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Synthesis and activity of 2-oxoamides containing long chain β-amino acids

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Abstract: 2-Oxoamides based on long chain β-amino acids were synthesized. 1-Benzyl substituted long chain amines, needed for such synthesis, were synthesized starting from Boc-phenylalaninol. The oxidative conversion of a phenyl group to a carboxyl group was used as the key transformation synthetic step. The compounds synthesized were studied for their activity against GIVA PLA_2, and were proven to be weak inhibitors.

Keywords: β-amino acids; inhibitors; long chain amines; 2-oxoamides; phospholipase A_2

INTRODUCTION

Phospholipase A_2(PLA_2) catalyses the hydrolysis of the *sn*-2 or middle fatty acyl chain from phospholipids [1]. Among the various phospholipases the Group IVA PLA_2 (also referred to as the 85 kDa cytosolic PLA_2) is a particularly attractive target for drug development, since it is the rate-limiting provider of free polyunsaturated fatty acids and lysophospholipids, precursors of the eicosanoids and PAF, respectively. Therefore, synthetic PLA_2 inhibitors are of great interest because they could be valuable for the treatment of various inflammatory conditions [2].

Group IVA PLA_2(GIVA PLA_2) possesses an unusual catalytic dyad involving a serine and an aspartate residue [3,4]. Taking the enzyme's catalytic mechanism and the structure of the substrate phospholipids into consideration, a novel class of inhibitors of GIVA PLA_2, 2-oxoamides based on γ-amino acids were proposed recently [5]. The 2-oxoamide functionality has been used successfully in the development of inhibitors of various lipolytic enzymes, which are characterized as serine hydrolases. It has been demonstrated that lipophilic 2-oxoamides [6,7], 2-oxoamide and bis-2-oxoamide triacylglycerol analogues [8,9] are efficient inhibitors of pancreatic and gastric lipases, enzymes containing a classic catalytic triad (Ser-His-Asp).

During the past decade the authors have been involved in the synthesis of non-natural lipidic α-amino acids [10–13] and have demonstrated that lipidic α-amino acid derivatives [14] and lipopeptides [15] act as PLA_2 inhibitors. This paper presents the synthesis of 2-oxoamides based on lipidic β-amino acids and the study of their activity against GIVA PLA_2.

RESULTS AND DISCUSSION

As shown in Scheme 1, the strategy for the synthesis of long chain β-amino acids was based on the oxidative conversion of the phenyl group of 1-benzyl substituted amines into a carboxy group. Such long chain amines may be prepared via a Wittig-type olefination reaction of *N*-protected phenylalaninal with the suitable long chain ylide.

Thus, Boc-protected phenylalaninol (**1**) was prepared from Boc-Phe-OH by the Kokotos method [16] and oxidized to the corresponding aldehyde with NaOCl in the presence of a catalytic amount of 4-acetamido-2,2,6,6-tetramethylpiperidin-1-yloxy radical (AcNH-TEMPO) [17,18] (Scheme 2). *N*-Protected α-amino aldehydes may be prepared either by reduction of a carboxy derivative of an amino acid or by oxidation of *N*-protected 2-amino alcohols [19]. The NaOCl/TEMPO oxidation method was chosen because it appears superior to reductive methods in terms of the preservation of the enantiomeric purity [20]. Aldehyde **2** was used immediately after its preparation without any purification and reacted with ylides that were generated by treatment of pentadecyl- and undecyl-triphenylphosphonium bromide with potassium bis(trimethylsilyl)amide (KHMDS) in toluene at 0 °C. Under these conditions, unsaturated compounds **3a,b** were isolated as *E*-isomers (>95%), as shown by

Abbreviations: AcNH-TEMPO, 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy free radical; GIVA-PLA_2, Group IVA phospolipase A_2; HOBt, 1-hydroxybenzotriazole; KHMDS, potassium bis(trimethylsilyl)amide; PAF, platelet activating factor; PLA_2, phospholipase A_2; WSCI, water soluble 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide.

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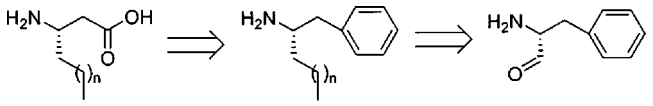


Scheme 1 Retrosynthetic analysis for long chain β-amino acids.

^{1}H NMR spectroscopic analysis. Amines **5a,b** were prepared by catalytic hydrogenation of compounds **3a,b** followed by treatment with HCl in ether.

The enantiomeric purity of long chain amines **5a,b** depends on the conditions used for both the synthesis of aldehyde **2** and the Wittig olefination reaction. Amines **5a,b** were almost quantitatively converted into amides with (*S*)-(−) and (*R*)-(+)-α-methoxy-(α-trifluoromethyl)phenylacetic acid [21]. ^{1}H and ^{19}F-NMR analysis of these Mosher amides indicated an enantiomeric excess >95%.

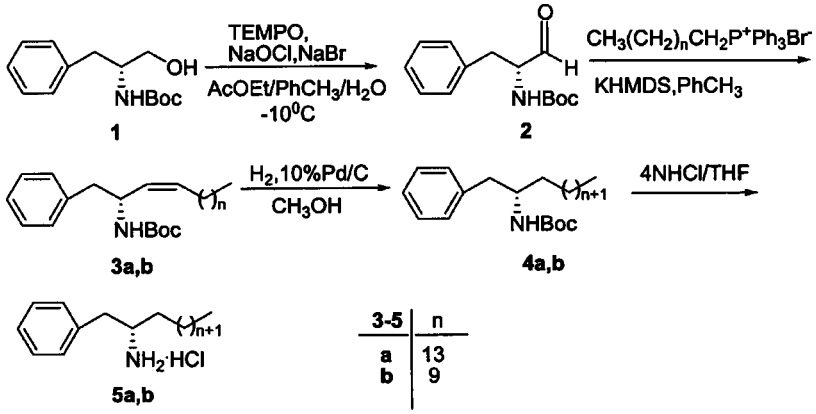
The target compounds **8a,b** may be prepared by two different routes, as depicted in Scheme 3. Commercially available 2-oxo-octanoic acid was coupled with amine **5a** by the mixed carbonic anhydride method [7,22]. The aromatic ring of **6** was converted into a carboxylic group by oxidation with $NaIO_4/RuCl_3 6H_2O$ [23]. Alternatively, amine **5b** was coupled with 2-hydroxy-dodecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCI) [24] as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt) to produce 2-hydroxy-amide **7**. 2-Oxo-amide **8b** was obtained by oxidation of **7** with $NaIO_4/RuCl_3 xH_2O$. Under these oxidative conditions, the phenyl group of **7** was converted into a carboxy group and simultaneously the 2-hydroxy-amide functionality was converted into a 2-oxoamide group.

Compounds **8a,b** were tested for their ability to inhibit human GIVA PLA_2 in a GIVA PLA_2 specific assay that uses mixed micelles of substrate, 1-palmitoyl-2-arachidonyl phosphatidylcholine, phosphatidylinositol 4,5-bisphosphate and detergent Triton X-100 (97 : 3:400 μM) [25,26]. 2-Oxoamides based on long chain β-amino acids were proven to be weak inhibitors of GIVA PLA_2. Compound **8a** exhibited 56% inhibition of GIVA PLA_2 activity at 0.091 molar fraction.

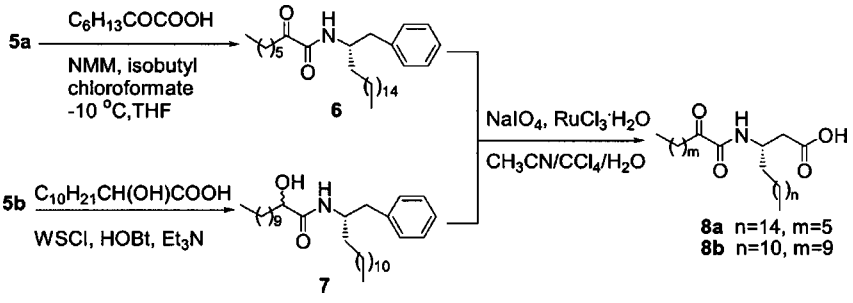
In conclusion, the present paper describes a method for the conversion of Boc-phenylalaninol into 1-benzyl substituted long chain amines. 2-Oxoamides based on β-amino acids were prepared from such amines using the oxidative conversion of a phenyl group into a carboxyl group as a key transformation reaction. These 2-oxoamides are weak inhibitors of human GIVA PLA_2.

EXPERIMENTAL SECTION

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. TLC plates (silica gel 60 F_{254}) and silica gel 60 (70–230 or



Scheme 2 Synthesis of 1-benzyl substituted long chain amines.



Scheme 3 Synthesis of 2-oxoamides.

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 and Et_2O were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were of reagent grade and used without further purification. All the products gave satisfactory elemental analyses.

General Method for the Wittig Olefination Reaction

To a solution of **1** (2.00 mmol) in a mixture of toluene–EtOAc (1 : 1, 12 ml), a solution of NaBr (0.22 g, 2.1 mmol) in H_2O (1 ml) was added, followed by AcNH-TEMPO (4 mg, 0.02 mmol). To the resulting biphasic system, which was cooled to 0 °C, an aqueous solution of 0.35 M NaOCl (6.2 ml, 2.2 mmol), containing $NaHCO_3$ (0.50 g, 6 mmol), was added dropwise with vigorous stirring at 0 °C over 1 h. The mixture was stirred for 15 min at 0 °C and H_2O (4 ml) was added. The aqueous layer was separated, acidified with 1 N HCl and extracted with EtOAc (2 × 12 ml). The combined organic layers were washed consecutively with 1% aqueous citric acid (12 ml) containing KI (0.08 g), 10% aqueous $Na_2S_2O_3$ (12 ml) and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude aldehyde was immediately used for the Wittig reaction.

To a stirred suspension of the phosphonium salt $CH_3(CH_2)_nCH_2PPh_3^+Br^-$ (2.00 mmol) in dry toluene (10 ml), a 0.5 M solution of KHMDS (4.00 ml) in toluene was added dropwise over a period of 5 min at 0 °C under N_2. The bright red solution was stirred for a further 15 min and cooled to −78 °C. A solution of the aldehyde in dry toluene (2 ml) was then added in one portion. The resulting light yellow mixture was stirred for 20 min at room temperature; then the reaction mixture was quenched with a saturated aqueous solution of NH_4Cl (20 ml) and extracted with Et_2O(3 × 5 ml). The combined organic layers were washed with brine and dried (Na_2SO_4). The solvents were removed and the residue was purified by column chromatography using petroleum ether : EtOAc 95 : 5 as the eluent.

(R)-(1-Benzyl-heptadec-2-enyl)-carbamic acid tert-butyl ester (3a). Yield 75%; white solid; mp 45–47 °C; $[\alpha]^{25}_D = +1.0$ (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (*t*, 3H, $J = 7.0$ Hz, CH_3), 1.05–1.40 (*m*, 24H, 12xCH_2), 1.50 [*s*, 9H, $C(CH_3)_3$], 1.97 (*m*,2H, CH_2CH=CH), 2.76 (*dd*, 1H, $J_1 = 7.2$ Hz, $J_2 = 13.2$, C_6H_5CHH), 2.99 (*dd*, 1H, $J_1 = 5.2$ Hz, $J_2 = 13.0$, C_6H_5CHH), 4.52–4.64 (*m*, 2H, OCONH, CH), 5.25 (*dd*, 1H, $J_1 = 10.2$ Hz, $J_2 = 11.0$ Hz, HC=$CHCH_2$), 5.47 (*m*, 1H, CH=$CHCH_2$), 7.31 (*m*, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (CH_3), 22.9, 28.0, 28.6, 29.5, 29.6, 29.7, 29.8, 29.9, 32.1, 42.4, 49.6 (CH), 79.5 (C), 126.5, 128.4, 129.1, 129.9, 133.4, 138.0, 155.2 (OCONH). Anal. Calcd for $C_{29}H_{49}NO_2$: C, 78.50; H, 11.13; N, 3.16. Found: C, 78.32; H, 11.03; N, 3.28.

(R)-(1-Benzyl-tridec-2-enyl)-carbamic acid tert-butyl ester (3b). Yield 63%; oil; $[\alpha]^{25}_D = +1.5$ (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (*t*, 3H, $J = 6.8$ Hz, CH_3), 1.18–1.40 (*m*, 16H, 8xCH_2), 1.50 [*s*, 9H, $C(CH_3)_3$], 1.98 (*m*, 2H,CH=CHCH_2), 2.77 (*dd*, 1H, $J_1 = 7.2$ Hz, $J_2 = 13.2$ Hz, C_6H_5CHH), 2.97 (*dd*, 1H, $J_1 = 5.2$ Hz, $J_2 = 13.0$ Hz, C_6H_5CHH), 4.55–4.66 (*m*, 2H, OCONH, CH), 5.25 (*dd*, 1H, $J_1 = 10.2$ Hz, $J_2 = 11.0$ Hz, C*H*=$CHCH_2$), 5.45 (*m*, 1H, CH=C*H*CH_2), 7.29 (*m*, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (CH_3), 22.9, 28.0, 28.6, 29.3, 29.5, 29.6, 29.7, 29.8, 32.1, 32.4, 42.4, 49.6 (CH), 79.4 (C), 126.5, 128.4, 129.1, 131.9, 133.1, 138.0, 155.2 (OCONH). Anal. Calcd for $C_{25}H_{41}NO_2$: C, 77.47; H, 10.66; N, 3.61. Found: C, 77.26; H, 10.78; N, 3.49.

General Method for Catalytic Hydrogenation

To a solution of **3a,b** (2.00 mmol) in MeOH (10 ml) (through which N_2 had been passed for 5 min), 10% Pd/C catalyst (22 mg, 0.02 mmol) was added. The reaction mixture was stirred under an H_2 atmosphere overnight at room temperature. The catalyst was removed by filtration through a pad of Celite and the organic solvent evaporated under reduced pressure.

(S)-(1-Benzyl-heptadecyl)-carbamic acid tert-butyl ester (4a). Yield 87%; white solid; mp 66–67 °C; $[\alpha]^{25}_D = -4.4$ (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (*t*, 3H, $J = 6.6$ Hz, CH_3), 1.10–1.42 (*m*, 30H, 15xCH_2), 1.43 [*s*, 9H, $C(CH_3)_3$], 2.67 (*d*, 2H, $J = 7.0$ Hz, $CH_2C_6H_5$), 3.65 (*m*, 1H, CH), 6.45 (*d*, 1H, $J = 9.0$ Hz, OCONH), 7.21 (m, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (CH_3), 22.9, 26.2, 28.6, 29.6, 29.7, 29.8, 29.9, 32.1, 34.4, 41.6, 51.8 (CH), 79.2 (C), 126.4, 128.5, 129.7, 138.6 (aromatic C), 155.7 (OCONH). Anal. Calcd for $C_{29}H_{51}NO_2$: C, 78.15; H, 11.53; N, 3.14. Found: C, 77.93; H, 11.67; N, 3.01.

(S)-(1-Benzyl-tridecyl)-carbamic acid tert-butyl ester (4b). Yield 92%; white solid; mp 56–58 °C; $[\alpha]^{25}_D = -8.2$ (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (*t*, 3H, $J = 6.6$ Hz, CH_3), 1.10–1.40 (*m*, 22H, 11xCH_2), 1.41 [*s*, 9H, $(CH_3)_3$], 2.74 (*d*, 2H, $J = 6.4$ Hz, $CH_2C_6H_5$), 3.77 (*m*, 1H, CH), 4.29 (*d*, IH, $J = 8.8$ Hz, OCONH), 7.23 (*m*, 5H, C_6H_5); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7, 26.0, 28.3, 29.3, 29.4, 29.5, 29.6, 31.9, 34.1, 41.3, 51.5 (CH), 79.5 (C), 126.1, 128.2, 129.5, 138.3 (aromatic C), 155.4 (OCONH). Anal. Calcd for $C_{25}H_{43}NO_2$: C, 77.07; H, 11.12; N, 3.60. Found: C, 76.84; H, 11.29; N, 3.51.

General Method for Boc Deprotection

Compound **4a,b** was added to a solution of 4N HCl in ether (5.0 ml, 20 mmol). After stirring at room temperature for 30 min, the mixture was evaporated, dry Et_2O was added, and the product was filtered. The solid was washed with ether and dried.

(S)-1-Benzyl-heptadecyl-ammonium chloride (5a). Yield 83%; white solid; mp 66–68 °C; $[\alpha]^{25}_D = -4.8$ (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, CD_3OD) δ 0.88 (*t*, 3H, $J = 6.6$ Hz, CH_3), 1.19–1.51 (*m*, 28H, 14xCH_2), 1.59 (*m*, 2H, CH_2CH), 2.93 (*d*, 2H, $J = 7.0$ Hz, $CH_2C_6H_5$), 3.43 (q, 1H, $J = 6.2$ Hz, CH), 7.33 (*m*, 5H, C_6H_5); ^{13}C NMR (50 MHz, CD_3OD) δ 13.3 (CH_3), 22.6, 24.9, 29.2, 29.3, 29.4, 29.6, 31.9, 32.1, 38.7, 46.9, 47.4, 47.8, 48.2, 48.7, 49.1, 53.1 (CH), 127.2, 128.8, 129.2, 136.1 (aromatic C). Anal. Calcd for $C_{24}H_{43}N{\cdot}HCl$: C, 75.45; H, 11.61; N, 3.67. Found: C, 75.12; H, 11.82; N, 3.51.

(S)-1-Benzyl-tridecyl-ammonium chloride (5b). Yield 94%; white solid; mp 50–52 °C; $[\alpha]^{25}_D = -2.8$ (*c*1, $CHCl_3$); ^{1}H NMR (200 MHz, CD_3OD) δ 0.91 (*t*, 3H, $J = 7.0$ Hz, CH_3), 1.16–1.45 (*m*, 20H, 10xCH_2), 1.60–1.82 (*m*, 2H, CH_2CH), 2.94 (*d*, 2H, $J = 7.0$ Hz, $CH_2C_6H_5$), 3.58 (*m*, 1H, CH), 7.31 (*m*, 5H, C_6H_5); ^{13}C NMR (50 MHz, CD_3OD) δ 13.3 (CH_3), 22.6, 24.9, 29.2,

29.3, 29.4, 29.6, 31.9, 32.1, 38.7, 47.0, 53.1 (CH), 127.2, 128.8, 129.2, 136.1 (aromatic C). Anal. Calcd for $C_{20}H_{35}N{\cdot}HCl$: C, 73.69; H, 11.13; N, 4.30. Found: C, 73.37; H, 11.32; N, 4.14.

(S)-2-Oxo-octanoic acid (1-benzyl-heptadecyl)-amide (6). To a stirred solution of **5a** (1.0 mmol) in THF (5 ml) cooled to −10 °C, *N*-methylmorpholine (0.11 ml, 1 mmol) and subsequently isobutyl chloroformate (0.13 ml, 1.0 mmol) were added dropwise. After stirring for 5 min at −10 °C, an ice-cooled solution of 2-oxooctanoic acid (1.0 mmol) in THF (5 ml) was added dropwise. The reaction mixture was stirred for 1 h at −10 °C and, then at room temperature overnight. The solvent was evaporated and the residue dissolved in EtOAc. The organic phase was washed with water, 0.5 N HCl, water, 5% $NaHCO_3$, brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue purified by column chromatography using petroleum ether 40–60 °C/EtOAc 95 : 5 as the eluent. Yield 79%; white solid; mp 55–57 °C; $[\alpha]^{25}_D = +0.70$ (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, CD_3OD) δ 0.86 (*t*, 6H, $J = 6.6$ Hz, 2xCH_3), 1.13–1.29 (*m*, 34H, 17xCH_2), 1.55 (m, 4H, CH_2CH$_2$COCONH, CH_2CH), 2.78–2.91 (m, 4H, CH_2C$_6$H$_5$, CH_2COCONH), 4.08 (*m*, 1H, CH), 6.78 (d, 1H, $J = 8$ Hz, NHCO) 7.22 (*m*, 5H, C$_6$H$_5$); ^{13}C NMR (50 MHz, CD_3OD) δ 14.2 (CH_3), 14.3 (CH_3), 22.7, 22.9, 23.4, 26.2, 28.9, 29.6, 29.8, 29.9, 31.7, 32.1, 34.1, 36.9, 41.2, 50.8 (CH), 126.7, 128.6, 129.6, 136.0 (aromatic C) 159.9 (NHCO), 192.8 (*C*OCONH). Anal. Calcd for $C_{32}H_{55}NO_2$: C, 79.12; H, 11.41; N, 2.88. Found: C, 78.91; H, 11.69; N, 2.73.

(S)-2-Hydroxy-dodecanoic acid(1-benzyl-tridecyl)-amide (7). To a stirred solution of 2-hydroxy-dodecanoic acid (2.0 mmol) and **5b** (2.0 mmol) in CH_2Cl_2 (20 ml), Et_3N (6.2 ml, 4.4 mmol) and subsequently WSCI (0.42 g, 2.2 mmol) and HOBt (0.32 g, 2.0 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The solvent was evaporated under reduced pressure and EtOAc (20 ml) 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 petroleum ether: EtOAc 9 : 1 as the eluent. Yield 85%; white solid; mp 65–67 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (*t*, 6H, $J = 6.2$ Hz, 2xCH_3), 1.29 (*m*, 38H, 19xCH_2), 1.72 (*m*, 2H, CH_2), 2.82 (*m*, 2H, CH_2C$_6$H$_5$), 4.03 (*m*, 1H, CH), 4.15 (*m*, 1H, CH), 6.10 (*d*, 1H, $J = 8.8$ Hz, NHCO), 7.21 (*m*, 5H, C$_6$H$_5$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7, 24.7, 26.0, 29.3, 29.5, 29.6, 29.6, 29.7, 30.2, 30.3, 31.9, 34.3, 35.0, 49.8 (CHNH), 72.1 (CHOH), 125.5, 126.4, 128.3, 129.4 (aromatic C), 173.0 (NHCO). Anal. Calcd for $C_{32}H_{57}NO_2$: C, 78.79; H, 11.78; N, 2.87. Found: C, 78.61; H, 11.91; N, 2.67.

General Method for the Oxidation of a Phenyl Group to a Carboxyl Group

To a solution of **6** or **7** (1.00 mmol) in a mixture of CCl_4–MeCN–H_2O (1 : 1 : 2, 30 ml), $NaIO_4$ (6.2 g, 29.0 mmol) and $RuCl_3 6H_2O$ (12 mg, 0.045 mmol) were added and the mixture was stirred overnight at room temperature. Dichloromethane (30 ml) was added and, after stirring for 10 min, the organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography using petroleum ether: EtOAc 1 : 1 as eluent.

(S)-3-(2-Oxo-octanoylamino)-nonadecanoic acid (8a). Yield 55%;white solid; mp 69–71 °C; $[\alpha]^{25}_D = +7.5$ (*c* 0.4, $CHCl_3$); ^{1}H NMR (200 MHz, CD_3OD) δ 0.88 (*t*, 6H, $J = 6.6$ Hz, 2xCH_3), 1.10–1.50 (*m*, 34H, 17xCH_2), 1.61 (*m*, 4H, CH_2CH, CH_2CH$_2$COCONH), 2.62 (*d*, 2H, $J = 4.6$ Hz, CH_2COOH), 2.92 (*t*, 2H, $J = 7.2$ Hz, CH_2COCO), 4.21 (*m*, 1H, C*H*NHCO), 7.29 (*d*, 1H, $J = 10.6$ Hz, NHCO); ^{13}C NMR (50 MHz, CD_3OD) δ 14.0 (CH_3), 14.1 (CH_3), 22.4, 22.7, 23.1, 26.1, 28.7, 29.2, 29.3, 29.4, 29.5, 29.7, 31.5, 31.7, 33.8, 36.8 , 38.2, 46.1, 159.6 (NHCO), 176.2 (COOH), 199.1 (*C*OCONH). Anal. Calcd for $C_{27}H_{51}NO_4$: C, 71.48; H, 11.33; N, 3.09. Found: C, 71.31; H, 11.54; N, 2.99.

(S)-3-(2 Oxo-dodecanoylamino)-pentadecanoic acid (8b). Yield 61%. White solid; ^{1}H NMR δ 0.90 (*t*, 6H, $J = 6.2$ Hz, 2xCH_3), 1.12–1.40 (*m*, 34H, 17xCH_2), 1.60–1.80 (*m*, 4H, CH_2CH$_2$COCO, CH_2CH), 2.61 (*d*, 2H, $J = 5.6$ Hz, CH_2COOH), 2.91 (*t*, 2H, $J = 7.4$ Hz, CH_2COCO), 4.2 (*m*, 1H, CH), 7.38 (*d*, 1H, $J = 8.0$ Hz, NHCO); ^{13}C NMR δ 14.1, 22.6, 23.1, 24.4, 24.8, 25.3, 26.0, 26.1, 29.0, 29.3, 29.4, 29.5, 29.6, 30.3, 30.6, 31.9, 32.2, 33.0, 33.6, 33.9, 36.8, 38.3, 46.2, 159.7 (NHCO), 176.0 (COOH), 199.2 (*C*OCONH). Anal. Calcd for $C_{27}H_{51}NO_4$: C, 71.48; H, 11.33; N, 3.09. Found: C, 71.34; H, 11.48; N, 3.01.

Phospholipase A_2 Activity Assays

GIVA PLA$_2$-specific assay. The PLA_2 activity assays were performed using phospholipid-detergent mixed micelles as previously described [5,25]. The 500 µl final assay solution was composed of 100 mM HEPES (pH 7.5), 80 µM $CaCl_2$, 0.1 mg/ml fatty acid-free bovine serum-albumin, and 2 mM dithiothreitol. The substrate mixed micelles contained 97 µM 1-palmitoyl-2-[^{14}C]-arachidonoyl phosphatidylcholine (100 000 cpm), 3 µM phosphatidylinositol 4,5-bisphosphate, and 100 µM Triton X-100 (TX-100). Up to 5 µl (up to 1% total volume to avoid perturbing the enzyme) of neat dimethyl sulfoxide (DMSO) was added to the control tubes, and 5 µl of DMSO containing the appropriate amount of inhibitor to be tested was added to the experimental tubes. The reaction was initiated by the addition of GIVA PLA_2 (4 ng) in 50 µl of assay buffer, followed by vortexing. Assays were kept at 40 °C for 30 min. The reaction was quenched and worked-up using a modified Dole protocol [5]. The activity of GIVA PLA_2 in the presence of inhibitor was always compared with the DMSO-treated control tube.

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