Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 634–641
Published online 16 July 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.889

JOURNAL OF PEPTIDE SCIENCE

Synthesis of lipophilic 2-oxoamides based on γ-aminobutyric and δ-aminovaleric analogues and their activity against phospholipase A_2

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Received 23 February 2007; Revised 11 May 2007; Accepted 18 May 2007

Abstract: A variety of lipophilic 2-oxoamides based on γ-aminobutyric and δ-aminovaleric analogues were synthesized. 2-oxoamides containing a tetrazole, a thioethyl or a thioacetyl group are weak inhibitors of GIVA cPLA$_2$, while derivatives containing a methyl tetrazole, a diethyl phosphonate or a thioethyl group are weak inhibitors of GV sPLA$_2$. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: inhibitors; 2-oxoamides; phospholipase A_2; phosphonates; tetrazoles

INTRODUCTION

The phospholipase A_2 (PLA$_2$) superfamily consists of a broad range of enzymes defined by their ability to catalyze the hydrolysis of the ester bond at the *sn*-2 position of membrane phospholipids, yielding free fatty acids, including arachidonic acid, and lysophospholipids [1–4]. In particular, the GIVA cPLA$_2$ has attracted much attention for drug discovery, since it is considered the main provider of arachidonic acid and lysophospholipids, which can be converted into prostaglandins, leukotrienes, and platelet activating factor, respectively [1–4]. A great variety of compounds have been studied for their activity against GIVA cPLA$_2$ and the synthetic inhibitors of GIVA cPLA$_2$ have been summarized in a recent review [5].

Recently, we have developed a novel class of GIVA cPLA$_2$ inhibitors designed to contain the 2-oxoamide functionality and a free carboxyl group [6,7]. The 2-oxoamide derivative based on γ-aminobutyric acid, AX006 (Figure 1) presented a strong inhibition activity (X_I (50) 0.017 mole fraction) [6]. We have also demonstrated that the ethyl ester derivative of AX006, compound AX048 (Figure 1), is the first systemically bioavailable compound with a significant affinity for GIVA cPLA$_2$, which produces potent antihyperalgesia [8]. AX048 inhibits not only GIVA cPLA$_2$ (X_I (50) 0.022 ± 0.009 mole fraction), but also calcium-independent GVIA iPLA$_2$ (X_I (50) 0.027 ± 0.009 mole fraction), which is the main other cytosolic PLA$_2$ isoform [8]. We also synthesized 2-oxoamides based on long-chain β-amino acids; however such derivatives were found inactive [9]. On the contrary, 2-oxoamides based on the unnatural amino acid δ-norleucine exhibit slightly higher activity than the corresponding analogue based on γ-norleucine [10].

The aim of this work was to synthesize analogues of γ-aminobutyric and δ-aminovaleric acid-containing groups isosteric to the carboxyl group or the carboxyl ester group, to incorporate them into a lipophilic 2-oxoamide backbone and to evaluate their activity on PLA$_2$.

RESULTS AND DISCUSSION

Bioisosterism is a useful approach for the design of more effective medicinal agents [11]. The design of the 2-oxoamide derivatives presented in this work was based on the replacement of the carboxyl or the carboxyl ester group of AX006 and AX048 by a tetrazole group, a phosphonate group and sulfur-containing groups (Figure 1). The tetrazole group is considered isosteric to the carboxyl group and there are several examples in medicinal chemistry, where the replacement of a carboxyl by tetrazole leads to products with improved biological properties [12,13]. The free phosphonate group, in addition to its acidic properties, may contribute to a decrease of the lipophilicity of

Abbreviations: AcNH-TEMPO, 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy free radical; DIAD, diisopropyl azodicarboxylate; GIVA cPLA$_2$, Group IVA phospholipase A_2; GVIA iPLA$_2$, Group VIA phospholipase A_2; GV sPLA$_2$, Group V phospholipase A_2; HOBt, 1-hydroxybenzotriazole; THF, tetrahydrofuran; TMSBr, trimethylsilyl bromide; WSCI, water soluble 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide.

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AX006. Apart from derivatives containing a free acidic group, *N*-alkylated tetrazole derivatives as well as phosphonate esters are of interest for comparison with AX048. In addition, we designed derivatives containing thioethyl or thioacetyl groups.

To synthesize phosphonate derivatives, two routes were followed. As depicted in Figure 2, the commercially available diethyl (2-cyanoethyl)phosphonate (**1**) was reduced by $NaBH_4$ in the presence of $NiCl_2.6H_2O$ [14]. The amino component **2** was coupled with 2-hydroxy-hexadecanoic acid using *N*-ethyl-*N*′-dimethylaminopropylcarbodiimide (WSCI) [15] in the presence of HOBt and oxidized to the target 2-oxoamide **4** by oxidation with NaOCl in the presence of a catalytic amount of AcNH-TEMPO [16]. The synthesis of derivative **10**, containing one additional carbon atom, started from benzyloxycarbonyl-γ-aminobutyric acid, which was reduced to alcohol [17] and converted to the corresponding bromide (Figure 3). Following a Michaelis–Arbuzov reaction [18] by refluxing with a five-fold excess of triethyl phosphite [19], bromide **6** produced the amino phosphonate derivative **7**. The target compound **9** was synthesized by deprotection of compound **7**, followed by coupling with 2-hydroxyhexadecanoic acid and oxidation. Ethyl ester groups were removed from compound **9** to produce the free acid derivative **10** after treatment with TMSBr [20].

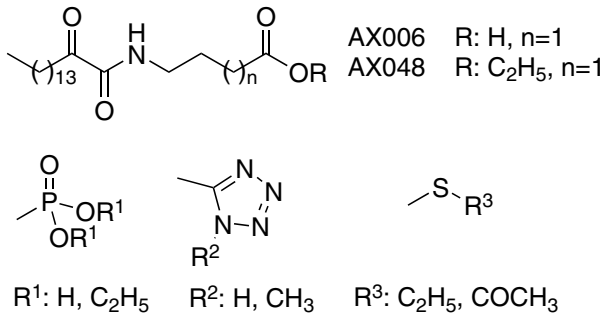


Figure 1 Structures of 2-oxoamide inhibitors of GIVA $cPLA_2$ and groups isosteric to carboxyl or carboxyl ester used for the synthesis of γ-aminobutyric ($n = 1$) and δ-aminovaleric ($n = 2$) acid analogues in the present work.

In a previous communication, we reported a general route for the synthesis of tetrazole analogues of γ- and δ-amino acids [21]. According to that method, amino tetrazoles **12**, **13** and **14** were prepared starting from benzyloxycarbonyl-β-alanine (**11**) (Figure 4). Compounds **12**–**14** were coupled with 2-hydroxyhexadecanoic acid and oxidized to target compounds **18**–**20** using the Dess–Martin reagent [22]. ^{13}C-NMR spectra of the constitutional isomeric compounds **19** and **20** revealed the expected difference for the C of the tetrazole moiety in accordance with the literature [21].

4-(*tert*-Butoxycarbonylamino)butanol (**21**), obtained by reduction of the corresponding acid [17], was converted to the acetylthio derivative by using the Mitsunobu reaction [23] with DIAD, triphenylphosphine and thioacetic acid in THF at 0 °C under argon [24] (Figure 5). After deprotection, compound **22** was coupled with 2-hydroxyhexadecanoic acid under conditions already described and oxidized to yield the target compound **24** using the Dess–Martin reagent.

In addition, the hydroxyl group of alcohol **21** was activated by mesylation and was replaced by sodium thioethoxide [25] to give hydrochloride **26** after acidic deprotection of Boc group. The target 2-oxoamide **28** was prepared following a reaction similar to those described above.

Compounds **4**, **10**, **18**, **19**, **20**, **24** and **28** were tested for their ability to inhibit human GIVA $cPLA_2$ in a GIVA $cPLA_2$-specific assay, which uses mixed micelles of substrate 1-palmitoyl-2-arachidonyl phosphatidylcholine, phosphatidylinositol 4,5-bisphosphate and detergent Triton X-100 (97 : 3 : 400 μM), as previously described [6,7]. In addition, their activity against GVIA $iPLA_2$ and the secreted PLA_2 isoform GV $sPLA_2$ was determined. The *in vitro* assay system for GVIA $iPLA_2$ has been previously described [26], while details on the assay for GV $sPLA_2$ will be published elsewhere (paper in preparation). The inhibition results obtained at a 0.091-mole fraction are summarized in Table 1. The tetrazole derivative **18** and the thio-containing derivatives **24** and **28** presented the highest activity against GIVA $cPLA_2$, but proved to be very weak inhibitors (56,

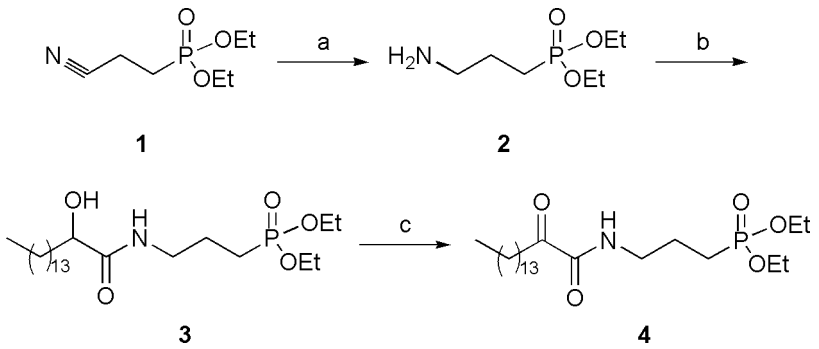
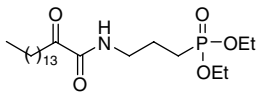
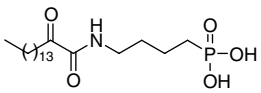
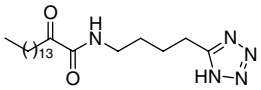
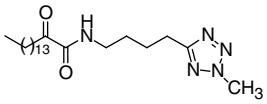
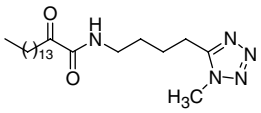
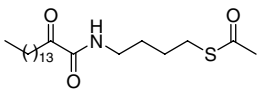
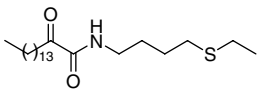
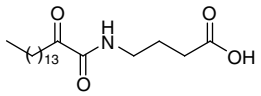
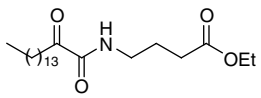


Figure 2 (a) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, 0 °C, then 30 min at room temperature (rt): (b) $CH_3(CH_2)_{13}CHOHCO_2H$, Et_3N, WSCl, HOBt, CH_2Cl_2, 1 h at 0 °C, then 24 h at rt; (c) NaOCl, AcNH-TEMPO, NaBr, $NaHCO_3$, CH_2Cl_2, 0 °C.

DOI: 10.1002/psc

Table 1 Effect of 2-oxoamide derivatives on GIVA cPLA$_2$, GVIA iPLA$_2$ and GV sPLA$_2$

Compound	Structure	Inhibition (% at 0.009 molar fraction)		
		GIVA cPLA$_2$	GVIA iPLA$_2$	GV sPLA$_2$
4		32	41	42
10		27	ND[a]	ND[a]
18		56	ND[a]	ND[a]
19		ND[a]	ND[a]	48
20		ND[a]	ND[a]	39
24		49	—	27
28		51	—	46
AX006		$X_I(50)$ 0.024 ± 0.015[b]	ND[a]	—
AX048		$X_I(50)$ 0.022 ± 0.009[b]	$X_I(50)$ 0.027 ± 0.009[b]	—

[a] Negligible inhibition (0–25%).
[b] $X_I(50)$ is the surface concentration of the inhibitor at which there is 50% inhibition.
Data taken from Ref. 8.

49 and 51% at 0.091 molar fraction, respectively). In accordance with our recent results [26], compounds **18** and **10**, containing a free acidic group, selectively inhibit GIVA cPLA$_2$, not affecting either GVIA iPLA$_2$ or GV sPLA$_2$ activity.

Interestingly, 2-oxoamide derivatives **4**, **19**, **20**, **24** and **28** inhibit weakly GV sPLA$_2$ (Table 1). In general, the 2-oxoamide derivatives have been designed and are inhibitors of GIVA cPLA$_2$, which uses a serine residue in the catalytic mechanism. GVIA iPLA$_2$ shares a common catalytic mechanism with GIVA cPLA$_2$, and therefore inhibition of GVIA iPLA$_2$ by 2-oxoamides may be expected. However, inhibition of GV sPLA$_2$ is not expected because the mechanism of the catalytic action of GV sPLA$_2$ is based on histidine instead of serine. It seems that lipophilic 2-oxoamides resemble phospholipids in their structure, and as a result may weakly inhibit GV sPLA$_2$.

In conclusion, this work describes the synthesis of lipophilic 2-oxoamides based on amino phosphonates,

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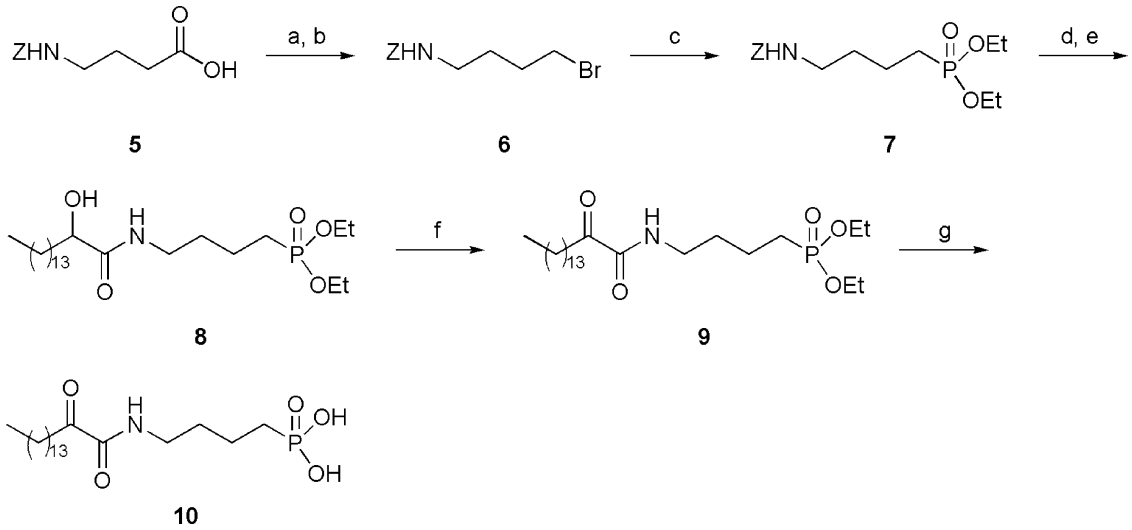

Figure 3 (a) (i) NMM, $ClCO_2Et$, THF, (ii) $NaBH_4$, MeOH; (b) PBr_3, THF, −10 °C, then rt; (c) $P(OEt)_3$, reflux; (d) H_2, Pd/C, EtOH, rt; (e) $CH_3(CH_2)_{13}CHOHCO_2H$, Et_3N, WSCl, HOBt, CH_2Cl_2, 1 h 0 °C, then 24 h rt; (f) NaOCl, AcNH-TEMPO, NaBr, $NaHCO_3$, CH_2Cl_2, 0 °C; (g) TMSBr, CH_2Cl_2, rt.

amino tetrazoles, thioacetate and thioethyl compounds. 2-Oxoamides containing a tetrazole or a thioethyl or a thioacetyl group are weak inhibitors of GIVA $cPLA_2$.

MATERIALS AND METHODS

Melting points are uncorrected. NMR spectra were recorded on a 200-MHz spectrometer. 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 and/or permanganate. THF was dried by standard procedures and stored over molecular sieves. DMF and CH_2Cl_2 were stored over molecular sieves. All other solvents and chemicals were reagent grade and used without further purification. Fast atom bombardment (FAB) mass spectra were recorded using a VG analytical ZAB-SE instrument. Electron spray ionization (ESI) mass spectra were recorded in a Finnigan, Surveyor MSQ plus spectrometer.

General Procedure for the Coupling Reaction of 2-Hydroxy-hexadecanoic Acid with Amino Components

To a stirred solution of 2-hydroxy-hexadecanoic acid (1 mmol) and the amino component (1 mmol), free or as hydrochloride, in CH_2Cl_2 (5 ml), Et_3N (1.1or 2.2 mmol in case of hydrochloride), WSCI (1.1 mmol) and HOBt (1 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at rt. The solvent was evaporated under reduced pressure and the residue was purified by SiO_2 column chromatography, using a mixture of $CHCl_3$: MeOH (9 : 1) as eluent. In case of compounds **15**, **17** and **23**, the isolated coupling products were used to the next oxidation step without purification.

Diethyl 3-((2-hydroxyhexadecanoyl)amino)propylphosphonate (3). Yield: 49%; oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80–0.85 (t, $J = 6.6$ Hz, 3H, CH_3), 1.03–1.20 (m, 30H, 12 × CH_2, 2 × OCH_2CH_3), 1.78–1.22 (m, 6H, 3 × CH_2), 3.28 (m, 2H, $NHCH_2$), 4.09–3.94 (m, 5H, 2 × OCH_2, CHOH), 7.13 (t, $J = 7.0$ Hz,1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 14.0, 16.2, 16.3, 21.3, 22.5, 25.1, 29.2, 29.4, 29.6, 31.8, 34.7, 38.7, 39.0, 61.7, 61.6, 71.9, 175.0. Anal. calcd for $C_{23}H_{48}NO_5P$: C, 61.44; H, 10.76; N, 3.12. Found: C, 61.40; H, 10.72; N, 3.09.

Diethyl 4-((2-hydroxyhexadecanoyl)amino)butylphosphonate (8). Yield 50%; oil; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.76–0.83 (t, $J = 6.6$ Hz, 3H, CH_3), 1.10–1.40 (m, 30H, 12 × CH_2, 2 × OCH_2CH_3), 1.40–1.82 (m, 8H, 4 × CH_2), 3.25 (m, 2H, $NHCH_2$), 3.55 (m, 2H, CH, OH), 3.97–4.15 (m, 4H, 2 × OCH_2), 6.95 (t, $J = 7.0$ Hz, 1H, NHCO). ^{13}C NMR (50 MHz, $CDCl_3$): 13.9, 16.2, 16.3, 19.5, 19.6, 22.6, 23.4, 25.0, 26.2, 29.2, 29.4, 29.5, 29.9, 30.2, 31.8, 34.8, 38.1, 61.4, 61.6, 71.7, 174.9. ^{31}P NMR (81 MHz, $CDCl_3$): δ 32.9. Anal. calcd for $C_{24}H_{50}NO_5P$: C, 62.17; H, 10.87; N, 3.02. Found: C, 61.98; H, 10.69; N, 3.17.

2-Hydroxy-N-(4-(2-methyl-2H-1,2,3,4-tetraazol-5-yl)butyl) hexadecanamide (16). Yield 43%; white solid; mp 107–109 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, $J = 6.4$ Hz, 3H, CH_3), 1.23 (s, 24H, 12 × CH_2), 1.54–1.89 (m, 6H, 3 × CH_2), 2.89 (t, $J = 7.4$ Hz, CH_2), 3.34 (m, 2H, CH_2NH), 4.07 (m, 1H, CHOH), 4.28 (s, 3H, CH_3N), 6.94 (t, 1H, $J = 5.4$ Hz, CONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 22.9, 25.1, 25.3, 25.4, 29.1, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 32.1, 35.1, 38.9, 39.4, 39.5, 72.4, 166.7, 174.3. Anal. calcd for $C_{22}H_{43}N_5O_2$: C, 64.51; H, 10.58; N, 17.10. Found: C, 64.48; H, 10.62; N, 16.98.

N-(4-(Ethylsulfanyl)butyl)-2-hydroxyhexadecanamide (27). Yield 55%; white amorphous solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, $J = 6.2$ Hz, 3H, CH_3), 1.27 (m, 27H, 12 × CH_2, CH_3CH_2S), 1.69 (m, 6H, 3 × CH_2), 2.53 (m, 4H, 2 × CH_2), 3.28 (m, 2H, CH_2NH), 4.07 (m, 1H, CHOH), 6.54 (m, 1H, CONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 14.9, 22.9, 25.2, 26.2, 27.01, 29.0, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 31.4, 32.2, 35.2, 38.8, 72.4, 174.0. Anal. calcd for $C_{22}H_{45}NO_2S$: C, 68.16; H, 11.70; N, 3.61. Found: C, 68.34; H, 11.58; N, 3.85

DOI: 10.1002/psc

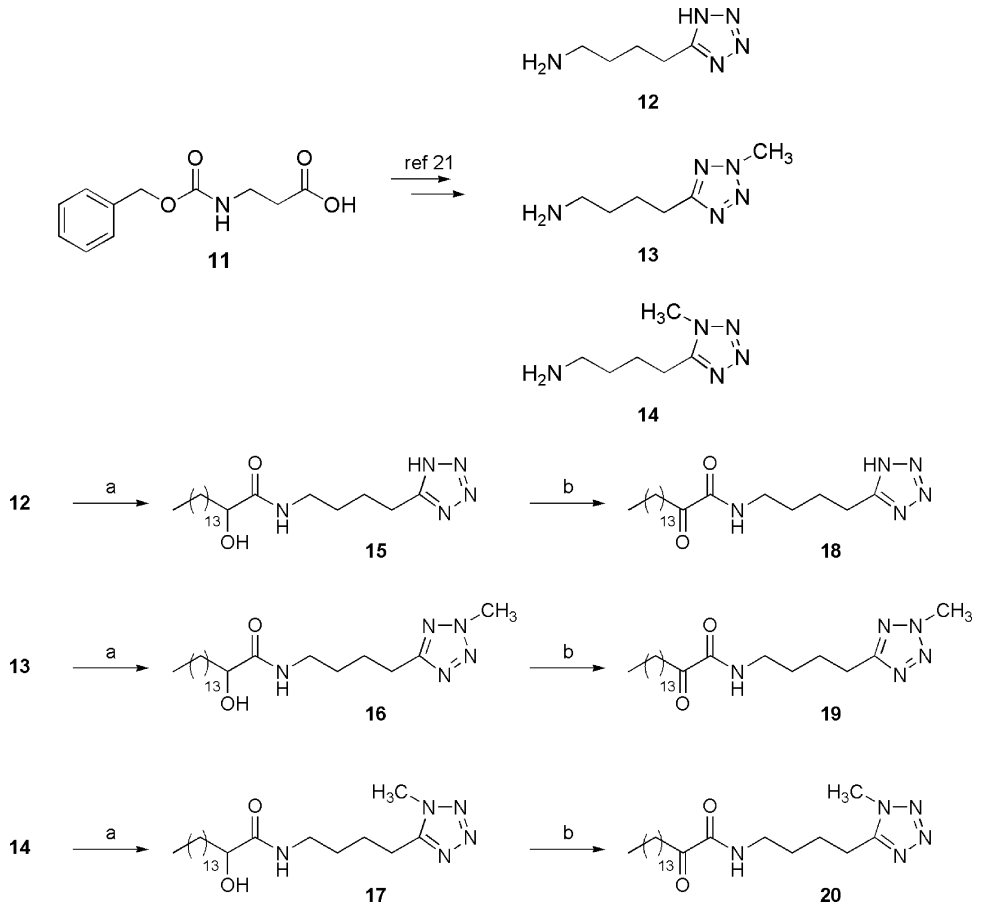

Figure 4 (a) $CH_3(CH_2)_{13}CHOHCO_2H$, Et_3N, WSCl, HOBt, CH_2Cl_2, 1 h, 0 °C then 24 h rt; (b) Dess–Martin reagent, CH_2Cl_2, 30 min.

General Procedures for the Oxidation of 2-Hydroxyamides to 2-Oxoamides

Method A. To a solution of 2-hydroxyamide (1 mmol) in CH_2Cl_2 (3 ml), a 2 M aqueous solution of NaBr (0.25 ml) was added, followed by AcNH-TEMPO (catalytic amount). To the resulting biphasic system, which was cooled to 0 °C, a 0.35 M aqueous solution of NaOCl (1.6 ml), containing $NaHCO_3$ (125 mg, 1.49 mmol) was added dropwise under vigorous stirring at 0 °C over 1 h. After the mixture had been stirred further for 15 min at 0 °C, EtOAc and H_2O were added and the aqueous layer was separated and washed twice with EtOAc. The combined organic layers were washed consecutively with 5% aqueous citric acid (6 ml) containing KI (20 mg), 10% aqueous $Na_2S_2O_3$ (6 ml) and brine. After drying with Na_2SO_4, the organic solvent was evaporated under reduced pressure, and the residue was purified by column chromatography using EtOAc as eluent.

Method B. To a solution of Dess–Martin periodinane (1.1 mmol) in dry CH_2Cl_2 (3 ml), a solution of 2-hydroxyamide (1 mmol) in dry CH_2Cl_2 (1 ml) was added and the mixture was stirred for 20–40 min at rt. Upon completion, Et_2O (6 ml) and a 5% aqueous solution of $NaHCO_3$ (6 ml) containing 1.5 g $Na_2S_2O_3$ were added under stirring until the solution became clear (5–10 min required). Then, Et_2O (5 ml) was added and the separated organic layer was washed by 5% aqueous $NaHCO_3$, followed by brine. After drying with Na_2SO_4, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography.

Diethyl 3-((2-oxohexadecanoyl)amino)propylphosphonate (4). Method A. Yield 90%; white solid; mp 44–46 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80–0.85 (t, 3H, CH_3), 1.20–1.90 (m, 28H, 11 × CH_2, 2 × OCH_2CH_3), 1.42–1.89 (m, 6H, 3 × CH_2), 1.82 (t, $J = 7.0$, Hz 2H, CH_2CO), 3.38 (m, 2H, $NHCH_2$), 3.98–4.19 (m, 4H, 2 × OCH_2), 7.38 (b, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.8, 16.1, 16.2, 21.4, 22.1. 22.2, 22.4, 22.9, 24.7, 28.8, 29.0, 29.1, 29.3, 31.6, 36.5, 39.0, 39.3, 61.3, 61.4, 160.2, 198.8. ^{31}P NMR (81 MHz, $CDCl_3$): δ 32.9. MS (ESI): $m/z = 447$ (100%) $[M]^+$. Anal. calcd for $C_{23}H_{46}NO_5P$: C, 61.72; H, 10.36; N, 3. 13. Found: C, 61.45; H, 10.52; N, 3.28

Diethyl 4-((2-oxohexadecanoyl)amino)butylphosphonate (9). Method A. Yield 81%; white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.69–0.75 (t, $J = 6.6$ Hz, 3H, CH_3), 1.10–1.21 (m, 30H, 2 × OCH_2CH_3, 12 × CH_2), 1.40–1.75 (m, 6H, 3 × CH_2), 2.72–2.79 (t, $J = 7.2$ Hz, 2H, CH_2COCO), 3.17 (m, 2H, $NHCH_2$), 3.87–4.01 (m, 4H, 2 × OCH_2), 7.27 (t, $J = 7.0$ Hz, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$): 13.8, 16.0, 16.2, 19.4, 19.5, 22.3, 22.8, 23.3, 26.1, 28.7, 29.0, 29.1, 29.2, 29.3, 29.7, 31.6, 36.4, 38.3, 61.1, 61.2, 160.1, 198.9. ^{31}P NMR (81 MHz, $CDCl_3$): δ 32.6. Anal. calcd for $C_{24}H_{48}NO_5P$: C, 62.45; H, 10.48; N, 3. 03. Found: C, 62.63; H, 10.50; N, 3.25

2-Oxo-N-(4-(1H-1,2,3,4-tetraazol-5-yl)butyl)hexadecan amide (18). Method B. Eluent system CH_2Cl_2: EtOH 95 : 5 then 9 : 1. Yield 50%; white solid; mp 115–118 °C. ^{1}H NMR (200 MHz, $CDCl_3/CD_3OD$ 2.5/1): δ 0.82 (t, $J = 6.2$ Hz, 3H,

DOI: 10.1002/psc

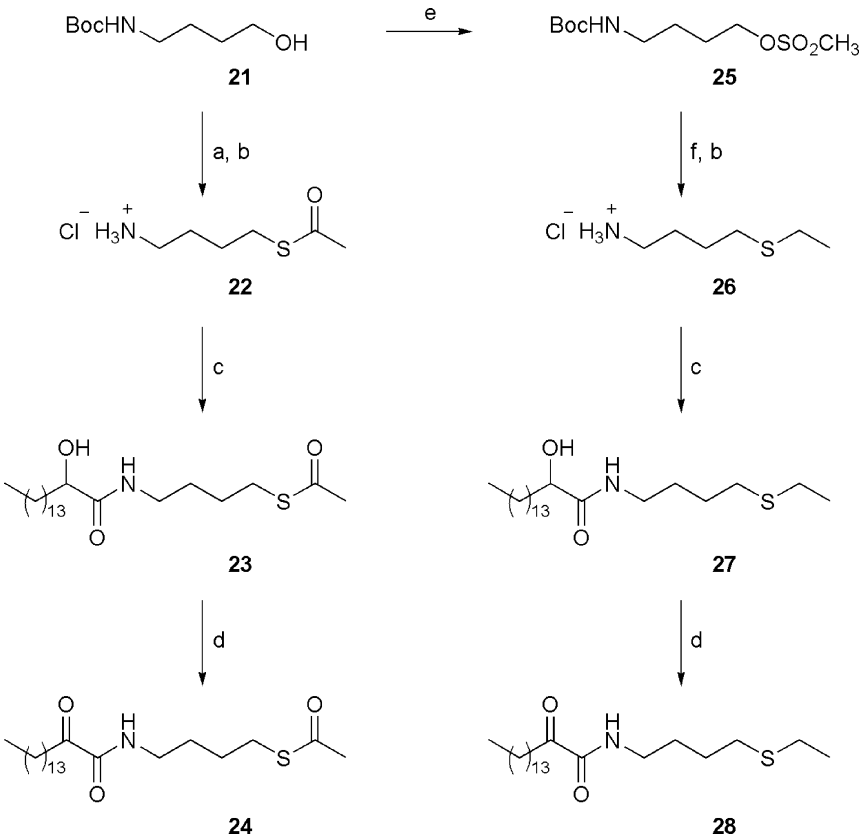

Figure 5 (a) Ph_3P, THF, DIAD, AcSH, 0 °C for 2 h, then rt overnight; (b) 4N HCl/dioxane; (c) $CH_3(CH_2)_{13}CHOHCO_2H$, Et_3N, WSCl, HOBt, CH_2Cl_2; 1 h, 0 °C then 24 h, rt; (d) Dess–Martin reagent, CH_2Cl_2, 30 min; (e) Et_3N, $MeSO_2Cl$, CH_2Cl_2, 0 °C for 30 min, then rt for another 30 min; (f) EtSNa, DMF, rt.

CH_3), 1.2 (s, 22H, $11 \times CH_2$), 1.5–1.8 (m, 6H, $3 \times CH_2$), 2.86 (m, 4H, $2 \times CH_2$), 3.25 (m, 2H, CH_2NH), 7.25 (bs, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3/CD_3OD$ 2.5/1): δ 14.2, 22.9, 23.0, 23.4, 24.8, 28.6, 29.3, 29.6, 29.7, 29.8, 29.9, 30.0, 32.2, 37.2, 38.8, 161.41, 161.42, 199.2. MS (FAB): $m/z = 395$ (100%) $[M + H]^+$, 273 (39%). Anal. calcd for $C_{21}H_{39}N_5O_2$: C, 64.09; H, 9.99; N, 17.79. Found: C, 64.27; H, 9.75; N, 17.52.

***N*-(4-(2-Methyl-2H-1,2,3,4-tetraazol-5-yl)butyl)-2-oxohexadecanamide (19).** Method B. Eluent system AcOEt : petroleum ether 1 : 1. Yield 65%; white solid; mp 79–81 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, $J = 6.2$ Hz, 3H, CH_3), 1.59 (s, 22H, $11 \times CH_2$), 1.62–1.86 (m, 6H, $3 \times CH_2$), 2.9 (m, 4H, $2 \times CH_2$), 3.32 (m, 2H, CH_2NH), 4.3 (s, 3H, CH_3N), 7.25 (bs, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 22.9, 23.4, 25.1, 25.4, 28.8, 29.3, 29.6, 29.7, 29.8, 29.9, 30.0, 32.1, 36.9, 39.1, 39.5, 160.4, 166.5, 199.6. MS (FAB): $m/z = 408$ (100%) $[M + H]^+$. Anal. calcd for $C_{22}H_{41}N_5O_2$: C, 64.83; H, 10.14; N, 17.18. Found: C, 64.75; H, 9.98; N, 17.25.

***N*-(4-(1-Methyl-1H-1,2,3,4-tetraazol-5-yl)butyl)-2-oxohexadecanamide (20).** Method B. Eluent system AcOEt. Yield 60%; white solid; mp 110–113 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, $J = 6.4$ Hz, 3H, CH_3), 1.23 (s, 22H, $11 \times CH_2$), 1.57–1.9 (m, 6H, $3 \times CH_2$), 2.87 (m, 4H, $2 \times CH_2$), 3.34 (m, 2H, CH_2NH), 3.99 (s, 3H, CH_3N), 7.25 (bs, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 22.7, 22.9, 23.4, 24.1, 28.9, 29.2, 29.6, 29.7, 29.8, 29.9, 32.1, 33.5, 37.0, 38.7, 158.1, 160.6, 199.4. MS (FAB): $m/z = 408$ (100%) $[M + H]^+$. Anal. calcd for $C_{22}H_{41}N_5O_2$: C, 64.83; H, 10.14; N, 17.18. Found: C, 64.68; H, 10.25; N, 17.12.

***S*-{4-((2-Oxohexadecanoyl)amino)butyl}ethanethioate (24).** Method B. Eluent system AcOEt: petroleum ether 1 : 1. Yield 65%; white solid; mp 67–69 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, $J = 6.2$ Hz, 3H, CH_3), 1.24 (s, 22H, $11 \times CH_2$), 1.59 (m, 6H, $3 \times CH_2$), 2.32 (s, 3H, CH_3COS), 2.87 (m, 4H, $2 \times CH_2$), 3.29 (m, 2H, CH_2NH), 6.99 (bs, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 22.9, 23.4, 27.2, 28.5, 28.7, 29.3, 29.6, 29.7, 29.8, 29.9, 30.9, 32.1, 36.9, 38.9, 160.4, 196.0, 199.6. MS (ESI): $m/z = 400$ (92%) $[M + H]^+$, 422 (63%) $[M + Na]^+$. Anal. calcd for $C_{22}H_{41}NO_3S$: C, 66.12; H, 10.34; N, 3.50. Found: C, 66.28; H, 10.29; N, 3.66.

***N*-(4-(Ethylsulfanyl)butyl)-2-oxohexadecanamide (28).** Method B. Eluent system CH_2Cl_2: EtOH 95 : 5. Yield 40%; white solid; mp 61–62 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, $J = 6.2$ Hz, 3H, CH_3), 1.24 (m, 25H, $11 \times CH_2$, CH_3CH_2S), 1.62 (m, 6H, $3 \times CH_2$), 2.52 (m, 4H, $2 \times CH_2$), 2.9 (m, 2H, CH_2), 3.31 (m, 2H, CH_2NH), 6.98 (bs, 1H, COCONH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.1, 14.8, 22.7, 23.2, 25.9, 26.7, 28.4, 29.1, 29.3, 29.4, 29.6, 29.7, 31.1, 31.9, 36.7, 38.9, 160.2, 199.4. MS (ESI): $m/z = 384$ (100%) $[M - H]^-$. Anal. calcd for $C_{22}H_{43}NO_2S$: C, 68.52; H, 11.24; N, 3.63. Found: C, 68.70; H, 11.19; N, 3.45.

Benzyl N-(4-bromobutyl)carbamate (6). The alcohol (645 mg, 2.89 mmol) derived from *N*-benzyloxycarbonyl-γ-aminobutyric acid [17] was dissolved in dry THF (5 ml) and

DOI: 10.1002/psc

the mixture was cooled at −10 °C. PBr_3 (391 mg, 1.45 mmol) was added and the reaction mixture was stirred for 3 h. Then, another portion of PBr_3 (391 mg, 1.45 mmol) was added at −10 °C. After stirring overnight at rt, water was added (5 ml) dropwise and the product was extracted with EtOAc (4 × 5 ml). After drying with Na_2SO_4, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography using EtOAc : petroleum ether (1 : 1) as eluent. Yield 40%; colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.45–1.98 (m, 4H, 2 × CH_2), 3.10–3.19 (m, 2H, CH_2), 3.37–3.31 (t, $J = 6.6$ Hz, 2H, CH_2), 5.06 (s, 2H, $C_6H_5CH_2$), 5.30 (m, 1H, NH), 7.30 (m, 5H, C_6H_5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.2, 29.5, 33.1, 39.7, 66.2, 127.6, 127.7, 128.1, 136.3, 158.2. Anal. calcd for $C_{12}H_{16}BrNO_2$: C, 50.37; H, 5.64; N, 4.89. Found: C, 50.52; H, 5.32; N, 4.70.

Diethyl 4-{[(benzyloxy)carbonyl]amino}butylphosphonate (7). Benzyl 4-bromobutylcarbamate (306 mg, 1.07 mmol) was added to triethyl phosphite (889 mg, 5.35 mmol) slowly at rt. The reaction mixture was refluxed overnight, and then excess triethyl phosphate and other volatile compounds were distilled out under reduced pressure (3 mm Hg) at 60 °C. The residue was purified by column chromatography using $CHCl_3$: MeOH (95 : 5) as eluent. Yield 52%; colorless viscous oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24–1.34 (t, $J = 7.8$ Hz, 6H, 2 × CH_3), 1.50–1.79 (m, 6H, $CH_2CH_2CH_2$P), 3.15 (m, 4H, NHCH_2), 3.96–4.12 (m, 4H, 2 × OCH_2), 5.16 (b, 1H, OCONH), 5.05 (s, 2H, $C_6H_5CH_2$), 7.30 (m, 5H, C_6H_5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.2, 16.3, 19.5, 23.6, 26.4, 30.3, 30.7, 40.3, 61.3, 61.4, 66.4, 127.9, 128.3, 136.5, 156.3. Anal. calcd for $C_{16}H_{26}NO_5P$: C, 55.97; H, 7.63; N, 4.08. Found: C, 56.15; H, 7.49; N, 4.33.

4-((2-Oxohexadecanoyl)amino)butylphosphonic acid (10). To a stirred solution of compound **9** (167 mg, 0.36 mmol) in dry CH_2Cl_2 (0.9 ml) bromotrimethylsilane (0.14 ml, 1.08 mmol) was added at 0–5 °C and stirring was continued overnight at rt. The solvent was evaporated under reduced pressure and a second portion of bromotrimethylsilane (0.14 ml, 1.08 mmol) in dry CH_2Cl_2 (0.9 ml) was added at 0–5 °C and the stirring was continued overnight again at rt. After evaporation of solvent, MeOH was added and evaporated twice. The oily residue was solidified by addition of Et_2O. Yield 70%; white solid; mp 124–126 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.82–0.88 (t, $J = 6.6$ Hz, 3H, CH_3), 1.23 (m, 22H, 11 × CH_2), 1.46–1.60 (m, 8H, 4 × CH_2), 2.74–2.81 (t, $J = 7.4$ Hz, 2H, CH_2COCO), 3.07–3.11 (m, 2H, NHCH_2), 8.55 (t, 1H, NHCOCO). ^{13}C NMR (50 MHz, DMSO-d_6): 14.0, 20.1, 22.1, 22.7, 25.8, 28.4, 28.6, 28.7, 28.8, 28.9, 29.0, 29.4, 31.3, 36.6, 161.2, 199.5. ^{31}P NMR (81 MHz, DMSO-d_6): δ 27.51. MS (ESI): $m/z = 404$ (100%) $[M - H]^-$. Anal. calcd for $C_{20}H_{40}NO_5P$: C, 59.24; H, 9.94; N, 3.45. Found: C, 59.42; H, 9.78; N, 3.52.

4-(Acetylsulfanyl)-1-butanaminium chloride (22). To a stirred solution of alcohol **21** (1.0 g, 5.3 mmol) and Ph_3P (1.53 g, 5.83 mmol) in dry THF (26 ml), DIAD (1.15 ml, 5.83 mmol) and AcSH (0.42 ml, 5.83 mmol) were added at 0 °C, under argon. The stirring was continued for 2 h at 0 °C and overnight at rt. After evaporation of the solvent, the residue was dissolved in EtOAc (30 ml) and H_2O (15 ml). The organic layer was further washed with 5% aqueous $NaHCO_3$ and brine. After drying over Na_2SO_4, the solvent was evaporated under reduced pressure to a small volume and precipitation of $Ph_3P{=}O$ was achieved by the addition of petroleum ether. After filtration and evaporation of the solvents, the product was finally purified by column chromatography using CH_2Cl_2 as eluent. Yield 75%; colorless oil. For the removal of Boc group, the above-mentioned product (0.25 g, 1 mmol) was treated with 4 N HCl/dioxane (5 ml). After stirring for 30 min at rt and evaporation of the solvent, the final hydrochloride was crystallized by addition of diethyl ether. Yield 90%; white solid; mp 134.5–136 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.78 (m, 4H, 2 × CH_2), 2.32 (s, 3H, CH_3COS), 2.89 (t, $J = 7.2$ Hz, 2H, CH_2SCOCH$_3$), 3.04 (bs, 2H, CH_2NH). ^{13}C NMR (50 MHz, $CDCl_3$): 26.6, 26.9, 28.5, 30.9, 39.7, 196.4. MS (ESI): $m/z = 148$ (100%) $[M + H]^+$. Anal. calcd for $C_6H_{14}ClNOS$: C, 39.23; H, 7.68; N, 7.62. Found: C, 39.18; H, 7.57; N, 7.72.

4-(Ethylsulfanyl)-1-butanaminium chloride (26). To an ice cooled solution of alcohol **21** (0.189 g, 1 mmol) and triethylamine (0.21 ml, 1.5 mmol) in CH_2Cl_2 (10 ml), methanesulfonyl chloride (0.12 ml, 1.5 mmol) was added dropwise. The reaction mixture was stirred for 30 min at 0 °C, then 30 min at rt. The organic phase was washed consecutively with brine, 5% aqueous H_2SO_4, brine, 5% aqueous $NaHCO_3$ and brine. After drying of the organic layer over Na_2SO_4 and evaporation of the solvent, the active ester **25** was used in the next step.

Compound **25** (0.29 g, 1.086 mmol) and EtSNa (0.13 g, 1.56 mmol) were dissolved in DMF (5 ml) and stirred for 1 h at rt. Another portion of EtSNa (50 mg, 0.6 mmol) was then added and the stirring was continued for 40 min. Upon completion of the reaction, DMF was evaporated, the residue was dissolved in CH_2Cl_2 and the organic layer was washed with aqueous solution of NH_4Cl and brine. After drying over Na_2SO_4 and evaporation of the solvent under reduced pressure, the product was obtained as a pure yellow oil. Yield 85%. For the removal of Boc-protecting group, the above-mentioned product (0.233 g, 1 mmol) was treated with 4N HCl/dioxane (5 ml). After stirring for 30 min at rt and evaporation of the solvent the final hydrochloride **26** was crystallized by addition of diethyl ether. Yield 95%; white amorphous solid. ^{1}H NMR (200 MHz, CD_3OD): δ 1.23 (t, $J = 7.5$ Hz, 3H, SCH_2CH_3), 1.73 (m, 4H, 2 × CH_2), 2.56 (m, 4H, 2 × CH_2), 2.93 (t, $J = 7.4$ Hz, 2H, CH_2NH). ^{13}C NMR (50 MHz, $CDCl_3$): 13.9, 25.3, 26.1, 26.5, 30.5, 39.2. Anal. calcd for $C_6H_{16}NSCl$: C, 42.46; H, 9.50; N, 8.25. Found: C, 42.32; H, 9.64; N, 8.17.

Acknowledgements

This work was supported by NIH Grant GM 20 501 (EAD), the AnalgesiX/UC Discovery Biotechnology Grant #B1002-10303 (EAD) and the Special Account for Research Grants of the University of Athens (GK).

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DOI: 10.1002/psc