, 29.5, 29.4, 29.3, 29.0, 24.4, 23.1, 22.7, 14.1; MS (ESI): m/z (%): 468 (100) $[M+Na]^+$; Anal. Calcd for $C_{27}H_{43}NO_4$: C, 72.77; H, 9.73; N, 3.14. Found: C, 72.86; H, 9.57; N, 3.23.

5.2.2.8. Allyl 4-(2-oxohexadecanamido)butanoate (8h). Yield 83%; White solid; mp 60–62 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.10 (t, J = 6.2 Hz, 1H), 6.02–5.82 (m, 1H), 5.37–5.22 (m, 2H), 4.61–4.58 (m, 2H), 3.41–3.31 (m, 2H), 2.91 (t, J = 7.6 Hz, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.05–1.84 (m, 2H), 1.64–1.56 (m, 2H), 1.26 (br s, 22H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.2, 172.5, 160.3, 132.0, 118.5, 65.3, 38.6, 36.7, 31.9, 31.5, 29.6, 29.5, 29.4, 29.3, 29.0, 24.4, 23.2, 22.7, 14.1; MS (ESI): m/z (%): 418 (100) $[M+Na]^+$; Anal. Calcd for $C_{23}H_{41}NO_4$: C, 69.83; H, 10.45; N, 3.54. Found: C, 69.72; H, 10.52; N, 3.47.

5.2.2.9. Methyl 5-(2-oxohexadecanamido)pentanoate (8i). Yield 93%; White solid; mp 68–70 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (t, J = 6.2 Hz, 1H), 3.68 (s, 3H), 3.36–3.27 (m, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.35 (t, J = 6.6 Hz, 2H), 1.70–1.56 (m, 6H), 1.25 (br s, 22H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 173.7, 160.2, 51.6, 38.8, 36.7, 33.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 28.6, 23.1, 22.7, 22.0, 14.1; MS (ESI): m/z (%): 406 (100) $[M+Na]^+$; Anal. Calcd for $C_{22}H_{41}NO_4$: C, 68.89; H, 10.77; N, 3.65. Found: C, 68.97; H, 10.71; N, 3.62.

5.2.2.10. Ethyl 5-(2-oxohexadecanamido)pentanoate (8j). Yield 87%; White solid; mp 59–61 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (t, J = 6.2 Hz, 1H), 4.13 (q, J = 7.2 Hz, 2H), 3.36–3.26 (m, 2 H), 2.91 (t, J = 7.4 Hz, 2H), 2.33 (t, J = 7 Hz, 2H), 1.75–1.56 (m, 6H), 1.25 (m, 25H), 0.88 (t, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 173.3, 160.2, 60.4, 38.8, 36.7, 33.7, 31.9, 29.62, 29.57, 29.4, 29.31, 29.33, 29.0, 28.6, 23.1, 22.7, 22.0, 14.2, 14.1; MS (FAB): m/z (%): 398 (100) $[M+H]^+$; Anal. Calcd for $C_{23}H_{43}NO_4$: C, 69.48; H, 10.90; N, 3.52. Found: C, 69.34; H, 10.97; N, 3.58.

5.2.2.11. *tert*-Butyl 5-(2-oxohexadecanamido)pentanoate (8k). Yield 77%; White solid; mp 48–50 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.04 (t, J = 6.2 Hz, 1 H), 3.34–3.24 (m, 2 H), 2.89 (t, J = 7.4 Hz, 2 H), 2.23 (t, J = 6.6 Hz, 2 H), 1.65–1.48 (m, 6 H), 1.43 (s, 9 H), 1.23 (br s, 22 H), 0.86 (t, J = 7 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 172.6, 160.1, 80.3, 38.9, 36.7, 34.8, 31.9, 29.6, 29.5, 29.4, 39.3, 29.0, 28.6, 28.0, 23.1, 22.6, 22.1, 14.1; MS (ESI): m/z (%): 448 (100) $[M+Na]^+$; Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.65; H, 11.05; N, 3.21.

5.2.2.12. Ethyl 4-(2-oxohexadecanamido)benzoate (8l). Yield 84%; White solid; mp 86–88 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.87 (s, 1 H), 8.19–7.43 (m, 4 H), 4.39 (q, J = 7.0 Hz, 2 H), 3.02 (t, J = 7.2 Hz, 2 H), 1.78–1.12 (m, 27 H), 0.88 (t, J = 6.6 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.2, 165.9, 157.7, 136.5, 131.5, 129.3, 126.2, 123.9, 120.6, 61.2, 36.3, 31.9, 29.63, 29.57, 29.4, 29.34, 29.30, 29.1, 23.3, 22.7, 14.3, 14.1; MS (FAB): m/z (%): 418 (100) $[M+H]^+$; Anal. Calcd for $C_{25}H_{39}NO_4$: C, 71.91; H, 9.41; N, 3.35. Found: C, 71.74; H, 9.57; N, 3.29.

5.2.2.13. (*R*)-Methyl 6-methyl-4-(2-oxohexadecanamido)heptanoate (12a). Yield 76%; Yellowish solid; mp 41–43 °C; $[\alpha]_D$ −9.0 (c 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.75–6.60 (m, 1 H), 4.10–3.85 (m, 1 H), 3.66 (s, 3 H), 2.91 (t, J = 7.4 Hz, 2 H), 2.32 (t, J = 7.4 Hz, 2 H), 1.95–1.40 (m, 5 H), 1.40–1.18 (m, 24 H), 0.95–0.81 (m, 9 H). Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.39; H, 11.35; N, 3.38.

5.2.2.14. (*S*)-Methyl 4-(2-oxohexadecanamido)octanoate (12b). Yield 92%; White solid; mp 56–58 °C; $[\alpha]_D$ −4.6 (c 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.80–6.60 (m, 1 H), 4.20–3.80 (m, 1 H),

3.66 (s, 3 H), 2.91 (t, J = 7.4 Hz, 2 H), 2.35 (t, J = 7.4 Hz, 2 H), 1.97–1.42 (m, 6 H), 1.38–1.15 (m, 26 H), 0.98-0.81 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.5, 173.6, 160.0, 51.7, 49.3, 36.8, 34.8, 31.9, 30.7, 29.9, 29.6, 29.5, 29.4, 29.3, 29.1, 27.9, 23.2, 22.7, 22.4, 14.1, 13.9. Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.25; H, 11.32; N, 3.25.

5.2.2.15. (*S*)-*tert*-Butyl 4-(2-oxohexadecanamido)pentanoate (12c). Yield 95%; White solid; mp 52–53 °C; $[\alpha]_D$ −1.6 (c 1.0, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.95–6.80 (m, 1 H), 4.05–3.80 (m, 1 H), 2.85 (t, J = 7.4 Hz, 2 H), 2.25 (t, J = 7.4 Hz, 2 H), 1.85–1.65 (m, 2 H), 1.65–1.45 (m, 2 H), 1.40 (s, 9 H), 1.40–1.10 (m, 25 H), 0.88 (t, J = 6.6 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 172.3, 159.6, 80.4, 45.2, 36.6, 32.1, 31.8, 31.3, 29.5, 29.4, 29.3, 29.2, 29.0, 28.0, 23.1, 22.6, 20.6, 14.0. Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.38; H, 11.25; N, 3.21.

5.2.2.16. (*S*,2*E*,4*E*)-Ethyl 6-(2-oxohexadecanamido)deca-2,4-dienoate (17). Yield 91%; White solid; mp 53–55 °C; $[\alpha]_D$ −11.2 (c 0.5 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.22 (dd, J_1 = 15.2 Hz, J_2 = 10.8 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 6.25 (dd, J_1 = 15.2 Hz, J_2 = 10.6 Hz, 1 H), 6.00 (dd, J_1 = 15.2 Hz, J_2 = 6.2 Hz, 1 H), 5.86 (d, J = 15.8 Hz, 1 H), 4.74 (m, 1 H), 4.19 (q, J = 7.2 Hz, 2 H), 2.91 (t, J = 7.4 Hz, 2 H), 1.73–1.44 (m, 4 H), 1.28 (br s, 29 H), 0.88 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 166.7, 159.4, 143.3, 141.0, 128.7, 122.0, 60.3, 50.9, 36.7, 34.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 27.7, 23.1, 22.6, 22.3, 14.2, 14.1, 13.9; MS (FAB): m/z (%): 464 (37) $[M+H]^+$; Anal. Calcd for $C_{28}H_{49}NO_4$: C, 72.53; H, 10.65; N, 3.02. Found: C, 72.28; H, 10.74; N, 3.09.

5.2.2.17. (*S*)-Ethyl 6-(2-oxohexadecanamido)decanoate (18). Yield 93%; White solid; mp 47–49 °C; $[\alpha]_D$ −1.6 (c 0.5 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.66 (d, J = 9.6 Hz, 1 H), 4.11 (q, J = 7.2 Hz, 2 H), 4.00–3.81 (m, 1 H), 2.92 (t, J = 7.4 Hz, 2 H), 2.28 (t, J = 7.4 Hz, 2 H), 1.80–1.21 (m, 39 H), 0.88 (t, J = 6.2 Hz, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.7, 173.5, 159.8, 60.2, 49.4, 36.7, 34.7, 34.1, 31.9, 29.6, 29.4, 29.3, 29.1, 27.9, 25.4, 24.7, 23.2, 22.7, 22.5, 14.2, 14.1, 13.9; MS (FAB): m/z (%): 468 (87) $[M+H]^+$; Anal. Calcd for $C_{28}H_{53}NO_4$: C, 71.90; H, 11.42; N, 2.99. Found: C, 71.77; H, 11.51; N, 3.08.

5.2.2.18. (*S*,2*E*,4*E*)-6-(2-Oxohexadecanamido)deca-2,4-dienoic acid (19). Saponification of **15** took place before oxidation. Yield 53%; White solid; mp 91–93 °C; $[\alpha]_D$ −35.1 (c 1 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.20 (dd, J_1 = 15.2 Hz, J_2 = 10.8 Hz, 1 H), 6.85 (d, J = 9 Hz, 1 H), 6.18 (dd, J_1 = 15.2 Hz, J_2 = 11 Hz, 1 H), 5.92 (dd, J_1 = 15.4 Hz, J_2 = 6.2 Hz, 1 H), 5.77 (d, J = 15.4 Hz, 1 H), 4.57–4.43 (m, 1 H), 2.82 (t, J = 7.2 Hz, 2 H), 1.76–1.43 (m, 4 H), 1.14 (br s, 26 H), 0.88 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.3, 171.8, 159.5, 145.7, 142.3, 128.4, 121.0, 50.9, 36.7, 34.3, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 27.7, 23.1, 22.6, 22.3, 14.1, 13.9; MS (ESI): m/z (%): 434 (100) $[M-H]^-$; Anal. Calcd for $C_{26}H_{45}NO_4$: C, 71.68; H, 10.41; N, 3.22. Found: C, 71.51; H, 10.52; N, 3.25.

5.2.2.19. 2-Oxo-N-(4-oxooctyl)hexadecanamide (26). Yield 79%; White solid; mp 73–76 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.09 (t, J = 6.4 Hz, 1 H), 3.35–3.25 (m, 2 H), 2.90 (t, J = 7.2 Hz, 2 H), 2.50–2.36 (m, 4 H), 1.98–1.76 (m, 2 H), 1.67–1.45 (m, 4 H), 1.25 (br s, 24 H), 0.88 (t, J = 6.6 Hz, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 210.3, 199.2, 160.3, 42.6, 39.7, 38.8, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 25.9, 23.2, 23.1, 22.7, 22.3, 14.1, 13.8; MS (FAB): m/z (%): 396 (100) $[M+H]^+$; Anal. Calcd for $C_{24}H_{45}NO_3$: C, 72.86; H, 11.46; N, 3.54. Found: C, 72.74; H, 11.51; N, 3.59.

5.2.2.20. *N*-(4-Ethoxybutyl)-2-oxohexadecanamide (27). Yield 88%; White solid; mp 55–57 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.19 (t, J = 6.4 Hz, 1 H), 3.52–3.25 (m, 6 H), 2.90 (t, J = 7.8 Hz, 2 H), 1.78–1.48 (m, 6 H), 1.40–1.15 (m, 25 H), 0.87 (t, J = 6.6 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 199.4, 160.2, 69.9, 66.2, 39.0, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 27.1, 26.2, 23.1, 22.6, 15.1, 14.1; MS (FAB): m/z (%): 370 (100) $[M+H]^+$; Anal. Calcd for $C_{22}H_{43}NO_3$: C, 71.50; H, 11.73; N, 3.79. Found: C, 71.43; H, 11.65; N, 3.91.

5.2.2.21. Methyl 2-(2-oxo-8-phenyloctanamido)acetate (37a). Yield 82%; Oily solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.55–7.42 (m, 1 H), 7.40–7.23 (m, 5 H), 4.16 (d, J = 5.4 Hz, 2 H), 3.86 (s, 3 H), 3.00 (t, J = 6.8 Hz, 2 H), 2.69 (t, J = 7.2 Hz, 2 H), 1.80–1.62 (m, 4 H), 1.55–1.35 (m, 4 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.1, 169.2, 160.2, 142.6, 128.3, 128.2, 125.6, 52.5, 40.8, 36.6, 35.8, 31.2, 28.9, 28.8, 23.0; MS (ESI) m/z (%): 328 (100) $[M+Na]^+$; Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.48; H, 7.78; N, 4.65.

5.2.2.22. *tert*-Butyl 3-(2-oxo-8-phenyloctanamido)propanoate (37b). Yield 83%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.58–7.42 (m, 1 H), 7.40–7.22 (m, 5 H), 3.61 (q, J = 6.2 Hz, 2 H), 2.99 (t, J = 7.0 Hz, 2 H), 2.68 (t, J = 7.4 Hz, 2 H), 2.56 (t, J = 6.2 Hz, 2 H), 1.79–1.60 (m, 4 H), 1.54 (s, 9 H), 1.49–1.40 (m, 4 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.9, 171.0, 160.0, 142.6, 128.3, 128.2, 125.5, 81.3, 36.6, 35.8, 34.8, 34.7, 31.2, 28.9, 28.8, 28.0, 23.0; MS (ESI) m/z (%): 384 (100) $[M+Na]^+$; Anal. Calcd for $C_{21}H_{31}NO_4$: C, 69.78; H, 8.64; N, 3.87. Found: C, 69.40; H, 8.89; N, 3.94.

5.2.3. (*R*,*E*)-Ethyl 4-(*tert*-butoxycarbonyl)oct-2-enoate (39a)

To a solution of **38** (0.22 g, 1.00 mmol) in a mixture of toluene–EtOAc (6 mL), a solution of NaBr (0.11 g, 1.05 mmol) in water (0.5 mL) was added, followed by AcNH-TEMPO (2.2 mg, 0.010 mmol). To the resulting biphasic system, which was cooled at −5 °C, an aqueous solution of 0.35 M NaOCl (3.14 mL, 1.10 mmol) containing $NaHCO_3$ (0.25 g, 3 mmol) was added dropwise while stirring vigorously at −5 °C over a period of 1 h. After the mixture had been stirred for a further 15 min at 0 °C, EtOAc (6 mL) and H_2O (2 mL) were added. The aqueous layer was separated and washed with EtOAc (4 mL). The combined organic layers were washed consecutively with 5% aqueous citric acid (6 mL) containing KI (0.04 g), 10% aqueous $Na_2S_2O_3$ (6 mL), and brine and dried over Na_2SO_4. The solvents were evaporated under reduced pressure, and the residue was used immediately in the next step without any purification. To the solution of the N-protected α-amino aldehyde in dry THF (5 mL), Ph_3P=CHCOOEt (1.1 mmol) was added and the reaction mixture was refluxed for 1 h. Saturated aqueous NH_4Cl (4 mL) was added and extracted with Et_2O (3 × 5 mL). The combined organic phases were washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography [EtOAc–petroleum ether (bp 40–60 °C) 2:8]. Yield 80% (0.23 g); White solid; mp 48-50 °C; $[\alpha]_D$ + 10.1 (c 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.84 (dd, J_1 = 5.6 Hz, J_2 = 15.8 Hz, 1 H), 5.90 (dd, J_1 = 1.4 Hz, J_2 = 15.8 Hz, 1 H), 4.52 (m, 1 H), 4.38–4.05 (m, 3 H), 1.80–1.10 (m, 18 H), 0.89 (t, J = 6.2 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.4, 155.1, 148.6, 120.5, 79.7, 60.4, 51.4, 34.3, 28.3, 27.7, 22.4, 14.2, 13.9; Anal. Calcd for $C_{15}H_{27}NO_4$: C, 63.13; H, 9.54; N, 4.91. Found: C, 63.02; H, 9.69; N, 4.85.

5.2.4. General method for catalytic hydrogenation

To a solution of the substrate (1.00 mmol) in MeOH (10 mL) (through which N_2 had been passed for 5 min), 10% Pd/C catalyst (10 mg, 0.01 mmol) was added. The reaction mixture was stirred under H_2 atmosphere overnight at room temperature. The catalyst was removed by filtration through a pad of Celite, and the organic solvent was evaporated under reduced pressure.

5.2.4.1. (*S*)-Ethyl 6-(*tert*-butoxycarbonylamino)decanoate (14). Yield 89%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.26 (d, J = 9.2 Hz, 1 H), 4.10 (q, J = 7 Hz, 2 H), 3.64–3.41 (m, 1 H), 2.28 (t, J = 7.4 Hz, 2 H), 1.71–1.16 (m, 24 H), 0.87 (t, J = 6.2 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.7, 155.7, 78.8, 60.1, 50.4, 35.2, 34.2, 28.3, 28.0, 25.4, 24.9, 22.6, 14.2, 14.0. Anal. Calcd for $C_{17}H_{33}NO_4$: C, 64.73; H, 10.54; N, 4.44. Found: C, 64.69; H, 10.61; N, 4.46.

5.2.4.2. (*R*)-Ethyl 4-(*tert*-butoxycarbonyl)octanoate (40a). Yield 99%; White solid; mp 32–34 °C; $[\alpha]_D$ +3.4 (c 1 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.29 (d, J = 9.2 Hz, 1 H), 4.13 (q, J = 6.4 Hz, 2 H), 3.62–3.45 (m, 1 H), 2.36 (t, J = 7.6 Hz, 2 H) 1.89–1.78 (m, 2 H), 1.76–1.58 (m, 2 H), 1.43 (s, 9 H), 1.40–1.21 (m, 7 H), 0.88 (t, J = 6.4 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.7, 155.7, 79.0, 60.4, 50.3, 35.5, 31.1, 30.5, 28.4, 28.0, 22.5, 14.2, 14.0; Anal. Calcd for $C_{15}H_{29}NO_4$: C, 62.69; H, 10.17; N, 4.87. Found: C, 62.55; H, 10.23; N, 4.95.

5.2.5. *tert*-Butyl 4-oxooctylcarbamate (22)

To a stirred solution of Boc-γ-aminobutyric acid (0.40 g, 2.0 mmol) in dry THF (2 mL), cooled at −78 °C, a solution of *n*-BuLi in hexane (3 mL, 2.5 M) was added dropwise. After completion of the addition, the reaction mixture was allowed to warm to room temperature and stirring was continued for 4 h. Then, saturated NH_4Cl (5 mL) and EtOAc (10 mL) were added. The aqueous layer was separated and extracted with EtOAc (2× 10 mL). The combined organic layers were dried over Na_2SO_4, the organic solvent was evaporated under reduced pressure and the residue was purified by column-chromatography using $CHCl_3$ as eluent. Yield 46%; White solid; mp 36–38 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.68 (br, 1 H), 3.13–3.03 (m, 2 H), 2.45–2.33 (m, 4 H), 1.87–1.65 (m, 2 H), 1.55–1.23 (m, 13 H), 0.86 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 210.7, 156.0, 79.0, 42.5, 40.0, 39.7, 28.3, 25.8, 23.9, 22.2, 13.7. Anal. Calcd for $C_{13}H_{25}NO_3$: C, 64.16; H, 10.36; N, 5.76. Found: C, 64.10; H, 10.42; N, 5.71.

5.2.6. *tert*-Butyl 4-ethoxybutylcarbamate (23)

To a stirred solution of *tert*-butyl 4-hydroxybutylcarbamate (0.38 g, 2.0 mmol) and ethyl bromide (0.65 g, 6 mmol) in benzene (2 mL) at 10 °C, aq 50% NaOH (2 mL) and phase transfer catalyst Bu_4NHSO_4 (0.08 g, 0.3 mmol) were added. The reaction mixture was vigorously stirred for 4 h at 10 °C and overnight at room temperature. The aqueous layer was separated and extracted with EtOAc (2× 5 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was purified by column-chromatography using $CHCl_3$ as eluent. Yield 95%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.72 (br, 1 H), 3.46–3.34 (m, 4 H), 3.19–3.06 (m, 2 H), 1.60–1.43 (m, 4 H), 1.38 (s, 9 H), 1.14 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 155.9, 78.7, 70.1, 66.0, 40.3, 28.3, 27.0, 26.8, 15.0. Anal. Calcd for $C_{11}H_{23}NO_3$: C, 60.80; H, 10.67; N, 6.45. Found: C, 60.55; H, 10.81; N, 6.32.

5.2.7. 2-Hydroxy-8-phenyloctanenitrile (34)

To a stirred solution of **33** (0.45 g, 2.4 mmol) in CH_2Cl_2 (3 mL), a solution of $NaHSO_3$ (0.37 g, 3.5 mmol) in water (0.6 mL) was added and the mixture was vigorously stirred for 30 min at room temperature. The organic solvent was evaporated under reduced pressure, water was added and the mixture was cooled at 0 °C. Then, a solution of KCN (0.23 g, 3.5 mmol) in water (0.6 mL) was added dropwise over a period of 3.5 h and then the mixture was left under stirring overnight at room temperature. The aqueous suspension was extracted with CH_2Cl_2 (2× 5 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by column-chromatography using $CHCl_3$ as eluent. Yield 75%; Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–7.22 (m, 5 H), 4.58–4.35 (m, 1 H), 3.90–3.78 (m, 1 H), 2.69 (t, J = 6.6 Hz, 2 H), 1.95–1.80 (m, 2 H), 1.80–1.60 (m, 2 H), 1.60–1.40 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 142.4, 128.2, 128.1, 125.5, 120.0, 60.9, 35.7, 34.8, 31.1, 28.8, 28.6, 24.3; Anal. Calcd for $C_{14}H_{19}NO$: C, 77.38; H, 8.81; N, 6.45. Found: C, 77.11; H, 8.95; N, 6.37.

5.2.8. 2-Hydroxy-8-phenyloctanoic acid (35)

A solution of **34** (0.36 g, 1.7 mmol) in concd HCl (4.2 mL) was stirred overnight at room temperature. Then, water was added and the aqueous solution was extracted with $CHCl_3$ (3× 5 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the resulting 2-hydroxy-8-phenyloctanamide was precipitated using Et_2O.

To a solution of 2-hydroxy-8-phenyloctanamide (0.32 g, 1.3 mmol) in a mixture of EtOH/water (2:1, 10 mL), KOH (0.75 g, 13.4 mmol) was added and the reaction mixture was refluxed for 4 h. After cooling, EtOH was removed under reduced pressure, water was added and the aqueous solution was acidified with concd H_2SO_4 until pH 1, followed by extraction with Et_2O (3× 5 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was purified by recrystallization using CH_2Cl_2/petroleum ether. Yield 85%; White solid; mp 84–86 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35–7.24 (m, 5 H), 4.40–4.25 (m, 1 H), 2.68 (t, J = 6.6 Hz, 2 H), 2.00–1.82 (m, 2 H), 1.80–1.58 (m, 3 H), 1.58–1.15 (m, 6 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 179.5, 142.6, 128.4, 128.2, 125.6, 70.2, 35.9, 34.1, 29.0, 24.6; Anal. Calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 70.92; H, 8.84.

5.2.9. (*R*)-4-(7-Phenylheptanamido)octanoic acid (42)

To a stirred solution of **41a** (or **41b**) (2.00 mmol) in a mixture of dioxane/H_2O (9:1, 20 mL), 1 N NaOH (2.2 mL, 2.2 mmol) was added, and the mixture was stirred for 12 h at room temperature. The organic solvent was evaporated under reduced pressure, and H_2O (10 mL) was added. The aqueous layer was washed with EtOAc, acidified with 1 N HCl, and extracted with EtOAc (3× 12 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified after recrystallization [EtOAc/petroleum ether (bp 40–60 °C)]. Yield 90%; White solid; mp 73–75 °C; $[\alpha]_D$ −5.7 (c 1 $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.10 (br s, 1 H), 7.39–7.23 (m, 5 H), 5.86 (d, J = 9.0 Hz, 1 H), 4.10–3.94 (m, 1 H), 2.68 (t, J = 7.4 Hz, 2 H), 2.46 (t, J = 7.4 Hz, 2 H), 2.26 (t, J = 7.4 Hz, 2 H), 2.04–1.87 (m, 1 H), 1.82–1.57 (m, 5 H), 1.57–1.09 (m, 10 H), 0.97 (t, J = 6.4 Hz, 3 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 177.4, 173.9, 142.5, 128.3, 128.1, 125.5, 49.0, 36.6, 35.8, 34.9, 31.2, 31.1, 30.2, 29.0, 28.8, 28.0, 25.7, 22.4, 13.9; MS (ESI) m/z (%): 370 (100) $[M+Na]^+$; Anal. Calcd for $C_{21}H_{33}NO_3$: C, 72.58; H, 9.57; N, 4.03. Found: C, 72.26; H, 9.74; N, 4.11.

Compounds **10a–c**,[26] **13**,[30] **29**,[49] **30**,[50] **31**,[35] **33**,[51] **38**,[52] **39b**,[26] **40b**[26] have been described previously.

5.3. In-vitro PLA_2 assays

Phospholipase A_2 activity was determined using the previously described modified Dole assay[25] with buffer and substrate conditions optimized for each enzyme as described previously:[25,26,29,30] (i) GIVA $cPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 97 μM PAPC, 1.8 μM ^{14}C-labeled PAPC, and 3 μM PIP_2 in buffer containing 100 mM HEPES pH 7.5, 90 μM $CaCl_2$, 2 mM DTT and 0.1 mg/ml BSA; (ii) GVI $iPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 99 μM DPPC, and 1.5 μM ^{14}C-labeled DPPC in buffer containing 200 mM HEPES pH

7.0, 1 mM ATP, 2 mM DTT and 0.1 mg/ml BSA; and (iii) GV $sPLA_2$ substrate mixed-micelles were composed of 400 μM Triton X-100, 99 μM DPPC, and 1.5 μM ^{14}C-labeled DPPC in buffer containing 50 mM Tris pH 8.0 and 5 mM $CaCl_2$.

5.4. In-vitro PLA_2 inhibition studies

Initial screening of compounds at 0.091 mole fraction inhibitor in mixed-micelles was carried out. We considered compounds displaying 25% or less inhibition to have no inhibitory effect (designated N.D.). We report average percent inhibition (and standard error, $n = 3$) for compounds displaying more than 25% and less than 90% enzyme inhibition. If percent inhibition was greater than 90%, we determined its $X_I(50)$ by plotting percent inhibition vs. inhibitor molar fraction (7 points; typically 0.005–0.091 mole fraction). Inhibition curves were modeled in Graphpad Prism using either a linear (x, y intercept = 0) or non-linear regression (one-site binding model—hyperbola, BMAX = 100) to calculate the reported $X_I(50)$ and associated error values.

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