Published in final edited form as:

Bioorg Med Chem. 2016 April 15; 24(8): 1683–1695. doi:10.1016/j.bmc.2016.02.040.

Development of a potent 2-oxoamide inhibitor of secreted phospholipase A₂ guided by molecular docking calculations and molecular dynamics simulations

Sofia Vasilakaki^a, Efrosini Barbayianni^a, Georgios Leonis^b, Manthos G. Papadopoulos^b, Thomas Mavromoustakos^a, Michael H. Gelb^c, and George Kokotos^{a,*}

^aLaboratory of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Greece

^bInstitute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Avenue, Athens 11635, Greece

^cDepartment of Chemistry and Biochemistry, University of Washington, Seattle, WA 98195, USA

Abstract

Inhibition of group IIA secreted phospholipase A₂ (GIIA sPLA₂) has been an important objective for medicinal chemists. We have previously shown that inhibitors incorporating the 2-oxoamide functionality may inhibit human and mouse GIIA sPLA₂s. Herein, the development of new potent inhibitors by molecular docking calculations using the structure of the known inhibitor **7** as scaffold, are described. Synthesis and biological evaluation of the new compounds revealed that the long chain 2-oxoamide based on (*S*)-valine **GK241** led to improved activity (IC₅₀ = 143 nM and 68 nM against human and mouse GIIA sPLA₂, respectively). In addition, molecular dynamics simulations were employed to shed light on **GK241** potent and selective inhibitory activity.

Keywords

Docking; Inhibitors; Molecular dynamics; 2-Oxoamides; Secreted phospholipase A₂

1. Introduction

Phospholipases A₂ (PLA₂) are a superfamily of enzymes whose main role is the hydrolysis of the ester bond of the membrane glycerophospholipids at the *sn*-2 position.¹ The products of this enzymatic activity are mainly unsaturated fatty acids which can act as bioactive mediators, correlating with numerous pathological conditions. PLA₂ enzymes are categorized into six types according to their dependency on calcium, their amino acid sequence domain, their molecular weight and their evolutionary relationship.¹

*Corresponding author. Tel.: +30 210 727 4462; fax: +30 210 727 4761. gkokotos@chem.uoa.gr (G. Kokotos).

Supplementary data

Supplementary data (code numbers of tested compounds, ¹H, ¹³C NMR and HRMS spectra of inhibitor **16a** are given. Docking results of the synthesized compounds and MD simulations of **16a** and **31c**) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2016.02.040>.

Approximately, one third of PLA₂ enzymes belong to the secreted phospholipase (sPLA₂) type, whose members are characterized by a low-molecular weight.^{2–4} They can act either by catalyzing reactions as enzymes or by binding to receptors and hence, they are involved in the activation of several biological pathways. To date, 11 gene products of sPLA₂s (IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB) have been identified in mammals. Their domains are highly stabilized by six common disulfide bonds and additionally one or two that are typical of each enzyme. Their active site contains the His/Asp dyad that is utilized in the catalytic mechanism. Their activation may require concentration of Ca²⁺ in the mM range and thus these proteins are localized in the extracellular space, targeting both cellular glycerophospholipids and soluble lipids.

The up-regulation or down-regulation of the expression of these enzymes is related to pathological conditions, such as atherosclerosis, cardiovascular disease and cancer, as it has been summarized in a number of recent reviews.^{5,6} As a consequence, a variety of synthetic inhibitors of sPLA₂ have been developed by pharmaceutical companies and research institutes.^{1,7–9} In particular, GIIA sPLA₂ has been related to inflammation since it was identified in 1989.¹⁰ GIIA sPLA₂ is highly expressed in synovial cells, while its concentration increases in the plasma of patients who suffer from coronary artery disease, making it a useful tool for prognostic purposes.

Many attempts for selective inhibition of GIIA sPLA₂ have been made with many positive outcomes. Varespladib¹¹ (**1a**, Fig. 1), which is a potent but not selective GIIA sPLA₂ inhibitor, was advanced into clinical trials as an intravenously-administered therapy for sepsis-induced systemic inflammatory response syndrome. Although it was found to have an acceptable safety profile in patients with severe sepsis, its development was terminated because Phase II studies resulted in little efficacy. Also, Varespladib methyl (**1b**, Fig. 1) which functions as a prodrug and is rapidly converted in vivo to Varespladib, was synthesized as an attempt to improve Varespladib efficacy. Both inhibitors were claimed by Anthera Pharmaceuticals as new agents for the treatment of cardiovascular diseases and underwent clinical trials from 2006 to 2012. However, on 2012 phase III clinical studies were terminated due to lack of efficacy.

The expression of the full set of human and mouse groups I, II, V, X, and XII sPLA₂s in *Escherichia coli* and insect cells provided pure recombinant enzymes for detailed comparative interfacial kinetic and binding studies.¹² Among the class of inhibitors of sPLA₂s based on substituted indoles, 6,7-benzoindoles and indolizines,^{13,14} it was found that compound **2** (Fig. 1) was a selectively potent inhibitor against hGX over all other human and mouse sPLA₂ enzymes, while compound **3** (Fig. 1) inhibited nearly all human and mouse sPLA₂s in the low nanomolar range. Another important class of sPLA₂ inhibitors is amides based on non-natural amino acids. Compound FPL67047XX¹⁵ (**4**, Fig. 1) was found to be a potent inhibitor of human platelet sPLA₂, and its precise binding interactions with the human nonpancreatic sPLA₂ were determined by high-resolution X-ray crystallography. The structurally related compound **5** (Fig. 1) was co-crystallized with GIIA hnpPLA₂ and the crystal structure revealed a chelation to a Ca²⁺ ion through its carboxylate and amide oxygen atoms, H-bonding through an amide NH group to His48, multiple hydrophobic contacts and a T-shaped aromatic group—His6 interaction.¹⁶ In addition, compound

GK115¹⁷ (**6**, Fig. 1) was found to inhibit GV sPLA₂ ($X_1(50) = 0.003 \pm 0.0004$) without affecting the activities of intracellular GIVA cPLA₂ and GVIA iPLA₂. In a continuation of our studies on a novel class of cPLA₂ inhibitors,^{18,19} the 2-oxoamide based on the natural α -amino acid (*S*)-leucine GK126,²⁰ (**7**, Fig. 1), demonstrated a promising IC₅₀ value of 0.30 μ M against GIIA sPLA₂.²⁰

Computer-aided drug design has recently offered powerful tools for the rational design of PLA₂ inhibitors, raising the potential for the development of new compounds with improved inhibitor properties. A recent review article demonstrates various applications of rational design on PLA₂ inhibitors.²¹ For example, docking calculations have led to the design of new indole inhibitors of sPLA₂.²² The aim of this work was to develop improved inhibitors of GIIA sPLA₂ by keeping the 2-oxoamide functionality of the lead 2-oxoamide inhibitor **7** and altering its other structural features. Using molecular docking simulations, we designed new 2-oxoamides based on α -amino acids. Herein, the synthesis of new 2-oxoamides derivatives and their in vitro activities against various sPLA₂s are presented. Moreover, we report our results from docking, molecular dynamics simulations and free energy calculations of the complexes, in an attempt to understand their structure–activity relationships.

2. Results and discussion

2.1. Design and docking of new 2-oxoamides

New 2-oxoamides were designed based on the structure of the lead inhibitor **7** (Fig. 2). Proteinogenic α -amino acids of both *S* and *R* configuration were used to replace the (*S*)-leucine part of **7** and the resulted derivatives were docked in the active site of GIIA sPLA₂ using GOLD v5.2²³ program by CCDC. The evaluation of the generated poses was mainly based on the number of interactions they formed with the residues of the active site upon binding. The E_{Clash} , E_{Internal} , *ChemScore.InternalCorrection.Weighted* and *ChemScore.Rot.Weighted* penalty terms of the *ChemScore* scoring function were also considered. The highly scored penalty terms usually indicate poor geometry of the bound inhibitors.

According to the docking results, the derivatives containing α -amino acids with *R* configuration did not generate favorable conformations. This, in combination with the low inhibitory activity of similar derivatives previously reported²⁰ led us to discard these structures.

The derivatives containing non-polar α -amino acids with *S* configuration frequently generated favorable binding modes with comparatively good scoring values. Therefore, in order to better understand this behavior, simulated annealing was employed to generate 30 possible conformations for each of these molecules, which subsequently were docked in GOLD. By docking multiple conformations of each ligand, improved statistical analysis of the docking results was achieved.

Indeed, the recurrence statistics of the desirable binding motif of (*S*)-valine, (*S*)-alanine and (*S*)-proline derivatives was high, according to their docking solutions. More specifically, the

carboxyl group of the derivatives chelates the calcium ion and also forms a hydrogen bond with either Gly31 or Lys62 residues. In addition, the 2-carbonyl group or the oxygen atom of the amide group point to Ca^{2+} ion. A hydrogen bond is formed between Gly29 and the 2-carbonyl group in most cases (Supporting info). Similar results were generated when derivatives of two non-natural amino acids ((*S*)-*tert*-leucine and 2-aminoisobutyric acid) were docked. Moreover, some 2-hydroxy amides were also docked and no significant differences between the generated poses and the corresponding 2-oxoamides ones were observed. The two (*S*)-Glu derivatives were docked for comparison.

Synthesis and biological evaluation of the docked ligands were accomplished. According to the experimental data, which are discussed later, the (*S*)-valine derivative **16a** (**GK241**) demonstrated inhibitory activity against GIIA sPLA₂. Therefore, an effort to modify its structure, in order to succeed a higher potency, was made. It is well established that when a ligand has multiple conformations while unbound, then narrowing down this number to the only favorable conformations in the binding pocket, is entropically expensive. Thus, the replacement of **16a** long aliphatic chain by a shorter, carrying an aromatic system (phenyl, biphenyl or naphthyl), was a reasonable concept. New analogs were designed and some of the most promising structures, according to the docking simulations, were synthesized and tested.

2.2. Synthesis of inhibitors

The synthesis of 2-oxoamides and 2-hydroxyamides is presented in Schemes 1–3. The protected (*S*)- α -amino acids **9a–d** and **13a–c** were coupled with 2-hydroxyhexadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCl-HCl) as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt). The α -hydroxyamide methyl esters **10a–d** were saponified to produce the acids **11a–d**. Compounds **11b–d** were then oxidized to the corresponding 2-oxoamide acids **12b–d** using Dess–Martin periodinane. In addition, α -hydroxyamide *tert*-butyl esters **14a–c** were first oxidized using Dess–Martin reagent to afford **15a–c** and subsequently, the protecting group was removed by trifluoroacetic acid to afford the desired 2-oxoamides **16a,c,d**. The synthesis of compound **21** is described in Scheme 3.

The synthesis of 2-oxoamide (*S*)-valine analogs, where the long chain has been replaced by a medium one carrying an aromatic system, is depicted in Scheme 4. Horner–Wadsworth–Emmons olefination of 1,1'-biphenyl-4-carbaldehyde and 2-naphthaldehyde **22a,b** led to esters **23a,b** and subsequently catalytic hydrogenation to saturated products **24a,b**. The ethyl esters were then converted to alcohols **25a,b** using the di-isobutyl aluminum hydride (DIBALH) reducing agent, followed by the NaOCl/AcNH-TEMPO oxidation method, to form the corresponding aldehydes **26a,b**. The synthesis of **31c** started from 5-phenyl-1-pentanol **25c**, which was converted to the corresponding aldehyde **26c**, as it described above. Cyanohydrin reaction followed by acid and then alkaline conditions afforded the desirable α -hydroxy acids **28a–c**. These acids were coupled with (*S*)-valine, followed by oxidation and removal of the protecting group to afford the final products **31a–c**. The synthesis of **37** was accomplished by similar methods (Scheme 5).

2.3. In vitro inhibition of GIIA sPLA₂

The new 2-oxoamide derivatives were tested for inhibitory activity against a panel of human and mouse sPLA₂ enzymes, using a previously described continuous fluorometric assay.²⁴ None of the compounds **12b–d**, **16c,d** and **21** presented inhibition of GIIA sPLA₂ higher than 25% at 1 μ M concentration and thus we did not further study them. However, the (*S*)-valine derivative **16a**, exhibited high and selective potency for inhibition of GIIA sPLA₂ and the IC₅₀ values are summarized in Table 1. Compound **16a** demonstrates a two times higher potency for GIIA sPLA₂ than the previously reported inhibitor **7** and it is ten times more selective for GIIA than GV sPLA₂. The IC₅₀ values for Varespladib (**1a**, Fig. 1) using the same assay conditions, are included in Table 1 for comparison.

In addition, **16a** does not exhibit any appreciable inhibition against various other human and mouse sPLA₂ (Table 2).

Moreover, the lack of inhibitory activity of 2-hydroxyamide derivatives (data not shown) indicates that indeed the 2-oxoamide functionality has a crucial contribution to the binding affinity of these compounds.

The analogs of **16a** were also tested against several sPLA₂s and the results are summarized in Table 3. The in vitro activities of **31a–c** and **37** failed to meet our docking predictions. Molecular dynamics simulations were applied on **16a** and on one of its analogs in an attempt to understand the in vitro data.

2.4. Molecular dynamics simulations

The lowest energy docking poses of **16a** and **31c** were subjected to MD simulations in AMBER v12 molecular dynamics package.²⁵ Poses were selected so that the key interactions between the ligands and the binding site to be present. The total simulation time was 200 ns in explicit solvent and RMSD calculations were performed with respect to the starting coordinates of each complex.

In the initial poses, the inhibitor interacts with the calcium ion via its carboxyl group and the 2-carbonyl group of the amide (Figs. 3 and 4). During the first 20 ns of the simulation, both molecules were reoriented within the active site in such a way that the carboxylate oxygen atoms chelated the metal in bidentate fashion (Figs. 3 and 4). This interaction lasted until the end of the simulation of both complexes.

During the MD simulation of **16a** complex, two hydrogen bonds are formed between the amide group and the residues His47 and Gly29 (Table 4), resulting in the stable binding of **16a** to the enzyme. The RMSD distribution further supports the low mobility of the **16a** functional groups, which allows the formation of this H-bonds network (Fig. 5). However, the 2-oxoamide group of **31c** does not form any H-bond with the residues of the catalytic centre, during the simulation time (Table 4). Due to conformational changes of **31c**, the 2-oxoamide group moves away from the catalytic centre and periodically interacts only with the solvent molecules.

The fluctuations of the RMSD values manifest the high mobility of the **31c** in the active site (Fig. 5). The RMSD values were calculated for the part of the molecules which form the key interactions and consists of the C α atom of (*S*)-valine, the two carbon atoms of the 2-oxoamide moiety and the nitrogen atom of the amide.

Using the MD trajectories, free energy calculations were performed with the Molecular Mechanics/Poisson–Boltzmann Surface Area method (MM–PBSA) (Table 5). Frames from the second half of the simulation were used for the calculations. Analysis of the energetic terms for each complex, revealed a high contribution from van der Waals forces.

16a creates two additional H-bonds within the cavity of the enzyme compared to **31c**, which is reflected on the difference between the *E* electrostatic term of the two complexes (Table 5). Moreover, the entropic term does not greatly differ between the two complexes. Partly, this could be due to the fact that both molecules are similarly flexible. Interestingly, in the case of **16a** the overall flexibility of the compound does not increase the mobility of the 2-oxoamide group, which creates the main interactions with the residues. The difference in binding energies between **16a** and **31c** is 10.96 kcal mol⁻¹. This indicates that **16a** complex formation is more favored over **31c** complex formation, in agreement with the in vitro results.

3. Conclusion

Structural modifications were applied on the GIIA sPLA₂ inhibitor **7** and the new compounds were docked using GOLD. The results suggested that the non-polar α -amino acids derivatives with *S* configuration could generate favorable conformations. Their in vitro evaluation revealed that the 2-oxoamide inhibitor based on (*S*)-valine **16a** is a potent and selective inhibitor of GIIA sPLA₂. Further attempts to optimize the **16a** activity by altering its structure, did not lead to any desirable outcomes.

Thus, molecular dynamics simulations were used in order to understand how the replacement of the long aliphatic chain of **16a** resulted in the decrease of its potency. It was suggested that the aliphatic chain, which creates van der Waals interactions, adopts a conformation that keeps the 2-oxoamide moiety close to the key residues of the catalytic centre. Subsequently, the 2-oxoamide moiety forms a permanent H-bond network which further stabilize the **16a** binding. The replacement of the long aliphatic chain by a four C atoms chain carrying a phenyl ring led to an increase of 2-oxoamide group's mobility and the loss of the H-bonding interactions it crated with the active side. Although its carboxylic group chelates the Ca²⁺ ion and creates an H-bond with Gly29, these interactions seems to be not enough to keep the derivative bound to the enzyme.

Further work will be needed to test the in vivo potency of **16a**. Molecular dynamics simulations are a useful tool for explaining and evaluating the experimental data and should be used towards this direction.

4. Experimental section

4.1. Computational methods

4.1.1. Preparation of ligands' structures—The preparation of the structures was accomplished using SYBYL v8.0.²⁶ The charges were defined at physiological pH and the *S* or *R* configuration was assigned. Several minimization steps were performed to accomplish optimization of the structures, using initially the steepest descent algorithm for 300 iteration steps followed by the conjugate gradients method for another 300 iteration steps and finally the Powell algorithm for 700 iteration steps.²⁷ The Tripos force field²⁸ was applied during optimization and each method was terminated when a convergence of 0.05 kcal mol⁻¹ Å⁻¹ was reached. For the Powell algorithm, the simplex method²⁹ was used for its initial optimization.

The simulated annealing method³⁰ was used in order to sample 30 relevant conformations for each structure. Molecules were heated at 600 K for 1500 fs and annealed at 250 K for 2000 fs. The Tripos force field was used and the non-bonded cutoff was set to 8.0 Å. The structures produced were optimized using 200 iteration steps with the Powell algorithm with a gradient of 0.05 kcal mol⁻¹ Å⁻¹. The simplex method was used as well.

4.1.2. Docking—The atomic coordinates of the human secreted phospholipase group IIA protein were downloaded from Protein Data Bank³¹ (PDB code: 1KQU).¹⁶ Missing loops were added with the Prime module of Schrödinger Suite.³² Hydrogen atoms were added in GOLD and atoms types were automatically assigned by the program.

Docking simulations were performed in GOLD. The cavity of the catalytic centre of GIIA sPLA₂ was oriented within 5 Å around the crystallographic ligand including Leu2, Phe5, His6, Gly29, Gly31, His47, Asp48, Lys52, Lys62 and Asp91 amino acids. Eight crystal water molecules in the spatial vicinity to the catalytic center were kept and assigned as toggle and spin, while the rest were deleted. His6 was assigned to have flexible side chain (1 rotamer). H-bond donors/acceptors were treated as solvent accessible. The options 'flip amide bonds and ring corners' were set on for ligand flexibility. The *ChemScore* scoring function (1) along with chemscore. p450_csd.params parameters file were used to rank the generated binding modes.

$$ChemScore = -(\Delta G + \Delta E(\text{clash}) + \Delta E(\text{int})) \quad (1)$$

where *G* is the free energy change upon ligand binding, *E*(clash) is the protein–ligand clash penalty and *E*(int) is the ligand internal torsion strain penalty. It was established via additional computational experiments that this combination of scoring function and parameters were suitable for the docking. Internal ligand energy offset was enabled and the preset genetic algorithm parameter settings were used and set to 100,000 operations.

4.1.3. Molecular dynamics simulations—MD simulations in explicit solvent were performed using AMBER.²⁵ The Antechamber tool was used to generate the partial atomic

charges of each ligand with the AM1-BCC³³ method. Modification of the force field parameters of each structure was made by Parmchk program. The AMBER force field *ff12SB*³⁴ and the general AMBER force field (GAFF)³⁵ were loaded in tLEaP module to create the parameters and topologies of the protein and the organic molecule, respectively. The complex was loaded in tLEaP along with the parameters file of calcium ions, which were created by a combination of crystallographic data,¹⁶ GAFF force field parameters³⁵ and the Aqvist et al. method.³⁶ Disulfide bonds between Cys83-Cys59, Cys124-Cys49, Cys117-Cys26, Cys50-Cys90, Cys28-Cys44, Cys43-Cys97 and Cys88-Cys77 were assigned, as well as the coordination between the metal ion of the catalytic centre and the residues His27, Gly29 and Gly31. The complex was then immersed in a truncated octahedral water box using the TIP3P water model with 12.0 Å periodic boundary conditions. Finally, the system was neutralized by adding 18 Cl⁻ counter ions using the Joung and Cheatham ion parameters³⁷ and its coordinate and parameter topology files were saved.

Prior to the MD simulation, an initial minimization was performed using 1500 iteration steps of steepest descent and 1500 iteration steps of conjugate gradient in SANDER,³⁸ keeping the complex almost fixed with a restraint set to 400 kcal mol⁻¹ Å⁻¹. In addition, four sets of steepest descent and conjugate gradient steps were applied to the system, with the strength of the restraint being gradually reduced to 8 kcal mol⁻¹ Å⁻¹. The system was then smoothly heated from 0 to 300 K in three steps under constant volume. During these steps of total time 100 ps, the restraint was retained to 8 kcal mol⁻¹ Å⁻¹, hydrogen atoms were constrained using the SHAKE algorithm and the Langevin thermostat was used for temperature control. Two steps of equilibration were applied keeping the restraint to 8 kcal mol⁻¹ Å⁻¹ for the first 50 ps and then removed it for the next 50 ps, under constant pressure. MD simulations were performed with the pmemd.cuda³⁹ implementation for 200 ns and a 8 Å nonbonded cutoff was applied. SHAKE was kept during the production runs to allow the use of 2 fs time step, and the long-range electrostatic interactions were accounted for using the particle mesh Ewald (PME) method.⁴⁰ Analysis on the produced trajectories (RMSD, distances, H-bond interactions) was performed with the ptraj analysis tool available in the AmberTools13.⁴¹

The relative binding energies of each system (Table 5) were estimated using the MM-PBSA.⁴² The E_{elect} term is the electrostatic interaction energy and the E_{vdw} term is the van der Waals interaction energy.

The Poisson-Boltzmann (PB) model⁴³ was used for the estimation of the electrostatic contribution to solvation energies (G_{PB}) and the solvent-accessible surface area (SASA) was used for the estimation of the non electrostatic contributions (G_{NP}). The SASA is calculated by the following equation:

$$\Delta G_{\text{NP}} = \gamma \text{SASA} + \beta \quad (2)$$

where the SASA term is the surface area of the solute, the γ term is 0.00542 kcal mol⁻¹ Å⁻² while β is -1.008000 kcal mol⁻¹.⁴⁴ The γ and β are constants related with the surface tension coefficient and the offset, respectively.

The entropic term $T \cdot S$ was calculated using the *nmode* program available in AMBER. The metal ions were removed during the calculation (strip_mask) to avoid misleading results.

4.2. Chemistry

Merck Silica Gel 60 (70–230 or 230–400 mesh) was used for the chromatographic purification of products and Silica Gel 60 F254 aluminum plates for the thin-layer chromatography (TLC). UV light and/or phosphomolybdic acid in EtOH was employed for visualizing spots. A Büchi 530 apparatus was used to estimate melting points and they were uncorrected. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury at 200 MHz or 300 MHz and 50 MHz respectively. Samples were diluted in CDCl_3 , CD_3OD or DMSO. Chemical shifts are given in ppm, and coupling constants (J) in Hz. Peak multiplicities are typified as: s, singlet, d, doublet, t, triplet and m, multiplet. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan Surveyor MSQ Plus spectrometer. Specific rotations of the compounds were measured at 25 °C on a Perkin-Elmer 343 polarimeter using a 10 cm cell. Dichloromethane, diethylether and toluene were dried by standard procedures and stored over molecular sieves. No further purification of other solvents and chemicals needed as they were reagent grade. HRMS spectra were recorded on a Bruker Maxis Impact QTOF Spectrometer.

Compounds **23b**,⁴⁵ **24b**,⁴⁵ **25a**,⁴⁶ **26a**,⁴⁶ **25b**,⁴⁷ **26b**⁴⁸ have been described elsewhere and their analytical data are in accordance with literature.

4.2.1. Coupling method—To a stirred solution of hydrochloride amino component (1.0 mmol) in CH_2Cl_2 (10 mL), Et_3N (0.3 mL, 2.2 mmol) and subsequently 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (WSCl·HCl) (0.21 g, 1.1 mmol) and 1-hydroxybenzotriazole (HOBt) (0.14 g, 1.0 mmol) were added at 0 °C. The acid reactant (1.0 mmol) was added and the reaction mixture was stirred for 1 h at 0 °C and then overnight at room temperature. After the completion of the reaction, 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. Purification by flash column chromatography eluting with the appropriate mixture of EtOAc/petroleum ether (bp 40–60 °C) afforded the product.

4.2.1.1. (2S)-Methyl 2-(2-hydroxyhexadecanamido)-3-(1H-indol-3-yl)propanoate

(mixture of diastereomers) (10a): Yield 52%; White solid; mp 78–85 °C; $[\alpha]_{\text{D}}^{20}$ 41.1 (c 1.00, CHCl_3); ^1H NMR (CDCl_3 , 200 MHz): δ 8.22 (s, 1H, NH), 7.61–7.50 (m, 1H, arom), 7.43–6.88 (m, 5H, arom & NH), 5.08–4.88 (m, 1H, NHCH), 4.14–3.97 (m, 1H, CHOH), 3.81–3.67 (m, 3H, COOCH_3), 3.34 (d, J = 5.6 Hz, 2H, NHCHCH_2), 1.87–1.64 (m, 2H, CH_2CHOH), 1.62–1.15 (m, 24H, CH_2), 0.89 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (CDCl_3 , 50 MHz): δ 173.6, 172.3, 169.0, 168.4, 135.9, 122.7, 122.2, 119.6, 119.5, 118.5, 118.3, 111.3, 109.8, 80.6, 72.0, 52.4, 34.6, 31.9, 29.6, 29.5, 29.4, 29.3, 27.6, 24.9, 22.6, 14.1; MS (ESI) m/z (%): 473.3 (100) $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{28}\text{H}_{44}\text{N}_2\text{O}_4$: C, 71.15; H, 9.38; N, 5.93. Found: C, 71.03; H, 9.45; N, 5.87.

4.2.1.2. (2S)-Methyl 1-(2-hydroxyhexadecanoyl)pyrrolidine-2-carboxylate (mixture of

diastereomers) (10b): Yield 64%; White solid; mp 42–55 °C; $[\alpha]_D^{20}$ –50.0 (*c* 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 4.75–4.43 (m, 1H, NCH), 4.29–4.14 (m, 1H, CHOH), 3.71 (s, 3H, CH₃), 3.62–3.44 (m, 2H, NCH₂), 3.06 (br, 1H, OH), 2.57–1.74 (m, 6H, NCH₂CH₂CH₂ & CH₂CHOH), 1.73–1.42 (m, 4H, CH₂), 1.23 (s, 20H, CH₂), 0.85 (t, *J* = 6.4 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 173.6, 172.4, 81.9, 69.5, 59.4, 59.1, 56.7, 52.5, 46.7, 45.6, 34.4, 32.7, 32.1, 29.9, 29.7, 29.6, 29.2, 28.9, 25.3, 24.9, 22.9, 14.3; MS (ESI) *m/z* (%): 384.4 (100) [M+H]⁺; Anal. Calcd for C₂₂H₄₁NO₄: C, 68.89; H, 10.77; N, 3.65. Found: C, 68.63; H, 10.84; N, 3.56.

4.2.1.3. (2S)-Methyl 2-(2-hydroxyhexadecanamido)-3,3-dimethylbutanoate (mixture of

diastereomers) (10c): Yield 84%; White solid; mp 73–75 °C; $[\alpha]_D^{20}$ 2.30 (*c* 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.29–7.07 (m, 1H, NH), 4.47–4.37 (m, 1H, NHCH), 4.21–4.04 (m, 1H, CHOH), 3.70 (s, 3H, CH₃), 1.93–1.70 (m, 2H, CH₂CHOH), 1.52–1.16 (m, 24H, CH₂), 0.96 (s, 9H, CH₃), 0.85 (t, *J* = 6.8 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.0, 172.1, 171.7, 72.2, 71.9, 59.5, 59.4, 51.7, 34.7, 31.8, 29.6, 29.3, 29.2, 26.4, 25.0, 24.9, 22.6, 14.0; MS (ESI) *m/z* (%): 400.2 (100) [M+H]⁺; Anal. Calcd for C₂₃H₄₅NO₄: C, 69.13; H, 11.35; N, 3.51. Found: C, 69.05; H, 11.51; N, 3.32.

4.2.1.4. Methyl 2-(2-hydroxyhexadecanamido)-2-methylpropanoate (racemic mixture)

(10d): Yield 54%; White solid; mp 53–56 °C; $[\alpha]_D^{20}$ 2.35 (*c* 1.02, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.13 (s, 1H, NH), 4.09–3.93 (m, 1H, CHOH), 3.72 (s, 3H, COOCH₃), 3.53 (br, 1H, OH), 1.84–1.58 (m, 2H, CH₂CHOH), 1.53 (s, 6H, CH₃), 1.34–1.10 (m, 24H, CH₂), 0.86 (t, *J* = 6.8 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.9, 173.7, 71.8, 55.9, 52.5, 34.5, 31.8, 29.6, 29.4, 29.3, 24.8, 24.6, 22.6, 14.0; MS (ESI) *m/z* (%): 372.2 (100) [M+H]⁺; Anal. Calcd for C₂₁H₄₁NO₄: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.66; H, 11.29; N, 3.72.

4.2.1.5. (2S)-tert-Butyl 2-(2-hydroxyhexadecanamido)-3-methylbutanoate (mixture of

diastereomers) (14a): Yield 62%; White solid; mp 49–53 °C; $[\alpha]_D^{20}$ 9.80 (*c* 1.01, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.17 (d, *J* = 9.0 Hz, ½H, NH), 7.05 (d, *J* = 9.0 Hz, ½H, NH), 4.46–4.31 (m, 1H, CH), 4.18–4.03 (m, 1H, CHOH), 4.00 (d, *J* = 4.8 Hz, ½H, OH), 3.83 (d, *J* = 4.8 Hz, ½H, OH), 2.24–2.03 (m, 1H, NHCHCH), 1.90–1.52 (m, 2H, CH₂CHOH), 1.44 (s, 9H, CH₃), 1.34–1.13 (m, 24H, CH₂), 0.97–0.75 (m, 9H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.1, 174.0, 171.3, 170.9, 82.0, 81.9, 72.2, 71.9, 57.0, 56.8, 34.8, 31.8, 31.3, 31.2, 29.6, 29.5, 29.3, 27.9, 25.0, 24.8, 22.6, 18.9, 18.8, 17.5, 17.4, 14.0; MS (ESI) *m/z* (%): 428.3 (95) [M+H]⁺; Anal. Calcd for C₂₅H₄₉NO₄: C, 70.21; H, 11.55; N, 3.28. Found: C, 70.03; H, 11.70; N, 3.19

4.2.1.6. (2S)-Di-tert-butyl 2-(2-hydroxyhexadecanamido)pentanedioate (mixture of

diastereomers) (14b): Yield 76%; Yellowish oil; $[\alpha]_D^{20}$ 8.1 (*c* 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.24–7.06 (m, 1H, NH), 4.61–4.38 (m, 1H, NHCH), 4.22–4.02 (m, 1H, CHOH), 3.61 (br, 1H, OH), 2.44–1.71 (m, 6H, CH₂), 1.53–1.38 (m, 18H, CH₃), 1.36–1.17 (m, 24H, CH₂), 0.97–0.78 (m, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.2, 174.0, 172.0, 170.8, 82.3, 82.2, 80.7, 71.9, 51.7, 34.7, 31.8, 31.5, 29.6, 29.3, 29.2, 27.9, 27.6, 24.9,

22.6, 14.0; MS (ESI) m/z (%): 514.6 (90) $[M+H]^+$; Anal. Calcd for $C_{29}H_{55}NO_6$: C, 67.80; H, 10.79; N, 2.73. Found: C, 67.62; H, 10.88; N, 2.67.

4.2.1.7. (2S)-tert-Butyl 2-(2-hydroxyhexadecanamido)propanoate (mixture of

diastereomers) (14c): Yield 40%; Colorless oil; $[\alpha]_D^{20}$ 7.7 (c 0.99, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.22–7.04 (m, 1H, NH), 4.56–4.35 (m, 1H, $NHCH$), 4.17–4.02 (m, 1H, $CHOH$), 3.45 (br, 1H, OH), 1.93–1.55 (m, 2H, CH_2CHOH), 1.45 (s, 9H, CH_3), 1.36 (d, J = 7.0 Hz, 3H, $NHCHCH_3$), 1.32–1.18 (s, 24H, CH_2), 0.86 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.9, 173.7, 172.3, 172.0, 81.9, 71.9, 48.1, 34.7, 34.6, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 27.8, 24.9, 22.6, 18.4, 14.0; MS (ESI) m/z (%): 400.4 (100) $[M+H]^+$; Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35; N, 3.51. Found: C, 68.91; H, 11.46; N, 3.43.

4.2.1.8. (2S)-5-tert-Butyl 1-ethyl-2-(2-hydroxyhexadecanamido) pentanedioate (mixture

of diastereomers) (19): Yield 53%; Yellow oil; $[\alpha]_D^{20}$ 5.80 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.33–7.19 (m, 1H, NH), 4.55–4.38 (m, 1H, $NHCH$), 4.18–3.95 (m, 3H, $CHOH$ & $COOCH_2$), 2.33–1.74 (m, 6H, CH_2 & CH_2CHOH), 1.34 (s, 9H, CH_3), 1.29–1.07 (m, 27H, CH_2 & CH_3), 0.76 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 174.6, 174.4, 171.8, 171.5, 80.6, 71.8, 61.4, 51.1, 34.5, 34.5, 34.4, 31.7, 31.2, 29.4, 29.2, 29.1, 27.8, 27.1, 24.8, 24.7, 22.4, 13.9; MS (ESI) m/z (%): 486.4 (100) $[M+H]^+$; Anal. Calcd for $C_{27}H_{51}NO_6$: C, 66.77; H, 10.58; N, 2.88. Found: C, 66.48; H, 10.72; N, 2.78.

4.2.1.9. (2S)-tert-Butyl 2-(2-hydroxy-6-(naphthalen-2-yl)hexanamido)-3-

methylbutanoate (mixture of diastereomers) (29a): Yield 73%; Colorless oil; $[\alpha]_D^{20}$ 10.1 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.86–7.68 (m, 3H, arom), 7.63–7.54 (m, 1H, arom), 7.51–7.24 (m, 3H, arom), 7.08 (d, J = 8.8 Hz, $\frac{1}{2}H$, NH), 6.91 (d, J = 8.8 Hz, $\frac{1}{2}H$, NH), 4.51–4.39 (m, 1H, $NHCH$), 4.21–4.06 (m, 1H, $CHOH$), 3.02 (br, 1H, OH), 2.78 (t, J = 7.4 Hz, 2H, CH_2), 2.27–2.06 (m, 1H, $NHCHCH$), 2.01–1.64 (m, 4H, CH_2), 1.62–1.40 (m, 11H, CH_2 & CH_3), 0.98–0.80 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.9, 173.8, 171.3, 170.9, 139.8, 133.5, 131.8, 127.7, 127.5, 127.3, 127.2, 126.2, 125.7, 124.9, 82.1, 82.0, 72.0, 71.8, 57.0, 56.8, 35.8, 35.8, 34.7, 34.6, 31.3, 31.2, 31.0, 27.9, 24.6, 18.9, 18.8, 17.5, 17.3; MS (ESI) m/z (%): 412.2 (100) $[M-H]^-$; Anal. Calcd for $C_{25}H_{35}NO_4$: C, 72.61; H, 8.53; N, 3.39. Found: C, 72.38; H, 8.69; N, 3.20.

4.2.1.10. (2S)-tert-Butyl 2-(6-([1,1'-biphenyl]-4-yl)-2-hydroxyhexanamido)-3-

methylbutanoate (mixture of diastereomers) (29b): Yield 68%; Colorless oil; $[\alpha]_D^{20}$ 10.8 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.64–6.90 (m, 10H, arom & NH), 4.47 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, $NHCH$), 4.25–4.05 (m, 1H, $CHOH$), 3.72–3.33 (m, 1H, OH), 2.67 (t, J = 7.4 Hz, 2H, CH_2), 2.31–2.08 (m, 1H, $NHCHCH$), 1.98–1.34 (m, 15H, CH_2 & CH_3), 1.00–0.81 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 174.0, 171.3, 170.9, 141.4, 141.0, 138.5, 128.8, 128.6, 126.8, 82.1, 82.0, 72.0, 71.8, 57.0, 56.8, 35.3, 34.6, 31.3, 31.2, 31.1, 27.9, 24.6, 18.9, 18.8, 17.5, 17.4; MS (ESI) m/z (%): 438.4 (100) $[M-H]^-$; Anal. Calcd for $C_{27}H_{37}NO_4$: C, 73.77; H, 8.48; N, 3.19. Found: C, 73.55; H, 8.65; N, 3.14.

4.2.1.11. (2S)-tert-Butyl 2-(2-hydroxy-6-phenylhexanamido)-3-methylbutanoate

(mixture of diastereomers) (29c): Yield 92%; Colorless oil; $[\alpha]_D^{20}$ 8.38 (*c* 0.99, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.33–6.96 (m, 6H, arom & NH), 4.42 (dd, $J_1 = 9.0$ Hz, $J_2 = 4.6$ Hz, 1H, NHCH), 4.21–4.03 (m, 1H, CHOH), 3.72 (br, 1H, OH), 2.61 (t, $J = 7.4$ Hz, 2H, CH₂), 2.30–2.06 (m, 1H, NHCHCH), 1.96–1.52 (m, 6H, CH₂), 1.47 (s, 9H, CH₃), 0.97–0.82 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.1, 174.0, 171.3, 170.8, 142.3, 128.2, 128.1, 125.5, 82.0, 81.9, 71.9, 71.8, 56.9, 56.7, 35.7, 34.5, 31.1, 27.9, 24.6, 18.8, 17.5, 17.4; MS (ESI) *m/z* (%): 362.4 (100) [M–H][–]; Anal. Calcd for C₂₁H₃₃NO₄: C, 69.39; H, 9.15; N, 3.85. Found: C, 69.27; H, 9.27; N, 3.75.

4.2.1.12. (2S)-tert-Butyl 2-(2-hydroxy-2-(naphthalen-2-yl)acetamido)-3-methylbutanoate (mixture of diastereomers) (35):

Yield 66%; Yellow oil; $[\alpha]_D^{20}$ 7.9 (*c* 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.88–7.68 (m, 4H, arom), 7.55–7.38 (m, 3H, arom), 7.29–6.77 (m, 1H, NH), 5.20 (d, $J = 3.0$ Hz, 1H, CHOH), 4.47–4.33 (m, 1H, NHCH), 2.20–1.97 (m, 1H, NHCHCH), 1.46–1.33 (m, 9H, CH₃), 0.93–0.66 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 172.1, 172.0, 170.7, 170.6, 136.7, 133.1, 133.0, 128.5, 128.3, 128.0, 127.5, 126.2, 126.1, 126.0, 125.9, 124.0, 123.9, 82.1, 74.3, 74.1, 57.3, 57.1, 31.3, 27.8, 18.7, 17.4, 17.2; MS (ESI) *m/z* (%): 356.2 (100) [M–H][–]; Anal. Calcd for C₂₁H₂₇NO₄: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.38; H, 7.70; N, 3.86.

4.2.2. Saponification of methyl esters—To a stirred solution of a methyl ester (1.0 mmol) in water, 1 N NaOH (1 mL, 1.0 mmol) was added and the mixture was left overnight at room temperature. After the completion of the reaction, the mixture was washed with EtOAc, acidified with 1 N HCl to pH 1 and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and evaporated under reduced pressure.

4.2.2.1. (2S)-2-(2-Hydroxyhexadecanamido)-3-(1H-indol-3-yl) propanoic acid (mixture of diastereomers) (11a):

Yield 80%; White solid; mp 144–146 °C; $[\alpha]_D^{20}$ 18.7 (*c* 1.00, MeOH); ¹H NMR (CD₃OD, 200 MHz): δ 7.63–7.51 (m, 1H, NH), 7.36–7.26 (m, 1H, arom), 7.15–6.92 (m, 3H, arom), 4.82–4.67 (m, 1H, NHCH), 4.01–3.88 (m, 1H, CHOH), 3.44–3.19 (m, 2H, NHCHCH₂), 1.77–1.50 (m, 2H, CH₂CHOH), 1.48–1.12 (m, 24H, CH₂), 0.89 (t, $J = 6.6$ Hz, 3H, CH₃); ¹³C NMR (CD₃OD, 50 MHz): δ 177.0, 175.0, 142.0, 128.9, 124.5, 122.4, 119.9, 119.4, 112.2, 110.4, 72.5, 53.3, 35.5, 33.1, 30.8, 30.5, 28.5, 25.9, 23.8, 14.5; HRMS (ESI) calcd for C₂₇H₄₂N₂NaO₄ [M+Na]⁺: 481.3037. Found: 481.3057; Anal. Calcd for C₂₇H₄₂N₂O₄: C, 70.71; H, 9.23; N, 6.11. Found: C, 70.59; H, 9.35; N, 6.01.

4.2.2.2. (2S)-1-(2-Hydroxyhexadecanoyl)pyrrolidine-2-carboxylic acid (mixture of diastereomers) (11b):

Yield 80%; White solid; mp 71–74 °C; $[\alpha]_D^{20}$ –5.2 (*c* 1.02, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 4.82–4.38 (m, 1H, NCH), 4.37–4.08 (m, 1H, CHOH), 3.82–3.30 (m, 2H, NCH₂), 2.56–1.76 (m, 4H, NCHCH₂CH₂), 1.73–1.00 (m, 26H, CH₂), 0.86 (t, $J = 6.8$ Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 174.6, 167.8, 81.5, 69.5, 59.5, 56.4, 46.7, 45.4, 33.9, 32.3, 31.8, 29.5, 29.4, 29.3, 28.9, 28.4, 25.0, 24.5, 22.6, 22.1, 14.0; HRMS (ESI) calcd for C₂₁H₃₉NNaO₄ [M+Na]⁺: 392.2771. Found: 392.2785;

Anal. Calcd for $C_{21}H_{39}NO_4$: C, 68.25; H, 10.64; N, 3.79. Found: C, 68.14; H, 10.73; N, 3.65.

4.2.2.3. (2S)-2-(2-Hydroxyhexadecanamido)-3,3-dimethylbutanoic acid (mixture of

diastereomers) (11c): Yield 80%; White solid; mp 99–101 °C; $[\alpha]_D^{20}$ 1.8 (*c* 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.52–7.01 (m, 1H, NH), 4.51–4.25 (m, 1H, $NHCH$), 4.24–4.03 (m, 1H, $CHOH$), 1.92–1.11 (m, 26H, CH_2), 0.96 (s, 9H, CH_3), 0.89–0.72 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 175.5, 174.6, 174.4, 74.5, 59.8, 34.6, 34.5, 31.9, 29.7, 29.6, 29.5, 29.3, 26.5, 25.1, 24.5, 22.6, 14.1; MS (ESI) m/z (%): 386.2 (100) $[M+H]^+$; Anal. Calcd for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.44; H, 11.35; N, 3.52.

4.2.2.4. 2-(2-Hydroxyhexadecanoylamino)-2-methylpropanoic acid (racemic mixture)

(11d): Yield 72%; White solid; mp 81–83 °C; $[\alpha]_D^{20}$ 1.7 (*c* 1.02, MeOH); 1H NMR ($CDCl_3$, 200 MHz): δ 7.20 (br s, 1H, NH), 4.21–3.92 (m, 1H, $CHOH$), 1.94–1.03 (m, 32H, CH_2 & CH_3), 0.88 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.1, 96.9, 96.4, 72.0, 34.3, 31.9, 29.8, 29.6, 29.5, 29.3, 25.1, 24.7, 24.5, 22.7, 14.1; MS (ESI) m/z (%): 358.3 (100) $[M+H]^+$; Anal. Calcd for $C_{20}H_{39}NO_4$: C, 67.19; H, 10.99; N, 3.92. Found: C, 67.02; H, 11.08; N, 3.86.

4.2.3. Oxidation of 2-hydroxyamides—To a solution of 2-hydroxyamide (1.0 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 1–3 h at room temperature. The organic solvent was evaporated under reduce pressure and Et_2O (30 mL) was added. The organic phase was washed with saturated aqueous $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (1.5 g, 9.5 mmol), H_2O (20 mL), dried over Na_2SO_4 and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [$EtOAc$ /petroleum ether (bp 40–60 °C), 2:8 or $CHCl_3$ /MeOH, 9:1].

4.2.3.1. (S)-1-(2-Oxohexadecanoyl)pyrrolidine-2-carboxylic acid (rotamers) (12b): Yield

80%; Colorless semi solid; $[\alpha]_D^{20}$ –3.2 (*c* 1.01, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.40 (br, 1H, $COOH$), 4.86–4.31 (m, 1H, NCH), 3.95–3.41 (m, 2H, NCH_2), 3.06–2.60 (m, 2H, CH_2CO), 2.46–1.12 (m, 28H, $NCHCH_2CH_2$ & CH_2), 0.88 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 200.0, 177.4, 163.0, 61.3, 48.3, 47.4, 39.1, 38.5, 31.9, 29.7, 29.5, 29.3, 29.1, 25.1, 23.1, 22.7, 22.6, 14.0; HRMS (ESI) calcd for $C_{21}H_{38}NO_4$ $[M+H]^+$: 368.2795. Found: 368.2794; Anal. Calcd for $C_{21}H_{37}NO_4$: C, 68.63; H, 10.15; N, 3.81. Found: C, 68.51; H, 10.22; N, 3.74.

4.2.3.2. (S)-3,3-Dimethyl 2-(2-oxohexadecanamido)butanoic acid (12c): Yield 62%;

Colorless oil; $[\alpha]_D^{20}$ 1.8 (*c* 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.50 (d, J = 9.2 Hz, 1H, NH), 4.31 (d, J = 9.2 Hz, 1H, $NHCH$), 2.91 (t, J = 6.8 Hz, 2H, CH_2CO), 1.74–1.49 (m, 2H, CH_2CH_2CO), 1.43–1.18 (m, 22H, CH_2), 1.01 (s, 9H, CH_3), 0.88 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.5, 175.7, 159.8, 60.8, 36.8, 34.7, 31.9, 29.6, 29.4, 29.3, 29.0, 26.5, 23.0, 22.6, 14.1; HRMS (ESI) calcd for $C_{22}H_{41}NNaO_4$ $[M+Na]^+$:

406.2928. Found: 406.2945; Anal. Calcd for $C_{22}H_{41}NO_4$: C, 68.89; H, 10.77; N, 3.65. Found: C, 69.11; H, 10.88; N, 3.59.

4.2.3.3. 2-Methyl 2-(2-oxo-hexadecanoylamino)propanoic acid (12d): Yield 95%; Colorless oil; 1H NMR ($CDCl_3$, 200 MHz): δ 7.77 (br s, 1H, COOH), 2.88 (t, J = 6.8 Hz, 2H, CH_2CO), 1.66–1.40 (m, 8H, CH_3 & CH_2CH_2CO), 1.39–1.15 (m, 22H, CH_2), 0.88 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 199.0, 198.8, 180.6, 160.0, 57.2, 36.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 24.2, 23.0, 22.6, 14.1; HRMS (ESI) calcd for $C_{20}H_{37}NNaO_4$ $[M+Na]^+$: 378.2615. Found: 378.2627; Anal. Calcd for $C_{20}H_{37}NO_4$: C, 67.57; H, 10.49; N, 3.94. Found: C, 67.36; H, 10.61; N, 3.87.

4.2.3.4. (S)-tert-Butyl 3-methyl-2-(2-oxohexadecanamido)butanoate (15a): Yield 92%; Yellow oil; $[\alpha]_D^{20}$ 12.7 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.40 (d, J = 9.0 Hz, 1H, NH), 4.40 (dd, J_1 = 9.2 Hz, J_2 = 4.4 Hz, 1H, $NHCH$), 2.91 (t, J = 7.2 Hz, 2H, CH_2COCO), 2.33–2.10 (m, 1H, $NHCHCH$), 1.71–1.53 (m, 2H, CH_2CH_2CO), 1.48 (s, 9H, CH_3) 1.40–1.16 (m, 22H, CH_2), 1.01–0.81 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.2, 169.6, 159.5, 81.9, 57.1, 36.4, 31.5, 31.1, 29.2, 29.0, 28.7, 27.6, 22.8, 22.3, 18.6, 17.1, 13.7; MS (ESI) m/z (%): 387.2 (100) $[M-tBu+NH_4]^+$; Anal. Calcd for $C_{25}H_{47}NO_4$: C, 70.54; H, 11.13; N, 3.29. Found: C, 70.43; H, 11.24; N, 3.21.

4.2.3.5. (S)-Di-tert-butyl 2-(2-oxohexadecanamido)pentanedioate (15b): Yield 84%; Yellow oil; $[\alpha]_D^{20}$ 9.18 (c 1.02, $CHCl_3$); 1H NMR ($CDCl_3$, 300 MHz): δ 7.47 (d, J = 8.4 Hz, 1H, NH), 4.49–4.37 (m, 1H, $NHCH$), 2.88 (t, J = 7.2 Hz, 2H, CH_2COCO), 2.38–2.08 (m, 3H, CH_2CHCH), 2.05–1.87 (m, 1H, CHH), 1.66–1.51 (m, 2H, CH_2-CH_2CO), 1.46 (s, 9H, CH_3), 1.42 (s, 9H, CH_3), 1.35–1.16 (m, 22H, CH_2), 0.86 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.3, 171.7, 170.0, 159.8, 82.7, 80.8, 52.1, 36.7, 31.8, 31.3, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.0, 27.9, 27.4, 23.0, 22.6, 14.0; MS (ESI) m/z (%): 529.5 (100) $[M+NH_4]^+$; Anal. Calcd for $C_{29}H_{53}NO_6$: C, 68.06; H, 10.44; N, 2.74. Found: C, 67.90; H, 10.55; N, 2.68.

4.2.3.6. (S)-tert-Butyl 2-(2-oxohexadecanamido)propanoate (15c): Yield 95%; White solid; mp 35–36 °C; $[\alpha]_D^{20}$ 5.0 (c 1.02, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.44 (d, J = 7.0 Hz, 1H, NH), 4.52–4.33 (m, 1H, $NHCH$), 2.90 (t, J = 7.4 Hz, 2H, CH_2CO), 1.76–1.38 (m, 14H, CH_3 & CH_2CH_2CO), 1.38–1.17 (m, 22H, CH_2), 0.86 (t, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.5, 171.1, 159.4, 82.4, 48.3, 36.6, 31.9, 29.6, 29.0, 27.9, 23.1, 22.6, 18.2, 14.1; MS (ESI) m/z (%): 415.3 (100) $[M+NH_4]^+$; Anal. Calcd for $C_{23}H_{43}NO_4$: C, 69.48; H, 10.90; N, 3.52. Found: C, 69.34; H, 10.96; N, 3.43.

4.2.3.7. (S)-5-tert-Butyl 1-ethyl 2-(2-oxohexadecanamido)pentanedioate (20): Yield 85%; Colorless oil; $[\alpha]_D^{20}$ 11.6 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 300 MHz): δ 7.52 (d, J = 8.2 Hz, 1H, NH), 4.60–4.49 (m, 1H, $NHCH$), 4.21 (q, J = 7.2 Hz, 2H, $COOCH_2$), 2.89 (t, J = 7.2 Hz, 2H, CH_2COCO), 2.41–2.17 (m, 3H, CH_2CHH), 2.08–1.92 (m, 1H, CHH), 1.66–1.52 (m, 2H, CH_2CH_2CO), 1.43 (s, 9H, CH_3), 1.37–1.17 (m, 25H, CH_2 & CH_3), 0.85 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.2, 171.6, 170.8, 159.9, 80.9, 61.7, 51.7,

36.7, 31.8, 31.2, 29.6, 29.4, 29.3, 29.0, 28.0, 27.2, 23.0, 22.6, 14.0; MS (ESI) m/z (%): 428.2 (100) $[M-tBu+H]^+$; Anal. Calcd for $C_{27}H_{49}NO_6$: C, 67.05; H, 10.21; N, 2.90. Found: C, 66.96; H, 10.29; N, 2.81.

4.2.3.8. (S)-tert-Butyl 3-methyl-2-(6-(naphthalen-2-yl)-2-oxohexanamido) butanoate

(30a): Yield 91%; Colorless oil; $[\alpha]_D^{20}$ 11.1 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.86–7.68 (m, 3H, arom), 7.65–7.53 (m, 1H, arom), 7.52–7.18 (m, 4H, arom & NH), 4.39 (dd, $J_1 = 9.2$ Hz, $J_2 = 4.6$ Hz, 1H, $NHCH$), 2.99 (t, $J = 7.2$ Hz, 2H, CH_2), 2.80 (t, $J = 7.0$ Hz, 2H, CH_2CO), 2.32–2.08 (m, 1H, $NHCHCH$), 1.86–1.58 (m, 4H, CH_2), 1.48 (s, 9H, CH_3), 1.00–0.82 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.3, 169.9, 159.8, 139.4, 133.5, 131.8, 127.8, 127.5, 127.3, 127.2, 126.3, 125.8, 125.0, 82.3, 57.4, 36.5, 35.6, 31.4, 30.6, 27.9, 22.7, 18.9, 17.4; MS (ESI) m/z (%): 356.2 (100) $[M-tBu+H]^+$; Anal. Calcd for $C_{25}H_{33}NO_4$: C, 72.96; H, 8.08; N, 3.40. Found: C, 72.85; H, 8.17; N, 3.26.

4.2.3.9. (S)-tert-Butyl 2-(6-([1,1'-biphenyl]-4-yl)-2-oxohexanamido)-3-methylbutanoate

(30b): Yield 82%; Yellow oil; $[\alpha]_D^{20}$ 12.2 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.75–7.16 (m, 10H, arom & NH), 4.48–4.29 (m, 1H, $NHCH$), 3.11–2.87 (m, 2H, CH_2), 2.80–2.55 (m, 2H, CH_2CO), 2.34–2.09 (m, 1H, $NHCHCH$), 1.85–1.60 (m, 4H, CH_2), 1.48 (s, 9H, CH_3), 1.04–0.82 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.3, 169.9, 159.8, 141.1, 141.0, 138.6, 128.7, 128.6, 127.0, 126.9, 82.3, 57.4, 36.5, 35.1, 31.4, 30.7, 27.9, 22.7, 18.9, 17.4; MS (ESI) m/z (%): 436.4 (100) $[M-H]^-$; Anal. Calcd for $C_{27}H_{35}NO_4$: C, 74.11; H, 8.06; N, 3.20. Found: C, 74.02; H, 8.17; N, 3.09.

4.2.3.10. (S)-tert-Butyl 3-methyl-2-(2-oxo-6-phenylhexanamido) butanoate (30c):

Yield 74%; Colorless oil; $[\alpha]_D^{20}$ 11.3 (c 1.00, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 7.50–6.94 (m, 6H, arom & NH), 4.36 (dd, $J_1 = 9.2$ Hz, $J_2 = 4.6$ Hz, 1H, $NHCH$), 2.93 (t, $J = 6.8$ Hz, 2H, CH_2), 2.62 (t, $J = 6.8$ Hz, 2H, CH_2CO), 2.31–2.06 (m, 1H, $NHCHCH$), 1.73–1.55 (m, 4H, CH_2), 1.46 (s, 9H, CH_3), 1.00–0.74 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 197.9, 169.7, 159.8, 142.0, 128.3, 125.6, 82.3, 57.1, 36.5, 35.5, 31.4, 30.7, 27.9, 22.6, 18.9, 17.4; MS (ESI) m/z (%): 360.4 (100) $[M-H]^-$; Anal. Calcd for $C_{21}H_{31}NO_4$: C, 69.78; H, 8.64; N, 3.87. Found: C, 69.61; H, 8.73; N, 3.81.

4.2.3.11. (S)-tert-Butyl 3-methyl-2-(2-(naphthalen-2-yl)-2-oxoacetamido) butanoate

(36): Yield 95%; Yellow oil; $[\alpha]_D^{20}$ 7.03 (c 1.01, $CHCl_3$); 1H NMR ($CDCl_3$, 200 MHz): δ 9.21–8.96 (m, 1H, arom), 8.23–7.05 (m, 7H, arom & NH), 4.55 (dd, $J_1 = 9.2$ Hz, $J_2 = 4.6$ Hz, 1H, $NHCH$), 2.43–2.08 (m, 1H, $NHCHCH$), 1.50 (s, 9H, CH_3), 1.09–0.79 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 186.6, 170.0, 161.6, 136.0, 134.8, 134.7, 132.2, 130.4, 130.2, 129.2, 128.2, 127.6, 126.7, 125.1, 82.3, 57.6, 31.5, 27.9, 19.0, 18.9, 17.6; MS (ESI) m/z (%): 354.1 (100) $[M-H]^-$; Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.74; H, 7.20; N, 3.86.

4.2.4. Cleavage of tert-butyl protecting group—A solution of the *tert*-butyl ester derivative (1.0 mmol) in 50% TFA/ CH_2Cl_2 (0.5 M) was stirred for 1–3 h at room

temperature. After the completion of the reaction, the organic solvent was evaporated under reduced pressure and the residue was purified by recrystallization.

4.2.4.1. (S)-3-Methyl 2-(2-oxohexadecanamido)butanoic acid (16a, GK241): Yield 72%;

White solid; mp 55–58 °C; $[\alpha]_D^{20}$ 5.04 (*c* 1.03, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.43 (d, *J* = 8.0 Hz, 1H, NH), 4.59–4.35 (m, 1H, NHCH), 2.91 (t, *J* = 6.8 Hz, 2H, CH₂CO), 2.42–2.16 (m, 1H, NHCHCH), 1.74–1.50 (m, 2H, CH₂CH₂CO), 1.47–1.15 (m, 22H, CH₂), 1.08–0.79 (m, 9H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.4, 176.0, 160.2, 57.4, 36.8, 31.9, 31.0, 29.7, 29.4, 29.3, 29.1, 23.1, 22.7, 19.1, 17.5, 14.1; HRMS (ESI) calcd for C₂₁H₄₀NO₄ [M+H]⁺: 370.2952. Found: 370.2938; Anal. Calcd for C₂₁H₃₉NO₄: C, 68.25; H, 10.64; N, 3.79. Found: C, 68.09; H, 10.76; N, 3.70.

4.2.4.2. (S)-2-(2-Oxohexadecanamido)propanoic acid (16c): Yield 90%; White solid; mp

98–101 °C; $[\alpha]_D^{20}$ 5.8 (*c* 1.03, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 8.92 (br, 1H, COOH), 7.43 (d, *J* = 7.6 Hz, 1H, NH), 4.69–4.50 (m, 1H, NHCH), 2.91 (t, *J* = 7.2 Hz, 2H, CH₂CO), 1.70–1.48 (m, 5H, CH₂CH₂CO & CH₃), 1.44–1.19 (s, 22H, CH₂), 0.88 (t, *J* = 6.6 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.3, 176.8, 159.6, 47.8, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.0, 22.6, 17.7, 14.1; HRMS (ESI) calcd for C₁₉H₃₆NO₄ [M+H]⁺: 342.2639. Found: 342.2650; Anal. Calcd for C₁₉H₃₅NO₄: C, 66.83; H, 10.33; N, 4.10. Found: C, 66.61; H, 10.45; N, 4.01.

4.2.4.3. (S)-2-(2-Oxohexadecanamido)pentanedioic acid (16d): Yield 90%; White solid;

mp 100–103 °C; $[\alpha]_D^{20}$ 30.5 (*c* 1.02, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 9.67 (br, 1H, COOH), 7.63 (d, *J* = 8.0 Hz, 1H, NH), 4.72–4.56 (m, 1H, NHCH), 2.91 (t, *J* = 7.0 Hz, 2H, CH₂CO), 2.64–2.43 (m, 2H, NHCHCH₂CH₂), 2.38–2.20 (m, 2H, NHCHCH₂), 1.72–1.51 (m, 2H, CH₂CH₂CO), 1.44–1.18 (m, 22H, CH₂), 0.88 (t, *J* = 6.4 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.1, 178.5, 176.2, 159.8, 51.2, 36.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 23.0, 22.6, 14.1; HRMS (ESI) calcd for C₂₁H₃₇NNaO₆ [M+Na]⁺: 422.2513. Found: 422.2513; Anal. Calcd for C₂₁H₃₇NO₆: C, 63.13; H, 9.33; N, 3.51. Found: C, 62.99; H, 9.44; N, 3.43.

4.2.4.4. (2S)-2-(2-Hydroxyhexadecanamido)-3-methylbutanoic acid (mixture of

diastereomers) (17): Yield 93%; White solid; mp 95–99 °C; $[\alpha]_D^{20}$ 17.1 (*c* 1.00, MeOH); ¹H NMR (CDCl₃, 200 MHz): δ 7.01 (d, *J* = 9.2 Hz, 1H, NH), 4.57–4.42 (m, 1H, NHCH), 4.24–4.10 (m, 1H, CHOH), 3.68 (br, 1H, OH), 2.39–2.15 (m, 1H, NHCHCH), 1.95–1.53 (m, 2H, CH₂CHOH), 1.51–1.12 (s, 24H, CH₂), 1.10–0.76 (m, 9H, 3xCH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 176.2, 175.3, 174.4, 72.2, 57.0, 56.7, 34.5, 34.4, 31.8, 30.6, 30.5, 29.6, 29.3, 25.0, 22.6, 19.0, 17.4, 14.1; HRMS (ESI) calcd for C₂₁H₄₁NNaO₄ [M+Na]⁺: 394.2928. Found: 394.2947; Anal. Calcd for C₂₁H₄₁NO₄: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.73; H, 11.23; N, 3.69.

4.2.4.5. (S)-5-Ethoxy-5-oxo-4-(2-oxohexadecanamido)pentanoic acid (21): Yield 93%;

White solid; mp 64–66 °C; $[\alpha]_D^{20}$ 13.4 (*c* 1.03, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 9.75 (br, 1H, COOH), 7.57 (d, *J* = 8.2 Hz, 1H, NH), 4.67–4.49 (m, 1H, NHCH), 4.22 (q, *J* = 7.0

Hz, 2H, COOCH₂), 2.89 (t, J = 7.2 Hz, 2H, CH₂CO), 2.36–1.94 (m, 2H, NHCHCH₂CH₂), 1.70–1.48 (m, 2H, NHCHCH₂), 1.46–1.08 (m, 24H, CH₂), 0.87 (t, J = 6.8 Hz, 3H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.1, 177.8, 170.7, 160.0, 61.9, 51.5, 36.7, 31.8, 29.9, 29.6, 29.3, 29.2, 29.0, 26.9, 23.0, 22.6, 14.0; HRMS (ESI) calcd for C₂₃H₄₁NNaO₆ [M + Na]⁺: 450.2826. Found: 450.2826; Anal. Calcd for C₂₃H₄₁NO₆: C, 64.61; H, 9.67; N, 3.28. Found: C, 64.49; H, 9.74; N, 3.22.

4.2.4.6. (S)-3-Methyl 2-(6-(naphthalen-2-yl)-2-oxohexanamido) butanoic acid

(31a): Yield 83%; Colorless solid; $[\alpha]_D^{20}$ –2.8 (c 0.5, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.85–7.69 (m, 3H, arom), 7.64–7.58 (m, 1H, arom), 7.51–7.28 (m, 4H, arom & NH), 5.94 (br, 1H, COOH), 4.52 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H, NHCH), 2.98 (t, J = 6.8 Hz, 2H, CH₂), 2.81 (t, J = 7.0 Hz, 2H, CH₂CO), 2.41–2.21 (m, 1H, NHCHCH), 1.88–1.59 (m, 4H, CH₂), 1.08–0.89 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.1, 175.4, 159.9, 139.4, 133.5, 131.9, 127.8, 127.5, 127.3, 127.2, 126.3, 125.8, 125.0, 56.9, 36.5, 35.6, 31.0, 30.5, 22.7, 19.0, 17.4; HRMS (ESI) calcd for C₂₁H₂₆NO₄ [M + H]⁺: 356.1856. Found: 356.1841; Anal. Calcd for C₂₁H₂₅NO₄: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.84; H, 7.16; N, 3.87.

4.2.4.7. (S)-2-(6-([1,1'-Biphenyl]-4-yl)-2-oxohexanamido)-3-methylbutanoic acid

(31b): Yield 92%; White solid; mp 123–124 °C; $[\alpha]_D^{20}$ –6.1 (c 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 7.70–7.08 (m, 10H, arom & NH), 4.56–4.26 (m, 1H, NHCH), 3.12–2.80 (m, 2H, CH₂), 2.76–2.46 (m, 2H, CH₂CO), 2.39–2.08 (m, 1H, NHCHCH), 1.83–1.44 (m, 4H, CH₂), 1.10–0.68 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.1, 176.2, 160.1, 141.0, 140.9, 138.6, 128.7, 128.6, 127.0, 126.9, 57.5, 36.6, 35.2, 30.7, 27.3, 22.6, 19.1, 17.5; HRMS (ESI) calcd for C₂₃H₂₈NO₄ [M + H]⁺: 382.2013. Found: 382.1995; Anal. Calcd for C₂₃H₂₇NO₄: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.64; H, 7.24; N, 3.25.

4.2.4.8. (S)-3-Methyl 2-(2-oxo-6-phenylhexanamido)butanoic acid (31c):

Yield 50%; Colorless oil; $[\alpha]_D^{20}$ 5.8 (c 1.00, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 9.43 (br, 1H, COOH), 7.37 (d, J = 9.0 Hz, 1H, NH), 7.32–7.03 (m, 5H, arom), 4.50 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHCH), 3.04–2.86 (m, 2H, CH₂), 2.72–2.50 (m, 2H, CH₂CO), 2.41–2.16 (m, 1H, NHCHCH), 1.79–1.50 (m, 4H, CH₂), 1.11–0.81 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 198.0, 175.8, 159.9, 141.9, 128.2, 125.7, 57.1, 36.5, 35.5, 30.9, 30.6, 22.5, 19.0, 17.4; HRMS (ESI) calcd for C₁₇H₂₄NO₄ [M + H]⁺: 306.1700. Found: 306.1695; Anal. Calcd for C₁₇H₂₃NO₄: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.65; H, 7.71; N, 4.52.

4.2.4.9. (S)-3-Methyl 2-(2-(naphthalen-2-yl)-2-oxoacetamido) butanoic acid (37):

Yield 80%; Yellow solid; mp 122–124 °C; $[\alpha]_D^{20}$ –25.3 (c 1.03, CHCl₃); ¹H NMR (CDCl₃, 200 MHz): δ 9.58 (br, 1H, COOH), 9.15 (s, 1H, arom), 8.17 (d, J = 8.6 Hz, 1H, NH), 8.00 (d, J = 7.8 Hz, 1H, arom), 7.94–7.76 (m, 2H, arom), 7.75–7.44 (m, 3H, arom), 4.71 (dd, J_1 = 9.0 Hz, J_2 = 4.6 Hz, 1H, NHCH), 2.54–2.26 (m, 1H, NHCHCH), 1.18–0.95 (m, 6H, CH₃); ¹³C NMR (CDCl₃, 50 MHz): δ 186.4, 176.4, 161.9, 136.0, 135.0, 132.2, 130.3, 130.2, 129.4, 128.4, 127.6, 126.8, 125.0, 57.1, 31.1, 19.1, 17.5; HRMS (ESI) calcd for C₁₇H₁₈NO₄ [M + H]⁺: 300.1230. Found: 300.1216; Anal. Calcd for C₁₇H₁₇NO₄: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.12; H, 5.80; N, 4.59.

4.2.4.10. (2E,4E)-Ethyl 5-(naphthalen-2-yl)penta-2,4-dienoate (23a): To a stirred solution of aldehyde (1.0 mmol) in dry THF (10 mL) and molecular sieves (1.5 g/mmol aldehyde) under Ar, $\text{C}_2\text{H}_5\text{OOCH}=\text{CHCH}_2\text{P}(=\text{O})(\text{OC}_2\text{H}_5)_2$ (1.5 mmol) was added at 0 °C. Then, $\text{LiOH}\cdot\text{H}_2\text{O}$ (1.5 mmol) was added dropwise and the reaction mixture heated at reflux for 24 h. The reaction was filtered over celite and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40–60 °C), 5:95]. Yield 50%; White solid; mp 100–102 °C; ^1H NMR (CDCl_3 , 200 MHz): δ 7.90–7.60 (m, 5H, arom), 7.55–7.45 (m, 3H, arom & CH), 7.10–6.95 (m, 2H, CH), 6.04 (s, 1H, CH), 4.26 (m, 2H, CH_2), 1.28 (t, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (CDCl_3 , 50 MHz): δ 167.1, 144.6, 140.4, 133.5, 133.4, 128.5, 128.2, 128.1, 127.7, 126.6, 126.5, 126.4, 123.3, 121.3, 60.4, 14.3; MS (ESI) m/z (%): 253 (100) $[\text{M}+\text{H}]^+$; Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{O}_2$: C, 80.93; H, 6.39. Found: C, 80.85; H, 6.55.

4.2.6. Ethyl 5-(naphthalen-2-yl)pentanoate (24a)—To a stirred solution of the alkene (1.0 mmol) in absolute EtOH (10 mL), Pd/C 10% was added. The reaction left overnight under H_2 at room temperature. The reaction was filtered over celite and the organic solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc/petroleum ether (bp 40–60 °C), 5:95]. Yield 80%; Colorless oil; ^1H NMR (CDCl_3 , 200 MHz): δ 8.03–7.18 (m, 7H, arom), 4.34–4.06 (m, 2H, $\text{COOCH}_2\text{CH}_3$), 3.03–2.69 (m, 2H, CH_2), 2.51–2.31 (m, 2H, CH_2), 1.91–1.69 (m, 4H, CH_2), 1.41–1.22 (m, 3H, CH_3); ^{13}C NMR (CDCl_3 , 50 MHz): δ 173.4, 139.4, 133.4, 131.8, 127.6, 127.4, 127.2, 127.1, 126.2, 125.7, 124.9, 60.0, 35.5, 34.0, 30.5, 24.4, 14.1; MS (ESI) m/z (%): 274.1 (100) $[\text{M}+\text{NH}_4]^+$; Anal. Calcd for $\text{C}_{17}\text{H}_{20}\text{O}_2$: C, 79.65; H, 7.86. Found: C, 79.39; H, 8.00.

4.2.7. General method of oxidation of primary alcohols to aldehydes and then to cyanohydrins—To a solution of primary alcohol (1.0 mmol) in a mixture of toluene (3 mL) and EtOAc (3 mL), a solution of NaBr (0.11 g, 1.1 mmol) in water (0.5 mL) was added and the mixture was cooled down to –5 °C. To the resulting biphasic system, AcNH-TEMPO (2.2 mg, 0.01 mmol) was added, followed by the drop-wise addition of an aqueous solution of 0.5 M NaOCl (2.2 mL, 1.1 mmol) and NaHCO_3 (0.24 g, 3.0 mmol) over a period of 1 h under vigorous stirring. After the mixture was stirred for a further 15 min at 0 °C, EtOAc (10 mL) and H_2O (10 mL) were added. The aqueous layer was separated and washed with EtOAc (2×10 mL). The combined organic layers were washed consecutively with 5% aqueous citric acid (10 mL) containing KI (0.04 g), 10% aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (10 mL), and brine and dried over Na_2SO_4 . The solvents were evaporated under reduced pressure and the aldehyde was used straight to the next reaction without further purification.

To a solution of the aldehyde (1.0 mmol) in CH_2Cl_2 (1.3 mL), an aqueous solution of NaHSO_3 (0.25 mL, 1.5 mmol) was added and the mixture was stirred for 30 min at room temperature. The organic solvent was evaporated under reduced pressure and H_2O (5 mL) was added. The mixture was cooled down to 0 °C and KCN 6 M (0.25 mL, 1.5 mmol) was added drop-wise under vigorous stirring. The reaction was left stirring for 18 h at room temperature. After the completion of the reaction, the organic layer was extracted with CH_2Cl_2 (2×20 mL), washed with brine and dried over Na_2SO_4 . The organic solvent was

evaporated under reduced pressure and the compound was purified with flash column chromatography [EtOAc/petroleum ether (bp 40–60 °C), 2:8].

4.2.8. 2-Hydroxy-6-(naphthalen-2-yl)hexanenitrile (27a)—Yield 56%; Yellow oil; ^1H NMR (CDCl_3 , 200 MHz): δ 7.95–7.19 (m, 7H, arom), 4.41 (t, J = 6.6 Hz, 1H, CHOH), 2.99 (br, 1H, OH), 2.88–2.66 (m, 2H, CH_2), 1.98–1.42 (m, 6H, CH_2); ^{13}C NMR (CDCl_3 , 50 MHz): δ 139.3, 133.4, 131.8, 127.8, 127.5, 127.3, 127.1, 126.3, 125.9, 125.1, 119.9, 61.0, 35.6, 34.9, 30.5, 24.1; MS (ESI) m/z (%): 257.1 (100) $[\text{M}+\text{NH}_4]^+$; Anal. Calcd for $\text{C}_{16}\text{H}_{17}\text{NO}$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.07; H, 7.32; N, 5.66.

4.2.9. 6-([1,1'-Biphenyl]-4-yl)-2-hydroxyhexanenitrile (27b)—Yield 80%; Pink solid; mp 78–82 °C; ^1H NMR (CDCl_3 , 200 MHz): δ 7.69–7.25 (m, 9H, arom), 4.50–4.46 (m, 1H, CHOH), 3.64 (br, 1H, OH), 2.83–2.70 (m, 2H, CH_2), 1.97–1.90 (m, 2H, CH_2), 1.80–1.61 (m, 4H, CH_2); ^{13}C NMR (CDCl_3 , 50 MHz): δ 140.9, 140.7, 138.5, 128.6, 126.9, 126.7, 120.0, 60.8, 35.0, 34.7, 30.5, 24.1; MS (ESI) m/z (%): 282.9 (100) $[\text{M}+\text{NH}_4]^+$; Anal. Calcd for $\text{C}_{18}\text{H}_{19}\text{NO}$: C, 81.47; H, 7.22; N, 5.28. Found: C, 81.25; H, 7.39; N, 5.23.

4.2.9.1. 2-Hydroxy-6-phenylhexanenitrile (27c): Yield 78%; Colorless oil; ^1H NMR (CDCl_3 , 200 MHz): δ 7.44–7.15 (m, 5H, arom), 4.56–4.32 (m, 1H, CHOH), 4.03 (br, 1H, OH), 2.81–2.59 (m, 2H, CH_2), 2.01–1.48 (m, 6H, CH_2); ^{13}C NMR (CDCl_3 , 50 MHz): δ 141.7, 128.1, 125.5, 119.9, 60.7, 35.3, 34.6, 30.4, 23.9; MS (ESI) m/z (%): 207.3 (90) $[\text{M}+\text{NH}_4]^+$; Anal. Calcd for $\text{C}_{12}\text{H}_{15}\text{NO}$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.04; H, 8.11; N, 7.30.

4.2.10. 2-Hydroxy-2-(naphthalen-2-yl)acetonitrile (33)—To a stirring solution of naphthalene-2-carbaldehyde (1.0 mmol) in EtOAc/THF/AcOH 4:4:2, aqueous solution of NaCN 6 M (0.25 mL, 1.5 mmol) was added. After the completion of the reaction, the organic layer was extracted with CH_2Cl_2 (2 $\times$ 20 mL), washed with brine and dried over Na_2SO_4 . The organic solvent was evaporated under reduced pressure and the compound was purified with flash column chromatography [EtOAc/petroleum ether (bp 40–60 °C), 2:8]. Yield 95%; White solid; mp 110–113 °C; ^1H NMR (CDCl_3 , 200 MHz): δ 7.99–7.24 (m, 7H, arom), 5.78–5.59 (m, 1H, CHOH), 2.08 (d, J = 2.4 Hz, 1H, OH); ^{13}C NMR (CDCl_3 , 50 MHz): δ 133.6, 132.8, 132.3, 129.4, 128.3, 127.7, 127.2, 123.6, 63.8; MS (ESI) m/z (%): 157.1 (73) $[\text{M}-\text{CN}]^+$; Anal. Calcd for $\text{C}_{12}\text{H}_9\text{NO}$: C, 78.67; H, 4.95; N, 7.65. Found: C, 78.49; H, 5.10; N, 7.56.

4.2.11. Converting the nitriles into corresponding carboxylic acids—The nitrile (1.0 mmol) was dissolved in HCl conc. (2.5 mL) and stirred for 18 h at room temperature. H_2O (5 mL) was then added and the organic layer was extracted with CH_2Cl_2 , washed with brine and dried over Na_2SO_4 . The organic solvent was evaporated under reduced pressure and the amide derivative was crystallized over cold petroleum ether.

To a solution of the amide derivative (0.8 mmol) in MeOH/ H_2O (2:1, 6 mL), KOH (8.0 mmol) was added and the reaction mixture was heated under reflux for 2 h. Then, methanol was evaporated under reduced pressure, H_2O was added and the aqueous layer was acidified with 1 N HCl to pH 1. The organic layer was extracted with EtOAc (3 $\times$ 15 mL), dried over

Na₂SO₄ and the solvent was evaporated under reduced pressure. The compound was purified by recrystallization [CH₂Cl₂/petroleum ether (bp 40–60 °C)].

4.2.11.1. 2-Hydroxy-6-(naphthalen-2-yl)hexanoic acid (28a): Yield 98%; White solid; mp 115–118 °C; ¹H NMR (CDCl₃, 200 MHz): δ 7.81–7.25 (m, 7H, arom), 4.35–4.10 (m, 1H, CHOH), 2.78 (t, *J* = 7.4 Hz, 2H, CH₂), 2.07–1.31 (m, 6H, CH₂); ¹³C NMR (CDCl₃, 50 MHz): δ 177.1, 139.8, 133.4, 131.7, 127.6, 127.4, 127.2, 127.2, 126.1, 125.7, 124.9, 69.9, 49.7, 49.2, 48.8, 35.7, 33.9, 30.9, 24.5; MS (ESI) *m/z* (%): 257.2 (100) [M–H][–]; Anal. Calcd for C₁₆H₁₈O₃: C, 74.39; H, 7.02. Found: C, 74.31; H, 7.18.

4.2.11.2. 6-([1,1'-Biphenyl]-4-yl)-2-hydroxyhexanoic acid (28b): Yield 93%; White solid; ¹H NMR (DMSO, 200 MHz): δ 7.48–7.11 (m, 9H, arom), 4.06–3.94 (m, 1H, CHOH), 2.58–2.50 (m, 2H, CH₂), 1.74–1.37 (m, 6H, CH₂); ¹³C NMR (CDCl₃, 50 MHz): δ 177.1, 141.4, 140.9, 138.4, 128.6, 128.5, 126.8, 126.8, 69.9, 35.2, 33.9, 31.0, 24.5; MS (ESI) *m/z* (%): 282.9 (100) [M–H][–]; Anal. Calcd for C₁₈H₂₀O₃: C, 76.03; H, 7.09. Found: C, 75.77; H, 7.23.

4.2.11.3. 2-Hydroxy-6-phenylhexanoic acid (28c): Yield 63%; White solid; mp 100–102 °C; ¹H NMR (CDCl₃, 200 MHz): δ 7.41–7.10 (m, 5H, arom), 4.36–4.20 (m, 1H, CHOH), 2.64 (t, *J* = 7.2 Hz, 2H, CH₂), 2.02–1.37 (m, 6H, CH₂); ¹³C NMR (CDCl₃, 50 MHz): δ 179.5, 142.3, 128.4, 128.3, 125.7, 70.1, 35.7, 34.0, 31.1, 24.4; MS (ESI) *m/z* (%): 208.1 (100) [M][–]; Anal. Calcd for C₁₂H₁₆O₃: C, 69.21; H, 7.74. Found: C, 69.04; H, 7.89.

4.2.11.4. 2-Hydroxy-2-(naphthalen-2-yl)acetic acid (34): Yield 90%; White solid; mp 57–60 °C; ¹H NMR (CDCl₃, 200 MHz): δ 7.89–7.24 (m, 7H, arom), 5.28 (s, 1H, CHOH), 3.32–3.08 (m, 1H, OH); ¹³C NMR (CDCl₃, 50 MHz): δ 175.1, 135.8, 133.1, 128.2, 127.9, 127.5, 126.2, 126.1, 126.1, 125.8, 124.1, 72.6; MS (ESI) *m/z* (%): 202.21 (100) [M+H]⁺; Anal. Calcd for C₁₂H₁₀O₃: C, 71.28; H, 4.98. Found: C, 71.11; H, 5.13.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research has been co-financed by the European Union (European Social Fund—ESF) and Greek national funds through the Operational Program ‘Education and Lifelong Learning’ of the National Strategic Reference Framework (NSRF)—Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

This work was supported in part by Grant HL36235 from the National Institutes of Health (to M.H.G.).

Partial support was offered by LinkSCEEM-2 project, funded by the European Commission under the 7th Framework Program through Capacities Research Infrastructure, INFRA-2010-1.2.3 Virtual Research Communities, Combination of Collaborative Project and Coordination and Support Actions (CP-CSA) under grant agreement no. RI-261600.

References and notes

1. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G. *Chem Rev.* 2011; 111:6130. [PubMed: 21910409]
2. Murakami M, Lambeau G. *Biochimie.* 2013; 95:43. [PubMed: 23022039]
3. Murakami M, Taketomi Y, Miki Y, Sato H, Yamamoto K, Lambeau G. *Biochimie.* 2014; 107(Pt A): 105. [PubMed: 25230085]
4. Murakami M, Sato H, Miki Y, Yamamoto K, Taketomi Y. *J Lipid Res.* 2015; 56:1248. [PubMed: 25805806]
5. Hui DY. *Curr Opin Lipidol.* 2012; 23:235. [PubMed: 22327613]
6. Quach ND, Arnold RD, Cummings BS. *Biochem Pharmacol.* 2014; 90:338. [PubMed: 24907600]
7. Magrioti V, Kokotos G. *Exp Opin Ther Pat.* 2010; 20:1.
8. Magrioti V, Kokotos G. *Exp Opin Ther Pat.* 2013; 3:333.
9. Ong WY, Farooqui T, Kokotos G, Farooqui AA. *ACS Chem Neurosci.* 2015; 6:814. [PubMed: 25891385]
10. Seilhamer JJ, Pruzanski W, Vadas P, Plant S, Miller JA, Kloss J, Johnson LK. *J Biol Chem.* 1989; 264:5335. [PubMed: 2925608]
11. Draheim SE, Bach NJ, Dillard RD, Berry DR, Carlson DG, Chirgadze NY, Clawson DK, Hartley LW, Johnson LM, Jones ND, McKinney ER, Mihelich ED, Olkowski JL, Schevitz RW, Smith AC, Snyder DW, Sommers CD, Wery JP. *J Med Chem.* 1996; 39:5159. [PubMed: 8978844]
12. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, Sadilek M, Nguyen E, Lazdunski M, Lambeau G, Gelb MH. *J Biol Chem.* 2002; 277:48535. [PubMed: 12359733]
13. Smart BP, Oslund RC, Walsh LA, Gelb MH. *J Med Chem.* 2006; 49:2858. [PubMed: 16686528]
14. Oslund RC, Cermak N, Gelb MH. *J Med Chem.* 2008; 51:4708. [PubMed: 18605714]
15. Beaton HG, Bennion C, Connolly S, Cook AR, Gensmantel NP, Hallam C, Hardy K, Hitchin B, Jackson CG, Robinson DH. *J Med Chem.* 1994; 37:557. [PubMed: 8126692]
16. Hansford KA, Reid RC, Clark CI, Tyndall JDA, Whitehouse MW, Guthrie T, McGearry RP, Schafer K, Martin JL, Fairlie DP. *ChemBioChem.* 2003; 4:181. [PubMed: 12616631]
17. Antonopoulou G, Barbayianni E, Magrioti V, Cotton N, Stephens D, Constantinou-Kokotou V, Dennis EA, Kokotos G. *Bioorg Med Chem.* 2008; 16:10257. [PubMed: 18993078]
18. Kokotos G, Six DA, Loukas V, Smith T, Constantinou-Kokotos V, Hadjipavlou-Litina D, Kotsovolou S, Chiou A, Beltzner CC, Dennis EA. *J Med Chem.* 2004; 47:3615. [PubMed: 15214789]
19. Six DA, Barbayianni E, Loukas V, Constantinou-Kokotou V, Hadjipavlou-Litina D, Stephens D, Wong AC, Magrioti V, Moutevelis-Minakakis P, Baker SF, Dennis EA, Kokotos G. *J Med Chem.* 2007; 50:4222. [PubMed: 17672443]
20. Mouchlis VD, Magrioti V, Barbayianni E, Cermak N, Oslund RC, Mavromoustakos TM, Gelb MH, Kokotos G. *Bioorg Med Chem.* 2011; 19:735. [PubMed: 21216150]
21. Mouchlis VD, Barbayianni E, Mavromoustakos TM, Kokotos G. *Curr Med Chem.* 2011; 18:2566. [PubMed: 21568891]
22. Mouchlis VD, Mavromoustakos TM, Kokotos G. *J Comput Aided Mol Des.* 2010; 24:107. [PubMed: 20130961]
23. GOLD 5.2. CCDC; Cambridge, UK: 2013.
24. Smart BP, Pan YH, Weeks AK, Bollinger JG, Bahnson BJ, Gelb MH. *Bioorg Med Chem.* 2004; 12:1737. [PubMed: 15028265]
25. Case, DA., Darden, T., Cheatham, TE., III, Simmerlilling, CL., Wang, J., Duke, RE., Luo, R., Walker, RC., Zhang, W., Merz, KM., Roberts, BP., Hayik, S., Roitberg, A., Seabra, G., Swails, J., Goetz, AW., Kolossvai, I., Wong, KF., Paesani, F., Vanicek, J., Wolf, RM., Liu, J., Wu, X., Brozell, SR., Steinbrecher, T., Gohlke, H., Cai, Q., Ye, X., Wang, B., Hsieh, MJ., Cui, G., Roe, DR., Mathews, DH., Seetin, MG., Salomon-Ferrer, R., Sagui, C., Babin, V., Luchko, T., Gusarov, S., Kovalenko, A., Kollman, PA. AMBER 12. University of California; San Francisco: 2012.
26. SYBYL. Molecular Modeling Software Packages, version 8.0. Tripos; 1699 South Hanley Rd., St. Louis, MO 63144-2917: 2007.

27. Powell DJM. Math Program. 1977; 12:241.
28. Clark M, Crammer DR III, Van Opdenbosch N. J Comput Chem. 1989; 10:982.
29. Murty, KG. Linear Programming. John Wiley & Sons; New York: 1983.
30. Kirkpatrick S, Gelatt CD Jr, Vecchi MP. Science. 1983; 220:671. [PubMed: 17813860]
31. RCSB Protein Data Bank. [accessed November 2015] <http://www.rcsb.org/pdb/home/home.do>
32. Schrödinger Suite 2009 Prime version 2.1. Schrödinger, LLC; New York, NY: 2009.
33. Jakalian A, Jack DB, Bayly CI. J Comput Chem. 2002; 23:1623. [PubMed: 12395429]
34. Cheatham TE III, Cieplak P, Kollman PA. J Biomol Struct Dyn. 1999; 16:845. [PubMed: 10217454]
35. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. J Comput Chem. 2004; 25:1157. [PubMed: 15116359]
36. Aqvist J. J Phys Chem. 1990; 94:8021.
37. Joong IS, Cheatham TE III. J Phys Chem B. 2008; 112:9020. [PubMed: 18593145]
38. Salomon-Ferrer, R., Case, DA., Walker, RC. Wiley Interdiscip Rev Comput Mol Sci. 2012. <http://dx.doi.org/10.1002/wcms.1121>
39. Salomon-Ferrer R, Goetz AW, Poole D, Le Grand S, Walker RC. J Chem Theory Comput. 2013; 9:3878. [PubMed: 26592383]
40. Darden T, York D, Pedersen L. J Chem Phys. 1993; 98:10089.
41. Case DA, Cheatham TE III, Darden T, Gohlke H, Luo R, Merz KM Jr, Onufriev A, Simmerling C, Wang B, Woods RJ. J Comput Chem. 2005; 26:1668. [PubMed: 16200636]
42. Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, Lee M, Lee T, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan J, Case DA, Cheatham TE III. Acc Chem Res. 2000; 33:889. [PubMed: 11123888]
43. Honig B, Nicholls A. Science. 1995; 268:1144. [PubMed: 7761829]
44. Sitkoff D, Sharp KA, Honig B. J Phys Chem. 1994; 98:1978.
45. Magrioti V, Nikolaou A, Smyrniotou A, Shah I, Constantinou-Kokotou V, Dennis AE, Kokotos G. Bioorg Med Chem. 2013; 21:5823. [PubMed: 23916152]
46. Nicolaou KC, Reingruber R, Sarlah D, Bräse S. J Am Chem Soc. 2009; 131:2086. [PubMed: 19173649]
47. Gribble AD, Dolle RE, Shaw A, McNair D, Novelli R, Novelli CE, Slingsby BP, Shah VP, Tew D, Saxty BA, Allen M, Groot PH, Pearce N, Yates J. J Med Chem. 1996; 39:3569. [PubMed: 8784456]
48. Bethesda Pharmaceuticals Inc. Patent. WO 2005/009437 A1. 2005.

Figure 1.
Common inhibitors of secreted PLA₂.

Figure 2.

The workflow of the development of new potential inhibitors based on the inhibitor **7** guided by molecular docking calculations.

Figure 3. Conformational changes of the complex **16a**–GIIA sPLA₂ at the beginning (up) and at the end (down) of the MD simulation.

Figure 4.
Conformational changes of the complex **31c**–GIIA sPLA₂ at the beginning (up) and at the end (down) of the MD simulation.

Figure 5.
RMSD for the **16a** and **31c**.

Scheme 1.

Reagents and conditions: (a) WSCI·HCl, HOBT, Et₃N, CH₂Cl₂; (b) 1 N aq NaOH, MeOH; (c) Dess–Martin periodinane, CH₂Cl₂.

Scheme 2.

Reagents and conditions: (a) WSCI-HCl, HOBT, Et₃N, CH₂Cl₂; (b) Dess–Martin periodinane, CH₂Cl₂; (c) CF₃COOH, CH₂Cl₂.

Scheme 3.

Reagents and conditions: (a) $\text{CH}_3(\text{CH}_2)_{13}\text{CHOHCOOH}$ (**8**), $\text{WSCI}\cdot\text{HCl}$, HOBt , Et_3N , CH_2Cl_2 ; (b) Dess–Martin periodinane, CH_2Cl_2 ; (c) CF_3COOH , CH_2Cl_2 .

Scheme 4.

Reagents and conditions: (a) $\text{C}_2\text{H}_5\text{OOCH}=\text{CHCH}_2\text{P}(=\text{O})(\text{OC}_2\text{H}_5)_2$, $\text{LiOH}\cdot\text{H}_2\text{O}$, dry THF; (b) H_2/Pd , THF; (c) DIBAL-H, Et_2O ; (d) $\text{NaBr}/\text{H}_2\text{O}$, toluene/ EtOAc , $\text{AcNH}\cdot\text{TEMPO}$, 0.5 M $\text{NaOCl}/\text{NaHCO}_3/\text{H}_2\text{O}$; (e) (i) NaHSO_3 , CHCl_3 (ii) H_2O , 6 M KCN ; (f) (i) HCl conc. (ii) $\text{EtOH}/\text{H}_2\text{O}$, KOH ; (g) (*S*)-Val-O tBu , $\text{WSCl}\cdot\text{HCl}$, HOBt , Et_3N , CH_2Cl_2 ; (i) Dess–Martin periodinane, CH_2Cl_2 ; (j) CF_3COOH , CH_2Cl_2 .

Scheme 5.

Reagents and conditions: (a) EtOAc/THF/80% CH_3COOH , 6 M KCN; (b) (i) HCl conc. (ii) EtOH/ H_2O , KOH; (c) (*S*)-Val-O tBu , WSCI $\cdot$ HCl, HOBT, Et_3N , CH_2Cl_2 ; (d) Dess–Martin periodinane, CH_2Cl_2 ; (e) CF_3COOH , CH_2Cl_2 .

Table 1IC₅₀ values of **1a** and **16a** on hGIIA, hGV, and mGIIA

Compound	IC ₅₀ value (nM)		
	hGIIA	hGV	mGIIA
1a	125 ± 20 ^{13,14}	500 ± 50 ^{13,14}	70 ± 20 ^{13,14}
16a	143 ± 50	1200 ± 50	68 ± 16

Table 2Inhibitory activity of **16a** on other sPLA₂ enzymes

Isoform	% Remaining activity of the enzymes at 1000 nM	Isoform	% Remaining activity of the enzymes at 1000 nM
hGX	95	mGX	98
hGIB	98	mIID	99
hGIE	93	mGV	90
hGIIF	94		

Table 3Inhibitory activity of analogs of **16a** on sPLA₂ enzymes

No	% Inhibition*			
	hGIIA	hGIB	hGV	hGX
31a	27	20	26	51
31b	21	30	50	54
31c	11	56	40	40
37	50	50	53	56

* 10 μ M final concentration of the inhibitor.

Table 4Main H-bond interactions between the ligands and the residues of the GIIA sPLA₂ active site

	16a	31c
GLY29	COO ⁻ group	COO ⁻ group
	3.05 Å	2.94 Å
	52%	75.8%
	NHCO group	
	2.9 Å	
HIS47	51%	
	NHCOCO group	
	3.18 Å	
	30.47%	

The numbers describe the H-bond distance and the occurrence as the percentage of simulation time that the interaction exists.

Table 5Energetic analysis for **16a**–GIIA sPLA₂ and **31c**–GIIA sPLA₂ complexes

Energy* (kcal mol ⁻¹)	16a	31c
E_{vdW}	-36.86 ± 4.34	-30.12 ± 3.85
E_{elec}	-515.69 ± 12.53	-501.60 ± 10.45
G_{PB}	519.78 ± 11.93	506.27 ± 9.42
G_{NP}	-4.90 ± 0.19	-3.76 ± 0.10
G_{solv}	514.88 ± 11.88	502.51 ± 9.407
$E_{\text{MM, gas}}$	-552.55 ± 12.51	-531.73 ± 8.69
$H_{(\text{MM+solv})}$	-37.67 ± 5.16	-29.21 ± 4.62
$-T \cdot S_{\text{tot}}$	20.09 ± 9.03	22.59 ± 11.10
$G_{\text{MM-PBSA}}$	-17.58 ± 3.37	-6.62 ± 1.81

* Errors represent the standard deviation error out of the total number of trajectory snapshots used in MM–PBSA calculations.