Structure−Activity Relationship of 2-Oxoamide Inhibition of Group IVA Cytosolic Phospholipase A_2 and Group V Secreted Phospholipase A_2

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The Group IVA cytosolic phospholipase A_2 (GIVA $cPLA_2$) is a key provider of substrates for the production of eicosanoids and platelet-activating factor. We explored the structure−activity relationship of 2-oxoamide-based compounds and GIVA $cPLA_2$ inhibition. The most potent inhibitors are derived from δ- and γ-amino acid-based 2-oxoamides. The optimal side-chain moiety is a short nonpolar aliphatic chain. All of the newly developed 2-oxoamides as well as those previously described have now been tested with the human Group V secreted PLA_2 (GV $sPLA_2$) and the human Group VIA calcium-independent PLA_2 (GVIA $iPLA_2$). Only one 2-oxoamide compound had appreciable inhibition of GV $sPLA_2$, and none of the potent GIVA $cPLA_2$ inhibitors inhibited either GV $sPLA_2$ or GVIA $iPLA_2$. Two of these specific GIVA $cPLA_2$ inhibitors were also found to have potent therapeutic effects in animal models of pain and inflammation at dosages well below the control nonsteroidal anti-inflammatory drugs.

Introduction

Group IVA cytosolic phospholipase A_2 (GIVA $cPLA_2$ [a]) catalyzes the preferential release of arachidonic acid (AA) from intracellular membrane phospholipids.[1,2] GIVA $cPLA_2$ is one of a large number of mammalian PLA_2 enzymes that share the ability to hydrolyze the central (*sn*-2) fatty acyl chain from substrate phospholipids.[1,3] GIVA $cPLA_2$ is an α/β hydrolase that utilizes a unique catalytic dyad of Ser-Asp, with the Ser hydroxyl positioned for nucleophilic attack on the substrate ester.[4,5] The resulting acyl−enzyme intermediate is rapidly hydrolyzed to yield a free fatty acid and a lysophospholipid. The two most important products of GIVA $cPLA_2$ action are AA and lyso-platelet-activating factor (lyso-PAF).[6−8] AA and its many eicosanoid metabolites are critical second messengers in inflammation, pain, and many pathophysiological processes.[6−10] Lyso-PAF is acetylated to form PAF, another important second messenger in inflammation as well as other pathophysiological processes.[7,8,11]

During times of inflammation, GIVA $cPLA_2$ inhibition would simultaneously block the up-regulated production of the AA and lyso-PAF metabolites.[8,11,12] The evidence for the key pathophysiological roles of GIVA $cPLA_2$ has come with detailed experimentation of the GIVA $cPLA_2$ knockout mouse[6,7,13,14] as well as confirmatory findings with existing inhibitors or antisense techniques that reduce GIVA $cPLA_2$ activity in vitro, ex vivo, and in vivo.[3,8,15] Together, this body of evidence suggests that GIVA $cPLA_2$ is a good target for the development of anti-inflammatory agents.[12,16,17]

There are several clear paralogues of GIVA $cPLA_2$ in mammalian genomes, but the expression of these paralogues is more restricted and the functions are limited or unknown.[3,14] The members of Group VI PLA_2 and GIVA $cPLA_2$ share distant sequence similarity in the active site residues.[3,18,19] The Group VIA calcium-independent phospholipase A_2 (GVIA $iPLA_2$) is the best-studied of this group and plays a key role in membrane homeostasis in many systems.[20,21] The other major class of PLA_2 enzymes includes the disulfide-rich small, secreted $sPLA_2$s and is characterized by a catalytic His/Asp dyad and catalytic Ca^{2+}.[1,3] These enzymes are present in a variety of tissues and, because they are secreted, can act in a transcellular manner.[22,23] While the active sites of diverse $sPLA_2$ paralogues are well conserved, the specific membrane targets and activities vary widely.[24] A well-studied, simple example of this class is the human Group V secreted phospholipase A_2 (GV $sPLA_2$).[24,25] GV $sPLA_2$ is able to bind to and hydrolyze phosphatidylcholine-containing membranes and to release AA.[26,27] In many cases the activity of GV $sPLA_2$ depends on or is linked to the activity of GIVA $cPLA_2$.[3,28]

A recently identified class of inhibitors of human GIVA $cPLA_2$ and GVIA $iPLA_2$ are the 2-oxoamides.[10,19,29−31] The 2-oxoamides were designed as phospholipid substrate analogues taking advantage of the noncleavable, electrophilic 2-oxoamide moiety. The 2-oxoamide inhibitors of GIVA $cPLA_2$ are char-

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[a] Abbreviations: AA, arachidonic acid; Boc, *N*-(*tert*-butoxycarbonyl); ClogP, calculated log of the octanol/water partition coefficient; DMAP, 4-dimethylaminopyridine; DMSO, dimethyl sulfoxide; DPPC, 1-palmitoyl-2-[^{14}C]palmitoylphosphatidylcholine; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EtOAc, ethyl acetate; Et_2O, diethyl ether; Et_3N; triethylamine; GIVA $cPLA_2$, Group IVA cytosolic phospholipase A_2; GV $sPLA_2$, Group V secreted phospholipase A_2; GVIA $iPLA_2$, Group VIA calcium-independent phospholipase A_2; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, 1-hydroxybenzotriazole; KHMDS, potassium bis(trimethylsilyl)amide; NSAIDs, nonsteroidal anti-inflammatory drugs; PAF, platelet-activating factor; PAPC, 1-palmitoyl-2-[^{14}C]arachidonoylphosphatidylcholine; PIP_2, phosphatidylinositol 4,5-bisphosphate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TEMPO, 2,2,6,6-tetramethylpiperidine-1-yloxy free radical; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tris-HCl, tris (hydroxymethyl)aminomethane HCl; WSCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; Z, benzyloxycarbonyl.

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Scheme 1. General Structure of 2-Oxoamide Inhibitors

R_1: alkyl group
R_2: long aliphatic chain
n: 0-4
X: H, CH_3, C_2H_5, *tert*-C_4H_9

acterized by a central 2-oxoamide moiety (Scheme 1) that is presumably acting as an electrophilic target for the nucleophilic active site serine.[29,30] Coming off the distal carbonyl is a hydrophobic R_2 group that likely binds the substrate fatty acyl chain binding site.[29,30] Attached to the amide nitrogen is a terminal charged or polar carboxy group that is likely interacting with the substrate polar head group binding site.[29,30] Branching off the linker to the polar carboxy is the R_1 side chain that introduces stereoselective enhancement of potency to the 2-oxoamide inhibitors of GIVA $cPLA_2$.[29,30] Because many inhibitors of GIVA $cPLA_2$ also inhibit GVIA $iPLA_2$, the 2-oxoamide compounds were tested on GVIA $iPLA_2$.[19] None of the potent GIVA $cPLA_2$ inhibitors inhibited GVIA $iPLA_2$, but several uncharged 2-oxoamides did inhibit GVIA $iPLA_2$.[19] Several 2-oxoamides have been shown to have systemic anti-inflammatory, analgesic, and/or antihyperalgesic properties in animal models.[10,30]

We have now explored the structure−activity relationship of 2-oxoamides and GIVA $cPLA_2$ inhibition showing definitively that δ- and γ-amino acid-based 2-oxoamides contain the optimal spacing between the 2-oxoamide moiety and the polar carboxy group.[32] In addition, the R_1 side chain was optimized. All of the newly developed 2-oxoamides as well as those previously described have now been tested with the human GV $sPLA_2$ and GVIA $iPLA_2$, demonstrating the specificity of the most potent GIVA $cPLA_2$ inhibitors. Two of these specific GIVA $cPLA_2$ inhibitors were found to have notably improved therapeutic effects in animal models of pain and inflammation above and beyond the control nonsteroidal anti-inflammatory drugs (NSAIDs).

Chemical Synthesis of 2-Oxoamides

The synthesis of norleucine- and glutamate-based inhibitors was carried out using methods previously described[29,30] and is depicted in Scheme 2. The AX series was initially described in ref 30 and subsequently in U.S. Patent Application 20050148549.

For the synthesis of the ϵ-norleucine-based inhibitors, *N*-(*tert*-butoxycarbonyl)norleucinol **7**[33] was oxidized to the aldehyde by NaOCl/2,2,6,6-tetramethylpiperidine-1-yloxy free radical (TEMPO),[34,35] which was directly reacted with triethyl phosphonocrotonate (Scheme 3).[36] Catalytic hydrogenation of compound **8**, removal of the *N*-(*tert*)-butoxycarbonyl (Boc) group, and coupling with 2-hydroxy acids produced compounds **9a**,**b**. Saponification, followed by oxidation with NaOCl/TEMPO, led to inhibitors **10a** (AX023) and **10b** (AX025).

For the synthesis of β- and δ-norleucine inhibitors, the conversion of a phenyl group to a carboxylic acid was the key reaction. Boc-D-phenylalaninol (**11**)[37,38] was oxidized to the aldehyde and treated with propanylidene phosphorane to produce compound **12** (Scheme 4). Removal of the protecting group and coupling with 2-hydroxypentadecanoic acid, followed by hydrogenation, led to compound **13**. The phenyl group of **13** was converted to a carboxylic acid by oxidation with $NaIO_4$, $RuCl_3$ in CH_3CN, ethyl acetate (EtOAc), H_2O (1:1:8).[39,40] Under these conditions the 2-hydroxyamide group was simultaneously converted into the 2-oxoamide group, leading to compound **14** (AX021). Compounds **17a** (AX022) and **17b** (AX109) were prepared by similar reactions (Scheme 5). The synthesis of various 2-oxoamides containing a carbamate or an amide group is depicted in Schemes 6−9.

In Vitro Inhibition of GIVA $cPLA_2$. Variable Carboxy Linker

Previous results have shown that a carboxy group in the 2-oxoamide structure is necessary to inhibit GIVA $cPLA_2$ in many 2-oxoamides,[29,30] but more recently a carboxymethyl ester analogue was shown to inhibit GIVA $cPLA_2$ with significant potency.[19] The polar carboxy or carboxymethyl ester group presumably acts as a mimic of the polar head group of a natural substrate phospholipid. For a natural substrate phospholipid, the number of bonds between the scissile *sn*-2 ester bond and the polar head group matches the number of bonds between the electrophilic 2-oxoamide and the polar group of a γ-amino butyric acid-based 2-oxoamide (compound **35**) or a γ-norleucine-based 2-oxoamide (compound **36**). The R_1 norleucine side chain of compound **36** gives the compound approximately 2-fold greater potency than the unsubstituted compound **35**.[29,30] To investigate whether the γ-spacing between the 2-oxoamide moiety and the free carboxyl is optimal, several α-, β-, δ-, and ϵ-amino acid variants of the γ-norleucine-containing 2-oxoamide (**36**) were synthesized and tested for inhibitor potency. The results, summarized in Table 1, show that the best inhibition is found with the δ and γ variants. Shorter and longer spacers to the carboxy group result in weaker inhibition of GIVA $cPLA_2$. An α-amino acid variant, compound **3**, was a very poor inhibitor (approximately 10-fold less potent than **36**). A β-norleucine compound, **14**, was a moderate inhibitor at approximately 3-fold less potent than **36**. Two δ-norleucine variants (**17a** and **17b**) were at least as potent as the γ-norleucine variants, compounds **36**−**38**. However, the ϵ-amino acid variants, **10a** and **10b**, were less potent than the γ-amino acid variants, indicating an ideal distance with the δ- or γ-norleucine variants. The appearance of a polar group in all potent 2-oxoamide inhibitors and the phospholipid-like γ- or δ-spacing between the polar group and the 2-oxamide moiety are consistent with occupation of the phospholipid substrate binding site of GIVA $cPLA_2$.

In Vitro Inhibition of GIVA $cPLA_2$. Side Chain Variation

GIVA $cPLA_2$ has a strong preference for unsaturated fatty acids such as AA at the *sn*-2 position of its substrate phospholipids.[41] This preference has also been reported for fatty alkyl trifluoromethyl ketone inhibitors.[42,43] However, the preference for unsaturated radyl chains in the substrates and inhibitors of GIVA $cPLA_2$ is not absolute. In addition to its PLA_2 activity, GIVA $cPLA_2$ also possesses comparable lysophospholipase activity toward substrates with saturated fatty acyl chains.[44−46] Inhibitors of GIVA $cPLA_2$ containing saturated radyl chains include trifluoromethyl ketone-based inhibitors,[43] tricarbonyl-based inhibitors,[47] and 2-oxoamide-based inhibitors.[19] Compound **39** has a R_2 oleate-based radyl chain in place of the saturated chain of compound **35**. As shown in Tables 1 and 2, there is no significant difference in potency between compound **35** and compound **39**. This suggests that there is no preference for unsaturated chains in the 2-oxoamide inhbitors of GIVA $cPLA_2$. Interestingly, the methyl ester variant (**40**) of compound **39** has comparable potency. This marks just the second uncharged 2-oxoamide to inhibit GIVA $cPLA_2$ with any potency. The first uncharged 2-oxoamide inhibitor of GIVA $cPLA_2$ (compound **11** from reference 19) is also a methyl ester variant of a free carboxy-containing GIVA $cPLA_2$ inhibitor.[19]

Previous studies have indicated that 2-oxoamide inhibitors of GIVA $cPLA_2$ had stereospecific enhancements of potency

Scheme 2. Synthesis of α-Norleucine- and Glutamic Acid-Based Inhibitors[a]

[a] Reagents and conditions: (a) HCl, H-L-Nle-OCH_3, WSCI, HOBt, Et_3N, CH_2Cl_2; (b) 1 N NaOH, dioxane/H_2O (9:1), then HCl; (c) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), 0 °C, then HCl; (d) H-Glu(OBu^t)-OBu^t, WSCI, HOBt, Et_3N, CH_2Cl_2; (e) 50% trifluoroacetic acid/CH_2Cl_2.

Scheme 3. Synthesis of ϵ-Norleucine-Based Inhibitors[a]

9a, n = 9
9b, n = 12
10a, n = 9
10b, n = 12

[a] Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), −5 °C; (b) $(EtO)_2P(=O)CH_2CH=CHCOOEt$, LiOH, $PhCH_3$, room temp; (c) H_2, 10% Pd/C, EtOH; (d) 4 N HCl/THF; (e) $CH_3(CH_2)_nCHOHCOOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (f) 1 N NaOH, dioxane/H_2O (9:1), then HCl; (g) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), 0 °C, then HCl.

Scheme 4. Synthesis of β-Norleucine-Based Inhibitor[a]

[a] Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), −5 °C; (b) $Ph_3P^+(CH_2)_2CH_3Br^-$, KHMDS, −15 °C, $PhCH_3$; (c) H_2, 10% Pd/C; (d) 4 N HCl/Et_2O; (e) $CH_3(CH_2)_{12}CHOHCOOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (f) $NaIO_4$, $RuCl_3$, EtOAc/MeCN/H_2O (1:1:8).

Scheme 5. Synthesis of δ-Norleucine-Based Inhibitors[a]

16a, n = 12
16b, n = 13
17a, n = 12
17b, n = 13

[a] Reagents and conditions: (a) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), −5 °C; (b) $Ph_3P^+(CH_2)_2PhBr^-$, KHMDS, −15 °C, $PhCH_3$; (c) H_2, 10% Pd/C; (d) 4 N HCl/Et_2O; (e) $CH_3(CH_2)_nCHOHCOOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (f) $NaIO_4$, $RuCl_3$, EtOAc/MeCN/H_2O (1:1:8).

with small hydrophobic aliphatic chains at the R_1 position (the side-chain).[29,30] To further characterize that side chain preference, several other γ-substituted 2-oxoamides were synthesized (**22a**−**c**, **26**, **31a**, and **31b**). Compounds **22b** and **22c** are enantiomers that contain a carbamate side chain. Neither compound presents any inhibition of GIVA $cPLA_2$. Interestingly, the addition of six extra methylenes off the end of the R_1 carbamate chain gives compound **22a** that does inhibit GIVA $cPLA_2$, though only 60% at the highest concentration tested (0.091 mole fraction). In general 2-oxoamides with longer R_1 or R_2 side chains seem to be relatively poor inhibitors of GIVA $cPLA_2$ and to lack stereospecificity.[30] One explanation for the inhibition by **22a** and not by **22b** or **22c** is that the short-chain carbamates **22c** and **22b** are not as hydrophobic. ClogP represents the calculated partition coefficient in octanol/water in a logarithmic scale. ClogP is a measure of hydrophobicity of particular use in comparisons of related compounds. ClogP values are calculated for the neutral form of partially ionized molecules. Compound **22a** has a ClogP of 7.7, while **22b** and **22c** each have a ClogP of 4.5.

Compounds **26** and **31a** are enantiomeric compounds that contain an amide side chain, analogous to the carbamate-containing **22c** and **22b**. Both of these compounds are very poor inhibitors of GIVA $cPLA_2$. The addition of eight extra methylenes off the end of the R_1 amide chain results in compound **31b** that does inhibit GIVA $cPLA_2$ with an $X_I(50)$ of 0.025 mole fraction. This modest increase in inhibitory potency may be due to an increase in hydrophobicity for these compounds from a ClogP of 2.9 up to a ClogP of 9.3. This same trend is seen for the carbamates, as described above.

Two other novel 2-oxoamide compounds were synthesized and tested. Compound **6** contains a second carboxylic acid in the R_1 side chain. This analogue is significantly more hydrophilic (ClogP = 3.1) than **35** and has no detectible inhibition (<10%) at up to 0.091 mole fraction. It is possible that the second carboxy group increases the solubility of the compound enough to reduce the fraction partitioning to the substrate micelles. Compound **34** is a carbamate analogue (containing a R_1 norleucine side chain) that is extremely hydrophobic (ClogP = 12.1) and gave no inhibition (<10%) of GIVA $cPLA_2$ at up to 0.091 mole fraction. If the polar carboxy or carboxy ester groups are interacting with the GIVA $cPLA_2$ phosphate-binding site, a polar carbamate should be sufficient. However, it is possible that the eight-carbon extension off the carbamate causes a conformational change or structural distortion, possibly causing a change in orientation in the micelle surface, or merely creates

Scheme 6. Synthesis of Inhibitors Containing a Carbamate Group[a]

20-22	n	
a	7	*S*
b	1	*S*
c	1	*R*

[a] Reagents and conditions: (a) $CH_3(CH_2)_nNCO$, DMAP, $CHCl_3$; (b) (i) 4 N HCl/MeOH; (ii) $CH_3(CH_2)_9CHOHCOOH$, Et_3N, WSCI, HOBt, CH_2Cl_2; (c) 1 N NaOH, CH_3OH; (d) NaOCl, TEMPO, NaBr, $NAHCO_3$, ACOEt/$PhCH_3$/H_2O (3:3:0.5), 0 °C, then HCl.

Scheme 7. Synthesis of Inhibitors Containing an Amide Group[a]

[a] Reagents and conditions: (a) $C_2H_5NH_2$, WSCI, HOBt Et_3N, CH_2Cl_2; (b) (i) 4 N HCl/MeOH; (ii) $CH_3(CH_2)_9CHOHCOOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (c) (i) 1 N NaOH, CH_3OH; (ii) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/$PhCH_3$/H_2O (3:3:0.5), 0 °C, then HCl.

Scheme 8. Synthesis of Inhibitors Containing an Amide Group[a]

28-31	m	n
a	1	9
b	9	13

[a] Reagents and conditions: (a) $CH_3(CH_2)_mNH_2$, WSCI, HOBt, Et_3N, CH_2Cl_2; (b) (i) H_2, Pd/C; (ii) $CH_3(CH_2)_nCHOHCOOH$, WSCI, HOBt, Et_3N, CH_2Cl_2; (c) NaOCl, TEMPO, NaBr, $NaHCO_3$, EtOAc/toluene/H_2O (3:3:0.5), 0 °C; (d) 50% trifluoroacetic acid/CH_2Cl_2.

Scheme 9. Synthesis of Norleucinol Carbamate-Based Inhibitor[a]

[a] Reagents and conditions: (a) $CH_3(CH_2)_7NCO$, DMAP, $CHCl_3$; (b) (i) 4 N HCl/MeOH; (ii) $CH_3(CH_2)_{13}CHOHCOOH$, Et_3N, WSCI, HOBt, CH_2Cl_2; (c) NaOCl, TEMPO, NaBr, $NAHCO_3$, ACOEt/$PhCH_3$/H_2O (3:3:0.5), 0 °C.

a steric clash in the active site. We have previously shown that 2-oxoamides based on long-chain β-amino acids are relatively weak inhibitors of GIVA $cPLA_2$.[31]

Specificity for GIVA $cPLA_2$ vs GV $sPLA_2$ and GVIA $iPLA_2$

Many inhibitors of GIVA $cPLA_2$ and GVIA $iPLA_2$ have been developed, though most have the problems that they lack specificity (or specificity has not been determined), are time-dependent and/or covalent, are membrane-impermeant, or are too large for pharmaceutical use.[48,49]

The 2-oxoamide compounds were designed as and are potent inhibitors of serine esterases like pancreatic lipase and gastric lipase,[50,51] GIVA $cPLA_2$,[29] and most recently GVIA $iPLA_2$.[19] Previously, activated ketones (hydrated ketones) have been shown to inhibit both serine proteases that utilize a nucleophilic

Table 1. Inhibition of GIVA $cPLA_2$ with α- through ε-Amino Acid-Based 2-Oxoamides

compound	structure	$X_I(50)$[a]	inhibition	ClogP
35		0.017 ± 0.009[b]	N/A[c]	6.6
3		N.D.[d]	0.091; 60%	6.1
14		0.027 ± 0.004	N/A	7.7
36		0.009 ± 0.004[b]	N/A	6.4
37		0.010 ± 0.004[e]	N/A	8.0
38		0.008 ± 0.003	N/A	8.5
17a		0.005 ± 0.001	N/A	7.9
17b		0.005 ± 0.002	N/A	8.4
10a		0.025 ± 0.013	N/A	5.3
10b		0.013 ± 0.005	N/A	8.4

[a] $X_I(50)$ is the concentration of the inhibitor at which 50% inhibition is detected, with units of mole fraction calculated by taking the number of moles of inhibitor divided by total number of moles of substrate plus inhibitor plus detergent. [b] Data taken from ref 29. [c] N/A signifies not applicable because an $X_I(50)$ value was determined. [d] N.D. signifies inhibition was not potent enough to determine an $X_I(50)$. [e] Data taken from ref 30.

Table 2. Inhibition of GIVA $cPLA_2$ by 2-Oxoamides with Varying Side Chains

compound	structure	$X_I(50)$	inhibition	ClogP
39		0.011 ± 0.003[a]	N/A	7.7
40		0.012 ± 0.014[a]	N/A	8.2
22c		N.D.	None[b]	4.5
22b		N.D.	None	4.5
26		N.D.	0.091; 12%	2.9
31a		N.D.	0.091; 30%	2.9
22a		N.D.	0.091; 60%	7.7
31b		0.025 ± 0.012	N/A	9.3
6		N.D.	None	3.1
34		N.D.	None	12.1
52		0.020 ± 0.011	N/A	8.7

[a] Data taken from ref 19. [b] None signifies no significant inhibition was detected at the highest concentration tested.

serine and also zinc metalloproteases and aspartylproteases that utilize an activated nucleophilic water molecule.[52] The small, secreted human GV $sPLA_2$ utilizes a catalytic histidine to activate a water molecule as the nucleophile in phospholipid hydrolysis.[53] Although there is no serine nucleophile in GV $sPLA_2$, the 2-oxoamides may resemble the substrate phospholipids or the transition state such that they would bind to the GV $sPLA_2$ active site and inhibit the enzyme. Additionally, some potent and selective $sPLA_2$ inhibitors are indole analogues that contain a primary 2-oxoamide moiety.[54,55] Long-running pharmaceutical research into inhibitors of the $sPLA_2$ paralogues has resulted in clinical trials for GIIA $sPLA_2$ inhibitors that has shown no benefit for sepsis, rheumatoid arthritis, or asthma.[54−58] Research is ongoing to design potent, specific inhibitors of the various human $sPLA_2$ paralogues, including the GV $sPLA_2$.[25,59−61]

To confirm the specificity of the 2-oxoamides, recombinant human GV $sPLA_2$ was expressed, folded, purified, and tested against 31 of the published 2-oxoamide compounds.[19,29−31] In addition, the 16 novel compounds described herein were also tested. These 47 compounds showed varying levels of inhibition of GV $sPLA_2$. A total of 27 compounds did not significantly inhibit GV $sPLA_2$ (<14% inhibition at the highest dose tested, Table 1 in Supporting Information), 19 compounds moderately inhibited GV $sPLA_2$ (14−50% inhibition, Table 3), and 1 compound significantly inhibited GV $sPLA_2$ with an $X_I(50)$ of 0.035 mole fraction (Table 3). All compounds that showed significant inhibition were maximally tested at 0.091 mole fraction except that compound **41** was tested at 0.01 mole fraction, **42** was tested at 0.02 mole fraction, **40** was tested at 0.04 mole fraction, and **43** was tested at 0.05 mole fraction, as indicated in Table 3.

The 2-oxoamides that showed moderate inhibition of GV $sPLA_2$ are shown in Table 3. The inhibition of GV $sPLA_2$ by **41** was low (22%), though the maximum concentration tested was only 0.01 mole fraction. Compound **42** has significant activity toward GV $sPLA_2$ at a relatively low 0.02 mole fraction but requires heating to 65 °C to solubilize it in dimethyl

Table 3. Notable Inhibition of GV $sPLA_2$ by 2-Oxoamides

compound[a]	structure	mole fraction; Inhibition	ClogP
41		0.01; 22%	10.6
42		0.02; 37%	14.9
43		0.048; 27%	2.4
44		0.091; 30%	7.9
45		0.091; 22%	7.1
46		0.091; 45%	0.8
47		0.091; 14%	3.4
17		0.091; 26%	7.9
10a		0.091; 22%	5.3
37		0.091; 30%	8.0
10b		0.091; 22%	8.4
31b		0.091; 33%	9.3
48		0.091; 27%	2.8
49		0.091; 34%	2.2
50		0.091; 48%	2.7
51		0.091; 31%	3.2
6		0.091; 15%	3.1
40		0.040; 24%	8.2
34		0.091; 20%	12.1
52		$X_I(50)$: 0.035 ± 0.018	8.7

[a] No inhibition (<14% inhibition) found for any other 2-oxoamide compounds with maximal concentration tested, typically 0.091 mole fraction.

sulfoxide (DMSO) and to integrate it into substrate mixed micelles. The extreme hydrophobic nature of both **41** and **42** make them unlikely candidates for practical use. Compound **45**, a poor inhibitor of GIVA $cPLA_2$ and GVIA $iPLA_2$ inhibitor,[19] also does not strongly inhibit GV $sPLA_2$. Compound **46** significantly inhibits GV $sPLA_2$ but not GIVA $cPLA_2$ or GVIA $iPLA_2$,[19,30] which may be because of its primary amide and/or its low hydrophobicity, unique features among the 2-oxoamides tested thus far. Compound **31b** also poorly, but significantly, inhibits GV $sPLA_2$ as well as GIVA $cPLA_2$ (Table 2) but not GVIA $iPLA_2$ (data not shown). Compound **31b** contains an amide in the R_1 side chain position. Phospholipid analogues with an *sn*-2 amide-linked acyl chain are potent inhibitors of secreted PLA_2 family members;[62,63] therefore, it is possible that the amide and not the 2-oxoamide of **31b** is responsible for the measurable inhibition of GV $sPLA_2$.

Table 4. In Vivo Anti-Inflammatory and Analgesic Activity of Selected 2-Oxoamide Inhibitors

name	structure	rat *in vivo*	
		anti-inflammatory activity ED_{50} (mmol/kg)[a]	analgesic activity[b]
10a		0.01	33%
31b		0.0165	11%
39		0.0001	53%
38		0.008	65%
17b		0.00005	71%

[a] Statistical significance of results was established using the Student's *t*-test, $P < 0.001$. [b] Analgesic activity (% analgesia) for the corresponding ED_{50} doses.

Compound **52** uniquely inhibited all three PLA_2 enzymes tested with no statistical preference for GV $sPLA_2$ (Table 3), GIVA $cPLA_2$ (Table 2), or GVIA $iPLA_2$ ($X_I(50) = 0.20 \pm 0.09$, data not shown). The inhibition of these diverse enzymes that utilize widely varying active sites and catalytic mechanisms suggests there may be an effect on the phospholipid substrate presentation. It is also possible that this compound most closely mimics a phospholipid containing two acyl chains and therefore binds the active site of all three enzymes. None of the other 15 2-oxoamides whose syntheses are described here for the first time (Schemes 2−9) significantly inhibited GVIA $iPLA_2$ (data not shown), consistent with a lack of inhibition of GVIA $iPLA_2$ by 2-oxoamides containing a free carboxy group.[19]

Animal Models of Inflammation and Pain

Previously, several 2-oxoamides that inhibited GIVA $cPLA_2$ were found to have potent anti-inflammatory and analgesic effects, comparable to those of indomethacin and acetylsalicylate, respectively.[30] These effects are consistent with the role of GIVA $cPLA_2$ as the rate-limiting provider of the free AA precursors of inflammatory prostaglandins.[6,7] Five additional 2-oxoamides have now been investigated in the same animal models of inflammation and pain (Table 4). All of the compounds inhibit GIVA $cPLA_2$ (Tables 1 and 2) with measurable $X_I(50)$ values ranging from 0.008 for compound **38** up to 0.025 for compounds **10a** and **31b**. Of these five compounds tested, compounds **39** and **17b** presented anti-inflammatory activity at very low doses, with ED_{50} values of 0.0001 and 0.00005 mmol/kg, respectively. These dosages are approximately 2 orders of magnitude lower than the best 2-oxoamides previously tested, including AX006 (compound

35) and AX012.[30] The control drug indomethacin, tested at 0.01 mmol/kg, gave a comparable reduction in edema to compounds **39** and **17b**, which were tested at 100-fold lower dosages. Moreover, at the ED_{50} values determined in the inflammation assay, there is significant analgesia for compounds **39** (53% analgesic activity) and **17b** (71% analgesic activity).

It is unclear why these two compounds present anti-inflammatory and analgesic activities at such low dosages. While compound **39** lacks a R_1 side chain, so does compound **35**, which is not nearly as potent.[30] Compound **39** is the only compound tested thus far with an unsaturated linear radyl chain in the R_2 main chain. It is possible that this facilitates delivery to the cytosol or affects the physiological half-life of the compound. Compound **17b** is the only δ-amino acid-based 2-oxoamide tested, with the γ- and ϵ-amino acid-based 2-oxoamides being significantly less potent (Table 4 and ref 30). We previously noted a disconnect between compounds with similar in vitro potency but disparate in vivo potency as seen with compounds **35** and **36**.[30]

None of the compounds tested have any effect on GVIA $iPLA_2$ activity (ref 19 and data not shown), and only compounds **10a** and **31b** present even weak GV $sPLA_2$ inhibition. The discrepancy between in vitro inhibition and in vivo effects may reflect differential bioavailability throughout the body, uptake, intracellular localization, and/or half-life of the compounds. One additional caveat is that the in vitro inhibition assays were done with the human GIVA $cPLA_2$, while the animal studies were done with rats. The human and rat GIVA $cPLA_2$ enzymes are 94% identical at the amino acid level, and both possess the three known regulated serine phosphorylation sites.[1] Besides the 6% amino acid sequence difference, there is a three-amino acid C-terminal extension in the rat enzyme. Also, there is no way to discern the critical post-translational phosphorylation pattern of the rat GIVA $cPLA_2$. These differences could also explain the lack of correlation of in vitro and in vivo potency.

In conclusion, potent 2-oxoamide inhibitors of GIVA $cPLA_2$ are γ- and δ-amino acid-based compounds with a free carboxy group that do not inhibit GVIA $iPLA_2$ or appreciably inhibit GV $sPLA_2$. Of the 47 2-oxoamides tested with GV $sPLA_2$, only one compound had appreciable inhibition. Two of the novel 2-oxoamide inhibitors of GIVA $cPLA_2$ have potent anti-inflammatory and analgesic effects in animal models. Effective dosages for these two compounds are significantly lower than those for indomethacin and acetylsalicylate, the control drugs. These 2-oxoamide compounds specifically inhibited GIVA $cPLA_2$ and not GVIA $iPLA_2$ or GV $sPLA_2$ when tested in vitro. Because GIVA $cPLA_2$ action is a rate-limiting step in the inflammatory cascade,[8,64] GIVA $cPLA_2$ is an important target for the development of safe anti-inflammatory and analgesic pharmaceuticals.[11,65] The overall reduction in stimulated AA production by selective inhibition of GIVA $cPLA_2$ is particularly important to avoid an imbalance of one branch of eicosanoid production over another through substrate shunting, a phenomenon seen with the use of NSAIDs.[66,67] Alternative anti-inflammatory targets are particularly relevant given the recently identified adverse cardiovascular side effects associated with the cyclooxygenase-2-specific inhibitors and the known gastrointestinal and renal side effects of traditional nonsteroidal anti-inflammatory drugs.[66,68] It may be expected that inhibitors of GIVA $cPLA_2$ would avoid the pitfalls of both nonspecific cyclooxygenase inhibitors and cyclooxygenase-2-selective inhibitors because the action of fatty acid biosynthesis, uptake, and liberation by other PLA_2 enzymes should not be affected by specific GIVA $cPLA_2$ inhibitors.[66,67,69] In particular, GVIA $iPLA_2$ seems to have a housekeeping role in membrane homeostasis that results in low-level release of free fatty acids from the cell membranes.[21] GV $sPLA_2$ and its many extracellular paralogues would also remain free to act. GV $sPLA_2$ and other close paralogues have been shown to release significant amounts of free fatty acids but generally do not lead to up-regulated inflammatory eicosanoid production without the activity of GIVA $cPLA_2$.[12,54,55] The potencies of the specific GIVA $cPLA_2$ 2-oxoamide inhibitors in animal models of inflammation and pain both validate the target and provide enticing leads for development of anti-inflammatory therapeutics.

Experimental Section

Synthesis of 2-Oxoamide Compounds. 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. All amino acid derivatives were purchased from Fluka Chemical Co. Thin layer chromatography (TLC) plates (silica gel 60 F_{254}) and silica gel 60 (70−230 or 230−400 mesh) for column chromatography were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain. Tetrahydrofuran (THF), toluene, and diethyl ether (Et_2O) were dried by standard procedures and stored over molecular sieves or Na. All other solvents and chemicals were reagent grade and used without further purification. All the products gave satisfactory elemental analysis results. The ClogP values were calculated using ChemDraw Ultra 8.0 (with the embedded C-QSAR program of Biobyte Corp.). The syntheses of 16 2-oxoamide compounds have been previously described: compounds **43** (AX005), **35** (AX006), **36** (AX007), **41** (AX003), and **42** (AX004) in ref 41; compounds **37** (AX024), **44** (AX009), **45** (AX010), **46** (AX015), and **47** (AX017) in ref 42; and compounds **39** (AX040), **40** (AX041), **48** (AX035), **49** (AX036), **50** (AX037), and **51** (AX038) in ref 21. The synthesis of **38** (AX062) was accomplished in the same fashion as the closely related compound **37**.[42] The complete set of structures are shown in Tables 1−3.

Coupling of Carboxylic Acids with Amino Components. To a stirred solution of 2-hydroxy acid (2.0 mmol) and the hydrochloride amino component (2.0 mmol) in CH_2Cl_2 (20 mL), triethylamine (Et_3N) (6.2 mL, 4.4 mmol) and subsequently 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (WSCI) (0.42 g, 2.2 mmol) and 1-hydroxybenzotriazole (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) was added. The organic layer was washed consecutively with brine, 1 N HCl, brine, 5% $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as the eluent.

(2*S*)-Methyl 2-(2-Hydroxydodecanamido)hexanoate (2). Yield 70%; yellow oil. ^{1}H NMR: δ 7.02 (1H, m, NH), 4.58 (1H, m, CH), 4.13 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.06 (1H, br, OH), 1.95−1.52 (4H, m, 2 × CH_2), 1.50−1.17 (20H, m, 10 × CH_2), 0.91−0.72 (6H, m, 2 × CH_3). ^{13}C NMR: δ 174.1, 173.2, 72.2, 52.3, 51.5, 34.8, 31.8, 29.5, 29.4, 29.3, 27.3, 24.8, 22.6, 22.2, 14.0, 13.8. Anal. ($C_{19}H_{37}NO_4$) C, H, N.

(2*S*)-Di-*tert*-butyl 2-(2-Hydroxydodecanamido)pentanedioate (4). Yield 96%; oil. ^{1}H NMR: δ 7.08 (1H, d, J = 7.0 Hz, NHCO), 4.48 (1H, m, CH), 4.11 (1H, m, CH), 2.31 (2H, m, CH_2COO), 2.20−1.63 (4H, m, 2 × CH_2), 1.46 [9H, s, $C(CH_3)_3$], 1.41 [9H, s, $C(CH_3)_3$], 1.25 (16H, br s, 8 × CH_2), 0.87 (3H, t, J = 7.0 Hz, CH_3). ^{13}C NMR: δ 174.1, 172.2, 171.1, 82.4, 80.8, 72.0, 51.9, 34.9, 31.9, 31.5, 29.6, 29.5, 29.3, 28.0, 27.9, 27.6, 25.0, 24.9, 22.6, 14.1. Anal. ($C_{25}H_{47}NO_6$) C, H, N.

(6*S*)-Ethyl 6-(2-Hydroxydodecanamido)decanoate (9a). Yield 70%; yellow oil. ^{1}H NMR: δ 6.31 (1H, d, J = 8.4 Hz, NH), 4.10 (2H, t, J = 7.0 Hz, OCH_2), 4.08 (1H, m, CH), 3.87 (1H, m, CH),

3.30 (1H, br, OH), 2.27 (2H, t, $J = 7.2$ Hz, CH_2COO), 1.87−1.14 (33H, m, 15 × CH_2, CH_3), 0.86 (6H, m, 2 × CH_3). ^{13}C NMR: δ 173.7, 173.5, 72.0, 60.2, 48.8, 34.9, 34.1, 31.8, 29.5, 29.4, 29.3, 28.0, 25.3, 24.9, 24.7, 22.6, 22.5, 14.1, 14.0, 13.9. Anal. ($C_{24}H_{47}NO_4$) C, H, N.

(6*S*)-Ethyl 6-(2-Hydroxypentadecanamido)decanoate (9b). Yield 72%; yellow oil. ^{1}H NMR: δ 6.33 (1H, m, NH), 4.02−4.15 (3H, m, OCH_2, CH), 3.88 (1H, m, CH), 3.18 (1H, br, OH), 2.27 (2H, t, $J = 7.4$ Hz, CH_2COO), 1.87 (1.14 (37H, m, 18 × CH_2, CH_3), 0.90−0.81 (6H, m, 2 × CH_3). ^{13}C NMR: δ 173.9, 173.5, 72.1, 60.3, 48.8, 34.9, 34.7, 34.1, 31.9, 29.6, 29.5, 29.4, 29.3, 28.0, 25.3, 24.9, 24.8, 22.6, 22.5, 14.1, 14.0, 13.9. Anal. ($C_{27}H_{53}NO_4$) C, H, N.

2-Hydroxy-*N*-((*S*)-1-phenylhexan-2-yl)pentadecanamide (13). Yield 89%; white solid. ^{1}H NMR: δ 7.24 (5H, m, C_6H_5), 6.19 (1H, m, NH), 4.18 (1H, m, CH), 4.02 (1H, m, CH), 2.55−2.83 (3H, m, $CH_2C_6H_5$, OH), 1.78−1.47 (4H, m, 2 × CH_2), 1.42−1.15 (26H, m, 13 × CH_2), 0.91−0.82 (6H, m, 2 × CH_3). ^{13}C NMR: δ 173.1, 137.9, 129.4, 128.3, 126.4, 72.0, 49.8, 41.0, 35.0, 33.9, 31.9, 29.6, 29.5, 29.4, 29.3, 28.1, 24.8, 22.7, 22.5, 14.1, 14.0. Anal. ($C_{27}H_{47}NO_2$) C, H, N.

2-Hydroxy-*N*-((S)-1-phenyloctan-4-yl)pentadecanamide (16a). Yield 68%; white solid. ^{1}H NMR ($CDCl_3$): δ 7.39−7.11 (5H, m, C_6H_5), 6.28 (1H, d, $J = 9.6$ Hz, NH), 4.05 (1H, m, CH), 3.91 (1H, m, CH), 2.99 (1H, br s, OH), 2.64 (2H, m, $CH_2C_6H_5$), 1.81−1.02 (34H, m, 17 × CH_2), 0.88 (6H, t, $J = 6.6$ Hz, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 173.7, 142.3, 128.3, 128.2, 125.7, 72.1, 48.6, 35.6, 35.0, 34.9, 31.8, 29.7, 29.6, 29.5, 29.3, 28.0, 27.6, 24.7, 22.7, 22.5, 14.1, 14.0. Anal. ($C_{29}H_{51}NO_2$) C, H, N.

2-Hydroxy-*N*-((*S*)-1-phenyloctan-4-yl)hexadecanamide (16b). Yield 73%; white solid; mp 43−45 °C. ^{1}H NMR ($CDCl_3$): δ 7.32−7.14 (5H, m, C_6H_5), 6.25 (1H, d, $J = 9.6$ Hz, NH), 4.08 (1H, m, CH), 3.95 (1H, m, CH), 3.08 (1H, br s, OH), 2.62 (2H, m, $CH_2C_6H_5$), 1.89−1.05 (36H, m, 18 × CH_2), 0.88 (6H, t, $J = 6.6$ Hz, 2 × CH_3). ^{13}C NMR ($CDCl_3$): δ 173.4, 142.1, 128.3, 128.2, 125.7, 72.0, 48.7, 35.6, 35.0, 34.8, 31.9, 29.7, 29.6, 29.5, 29.3, 28.0, 27.6, 24.8, 22.6, 22.5, 14.1, 14.0. Anal. ($C_{30}H_{53}NO_2$) C, H, N.

(4*S*)-Methyl 4-(2-Hydroxydodecanamido)-5-(octylcarbamoyloxy)pentanoate (20a). Yield 47%; white solid; mp 70−73 °C. ^{1}H NMR: δ 6.69 (1H, m, CONH), 4.79 (1H, m, OCONH), 4.08 (4H, m, 2 × CH, CH_2OCONH), 3.68 (3H, s, CH_3O), 3.17 (2H, m, CH_2NH), 2.40 (2H, m, CH_2COO), 1.85 (4H, m, 2 × CH_2), 1.49−1.26 (28H, m, 14 × CH_2), 0.88 (6H, t, $J = 6.6$ Hz, 2 × CH_3). ^{13}C NMR: δ 174.5, 173.7, 156.5, 72.1, 66.1, 51.8, 48.1, 41.1 34.7, 31.9, 30.4, 29.8, 29.6, 29.4, 29.3, 29.2, 26.7, 25.0, 22.6, 14.0. Anal. ($C_{27}H_{52}N_2O_6$) C, H, N.

(4*S*)-Methyl 5-(Ethylcarbamoyloxy)-4-(2-hydroxydodecanamido)pentanoate (20b). Yield 61%; white solid; mp 79−81 °C. ^{1}H NMR: δ 6.75 (1H, m, CONH), 4.91 (1H, m, OCONH), 4.11 (4H, m, 2 × CH, CH_2OCONH), 3.68 (3H, s, CH_3O), 3.21 (2H, m, CH_2NH), 2.40 (2H, m, CH_2COO), 1.83 (4H, m, 2 × CH_2), 1.60−1.26 (16H, m, 8 × CH_2), 1.18 (3H, t, $J = 6.0$ Hz, CH_3), 0.88 (3H, t, $J = 6.0$ Hz, CH_3). ^{13}C NMR: δ 174.6, 173.7, 156.4, 72.1, 66.1, 51.7, 48.1, 35.1, 34.7, 31.8, 30.5, 30.4, 29.4, 29.3, 26.5, 26.4, 25.1, 25.0, 22.6, 15.0, 14.0. Anal. ($C_{21}H_{40}N_2O_6$) C, H, N.

(4*R*)-Methyl 5-(Ethylcarbamoyloxy)-4-(2-hydroxydodecanamido)pentanoate (20c). Yield 58%; white solid; mp 78−80 °C. ^{1}H NMR: δ 6.75 (1H, m, CONH), 4.91 (1H, m, OCONH), 4.10 (4H, m, 2 × CH, CH_2OCONH), 3.68 (3H, s, CH_3O), 3.21 (2H, m, CH_2NH), 2.40 (2H, m, CH_2COO), 1.83 (4H, m, 2 × CH_2), 1.62−1.26 (16H, m, 8 × CH_2), 1.18 (3H, t, $J = 7.2$ Hz, CH_3), 0.88 (3H, t, $J = 7.2$ Hz, CH_3). ^{13}C NMR: δ 174.5, 173.8, 156.4, 72.1, 66.1, 51.8, 48.1, 35.9, 34.7, 31.8, 30.4, 29.6, 29.5, 29.3, 26.5, 26.4, 25.1, 25.0, 22.6, 15.1, 14.1.

(*R*)-Methyl 4-(*tert*-Butoxycarbonyl)-5-(ethylamino)-5-oxopentanoate (24). Yield 69%; white solid; mp 64−66 °C; $[\alpha]_D$ +7.1 (*c* 1, $CHCl_3$). ^{1}H NMR: δ 6.45 (1H, m, CONH), 5.39 (1H, d, $J = 9.0$ Hz, OCONH), 4.11 (1H, m, CH), 3.67 (3H, s, CH_3O), 3.27 (2H, m, CH_2NH), 2.41 (2H, m, CH_2COO), 2.12 (1H, m, CHHCH), 1.92 (1H, m, CHHCH), 1.42 [9H, s, $(CCH_3)_3$], 1.12 (3H, t, $J = 7.4$ Hz, CH_3). ^{13}C NMR: δ 173.8, 171.2, 155.7, 79.9, 53.8, 51.8, 34.3, 30.2, 28.2, 28.1, 14.7. Anal. ($C_{13}H_{24}N_2O_5$) C, H, N.

(4*R*)-Methyl 5-(Ethylamino)-4-(2-hydroxydodecanamido)-5-oxopentanoate (25). Yield 67%; white solid; mp 83−85 °C. ^{1}H NMR: δ 7.48 (1H, m, CONH), 6.83 (1H, m, CONH), 4.48 (2H, m, CH), 4.10 (1H, m, CH), 3.68 (3H, s, CH_3O), 3.26 (2H, m, CH_2NH), 2.41 (2H, m, CH_2COO), 2.17−2.09 (2H, m, CH_2), 1.80−1.62 (2H, m, CH_2), 1.25 (16H, br s, 8 × CH_2), 1.13 (3H, t, $J = 7.2$ Hz, CH_3), 0.87 (3H, t, $J = 7.0$ Hz, CH_3). ^{13}C NMR: δ 177.1, 173.6, 171.2, 72.0, 51.8, 34.7, 31.8, 29.6, 29.4, 29.3, 27.8, 25.1, 22.6, 14.2, 14.1. Anal. ($C_{20}H_{38}N_2O_5$) C, H, N.

(*S*)-*tert*-Butyl 4-(Benzyloxycarbonyl)-5-(ethylamino)-5-oxopentanoate (28a). Yield 85%; white solid; mp 69−71 °C; $[\alpha]_D$ −5.4 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 7.35 (5H, m, C_6H_5), 6.25 (1H, m, NHCO), 5.67 (1H, d, $J = 8.0$ Hz, OCONH), 5.10 (2H, s, $CH_2C_6H_5$), 4.14 (1H, m, CH), 3.29 (2H, m, CH_2NH), 2.36 (2H, m, CH_2COO), 1.96 (2H, m, CH_2CH), 1.44 [9H, s, $C(CH_3)_3$], 1.13 (3H, t, $J = 7.4$ Hz, CH_3). ^{13}C NMR: δ 172.8, 171.0, 156.0, 136.2, 128.5, 128.1, 128.0, 80.9, 66.9, 54.3, 34.4, 31.6, 28.1, 28.0, 14.6. Anal. ($C_{19}H_{28}N_2O_5$) C, H, N.

(*S*)-*tert*-Butyl 4-(Benzyloxycarbonyl)-5-(decylamino)-5-oxopentanoate (28b). Yield 98%; oil; $[\alpha]_D$ −3.4 (*c* 1, $CHCl_3$). ^{1}H NMR: δ 7.34 (5H, m, C_6H_5), 6.40 (1H, m, CONH), 5.75 (1H, d, $J = 7.2$ Hz, OCONH), 5.09 (2H, s, $CH_2C_6H_5$), 4.19 (1H, m, CH), 3.24 (2H, m, CH_2NH), 2.32 (2H, m, CH_2COO), 1.96 (4H, m, 2 × CH_2), 1.44 [9H, s, $C(CH_3)_3$], 1.25 (14H, br s, 7 × CH_2), 0.88 (3H, t, $J = 6.6$ Hz, CH_3). ^{13}C NMR: δ 172.9, 171.0, 156.2, 136.1, 128.5, 128.1, 128.0, 81.0, 66.9, 54.3, 39.5, 31.8, 29.5, 29.4, 29.3, 29.2, 28.2, 28.0, 26.8, 22.6, 14.1. Anal. ($C_{27}H_{44}N_2O_5$) C, H, N.

(4*S*)-*tert*-Butyl 5-(Ethylamino)-4-(2-hydroxydodecanamido)-5-oxopentanoate (29a). Yield 55%; oil. ^{1}H NMR: δ 7.45 (1H, d, $J = 8.6$ Hz NHCO), 6.85 (1H, m, NHCO), 4.41 (1H, m, CH), 4.11 (1H, m, CH), 3.25 (2H, m, $NHCH_2$), 2.30 (2H, m, CH_2COO), 2.05 (2H, m, CH_2), 1.80−1.62 (2H, m, CH_2), 1.43 (9H, s, 3 × CH_3), 1.29 (16H, br s, 8 × CH_2), 1.09 (3H, t, $J = 7.2$ Hz, CH_3), 0.88 (3H, t, $J = 7.0$ Hz, CH_3). ^{13}C NMR: δ 174.8, 172.6, 171.1, 80.8, 72.0, 51.9, 34.7, 34.4, 31.8, 31.5, 29.5, 29.4, 29.3, 28.0, 25.1, 22.6, 14.5, 14.0. Anal. ($C_{23}H_{44}N_2O_5$) C, H, N.

(4*S*)-*tert*-Butyl 5-(Decylamino)-4-(2-hydroxyhexadecanamido)-5-oxopentanoate (29b). Yield 52%; white solid; mp 73−75 °C. ^{1}H NMR: δ 7.35 (1H, m, CONH), 6.59 (1H, m, CONH), 4.41 (1H, m, CH), 4.11 (1H, m, CH), 3.25 (2H, m, CH_2NH), 2.39 (2H, m, CH_2COO), 2.08−1.63 (6H, m, CH_2CH, CH_2CH_2NH, CH_2CHOH), 1.45 [9H, s, $C(CH_3)_3$], 1.25 (38H, br s, 19 × CH_2), 0.88 (6H, t, $J = 6.6$ Hz, 2 × CH_3). ^{13}C NMR: δ 174.6, 172.9, 170.9, 81.1, 72.0, 52.1, 39.6, 34.8, 31.9, 29.7, 29.5, 29.4, 29.3, 28.0, 27.8, 26.9, 25.0, 22.7, 14.1. Anal. ($C_{35}H_{68}N_2O_5$) C, H, N.

(*S*)-2-(2-Hydroxyhexadecanamido)hexyl Octylcarbamate (33). Yield 59%; white solid; mp 65−66 °C. ^{1}H NMR: δ 6.62 (1H, m, NHCO), 4.99 (1H, m, OCONH), 4.12 (3H, m, 2 × CH, CH_2OCONH), 3.12 (2H, m, CH_2NH), 1.79 (1H, m, CHHCH), 1.66−1.44 (5H, m, CHHCH, 2 × CH_2), 1.25 (28H, br s, 14 × CH_2), 0.88 (3H, t, $J = 6.6$ Hz, CH_3). ^{13}C NMR: δ 174.0, 156.8, 72.2, 66.4, 66.1, 48.7, 41.1, 34.9, 34.7, 31.9, 31.8, 31.1, 29.9, 29.7, 29.5, 29.3, 29.2, 27.9, 26.7, 25.0, 22.7, 22.6, 22.5, 14.1, 13.9. Anal. ($C_{31}H_{62}N_2O_4$) C, H, N.

Oxidation of 2-Hydroxyamides. To a solution of 2-hydroxyamide (5.00 mmol) in a mixture of toluene−EtOAc (30 mL), a solution of NaBr (0.54 g, 5.25 mmol) in water (2.5 mL) was added followed by TEMPO (11 mg, 0.050 mmol). To the resulting biphasic system, which was cooled at −5 °C, an aqueous solution of 0.35 M NaOCl (15.7 mL, 5.50 mmol) containing $NaHCO_3$ (1.26 g, 15 mmol) was added dropwise under vigorous stirring at −5 °C over a period of 1 h. After the mixture had been stirred for a further 15 min at 0 °C, EtOAc (30 mL) and H_2O (10 mL) were added. The aqueous layer was separated and washed with EtOAc (20 mL). The combined organic layers were washed consecutively with 5% aqueous citric acid (30 mL) containing KI (0.18 g), 10% aqueous $Na_2S_2O_3$ (30 mL), and brine and dried over Na_2SO_4. The solvents

were evaporated under reduced pressure, and the residue was purified by column chromatography [EtOAc−petroleum ether (bp 40−60 °C)].

(*S*)-2-(2-Oxododecanamido)hexanoic Acid (3). Yield 55%; pale-yellow solid; mp 42−43 °C; $[\alpha]_D$ +13.5 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 7.41 (1H, d, *J* = 9 Hz, NH), 4.52 (1H, m, CH), 2.90 (2H, t, *J* = 7.8 Hz, CH_2COCO), 2.00−1.56 (6H, m, 3 × CH_2), 1.25 (16H, m, 8 × CH_2), 0.88 (6H, t, *J* = 6.7 Hz, 2 × CH_3). ^{13}C NMR: δ 198.5, 176.4, 159.9, 52.8, 36.8, 31.9, 29.6, 29.4, 29.3, 29.0, 27.4, 23.1, 22.7, 22.3, 14.1, 13.8. MS (FAB), *m/z* (%): 372 (100) $[M + 2Na - H]^+$. Anal. ($C_{18}H_{33}NO_4$) C, H, N.

(*S*)-Di-*tert*-butyl 2-(2-Oxododecanamido)pentanedioate (5). Yield 93%; oil; $[\alpha]_D$ +10.9 (*c* 1.15, $CHCl_3$). ^1H NMR: δ 7.45 (1H, d, *J* = 9.2 Hz, NH), 4.45 (1H, m, CH), 2.89 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.30−1.95 (4H, m, 2 × CH_2), 1.60 (2H, m, CH_2), 1.48 [9H, s, $C(CH_3)_3$], 1.44 [9H, s, $C(CH_3)_3$], 1.26 (16H, br s, 8 × CH_2), 0.88 (3H, t, *J* = 7.0 Hz, CH_3). ^{13}C NMR: δ 198.4, 171.7, 170.0, 159.9, 82.7, 80.8, 52.1, 36.7, 31.8, 31.3, 29.5, 29.4, 29.3, 29.0, 28.0, 27.9, 27.5, 23.1, 22.6, 14.1. Anal. ($C_{25}H_{45}NO_6$) C, H, N.

(*S*)-6-(2-Oxododecanamido)decanoic Acid (10a). Yield 73%; white solid; mp 58−59 °C; $[\alpha]_D$ −0.9 (*c* 2.0, $CHCl_3$). ^1H NMR: δ 6.72 (1H, d, *J* = 9 Hz, NH), 3.85 (1H, m, CH), 2.90 (2H, t, *J* = 7.8 Hz, CH_2COCO), 2.31 (2H, t, *J* = 7.4 Hz, CH_2COOH), 1.78−1.23 (28H, m, 14 × CH_2), 0.88 (6H, t, *J* = 6.8 Hz, 2 × CH_3). ^{13}C NMR: δ 199.7, 179.3, 159.8, 49.4, 36.8, 34.6, 33.7, 31.8, 29.5, 29.4, 29.3, 29.0, 27.9, 25.3, 24.4, 23.2, 22.6, 22.5, 14.1, 13.9. MS (FAB), *m/z* (%): 428 (100) $[M + 2Na - H]^+$, 406 (63) $[M + Na]^+$. Anal. ($C_{22}H_{41}NO_4$) C, H, N.

(*S*)-6-(2-Oxopentadecanamido)decanoic Acid (10b). Yield 80%; pale-yellow solid; mp 63−64 °C; $[\alpha]_D$ −0.5 (*c* 1.0, $CHCl_3$). ^1H NMR: δ 6.72 (1H, d, *J* = 9.8 Hz, NH), 3.85 (1H, m, CH), 2.93 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.34 (2H, t, *J* = 7.4 Hz, CH_2COOH), 1.70−1.12 (34H, m, 17 × CH_2), 0.91−0.82 (6H, m, 2 × CH_3). ^{13}C NMR: δ 199.7, 179.0, 159.8, 49.4, 36.8, 34.6, 33.7, 31.9, 29.6, 29.4, 29.3, 29.0, 28.0, 25.3, 24.4, 23.2, 22.7, 22.5, 14.1, 13.9. MS (FAB), *m/z* (%): 426 (100) $[M + H]^+$. Anal. ($C_{25}H_{47}NO_4$) C, H, N.

(*S*)-5-(Octylcarbamoyloxy)-4-(2-oxododecanamido)pentanoic Acid (22a). Yield 42%; oily solid; $[\alpha]_D$ −16.8 (*c* 0.26, $CHCl_3$). ^1H NMR: δ 7.22 (1H, m, NHCOCO), 4.85 (1H, m, OCONH), 4.15 (3H, m, CH, CH_2OCONH), 3.15 (2H, m, $NHCH_2$), 2.91 (2H, t, *J* = 7.8 Hz, CH_2COCO), 2.40 (2H, m, CH_2COOH), 1.90 (2H, m, CH_2), 1.70−1.40 (4H, m, 2 × CH_2), 1.27 (24H, m, 12 × CH_2), 0.89 (6H, t, *J* = 6.8 Hz, 2 × CH_3). ^{13}C NMR: δ 198.9, 176.9, 160.2, 156.0, 65.5, 48.6, 41.2, 36.8, 31.9, 31.8, 30.3, 29.8, 29.5, 29.4, 29.3, 29.2, 29.0, 26.7, 26.3, 23.1, 22.6, 14.1. MS (FAB), *m/z* (%): 485 (40) $[M + H]^+$. Anal. ($C_{26}H_{48}N_2O_6$) C, H, N.

(*S*)-5-(Ethylcarbamoyloxy)-4-(2-oxododecanamido)pentanoic Acid (22b). Yield 51%; white solid; mp 70−72 °C; $[\alpha]_D$ −25.6 (*c* 1, $CHCl_3$). ^1H NMR: δ 7.25 (1H, m, NHCOCO), 4.85 (1H, m, OCONH), 4.15 (3H, m, CH_2OCONH, CH), 3.20 (2H, m, $NHCH_2$), 2.90 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.42 (2H, m, CH_2COOH), 1.93 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.29 (14H, m, 7 × CH_2), 1.14 (3H, t, *J* = 7.4 Hz, $NHCH_2CH_3$), 0.88 (3H, t, *J* = 7.0 Hz, CH_3). ^{13}C NMR: δ 198.8, 177.3, 160.1, 156.0, 65.5, 48.4, 36.7, 35.9, 31.8, 30.3, 29.4, 29.3, 29.2, 29.0, 28.9, 26.2, 23.0, 22.6, 15.0, 14.1. MS (FAB), *m/z* (%): 423 (50) $[M + Na]^+$.

(*R*)-5-(Ethylcarbamoyloxy)-4-(2-oxododecanamido)pentanoic Acid (22c). Yield 56%; white solid; mp 70−72 °C; $[\alpha]_D$ +26.1 (*c* 1, $CHCl_3$). ^1H NMR: δ 7.25 (1H, m, NHCOCO), 4.82 (1H, m, OCONH), 4.19 (3H, m, CH_2OCONH, CH), 3.21 (2H, m, $NHCH_2$), 2.90 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.42 (2H, m, CH_2COOH), 1.98 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.29 (14H, m, 7 × CH_2), 1.14 (3H, t, *J* = 7.4 Hz, $NHCH_2CH_3$), 0.88 (3H, t, *J* = 7.0 Hz, CH_3). ^{13}C NMR: δ 198.7, 177.0, 160.0, 155.9, 65.4, 48.4, 36.6, 35.8, 31.7, 30.2, 29.4, 29.2, 29.1, 28.9, 26.1, 22.9, 22.5, 15.0, 13.9. Anal. ($C_{20}H_{36}N_2O_6$) C, H, N.

(*R*)-5-(Ethylamino)-5-oxo-4-(2-oxododecanamido)pentanoic Acid (26). Yield 57%; white solid; mp 109−112 °C; $[\alpha]_D$ +11.6 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 8.02 (1H, d, *J* = 9.0 Hz, NHCOCO), 7.03 (1H, m, CONH), 4.63 (1H, m, CH), 3.32 (2H, m, $NHCH_2$), 2.89 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.49 (2H, m, CH_2COOH), 2.12 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.23 (14H, m, 7 × CH_2), 1.17 (3H, t, *J* = 7.0 Hz, $NHCH_2CH_3$), 0.88 (3H, t, *J* = 6.6 Hz, CH_3). ^{13}C NMR: δ 197.9, 175.9, 170.1, 160.2, 52.0, 36.8, 34.5, 31.7, 29.7, 29.4, 29.3, 29.2, 29.1, 28.9, 27.6, 22.9, 22.5, 14.3, 14.0. Anal. ($C_{19}H_{34}N_2O_5$) C, H, N.

(*S*)-*tert*-Butyl 5-(Ethylamino)-5-oxo-4-(2-oxododecanamido)pentanoate (30a). Yield 81%; oil; $[\alpha]_D$ −6.4 (*c* 1, $CHCl_3$). ^1H NMR: δ 7.69 (1H, d, *J* = 9.0 Hz, NHCOCO), 7.42 (1H, m, CONH), 4.38 (1H, m, CH), 3.29 (2H, m, $NHCH_2$), 2.87 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.36 (2H, m, CH_2COO), 2.09 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.44 [9H, s, $C(CH_3)_3$], 1.25 (14H, m, 7 × CH_2), 1.18 (3H, t, *J* = 7.0 Hz, CH_3), 0.88 (3H, t, *J* = 6.6 Hz, CH_3). ^{13}C NMR: δ 198.2, 172.5, 169.8, 160.2, 81.1, 52.3, 36.8, 34.5, 31.8, 31.5, 29.5, 29.4, 29.2, 29.0, 28.0, 23.0, 22.6, 14.6, 14.1. Anal. ($C_{23}H_{42}N_2O_5$) C, H, N.

(*S*)-*tert*-Butyl 5-(Decylamino)-5-oxo-4-(2-oxohexadecanamido)pentanoate (30b). Yield 90%; white solid; mp 43−45 °C; $[\alpha]_D$ −6.0 (*c* 1, $CHCl_3$). ^1H NMR: δ 7.95 (1H, d, *J* = 8.0 Hz, NHCOCO), 6.31 (1H, m, CONH), 4.38 (1H, m, CH), 3.24 (2H, m, $NHCH_2$), 2.89 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.32 (2H, m, CH_2COO), 2.09 (2H, m, CH_2), 1.55 (4H, m, 2 × CH_2), 1.46 [9H, s, $C(CH_3)_3$], 1.26 (36H, m, 18 × CH_2), 0.88 (6H, t, *J* = 6.6 Hz, 2 × CH_3). ^{13}C NMR: δ 198.2, 172.6, 169.9, 160.2, 81.2, 52.4, 39.7, 36.8, 31.9, 31.8, 31.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 28.1, 28.0, 26.8, 23.1, 22.7, 14.1. Anal. ($C_{35}H_{66}N_2O_5$) C, H, N.

(*S*)-2-(2-Oxohexadecanamido)hexyl Octylcarbamate (34). Yield 66%; white solid; mp 73−75 °C; $[\alpha]_D$ −22.8 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 7.03 (1H, m, NHCOCO), 4.71 (1H, m, OCONH), 4.10 (3H, m, CH_2OCONH, CH), 3.16 (2H, m, CH_2NH), 2.92 (2H, t, *J* = 7.6 Hz, CH_2COCO), 1.62−1.51 (6H, m, 3 × CH_2), 1.26 (28H, br s, 14 × CH_2), 0.85 (3H, t, *J* = 5.6 Hz, CH_3). ^{13}C NMR: δ 199.5, 160.1, 156.1, 66.0, 49.3, 41.4, 37.0, 32.1, 32.0, 31.2, 30.1, 29.9, 29.7, 29.6, 29.4, 29.3, 28.1, 27.0, 23.4, 22.9, 22.8, 22.7, 14.3, 14.1. MS (FAB), *m/z* (%): 525 (20) $[M + H]^+$. Anal. ($C_{31}H_{60}N_2O_4$) C, H, N.

(*S*)-Methyl 5-(Octylcarbamoyloxy)-4-(2-oxododecanamido)pentanoate (52). This compound was prepared by oxidation of compound **20a.** Yield 63%; white solid; mp 55−57 °C; $[\alpha]_D$ −20.8 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 7.14 (1H, m, NHCOCO), 4.76 (1H, m, OCONH), 4.12 (3H, m, CH, CH_2OCONH), 3.67 (3H, s, CH_3O), 3.15 (2H, m, $NHCH_2$), 2.90 (2H, t, *J* = 7.2 Hz, $COCOCH_2$), 2.37 (2H, m, CH_2COO), 1.90 (2H, m, CH_2), 1.59−1.48 (4H, m, 2 × CH_2), 1.26 (24H, m, 12 × CH_2), 0.88 (6H, t, 2 × CH_3, *J* = 7.0 Hz). ^{13}C NMR: δ 198.9, 173.2, 160.0, 156.0, 65.5, 51.8, 48.5, 41.1, 36.7, 31.8, 31.7, 30.4, 29.8, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.7, 26.4, 23.1, 22.6, 14.0. MS (FAB), *m/z* (%): 499 (45) $[M + H]^+$. Anal. ($C_{27}H_{50}N_2O_6$) C, H, N.

Cleavage of the *tert*-Butyl Protecting Group. A solution of the compound (1 mmol) in 50% trifluoroacetic acid/CH_2Cl_2 (10 mL) was stirred for 1 h at room temperature. The organic solvent was evaporated under reduced pressure. The residue was purified by recrystallization [EtOAc−petroleum ether (bp 40−60 °C)].

(*S*)-2-(2-Oxododecanamido)pentanedioic Acid (6). Yield 92%; white solid; mp 97−98 °C; $[\alpha]_D$ +24.4 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 7.61 (1H, d, *J* = 9.2 Hz, NHCOCO), 4.61 (1H, m, CH), 2.91 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.54 (2H, m, CH_2COOH), 2.31 (2H, m, CH_2), 1.61 (2H, m, CH_2), 1.26 (14H, m, 7 × CH_2), 0.88 (3H, t, *J* = 6.6 Hz, CH_3). ^{13}C NMR: δ 198.1, 178.7, 176.5, 159.8, 51.2, 36.7, 31.9, 29.5, 29.4, 29.3, 29.0, 26.1, 23.1, 22.7, 14.1. MS (FAB), *m/z* (%): 366 (15) $[M + Na]^+$, 344 (65) $[M + H]^+$. Anal. ($C_{17}H_{29}NO_6$) C, H, N.

(*S*)-5-(Ethylamino)-5-oxo-4-(2-oxododecanamido)pentanoic Acid (31a). Yield 90%; white solid; mp 103−104 °C; $[\alpha]_D$ −14.2 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 8.10 (1H, d, *J* = 9.0 Hz, NHCOCO), 7.20 (1H, m, CONH), 4.65 (1H, m, CH), 3.30 (2H, m, $NHCH_2$), 2.87 (2H, t, *J* = 7.4 Hz, CH_2COCO), 2.45 (2H, m, CH_2COOH), 2.11 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.25 (14H, m, 7 × CH_2), 1.15 (3H, t, *J* = 7.0 Hz, $NHCH_2CH_3$), 0.87 (3H, t, *J* = 6.6 Hz, CH_3). ^{13}C NMR: δ 198.0, 176.2, 170.6, 160.5, 52.1, 37.0, 34,7,

31.8, 29.8, 29.5, 29.4, 29.3, 29.2, 29.0, 27.5, 23.0, 22.6, 14.4, 14.1. MS (FAB), *m/z* (%): 393 (86) [M + Na]$^+$. Anal. ($C_{19}H_{34}N_2O_5$) C, H, N.

(*S*)-5-(Decylamino)-5-oxo-4-(2-oxohexadecanamido)pentanoic Acid (31b). Yield 59%; white solid; mp 91−93 °C; $[\alpha]_D$ −10.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 7.95 (1H, d, *J* = 9.2 Hz, NHCOCO), 6.99 (1H, m, CONH), 4.67 (1H, m, CH), 3.25 (2H, m, NHCH_2), 2.88 (2H, t, *J* = 7.2 Hz, CH_2COCO), 2.46 (2H, m, CH_2COOH), 2.10 (2H, m, CH_2), 1.54 (4H, m, 2 × CH_2), 1.26 (36H, m, 18 × CH_2), 0.88 (6H, t, *J* = 6.6 Hz, 2 × CH_3). ^{13}C NMR: δ 197.8, 175.9, 170.2, 160.3, 52.1, 39.8, 37,1, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 27.7, 26.8, 23.0, 22.7, 14.1. MS (FAB), *m/z* (%): 539 (10) [M + H]$^+$. Anal. ($C_{31}H_{58}N_2O_5$) C, H, N.

(4*S*)-4-(2-Oxohexadecanamido) octanoic Acid (38). Yield 72%; white solid; mp 67−68 °C; $[\alpha]_D$ −1.6 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 6.86 (1H, d, *J* = 9.4 Hz, NH), 3.93 (1H, m, C*H*NH), 2.92 (2H, t, *J* = 7.8 Hz, CH_2COCO), 2.35 (2H, t, *J* = 6.3 Hz, C*H*2COOH), 2.10−1.41 (6H, m, 3 × CH_2), 1.40−1.05 (26H, m, 13 × CH_2), 0.87 (6H, t, *J* = 6.0 Hz, 2 × CH_3). ^{13}C NMR: δ 199.4, 178.3, 160.1, 49.3, 36.8, 34.7, 31.9, 30.7, 29.9, 29.6, 29.4, 29.3, 29.1, 29.0, 28.0, 23.2, 22.7, 22.4, 14.1, 13.9. Anal. ($C_{24}H_{45}NO_4$) C, H, N.

Synthesis of Carbamates. To a solution of alcohol (1 mmol) and 4-dimethylaminopyridine (DMAP) (0.24 g, 2 mmol) in dry $CHCl_3$ (10 mL) was added dropwise, under nitrogen atmosphere, the isocyanate component (2.4 mmol), and the mixture was stirred overnight at room temperature. The organic solution was washed with aqueous HCl (2.5%) and water. The organic layer was dried over Na_2SO_4, and the organic solvent was evaporated under reduced pressure. The residue was purified by column chromatography [EtOAc−petroleum ether (bp 40−60 °C)].

(*S*)-Methyl 4-(*tert*-Butoxycarbonyl)-5-(octylcarbamoyloxy)-pentanoate (19a). Yield 67%; white solid; mp 64−66 °C; $[\alpha]_D$ −16.1 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 4.68 (2H, m, 2 × OCONH), 4.05 (2H, m, CH_2OCONH), 3.85 (1H, m, CH), 3.68 (3H, s, CH_3O), 3.14 (2H, m, CH_2NH), 2.42 (2H, t, *J* = 7.6 Hz, CH_2COO), 1.79 (2H, m, CH_2CH), 1.44 [9H, s, C(CH_3)$_3$], 1.28 (12H, m, 6 × CH_2), 0.88 (3H, t, *J* = 6.6 Hz, CH_3). Anal. ($C_{20}H_{38}N_2O_6$) C, H, N.

(*S*)-Methyl 4-(*tert*-Butoxycarbonyl)-5-(ethylcarbamoyloxy)-pentanoate (19b). Yield 72%; white solid; mp 70−71 °C; $[\alpha]_D$ −20.5 (*c* 1, $CHCl_3$). ^{1}H NMR: δ 4.68 (2H, m, 2 × OCONH), 4.06 (2H, m, CH_2OCONH), 3.83 (1H, m, CH), 3.67 (3H, s, CH_3O), 3.21 (2H, m, CH_2NH), 2.41 (2H, t, *J* = 7.8 Hz, CH_2COO), 1.80 (2H, m, CH_2CH), 1.44 [9H, s, C(CH_3)$_3$], 1.13 (3H, t, *J* = 7.4 Hz, CH_3). ^{13}C NMR: δ 173.7, 156.2, 155.5, 79.5, 66.3, 51.7, 49.6, 35.9, 30.6, 28.3, 27.0, 15.1. Anal. ($C_{14}H_{26}N_2O_6$) C, H, N.

(*R*)-Methyl 4-(*tert*-Butoxycarbonyl)-5-(ethylcarbamoyloxy)-pentanoate (19c). Yield 75%; white solid; mp 70−71 °C; $[\alpha]_D$ +20.6 (*c* 1, $CHCl_3$). ^{1}H NMR: δ 4.75 (2H, m, 2 × OCONH), 4.06 (2H, m, CH_2OCONH), 3.81 (1H, m, CH), 3.67 (3H, s, CH_3O), 3.20 (2H, m, CH_2NH), 2.40 (2H, t, *J* = 7.6 Hz, CH_2COO), 1.80 (2H, m, CH_2CH), 1.42 [9H, s, C(CH_3)$_3$], 1.13 (3H, t, *J* = 7.2 Hz, CH_3). ^{13}C NMR: δ 173.7, 156.1, 155.5, 79.4, 66.3, 51.7, 49.6, 35.8, 30.5, 28.3, 27.0, 15.1.

(*S*)-(2-*tert*-Butoxycarbonyl)hexyl Octylcarbamate (32). Yield 68%; white solid; mp 43−44 °C; $[\alpha]_D$ −20.8 (*c* 1, $CHCl_3$). ^{1}H NMR: δ 4.72 (1H, m, OCONH), 4.61 (1H, m, OCONH), 4.04 (2H, m, CH_2O), 3.78 (1H, m, CH), 3.14 (2H, m, CH_2NH), 1.44 [11H, m, CH_2CH, C(CH_3)$_3$], 1.27 (16H, br s, 8 × CH_2), 0.88 (6H, t, *J* = 6.6 Hz, 2 × CH_3). ^{13}C NMR: δ 156.5, 155.5, 79.2, 66.4, 50.0, 41.0, 31.7, 31.6, 29.9, 29.2, 29.1, 28.3, 27.9, 26.7, 22.6, 22.5, 14.0, 13.9. Anal. ($C_{20}H_{40}N_2O_4$) C, H, N.

Saponification of 2-Hydroxyamides Containing Ester Groups. To a stirred solution of a 2-hydroxyamide containing an ester group (2.00 mmol) in a mixture of dioxane−H_2O (9:1, 20 mL) was added 1 N NaOH (2.2 mL, 2.2 mmol), and the mixture was stirred for 12 h at room temperature. The organic solvent was evaporated under reduced pressure, and H_2O (10 mL) was added. The aqueous layer was washed with EtOAc, acidified with 1 N HCl, and extracted with EtOAc (3 × 12 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified after recrystallization [EtOAc−petroleum ether (bp 40−60 °C)].

(4*S*)-4-(2-Hydroxydodecanamido)-5-(octylcarbamoyloxy)pentanoic Acid (21a). Yield 69%; white solid; mp 96−98 °C. ^{1}H NMR: δ 7.13 (1H, m, CONH), 5.20 ($^2/_3$H, t, *J* = 7.0 Hz, OCONH), 5.16 ($^1/_3$H, t, *J* = 7.0 Hz, OCONH), 4.09 (4H, m, 2 × CH, CH_2OCONH), 3.12 (2H, m, CH_2NH), 2.40 (2H, m, CH_2COOH), 1.84 (4H, m, 2 × CH_2), 1.49−1.26 (28H, m, 14 × CH_2), 0.88 (6H, t, *J* = 6.6 Hz, 2 × CH_3). ^{13}C NMR: δ 176.6, 175.2, 156.4, 72.2, 48.4, 41.2, 34.6, 31.9, 31.8, 29.7, 29.3, 29.2, 26.8, 22.7, 22.6, 14.0. Anal. ($C_{26}H_{50}N_2O_6$) C, H, N.

(4*S*)-5-(Ethylcarbamoyloxy)-4-(2-hydroxydodecanamido)pentanoic Acid (21b). Yield 99%; white solid; mp 84−86 °C. ^{1}H NMR: δ 7.20 (1H, m, CONH), 5.31 (1/2H, t, *J* = 7.0 Hz, OCONH), 5.19 ($^1/_2$H, t, *J* = 7.0 Hz, OCONH), 4.08 (4H, m, 2 × CH, CH_2OCONH), 3.10 (2H, m, CH_2NH), 2.42 (2H, m, CH_2COOH), 1.83 (4H, m, 2 × CH_2), 1.60−1.25 (16H, m, 8 × CH_2), 1.18 (3H, t, *J* = 6.0 Hz, CH_3), 0.88 (3H, t, *J* = 6.0 Hz, CH_3). ^{13}C NMR: δ 176.8, 175.9, 156.7, 72.1, 66.1, 48.1, 35.9, 34.5, 31.9, 30.9, 30.4, 29.6, 26.2, 25.2, 22.6, 15.0, 14.1. Anal. ($C_{20}H_{38}N_2O_6$) C, H, N.

(4*R*)-5-(Ethylcarbamoyloxy)-4-(2-hydroxydodecanamido)pentanoic Acid (21c). Yield 99%; white solid; mp 74−76 °C. ^{1}H NMR: δ 7.22 (1H, m, CONH), 5.40 ($^2/_3$H, t, *J* = 7.0 Hz, OCONH), 5.22 ($^1/_3$H, t, *J* = 7.0 Hz, OCONH), 4.11 (4H, m, 2 × CH, CH_2OCONH), 3.18 (2H, m, CH_2NH), 2.40 (2H, m, CH_2COOH), 1.83 (4H, m, 2 × CH_2), 1.60−1.25 (16H, m, 8 × CH_2), 1.13 (3H, t, *J* = 7.0 Hz, CH_3), 0.88 (3H, t, *J* = 7.0 Hz, CH_3). ^{13}C NMR: δ 176.8, 175.9, 156.7, 72.1, 66.1, 48.1, 35.9, 34.5, 31.9, 30.9, 30.4, 29.6, 26.2, 25.2, 22.6, 15.0, 14.1.

Cleavage of the Benzyloxycarbonyl (Z) Protecting Group. To a solution of the compound (1 mmol) in EtOH (10 mL) catalyst 10% Pd/C was added, and the mixture was stirred overnight at room temperature under H_2 atmosphere. The catalyst was removed by filtration over Celite, and the organic solvent was evaporated under reduced pressure.

Oxidation of N-Protected Amino Alcohols to Aldehydes. The procedure described above for the oxidation of 2-hydroxyamides was followed except that the residue was used immediately in the next step without any purification.

Wittig−Horner−Emmons Reaction. A suspension of the aldehyde (2 mmol), triethyl 4-phosphonocrotonate (2.2 mmol), LiOH·H_2O (2.2 mmol), and activated molecular sieves (beads, 4−8 mesh, 1.5 g/mmol aldehyde) in THF (overall 0.1 M aldehyde) was stirred under a nitrogen atmosphere for 12 h at room temperature. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography [EtOAc−petroleum ether (bp 40−60 °C), 2:8].

(*S*,2*E*,4*E*)-Ethyl 6-(*tert*-Butoxycarbonyl)deca-2,4-dienoate (8). Yield 66%; colorless oil; $[\alpha]_D$ −5.2 (*c* 1.2, $CHCl_3$). ^{1}H NMR: δ 7.24 (1H, dd, J_1 = 15.4 Hz, J_2 = 11.0 Hz, CH=CHC*H*=CHCOO), 6.25 (1H, dd, J_1 = 15.4 Hz, J_2 = 11.0 Hz, CH=C*H*CH=CHCOO), 5.97 (1H, dd, J_1 = 15.4 Hz, J_2 = 6.2 Hz, C*H*=CHCH=CHCOO), 5.85 (1H, d, *J* = 15.4 Hz, CHCOO), 4.46 (1H, d, *J* = 8.0 Hz, NH), 4.23−4.06 (3H, m, OCH_2, C*H*NH), 1.56−1.17 [18H, m, CH_3, C(CH_3)$_3$, 3 × CH_2], 0.91 (3H, t, *J* = 6.0 Hz, CH_3). ^{13}C NMR: δ 167.0, 155.2, 144.0, 143.4, 127.6, 121.2, 79.6, 60.3, 52.3, 34.8, 28.3, 27.8, 22.4, 14.3, 14.0. Anal. ($C_{17}H_{29}NO_4$) C, H, N.

Wittig Reaction of N-Protected α-Aminoaldehydes with Nonstabilized Ylides. To a stirred suspension of the phosphonium salt $Br^-Ph_3P^+CH_2CH_2CH_3$ or $Br^-Ph_3P^+CH_2CH_2C_6H_5$ (2.40 mmol) in dry toluene (12 mL), a solution of 0.5 M potassium bis-(trimethylsilyl)amide (KHMDS) (4.8 mL, 2.40 mmol) in toluene was added dropwise over a period of 5 min at 0 °C under N_2. The bright-red solution was stirred for another 15 min and cooled to −78 °C, when the solution of the aldehyde in dry toluene (4 mL) was instantly added, and the temperature was left to rise from −78 °C to room temperature. The light-yellow mixture was stirred at room temperature for 20 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (20 mL) and extracted with Et_2O (3 × 6 mL). The combined organic phases were washed with brine

and dried over Na_2SO_4. The solvent was removed, and the residue was purified by column chromatography [EtOAc−petroleum ether (bp 40−60 °C), 1:9].

(*R,Z*)-*tert*-Butyl 1-Phenylhex-3-en-2-ylcarbamate (12). Yield 75%; white solid; mp 56−58 °C; $[\alpha]_D$ +2.2 (*c* 1, $CHCl_3$). ^1H NMR: δ 7.27 (5H, m, C_6H_5), 5.43 (1H, dt, J_1 = 10.6 Hz, J_2 = 7.2 Hz, $CHCH{=}CH_2$), 5.23 (1H, dd, J_1 = 10.6 Hz, J_2 = 9.6 Hz, $CHCH{=}CH_2$), 4.40−4.72 (2H, m, NH, CH), 2.95 (1H, dd, J_1 = 13.2 Hz, J_2 = 5.2 Hz, C_6H_5CHH), 2.72 (1H, dd, J_1 = 13.2 Hz, J_2 = 7.4 Hz, C_6H_5CHH), 2.10−1.80 (2H, m, CH_2CH_3), 1.46 [9H, s, $C(CH_3)_3$], 0.82 (3H, t, J = 7.8 Hz, CH_3). ^{13}C NMR: δ 154.7, 137.3, 133.9, 129.3, 128.1, 127.8, 125.9, 78.9, 48.9, 41.8, 28.0, 20.6, 13.5. Anal. ($C_{17}H_{25}NO_2$) C, H, N.

(*S,Z*)-*tert*-Butyl 1-Phenyloct-2-en-4-ylcarbamate (15). Yield 71%; white solid; $[\alpha]_D$ +1.9 (*c* 2.1, $CHCl_3$). ^1H NMR: δ 7.26 (5H, m, C_6H_5), 5.65 (1H, dt, J_1 = 11.0 Hz, J_2 = 7.4 Hz, $CH_2CH{=}CH$), 5.31 (1H, dd, J_1 = 11.0 Hz, J_2 = 10.0 Hz, $CH_2CH{=}CH$), 4.69−4.40 (2H, m, NH, CH), 3.53 (2H, m, $CH_2C_6H_5$), 1.61 (2H, m, CH_2), 1.45 [9H, s, $C(CH_3)_3$], 1.31 (4H, m, 2 × CH_2), 0.91 (3H, t, J = 6.4 Hz, CH_3). ^{13}C NMR: δ 154.9, 140.4, 131.6, 130.4, 128.4, 125.9, 79.1, 47.9, 35.8, 34.0, 28.4, 27.8, 22.5, 14.0. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

Oxidation of a Phenyl Group to a Carboxylic Group. To a solution of **13** or **16a**,**b** (1.00 mmol) in a mixture of EtOAc−CH_3CN−H_2O (1:1:8, 30 mL) were added $NaIO_4$ (6.2 g, 29.0 mmol) and $RuCl_3$ (10 mg, 0.05 mmol). After being stirred overnight at room temperature, the mixture was diluted with H_2O (25 mL) and EtOAc (50 mL). The organic layer was separated, washed with H_2O (25 mL), dried over Na_2SO_4, and filtered through a pad of silica gel 60 (230−400 mesh) and a short pad of Celite. The filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography using EtOAc as the eluent.

(*S*)-3-(2-Oxopentadecanamido)heptanoic Acid (14). Yield 61%; white solid; mp 81−82 °C; $[\alpha]_D$ −14.5 (*c* 0.4, $CHCl_3$). ^1H NMR: δ 7.31 (1H, d, J = 9 Hz, NHCOCO), 4.20 (1H, m, CH), 2.91 (2H, t, J = 7.8 Hz, CH_2COCO), 2.61 (2H, d, J = 5 Hz, CH_2COOH), 1.60 (4H, m, 2 × CH_2), 1.25 (24H, m, 12 × CH_2), 0.88 (6H, t, J = 6.8 Hz, 2 × CH_3). ^{13}C NMR: δ 199.2, 176.4, 159.7, 46.1, 38.2, 36.8, 33.5, 31.9, 29.6, 29.4, 29.3, 29.0, 28.1, 23.1, 22.7, 22.3, 14.1, 13.9. MS (FAB), m/z (%): 384 (100) $[M + H]^+$. Anal. ($C_{22}H_{41}NO_4$) C, H, N.

(*S*)-5-(2-Oxopentadecanamido)nonanoic Acid (17a). Yield 69%; white solid; mp 76−77 °C; $[\alpha]_D$ +0.9 (*c* 0.8, $CHCl_3$). ^1H NMR: δ 6.73 (1H, d, J = 9 Hz, NHCOCO), 3.87 (1H, m, CH), 2.92 (2H, t, J = 7.8 Hz, CH_2COCO), 2.35 (2H, m, CH_2COOH), 1.72−1.46 (8H, m, 4 × CH_2), 1.25 (24H, m, 12 × CH_2), 0.88 (6H, t, J = 6.8 Hz, 2 × CH_3). ^{13}C NMR: δ 199.6, 178.3, 159.9, 49.3, 36.8, 34.6, 34.2, 33.4, 31.9, 29.6, 29.4, 29.3, 29.0, 27.9, 23.2, 22.7, 22.5, 20.9, 14.1, 13.9. MS (FAB), m/z (%): 412 (100) $[M + H]^+$. Anal. ($C_{24}H_{45}NO_4$) C, H, N.

(*S*)-5-(2-Oxohexadecanamido)nonanoic Acid (17b). Yield 37%; white solid; mp 87−89 °C; $[\alpha]_D$ +0.8 (*c* 0.5, $CHCl_3$). ^1H NMR: δ 6.75 (1H, d, J = 9.6 Hz, NHCOCO), 3.87 (1H, m, CH), 2.92 (2H, t, J = 7.6 Hz, CH_2COCO), 2.37 (2H, m, CH_2COOH), 1.75−1.40 (8H, m, 4 × CH_2), 1.39−1.20 (26H, br s, 13×CH_2), 0.88 (6H, t, J = 6.6 Hz, 2 × CH_3). ^{13}C NMR: δ 199.6, 178.9, 159.9, 49.3, 36.8, 34.6, 34.2, 33.5, 31.9, 29.6, 29.5, 29.4, 29.3, 29.0, 27.9, 23.1, 22.7, 22.4, 21.0, 14.1, 13.9. MS (FAB), m/z (%): 426 (100) $[M + H]^+$. Anal. ($C_{25}H_{47}NO_4$) C, H, N.

Human Group V sPLA$_2$ Expression and Purification. The wild-type human GV sPLA$_2$ cDNA[70] was cloned into the pET-21a(+) vector using the NdeI and EcoRI restriction sites and transformed into BL21 (DE3) *E. coli*. The bacteria were streaked onto LB/ampicillin (100 μg/mL) plates and grown overnight at 37 °C. Single colonies were picked to innoculate two 25-mL LB/ampicillin (100 μg/mL) precultures, which were grown at 37 °C overnight. Each 25 mL preculture was used to innoculate 1 L of LB/ampicillin (100 μg/mL). Following 4 h of incubation at 37 °C, protein expression was induced by the addition of isopropyl β-D-thiogalactopyranoside to a concentration of 400 μM. Following an additional 3 h of incubation at 37 °C, the cultures were centrifuged at 1000*g* for 15 min at 4 °C, the media were decanted, and the cell pellet was stored at −20 °C.

GV sPLA$_2$ was purified in a manner analogous to that of the human group IIA sPLA$_2$.[71,72] The cells were thawed and resuspended in 100 mL of buffer containing 100 mM tris (hydroxymethyl)aminomethane HCl (Tris-HCl), pH 8.0, 5 mM ethylenediamine tetraacetic acid (EDTA), 50 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.4% (w/v) deoxycholic acid, and 0.4% (v/v) Triton X-100. Following 20 min of stirring at 4 °C, the suspension was disrupted by homogenization (Kinematica Polytron, Brinkmann Instruments) for 15 min on ice and then centrifuged at 12000*g* for 10 min at 4 °C. The pellet was collected and resuspended in 100 mL of buffer containing 100 mM Tris-HCl, pH 8.0, 5 mM EDTA, 50 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.8% w/v deoxycholic acid, and 0.8% v/v Triton X-100. After being stirred at 4 °C for 20 min, the suspension was again disrupted by homogenization for 15 min on ice and then centrifuged at 12000*g* for 10 min at 4 °C. The pellet was resuspended a third time in the same buffer but this time containing 1% v/v Triton X-100 and no deoxycholic acid. This suspension was stirred at room temperature for 20 min and then centrifuged at 12000*g* for 10 min at 4 °C, resulting in a pelleting of the bacterial inclusion bodies containing GV sPLA$_2$. The inclusion body pellet was washed with 50 mL of 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, and 5 M urea for 30 min at 4 °C and then collected by centrifugation at 12000*g* at 4 °C. The inclusion body pellet was solubilized by stirring overnight at 4 °C in 25 mL of 50 mM Tris-HCl, pH 8.5, 0.3 M sodium sulfite, and 8 M guanidine HCl. Insoluble material was discarded after centrifugation at 15000*g* for 30 min at 4 °C. To this clarified protein solution, 5 mL of 50 mM 2-nitro-5-(sulfothio)benzoate solution was added and stirred for 45 min at room temperature.

The sulfonated protein mixture was loaded onto a G25 Sephadex (Amersham Pharmacia) column (4.5 cm × 45 cm) equilibrated with 25 mM Tris-HCl, pH 8.0, 5 M urea, and 5 mM EDTA. The elution was monitored spectrophotometrically at 280 nm, and the first major protein peak was collected (30 mL). This sulfonated protein solution was then loaded onto a G75 Sephadex (Amersham Pharmacia) column (4.5 cm × 45 cm) pre-equilibrated with 25 mM Tris-HCl, pH 8.0, 5 M urea, and 5 mM EDTA. The elution was monitored spectrophotometrically at 280 nm. The fractions were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), and those containing GV sPLA$_2$ (the second major protein peak) were pooled (50 mL). GV sPLA$_2$ was precipitated after dialyzing against deionized water for 8 h and against 0.4% v/v glacial acetic acid overnight at 4 °C. The precipitated GV sPLA$_2$ was washed twice with 50 mL of deionized water and then resuspended in 5 mL of 50 mM Tris-HCl, pH 7.5, 5 M guanidine HCl, and 5 mM EDTA. This concentrated protein solution was then loaded onto a G75 Sephadex column (4.5 cm × 45 cm) pre-equilibrated with 50 mM Tris-HCl, pH 7.5, 5 M guanidine HCl, and 5 mM EDTA, and elution was monitored spectrophotometrically at 280 nm. The fractions were analyzed with SDS−PAGE, and those containing GV sPLA$_2$ (the major protein peak) were collected and pooled (40 mL).

To 10 mL of this sulfonated GV sPLA$_2$ protein solution, 10 mL of 50 mM Tris-HCl, pH 8.5, 8 mM reduced glutathione, 7 mM oxidized glutathione, and 10% glycerol buffer were added dropwise over 3 h with stirring. Stirring was continued for 20 h at room temperature. The GV sPLA$_2$ solution was dialyzed against 3 volumes of 4 L of 25 mM Tris-HCl, pH 8.5, 0.2 M guanidine HCl, and 10% glycerol at 4 °C. The purity of the refolded GV sPLA$_2$ was assessed by SDS−PAGE and judged to be higher than 90%.

Group IVA cPLA$_2$ Activity Assays. GIVA cPLA$_2$ activity was measured as described previously.[29,30,73] Pure, native human GIVA cPLA$_2$ was a generous gift from Dr. Ruth Kramer of Lilly Research Laboratories. In brief, the liberation of [^{14}C]-AA from phospholipid-detergent mixed micelles containing 1-palmitoyl-2-[^{14}C]arachidonoyl phosphatidylcholine (PAPC), phosphatidylinositol 4,5-bisphosphate (PIP$_2$), and Triton X-100 (97:3:400 μM, respectively) was quantitated. The various 2-oxoamides, dissolved at appropriate concentrations in DMSO, were added to the mixed micelle solution

to give a final DMSO concentration of 1% (v/v). The specific activity of GIVA $cPLA_2$ was calculated and compared to the control reaction where only DMSO was added. In addition to any 2-oxamide compounds, the final assay buffer contained 10 ng of GIVA $cPLA_2$, 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 80 μM $CaCl_2$, 0.1 mg/mL fatty acid-free bovine serum albumin, 2 mM dithiothreitol (DTT), 97 μM PAPC (100 000 cpm/assay), 3 μM PIP_2, 400 μM Triton X-100, and 1% (v/v) DMSO. The reaction was initiated with the addition of enzyme or buffer control, incubated at 40 °C for 10−60 min, and quenched, extracted, and anlyzed using the modified Dole assay.[74] Because there are no commercially available inhibitors of GIVA $cPLA_2$ that possess the same mode of inhibition as the 2-oxoamide inhibitors (fast, reversible), we assayed pyrrophenone, a well-studied inhibitor of GIVA $cPLA_2$,[75] received as a generous gift from Dr. Kohji Hanasaki (Shionogi Research Laboratories of Shionogi & Co., Ltd.). While an excellent tool, pyrrophenone is not itself being pursued as a drug at least in part because of its large size.[76] When the GIVA $cPLA_2$ assay conditions described above were used, pyrrophenone gave an $X_I(50)$ of 0.0005 ± 0.0003 mole fraction. In this specific in vitro assay, the most potent 2-oxoamide inhibitor of GIVA $cPLA_2$ is somewhat less potent with an $X_I(50)$ of 0.003 mole fraction,[19] and the most potent newly described 2-oxoamides have $X_I(50)$ values of 0.005 mole fraction (compounds **17a** and **17b**).

Group V $sPLA_2$ Activity Assays. GV $sPLA_2$ activity was measured in an assay similar to the assays for GIVA $cPLA_2$ and GVIA $iPLA_2$. Briefly, the reaction monitored the release of [^{14}C]-palmitic acid from phospholipid-detergent mixed micelles containing 1-palmitoyl-2-[^{14}C]palmitoyl phosphatidylcholine (DPPC) and Triton X-100 (1:4 ratio). Each assay was performed in 500 μL total volume made up of 100 μL of 5× substrate solution (20 μL of 10 mM Triton X-100 and 80 μL of assay buffer), 390 μL of assay buffer, 10 μL of GV $sPLA_2$ solution (1 μL of 40 ng/μL stock and 9 μL of assay buffer), and 5 μL of DMSO or 2-oxoamide in DMSO. The assay buffer was composed of 50 mM Tris-HCl (pH 8.0) and 5 mM $CaCl_2$. The 5× substrate solution was prepared by drying down the phospholipids (from organic solvent) with N_2. The appropriate volume of 10 mM Triton X-100 was added, heated, and vortexed until clear. Then assay buffer was added to make a 5× substrate solution. The final assay buffer contained 40 ng of GV $sPLA_2$, 50 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$, 100 μM DPPC (100 000 cpm/assay), 400 μM Triton X-100, and 1% (v/v) DMSO. The reaction was initiated and terminated as described for GIVA $cPLA_2$.

Group VIA $iPLA_2$ Activity Assays. Human GVIA $iPLA_2$ was expressed, purified, and assayed as described previously.[19] The assay is similar to that described for GV $sPLA_2$. The final assay buffer was composed of 190 ng of GVIA $iPLA_2$, 10 mM HEPES, pH 7.5, 5 mM EDTA, 2 mM DTT, 1 mM ATP, 100 μM DPPC, 400 μM Triton X-100, and 1% (v/v) DMSO. The final volume for each assay was 500 μL and contained 100 000 cpm of DPPC. The reaction was initiated and terminated as described for GIVA $cPLA_2$.

Rat-Paw Carrageenan-Induced Edema Assay. The anti-inflammatory activities of specific 2-oxoamides were determined using the rat-paw carrageenan-induced edema assay as described previously.[30,77] Briefly, the compounds were given intraperitoneally at the same time as carrageenan was given by intradermal injection. After 3.5 h the rats were euthanized. Each compound was tested at four different concentrations on two groups of five animals. The weight of the uninjected paw was subtracted from the weight of the injected paw for each animal. The change in paw weight for treated animals was compared to the control animals and expressed as percent inhibition of edema. Values for percent inhibition at each concentration were an average from two different experiments with a standard error of the mean that was less than 10%. Statistical significance of the results was established with the Student's *t*-test, $P < 0.001$. The ED_{50} value for each compound was determined using the values for percent inhibition of edema at the four concentrations tested. Indomethacin was administered at 0.01 mmol/kg as a standard comparative drug and gave 47% inhibition of edema.[30]

Analgesis Screen. The analgesic properties of specific 2-oxoamides were determined using the writhing reflex as described previously.[30,78] Briefly, the compounds were given intraperitoneally with a dose equivalent to the ED_{50} (derived from the edema assay) 30 min prior to intraperitoneal administration of acetic acid. The total number of writhes exhibited by each animal in the test group was compared to that of the vehicle-treated control group. The percent decrease in writhes was used as a measure of the analgesic activity of the compound. Sodium acetylsalicylate was administered intraperitoneally as a control drug and gave 93.4% analgesis at 1 mmol/kg.[30]

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Note Added in Proof. A paper by Lee et al. (*J. Med. Chem.* **2007**, *50*, 1380−1400) describing the discovery and characterization of ecopladib, an indole inhibitor of GIVA $cPLA_2$, appeared after this mancuscript was reviewed. The paper confirms that a 2-oxoamide (compound **35**) inhibits GIVA $cPLA_2$ with potency comparable to that of a previously published indole inhibitor of GIVA $cPLA_2$ that was the lead compound for their study. The potency of ecopladib in animal models of edema cannot be directly compared to the results reported herein because of the differential timing and modes of administration of the compounds in their studies, but the efficacy of ecopladib confirms that specific GIVA $cPLA_2$ inhibitors have significant promise as anti-inflammatory drugs.

Supporting Information Available: Elemental analysis results for the compounds and a table of the 2-oxoamides that do not inhibit GV $sPLA_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Six, D. A.; Dennis, E. A. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. *Biochim. Biophys. Acta* **2000**, *1488*, 1−19.
(2) Leslie, C. C. Regulation of the specific release of arachidonic acid by cytosolic phospholipase A2. *Prostaglandins, Leukotrienes, Essent. Fatty Acids* **2004**, *70*, 373−376.
(3) Schaloske, R. H.; Dennis, E. A. The phospholipase A(2) superfamily and its group numbering system. *Biochim. Biophys. Acta* **2006**, *1761*, 1246−1259.
(4) Pickard, R. T.; Chiou, X. G.; Strifler, B. A.; DeFelippis, M. R.; Hyslop, P. A.; Tebbe, A. L.; Yee, Y. K.; Reynolds, L. J.; Dennis, E. A.; Kramer, R. M.; Sharp, J. D. Identification of essential residues for the catalytic function of 85-kDa cytosolic phospholipase A_2. Probing the role of histidine, aspartic acid, cysteine, and arginine. *J. Biol. Chem.* **1996**, *271*, 19225−19231.
(5) Dessen, A.; Tang, J.; Schmidt, H.; Stahl, M.; Clark, J. D.; Seehra, J.; Somers, W. S. Crystal structure of human cytosolic phospholipase A2 reveals a novel topology and catalytic mechanism. *Cell* **1999**, *97*, 349−360.
(6) Bonventre, J. V.; Huang, Z.; Taheri, M. R.; O'Leary, E.; Li, E.; Moskowitz, M. A.; Sapirstein, A. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* **1997**, *390*, 622−625.
(7) Uozumi, N.; Kume, K.; Nagase, T.; Nakatani, N.; Ishii, S.; Tashiro, F.; Komagata, Y.; Maki, K.; Ikuta, K.; Ouchi, Y.; Miyazaki, J.; Shimizu, T. Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* **1997**, *390*, 618−622.
(8) Rubin, B. B.; Downey, G. P.; Koh, A.; Degousee, N.; Ghomashchi, F.; Nallan, L.; Stefanski, E.; Harkin, D. W.; Sun, C.; Smart, B. P.; Lindsay, T. F.; Cherepanov, V.; Vachon, E.; Kelvin, D.; Sadilek, M.; Brown, G. E.; Yaffe, M. B.; Plumb, J.; Grinstein, S.; Glogauer, M.; Gelb, M. H. Cytosolic phospholipase A2-alpha is necessary for platelet-activating factor biosynthesis, efficient neutrophil-mediated bacterial killing, and the innate immune response to pulmonary infection: cPLA2-alpha does not regulate neutrophil NADPH oxidase activity. *J. Biol. Chem.* **2005**, *280*, 7519−7529.

(9) Funk, C. D. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* **2001**, *294*, 1871−1875.
(10) Yaksh, T. L.; Kokotos, G.; Svensson, C. I.; Stephens, D.; Kokotos, C. G.; Fitzsimmons, B.; Hadjipavlou-Litina, D.; Hua, X. Y.; Dennis, E. A. Systemic and intrathecal effects of a novel series of phospholipase A2 inhibitors on hyperalgesia and spinal prostaglandin E2 release. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 466−475.
(11) Meyer, M. C.; Rastogi, P.; Beckett, C. S.; McHowat, J. Phospholipase A2 inhibitors as potential anti-inflammatory agents. *Curr. Pharm. Des.* **2005**, *11*, 1301−1312.
(12) Yedgar, S.; Cohen, Y.; Shoseyov, D. Control of phospholipase A(2) activities for the treatment of inflammatory conditions. *Biochim. Biophys. Acta* **2006**, *1761*, 1373−1382.
(13) Uozumi, N.; Shimizu, T. Roles for cytosolic phospholipase A2alpha as revealed by gene-targeted mice. *Prostaglandins Other Lipid Mediators* **2002**, *68−69*, 59−69.
(14) Ghosh, M.; Tucker, D. E.; Burchett, S. A.; Leslie, C. C. Properties of the group IV phospholipase A(2) family. *Prog. Lipid Res.* **2006**, *45*, 487−510.
(15) Ghomashchi, F.; Stewart, A.; Hefner, Y.; Ramanadham, S.; Turk, J.; Leslie, C. C.; Gelb, M. H. A pyrrolidine-based specific inhibitor of cytosolic phospholipase A(2)alpha blocks arachidonic acid release in a variety of mammalian cells. *Biochim. Biophys. Acta* **2001**, *1513*, 160−166.
(16) Bellido-Reyes, Y. A.; Akamatsu, H.; Kojima, K.; Arai, H.; Tanaka, H.; Sunamori, M. Cytosolic phospholipase A2 inhibition attenuates ischemia-reperfusion injury in an isolated rat lung model. *Transplantation* **2006**, *81*, 1700−1707.
(17) Malaviya, R.; Ansell, J.; Hall, L.; Fahmy, M.; Argentieri, R. L.; Olini, G. C., Jr.; Pereira, D. W.; Sur, R.; Cavender, D. Targeting cytosolic phospholipase A2 by arachidonyl trifluoromethyl ketone prevents chronic inflammation in mice. *Eur. J. Pharmacol.* **2006**, *539*, 195−204.
(18) Phillips, R. M.; Six, D. A.; Dennis, E. A.; Ghosh, P. In vivo phospholipase activity of the *Pseudomonas aeruginosa* cytotoxin ExoU and protection of mammalian cells with phospholipase A2 inhibitors. *J. Biol. Chem.* **2003**, *278*, 41326−41332.
(19) Stephens, D.; Barbayianni, E.; Constantinou-Kokotou, V.; Peristeraki, A.; Six, D. A.; Cooper, J.; Harkewicz, R.; Deems, R. A.; Dennis, E. A.; Kokotos, G. Differential inhibition of group IVA and group VIA phospholipases A(2) by 2-oxoamides. *J. Med. Chem.* **2006**, *49*, 2821−2828.
(20) Winstead, M. V.; Balsinde, J.; Dennis, E. A. Calcium-independent phospholipase A(2): structure and function. *Biochim. Biophys. Acta* **2000**, *1488*, 28−39.
(21) Balsinde, J.; Balboa, M. A. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A(2) in activated cells. *Cell. Signalling* **2005**, *17*, 1052−1062.
(22) Masuda, S.; Murakami, M.; Ishikawa, Y.; Ishii, T.; Kudo, I. Diverse cellular localizations of secretory phospholipase A2 enzymes in several human tissues. *Biochim. Biophys. Acta* **2005**, *1736*, 200−210.
(23) Wijewickrama, G. T.; Kim, J. H.; Kim, Y. J.; Abraham, A.; Oh, Y.; Ananthanarayanan, B.; Kwatia, M.; Ackerman, S. J.; Cho, W. Systematic evaluation of transcellular activities of secretory phospholipases A2. High activity of group V phospholipases A2 to induce eicosanoid biosynthesis in neighboring inflammatory cells. *J. Biol. Chem.* **2006**, *281*, 10935−10944.
(24) Singer, A. G.; Ghomashchi, F.; Le Calvez, C.; Bollinger, J.; Bezzine, S.; Rouault, M.; Sadilek, M.; Nguyen, E.; Lazdunski, M.; Lambeau, G.; Gelb, M. H. Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. *J. Biol. Chem.* **2002**, *277*, 48535−48549.
(25) Winget, J. M.; Pan, Y. H.; Bahnson, B. J. The interfacial binding surface of phospholipase A2s. *Biochim. Biophys. Acta* **2006**, *1761*, 1260−1269.
(26) Cho, W. Structure, function, and regulation of group V phospholipase A(2). *Biochim. Biophys. Acta* **2000**, *1488*, 48−58.
(27) Satake, Y.; Diaz, B. L.; Balestrieri, B.; Lam, B. K.; Kanaoka, Y.; Grusby, M. J.; Arm, J. P. Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. *J. Biol. Chem.* **2004**, *279*, 16488−16494.
(28) Shirai, Y.; Balsinde, J.; Dennis, E. A. Localization and functional interrelationships among cytosolic group IV, secreted group V, and Ca^{2+}-independent group VI phospholipase A2s in P388D1 macrophages using GFP/RFP constructs. *Biochim. Biophys. Acta* **2005**, *1735*, 119−129.
(29) Kokotos, G.; Kotsovolou, S.; Six, D. A.; Constantinou-Kokotou, V.; Beltzner, C. C.; Dennis, E. A. Novel 2-oxoamide inhibitors of human group IVA phospholipase A(2). *J. Med. Chem.* **2002**, *45*, 2891−2893.
(30) Kokotos, G.; Six, D. A.; Loukas, V.; Smith, T.; Constantinou-Kokotou, V.; Hadjipavlou-Litina, D.; Kotsovolou, S.; Chiou, A.; Beltzner, C. C.; Dennis, E. A. Inhibition of group IVA cytosolic phospholipase A2 by novel 2-oxoamides in vitro, in cells, and in vivo. *J. Med. Chem.* **2004**, *47*, 3615−3628.
(31) Constantinou-Kokotou, V.; Peristeraki, A.; Kokotos, C. G.; Six, D. A.; Dennis, E. A. Synthesis and activity of 2-oxoamides containing long chain beta-amino acids. *J. Pept. Sci.* **2005**, *11*, 431−435.
(32) Preliminary results from this paper were from the following: (a) Loukas, V.; Six, D. A.; Constantinou-Kokotou, V.; Moutevelis-Minakakis, P.; Dennis, E. A.; Kokotos, G. Synthesis of 2-Oxoamides Based on δ-Amino Acids and Study of Their Activity on Human GIVA Phospholipase A_2. In *Peptides 2004*, Proceedings of the 3rd International and 28th European Peptide Symposium, Prague, Czech Republic, September 5−10, 2004; Flegel, M., Fridkin, M., Gilon, C. Slaninova, J., Eds.; KENES International: Geneva, Switzerland, 2005; pp 615−616. (b) Kokotos, G.; Loukas, V.; Six, D. A.; Hadjipavlou-Litina, D.; Kotsovolou, S.; Constantinou-Kokotou, V.; Dennis, E. A. 2-Oxoamides Based on γ-Amino Acids Inhibit Human GIVA Phospholipase A_2 and Present in Vivo Anti-Inflammatory Activity. In *Peptides 2004*, Proceedings of the 3rd International and 28th European Peptide Symposium, Prague, Czech Republic, September 5−10, 2004; Flegel, M., Fridkin, M., Gilon, C., Slaninova, J., Eds.; KENES International: Geneva, Switzerland, 2005; pp 643−644. (c) Barbayianni, E.; Six, D. A.; Dennis, E. A.; Kokotos, G. 2-Oxoamides Based on Glutamic acid as Inhibitors of Human GIVA Phospholipase A_2. In *4th Hellenic Forum on Bioactive Peptides*; Cordopatis, P. A., Manessi-Zoupa, E., Pairas, G., Eds.; TYPORAMA: Patras, Greece, 2005; pp 247−251.
(33) Kokotos, G.; Constantinou-Kokotou, V.; Noula, C.; Nicolaou, A.; Gibbons, W. A. Synthesis of lipidic amino acid and dipeptide inhibitors of human platelet phospholipase A2. *Int. J. Pept. Protein Res.* **1996**, *48*, 160−166.
(34) Leanna, M. R.; Sowin, T. J.; Morton, H. E. Synthesis of alpha-amino and alpha-alkoxy aldehydes via oxoammonium oxidation. *Tetrahedron Lett.* **1992**, *33*, 5029−5032.
(35) Ma, Z.; Bobbitt, J. M. Organic oxoammonium salts: a new convenient method for the oxidation of alcohols to aldehydes and ketones. *J. Org. Chem.* **1991**, *56*, 6110−6114.
(36) Takacs, J. M.; Jaber, M. R.; Clement, F.; Walters, C. A useful procedure for the preparation of (*E*,*E*)-2,4-dienoates: lithium hydroxide-promoted dienylation by 4-phosphonocrotonate. *J. Org. Chem.* **1998**, *63*, 6757−6760.
(37) Kokotos, G.; Noula, C. Selective one-pot conversion of carboxylic acids into alcohols. *J. Org. Chem.* **1996**, *61*, 6994−6996.
(38) Kokotos, G. A convenient one-pot conversion of N-protected amino-acids and peptides into alcohols. *Synthesis* **1990**, 299−301.
(39) Nunez, M. T.; Martin, V. S. Efficient oxidation of phenyl groups to carboxylic-acids with ruthenium tetraoxide—a simple synthesis of (*R*)-gamma-caprolactone, the pheromone of trogoderma-granarium. *J. Org. Chem.* **1990**, *55*, 1928−1932.
(40) Moutevelis-Minakakis, P.; Sinanoglou, C.; Loukas, V.; Kokotos, G. Synthesis of non-natural amino acids based on the ruthenium-catalysed oxidation of a phenyl group to carboxylic acid. *Synthesis* **2005**, 933−938.
(41) Clark, J. D.; Lin, L. L.; Kriz, R. W.; Ramesha, C. S.; Sultzman, L. A.; Lin, A. Y.; Milona, N.; Knopf, J. L. A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. *Cell* **1991**, *65*, 1043−1051.
(42) Street, I. P.; Lin, H. K.; Laliberte, F.; Ghomashchi, F.; Wang, Z.; Perrier, H.; Tremblay, N. M.; Huang, Z.; Weech, P. K.; Gelb, M. H. Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A2. *Biochemistry* **1993**, *32*, 5935−5940.
(43) Ghomashchi, F.; Loo, R. W.; Balsinde, J.; Bartoli, F.; Apitz-Castro, R.; Clark, J. D.; Dennis, E. A.; Gelb, M. H. Trifluoromethyl ketones and fluoromethyl phosphonates as selective inhibitors of group IV and VI phospholipases A_2: structure−function studies with vesicle, micelle, and membrane assays. *Biochim. Biophys. Acta* **1999**, *1420*, 45−56.
(44) Leslie, C. C. Kinetic properties of a high molecular mass arachidonoyl-hydrolyzing phospholipase A2 that exhibits lysophospholipase activity. *J. Biol. Chem.* **1991**, *266*, 11366−11371.
(45) Reynolds, L.; Hughes, L.; Louis, A. I.; Kramer, R. A.; Dennis, E. A. Metal ion and salt effects on the phospholipase A_2, lysophospholipase, and transacylase activities of human cytosolic phospholipase A_2. *Biochim. Biophys. Acta* **1993**, *1167*, 272−280.
(46) Loo, R. W.; Conde-Frieboes, K.; Reynolds, L. J.; Dennis, E. A. Activation, inhibition, and regiospecificity of the lysophospholipase activity of the 85 kDa cytosolic group IV phospholipase A_2. *J. Biol. Chem.* **1997**, *272*, 19214−19219.

(47) Conde-Frieboes, K.; Reynolds, L. J.; Lio, Y.; Hale, M.; Wasserman, H. H.; Dennis, E. A. Activated ketones as inhibitors of intracellular Ca^{2+}-dependent and Ca^{2+}-independent phospholipase A_2. *J. Am. Chem. Soc.* **1996**, *118*, 5519−5525.

(48) Magrioti, V.; Kokotos, G. Synthetic inhibitors of group IVA and group VIA phospholipase A2. *Anti-Inflammatory Anti-Allergy Agents Med. Chem.* **2006**, *5*, 189−203.

(49) McKew, J. C.; Foley, M. A.; Thakker, P.; Behnke, M. L.; Lovering, F. E.; Sum, F. W.; Tam, S.; Wu, K.; Shen, M. W.; Zhang, W.; Gonzalez, M.; Liu, S.; Mahadevan, A.; Sard, H.; Khor, S. P.; Clark, J. D. Inhibition of cytosolic phospholipase A2alpha: hit to lead optimization. *J. Med. Chem.* **2006**, *49*, 135−158.

(50) Kokotos, G.; Verger, R.; Chiou, A. Synthesis of 2-oxo amide triacylglycerol analogues and study of their inhibition effect on pancreatic and gastric lipases. *Chem.*−*Eur. J.* **2000**, *6*, 4211−4217.

(51) Kotsovolou, S.; Chiou, A.; Verger, R.; Kokotos, G. Bis-2-oxo amide triacylglycerol analogues: a novel class of potent human gastric lipase inhibitors. *J. Org. Chem.* **2001**, *66*, 962−967.

(52) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. Fluoro ketone inhibitors of hydrolytic enzymes. *Biochemistry* **1985**, *24*, 1813−1817.

(53) White, S. P.; Scott, D. L.; Otwinowski, Z.; Gelb, M. H.; Sigler, P. B. Crystal structure of cobra-venom phospholipase A2 in a complex with a transition-state analogue. *Science* **1990**, *250*, 1560−1563.

(54) Reid, R. C. Inhibitors of secretory phospholipase A2 group IIA. *Curr. Med. Chem.* **2005**, *12*, 3011−3026.

(55) Smart, B. P.; Pan, Y. H.; Weeks, A. K.; Bollinger, J. G.; Bahnson, B. J.; Gelb, M. H. Inhibition of the complete set of mammalian secreted phospholipases A(2) by indole analogues: a structure-guided study. *Bioorg. Med. Chem.* **2004**, *12*, 1737−1749.

(56) Zeiher, B. G.; Steingrub, J.; Laterre, P. F.; Dmitrienko, A.; Fukiishi, Y.; Abraham, E. LY315920NA/S-5920, a selective inhibitor of group IIA secretory phospholipase A2, fails to improve clinical outcome for patients with severe sepsis. *Crit. Care Med.* **2005**, *33*, 1741−1748.

(57) Bowton, D. L.; Dmitrienko, A. A.; Israel, E.; Zeiher, B. G.; Sides, G. D. Impact of a soluble phospholipase A2 inhibitor on inhaled allergen challenge in subjects with asthma. *J. Asthma* **2005**, *42*, 65−71.

(58) Bradley, J. D.; Dmitrienko, A. A.; Kivitz, A. J.; Gluck, O. S.; Weaver, A. L.; Wiesenhutter, C.; Myers, S. L.; Sides, G. D. A randomized, double-blinded, placebo-controlled clinical trial of LY333013, a selective inhibitor of group II secretory phospholipase A2, in the treatment of rheumatoid arthritis. *J. Rheumatol.* **2005**, *32*, 417−423.

(59) OmPraba, G.; Velmurugan, D. Quantitative structure−activity relationship (QSAR) analysis of a series of indole analogues as inhibitor for human group V secretory phospholipase A2. *Indian J. Biochem. Biophys.* **2006**, *43*, 154−159.

(60) Smart, B. P.; Pan, Y. H.; Weeks, A. K.; Bollinger, J. G.; Bahnson, B. J.; Gelb, M. H. Inhibition of the complete set of mammalian secreted phospholipases A(2) by indole analogues: a structure-guided study. *Bioorg. Med. Chem.* **2004**, *12*, 1737−1749.

(61) Smart, B. P.; Oslund, R. C.; Walsh, L. A.; Gelb, M. H. The first potent inhibitor of mammalian group X secreted phospholipase A2: elucidation of sites for enhanced binding. *J. Med. Chem.* **2006**, *49*, 2858−2860.

(62) Yu, L.; Deems, R. A.; Hajdu, J.; Dennis, E. A. The interaction of phospholipase A_2 with phospholipid analogues and inhibitors. *J. Biol. Chem.* **1990**, *265*, 2657−2664.

(63) Yu, L.; Dennis, E. A. Defining the dimensions of the catalytic site of phospholipase A_2 using amide substrate analogues. *J. Am. Chem. Soc.* **1992**, *114*, 8757−8763.

(64) Sapirstein, A.; Bonventre, J. V. Specific physiological roles of cytosolic phospholipase A(2) as defined by gene knockouts. *Biochim. Biophys. Acta* **2000**, *1488*, 139−148.

(65) Serhan, C. N. Preventing injury from within, using selective cPLA2 inhibitors. *Nat. Immunol.* **2000**, *1*, 13−15.

(66) Cicero, A. F.; Derosa, G.; Gaddi, A. Combined lipoxygenase/cyclooxygenase inhibition in the elderly: the example of licofelone. *Drugs Aging* **2005**, *22*, 393−403.

(67) Martel-Pelletier, J.; Lajeunesse, D.; Reboul, P.; Pelletier, J. P. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Ann. Rheum. Dis.* **2003**, *62*, 501−509.

(68) Mitchell, J. A.; Warner, T. D. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. *Nat. Rev. Drug Discovery* **2006**, *5*, 75−86.

(69) Moreau, M.; Daminet, S.; Martel-Pelletier, J.; Fernandes, J.; Pelletier, J. P. Superiority of the gastroduodenal safety profile of licofelone over rofecoxib, a COX-2 selective inhibitor, in dogs. *J. Vet. Pharmacol. Ther.* **2005**, *28*, 81−86.

(70) Chen, Y.; Dennis, E. A. Expression and characterization of human group V phospholipase A_2. *Biochim. Biophys. Acta* **1998**, *1394*, 57−64.

(71) Othman, R.; Baker, S.; Li, Y.; Worrall, A. F.; Wilton, D. C. Human non-pancreatic (group II) secreted phospholipase A2 expressed from a synthetic gene in *Escherichia coli*: characterisation of N-terminal mutants. *Biochim. Biophys. Acta* **1996**, *1303*, 92−102.

(72) Baker, S. F.; Othman, R.; Wilton, D. C. Tryptophan-containing mutant of human (group IIa) secreted phospholipase A2 has a dramatically increased ability to hydrolyze phosphatidylcholine vesicles and cell membranes. *Biochemistry* **1998**, *37*, 13203−13211.

(73) Yang, H.-C.; Mosior, M.; Johnson, C. A.; Chen, Y.; Dennis, E. A. Group-specific assays that distinguish between the four major types of mammalian phospholipase A_2. *Anal. Biochem.* **1999**, *269*, 278−288.

(74) Reynolds, L. J.; Washburn, W. N.; Deems, R. A.; Dennis, E. A. Assay Strategies and Methods for Phospholipases. In *Methods in Enzymology*; Dennis, E. A., Ed.; Academic Press: Orlando, FL, 1991; Vol. 197, pp 3−23.

(75) Ono, T.; Yamada, K.; Chikazawa, Y.; Ueno, M.; Nakamoto, S.; Okuno, T.; Seno, K. Characterization of a novel inhibitor of cytosolic phospholipase A2alpha, pyrrophenone. *Biochem. J.* **2002**, *363*, 727−735.

(76) Seno, K.; Okuno, T.; Nishi, K.; Murakami, Y.; Yamada, K.; Nakamoto, S.; Ono, T. Pyrrolidine inhibitors of human cytosolic phospholipase A2. Part 2: synthesis of potent and crystallized 4-triphenylmethylthio derivative "pyrrophenone". *Bioorg. Med. Chem. Lett.* **2001**, *11*, 587−590.

(77) Kontogiorgis, C.; Hadjipavlou-Litina, D. Biological evaluation of several coumarin derivatives designed as possible anti-inflammatory/antioxidant agents. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 63−69.

(78) Hadjipavlou-Litina, D.; Rekka, E.; Hadjipetrou-Kourounakis, L.; Kourounakis, P. Synthesis and biological studies of some novel antiinflammatory aryl-hydroxy-amino-ketones. *Eur. J. Med. Chem.* **1991**, *26*, 85−90.

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