Synthesis of 2-Oxo Amide Triacylglycerol Analogues and Study of Their Inhibition Effect on Pancreatic and Gastric Lipases

George Kokotos,*[a] Robert Verger,[b] and Antonia Chiou[a]

Abstract: A general method for the synthesis of chiral 2-oxo amide triacylglycerol analogues, from (*R*)- or (*S*)-3-aminopropane-1,2-diol, was developed. These novel inhibitors of digestive lipases are analogues of the triacylglycerol molecule, a natural substrate of lipases, and they were designed to contain the 2-oxo amide functionality in place of the scissile ester bond at the *sn*-1 or *sn*-3 position and nonhydrolysable ether bonds instead of ester bonds at the other two remaining positions. The 2-oxo amide derivatives synthesised were tested for their ability to form stable monomolecular films at the air/water interface by recording their surface pressure/molecular area compression isotherms. The inhibition of porcine pancreatic and human gastric lipases by the 2-oxo amides was studied by means of the monolayer technique with mixed films of 1,2-dicaprin and with variable proportions of each inhibitor. The α_{50} values of these triacylglycerol analogues for PPL and HGL varied between 4.4 to 7.0 % and 5.6 to 15.9 %, respectively. The chirality at the *sn*-2 position of 2-oxo amide triacylglycerol analogues affected the α_{50} value for HGL, but not for PPL.

Keywords: amides · enzyme inhibitors · enzymes · lipases · monolayers · triacylglycerol analogues

Introduction

Lipases—triacylglycerol hydrolases—are enzymes that have attracted much attention because two of them (pancreatic and gastric) are essential enzymes for fat digestion, and they are also flexible biocatalysts for the acylation or deacylation of a wide range of unnatural substrates.[1] Human pancreatic lipase (HPL) contains an active site with a catalytic triad formed by serine 152, aspartate 176 and histidine 263.[2] This catalytic triad is homologous to that described in serine proteases such as chymotrypsin.[3] The hydrolytic mechanism catalysed by pancreatic lipase is driven by the nucleophilic attack of serine 152, and the resulting tetrahedral intermediate is stabilised by an oxy anion hole. The acyl enzyme transiently formed is further attacked by a water molecule, and the enzyme is regenerated and the acyl moiety liberated. The crystal structure of human gastric lipase has recently been reported.[4]

Specific and covalent inhibition of lipolytic enzymes is a difficult task, because of nonmutually exclusive processes such as interfacial denaturation, changes in interfacial quality[5] and surface dilution phenomena.[6] A few families of Ser reagents which inactivate lipases and include phosphorus-containing inhibitors and β-lactone containing inhibitors have been reported and reviewed.[7] Among them phosphonate-derived inhibitors have been found to irreversibly inactivate HPL and HGL[8–10] as well as microbial lipases.[11] These compounds mimic the transition state that occurs during carboxyester hydrolysis in both their charge distribution and configuration. On the other hand, the β-lactone containing inhibitor tetrahydrolipstatin is already in clinical use for the treatment of obesity.[12]

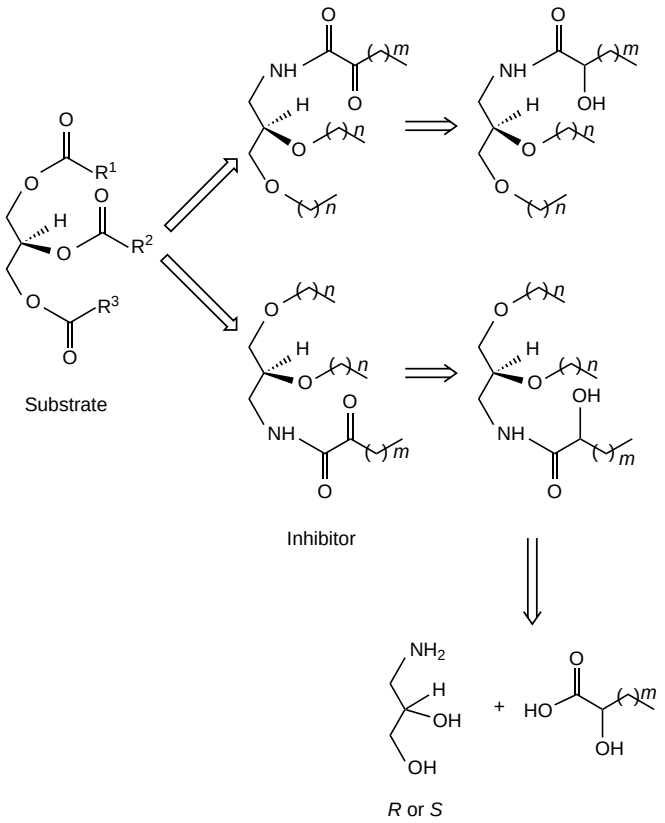
In the case of proteases, progress in drug design has led to the development of small synthetic inhibitors as therapeutic agents. Many inhibitors of serine proteases consist of a substrate-like structure with an activated carbonyl group at the site of the scissile amide bond, capable of forming a covalent intermediate. A number of reactive carbonyl groups, such as fluorinated ketones,[13] α-keto esters,[14] α-keto amides,[15] 1,2-diketones,[16] α-keto heterocycles[17] have been successfully used in the design of protease inhibitors. The mechanism of action of these electrophilic inhibitors most likely involves a nucleophilic addition of the active-site enzymatic serine hydroxy group to the carbonyl group of the inhibitor, with formation of a metastable hemiacetal adduct which mimics the tetrahedral species involved in the enzymatic cleavage of peptide bonds. In the case of some proteases X-ray[18] and ^{13}C NMR[19] studies have demonstrated the formation of such enzyme – inhibitor hemiacetal adducts.

[a] Assoc. Prof. G. Kokotos, A. Chiou
Laboratory of Organic Chemistry
Department of Chemistry
University of Athens
Panepistimiopolis, Athens 15771 (Greece)
Fax: (+301) 724-9101
E-mail: gkokotos@cc.uoa.gr

[b] Dr. R. Verger
Laboratoire de Lipolyse Enzymatique, CNRS, UPR 9025
31 Chemin Joseph-Aiguier, 13402 Marseile, Cedex 20 (France)

  0947-6539/00/0622-4211 $ 17.50+.50/0

To develop potent and specific inhibitors of lipases, we chose the 2-oxo amide functionality as the group to replace the scissile ester group of the natural lipases substrates. Since the interactions between an enzyme and a substrate, or an inhibitor, are very important for determination of its affinity, we decided to incorporate this 2-oxo amide group into a substrate-like structure. Ideally, in a triacylglycerol analogue only one 2-oxo amide functionality should be introduced, and in the other two positions ester bonds should be maintained. Such a combination of substrate and inhibitor functions in the same molecule complicates the interpretation of the inhibition kinetics. Therefore, the use of nonhydrolysable ether bonds seems to be unavoidable. Taking into account the structure of triacylglycerols, which are the natural substrates of lipases, we first replaced the ester bond at the *sn*-1 or *sn*-3 position of the substrate with the 2-oxo amide group. The two remaining ester bonds were replaced by ether bonds in order to avoid hydrolysis at these positions (Scheme 1). Furthermore, the synthesis of 2-oxo amide at the *sn*-1 or *sn*-3 position of triacylglycerol analogues was designed in order to study the stereoselectivity of the inhibition. To ensure the lipophilicity of these analogues, compounds with long alkoxy chains were selected. Retrosynthetic analysis for these 2-oxo amide triacylglycerol analogues (Scheme 1) leads to the corresponding 2-hydroxy amides. These latter compounds can be prepared by the coupling of a 2-hydroxy fatty acid with the appropriate alkyl chain with an enantiomerically pure amino component of either configuration, which in turn may be prepared from 3-aminopropane-1,2-diol.



Scheme 1. Retrosynthetic approach for the design of 2-oxo amide triacylglycerol analogues.

Results and Discussion

Synthesis: Compounds (*R*)- and (*S*)-3-aminopropane-1,2-diol (**1**), which are commercially available in optically pure form, were used as starting materials for the preparation of the target compounds (Scheme 2, here only the *R* enantiomers are shown). The amino group was protected with *tert*-butoxycarbonyl (Boc) using di-*tert*-butyl dicarbonate (Boc_2O) and triethylamine (Et_3N).[20] Treatment of the N-protected derivatives **2** with the appropriate alkyl bromide in a biphasic system of benzene/aqueous sodium hydroxide in the presence of a catalytic amount of Bu_4NHSO_4 afforded the ether derivatives **3a–c** in good yield (Scheme 2). Removal of the Boc group using HCl/THF led to the corresponding free amino compounds **4a–c**, and coupling with the suitable 2-hydroxy fatty acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (WSCI)[21] as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt) afforded the 2-hydroxy amides **5–f**. The racemic 2-hydroxy fatty acids were prepared by deamination of the corresponding 2-amino fatty acids[22] with $NaNO_2$ under acidic conditions. The 2-hydroxy amides were oxidised to the corresponding 2-oxo amides **6a–f** using either pyridinium dichromate (PDC) or NaOCl in the presence of 4-acetamido-2,2,6,6-tetramethylpiperidin-1-yloxy free radical (AcNH/TEMPO).[23] The use of PDC in acetic acid proved to be effective for the oxidation in most cases, and afforded the desired products in satisfactory yield. However, a decrease in the chemical yield was observed as the length of the aliphatic chains introduced in the molecules was increased. In the case of 2-hydroxy amides with alkoxy chain lengths of sixteen carbons, the oxidation to the corresponding 2-oxo amides proceeded only in moderate yield. In our hands, the use of NaOCl in the presence of catalytic amounts of AcNH/TEMPO in a biphasic system of toluene, ethyl acetate and aqueous NaBr, not only proved to be very effective for the cases where PDC failed to afford the products in high yield, but also afforded the oxidised products in almost quantitative yield in all the other cases tested. For the substrates with longer chains, the use of small quantities of CH_2Cl_2 in the solvent system to improve solubility, proved to be advantageous.

Abstract in Greek:
Στην παρούσα εργασία αναπτύχθηκε μία γενική μέθοδος σύνθεσης οπτικά ενεργών 2-οξο αμιδίων αναλόγων της τριακυλογλυκερόλης χρησιμοποιώντας ως πρώτη ύλη την (*R*)- ή (*S*)-3-αμινο-1,2-προπανοδιόλη. Οι ενώσεις αυτές, οι οποίες αποτελούν μία νέα κατηγορία αναστολέων των λιπασών της πέψης, είναι ανάλογες του μορίου της τριακυλογλυκερόλης, φυσικού υποστρώματος των λιπασών, και σχεδιάστηκαν έτσι ώστε να περιλαμβάνουν την 2-οξο αμιδική λειτουργική ομάδα στη θέση του υδρολυόμενου εστερικού δεσμού της *sn*-1 ή *sn*-3 θέσης και μη υδρολυόμενους αιθερικούς δεσμούς αντί των εστερικών στις δύο θέσεις που απομένουν. Τα παράγωγα 2-οξο αμιδίου που παρασκευάστηκαν μελετήθηκαν ως προς την ικανότητα τους να σχηματίζουν σταθερά μονομοριακά υμένια στη μεσεπιφάνεια αέρα/νερού με καταγραφή των ισοθέρμων καμπυλών επιφανειακής πίεσης/μοριακής επιφάνειας. Η αναστολή που προκαλούν οι ενώσεις αυτές στη χοίρια παγκρεατική και την ανθρώπινη γαστρική λιπάση μελετήθηκε με την τεχνική της μονοστοιβάδας και με χρήση μικτών μονομοριακών υμενίων 1,2-δικαπρίνης που περιείχαν τον κάθε αναστολέα σε διάφορες αναλογίες. Οι τιμές α_{50} για την PPL και HGL βρέθηκαν μεταξύ 4.4 έως 7.0 % και 5.6 έως 15.9 % αντίστοιχα. Η στερεοχημεία του άνθρακα στην *sn*-2 θέση των 2-οξο αμιδίων αναλόγων της τριακυλογλυκερόλης βρέθηκε ότι επηρεάζει την τιμή α_{50} για την HGL, όχι όμως για την PPL.

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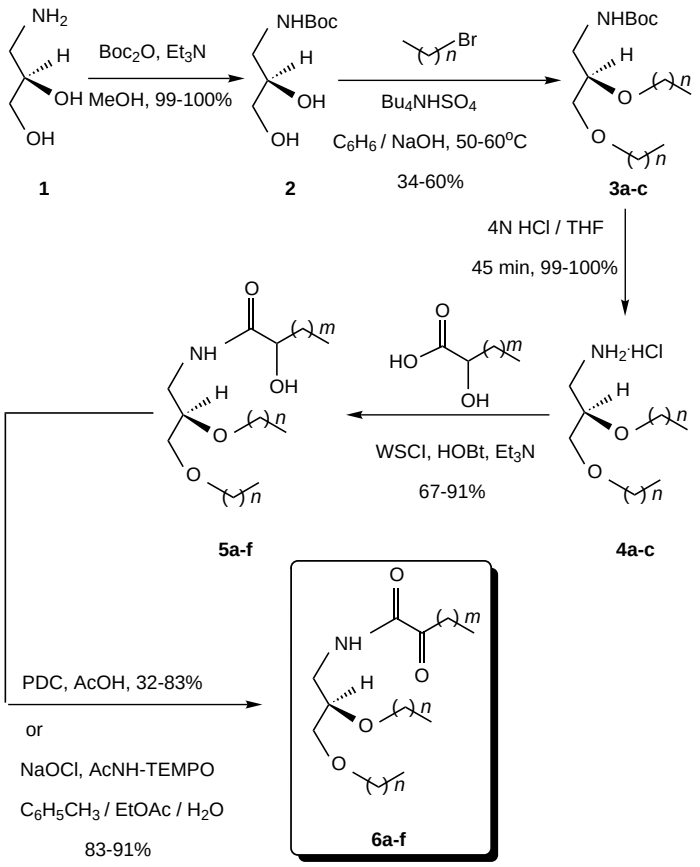

3, 4	*n*
a	9
b	13
c	15

5, 6	*n*	*m*
a	9	9
b	9	13
c	13	9
d	13	13
e	15	9
f	15	13

Scheme 2. General procedure for the synthesis of 2-oxo amide triacylglycerol analogues (only the *R* enantiomers are shown).

All intermediates and final products gave satisfactory analytical and spectroscopic data (see Experimental Section). In the ^{13}C NMR spectra of the 2-oxo amide derivatives two signals corresponding to carbon atoms of COCONH were clearly assigned. The carbon atom of the 2-oxo group was shifted to $\delta = 199$ owing to the presence of the adjacent amide function. The signal of the amide group carbon atom appeared at $\delta = 160$, while in the case of 2-hydroxy amides, the amide carbon appeared at $\delta = 174$. Furthermore, ^{13}C NMR spectra of 2-hydroxy amides showed a signal at $\delta = 72$, which was assigned to the carbon atom of CHOH. In the series of 3-aminopropane-1,2-diol derivatives, three signals corresponding to the carbon atoms of the 3-aminopropane-1,2-diol backbone, appeared at $\delta = 42-40$, $\delta = 77$ and $\delta = 72-70$ and were assigned to CH_2NH, CHO and CH_2O carbon atoms, respectively.

The enantiomeric purities of (*R*)-**4b** and (*S*)-**4b** were checked by NMR analysis of their amides with (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (Mosher acid).[24] In the ^{19}F NMR spectra of Mosher amides of (*R*)-**4b** and (*S*)-**4b**, signals at $\delta = 8.82$ and $\delta = 8.78$ (using CF_3COOH as an external reference) were observed, respectively. Thus, the absence of any diastereomeric fluorine signal indicated enantiomeric excess $> 95\,\%$.

Force/area curves of 2-oxo amide triacylglycerol analogues: The use of the monolayer technique, which is based upon surface pressure decrease owing to lipid-film hydrolysis, is advantageous for the study of lipase inhibitors since with conventional emulsified systems it is not possible to control the interfacial quality. This former technique is applicable to those cases where the lipid forms a stable monomolecular film at the air/water interface and where the reaction products are freely soluble and diffuse away rapidly into the aqueous phase.[5]

In order to determine the film stability and the interfacial properties at the air/water interface of the various compounds synthesised, we recorded their force/area curves. A force/area curve was obtained after a small volume of lipid solution was spread at the air/water interface in a volatile solvent (chloroform). The surface of the trough was progressively reduced by moving a mobile barrier at a constant rate, and the surface pressure was continuously recorded during compression. Unique information can be deduced from a force/area curve, that is, the area per molecule, collapse pressure, compressibility of the film and possibly phase transition, etc. Experiments were performed in the reservoir compartment of a zero-order trough as described under the materials and methods.

For compounds **6a–f** the molecular area dependency as a function of the surface pressure of a film spread over a buffered subphase at pH 8.0 is shown in Figure 1. As expected, the surface pressure/area curves obtained for all pairs of enantiomers were identical (within the range of experimental error). One can notice a decrease in the molecular area occupied by the 2-oxo amide compounds as the alkoxy chains increase (see Figure 1). This decrease is very clearly illustrated in the case of compounds **6d**, **f** with a chain

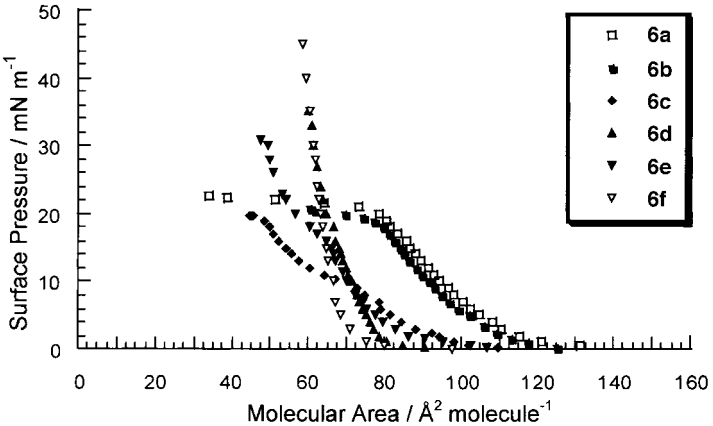

Figure 1. Force/area curves of the 2-oxo amide triacylglycerol analogues. The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The continuous compression experiments were performed in the rectangular reservoir of the zero-order trough.[26]

of thirteen carbon atoms in the 2-oxo amide moiety. Furthermore, the collapse pressures of compounds **6a**, **b**, **c** are observed at surface-pressure values of 20.9, 19.2, 19.8 mN m^{-1}, respectively, which are in the same range as the collapse pressure of a trioctanoyl glycerol film. In the case of smaller alkoxy chains, liquid-expanded films were obtained, and with increased chain lengths liquid-condensed (or even solid) force/area curves were recorded. In the case of compounds **6c**, **e** one can notice a clear transition from liquid-expanded to liquid-condensed state around surface pressures of 10 mN m^{-1} and 18 mN m^{-1}, respectively.

Pancreatic and gastric lipase activity on mixed films containing 2-oxo amide triacylglycerol analogues: The inhibition of pancreatic lipase was studied by means of the monomolecular film technique[25, 26] with mixed films of 1,2-dicaprin containing variable proportions of each 2-oxo amide triacylglycerol analogue. For most of the compounds the inhibition studies were performed at a constant surface pressure of 15 mN m^{-1}. This surface pressure was chosen for compounds **6b** and **6a** which have a collapse pressure around 19 – 21 mN m^{-1}, as well as for compounds **6d**, **f** in order to compare the data under identical experimental conditions. Inhibition studies with compounds **6c**, **e** were carried out at 10 mN m^{-1}, since above this value the monomolecular films were unstable with time. At both 10 and 15 mN m^{-1}, PPL was active and linear kinetics were recorded (data not shown).

Remaining lipase activity was plotted as a function of the inhibitor molar fraction (α). Lipase hydrolysis of 1,2-dicaprin decreased sharply as the molar fraction of inhibitors increased, and Figure 2 presents typical plots of data. The

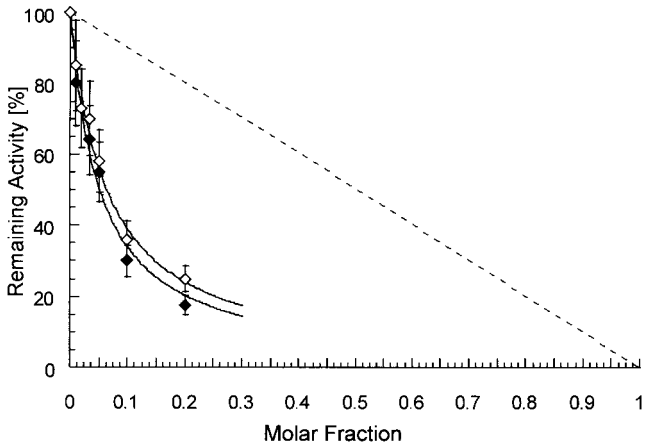

Figure 2. Effect of increasing concentration of (*S*)-**6b** (◆) and (*R*)-**6b** (◇) on the remaining activity of PPL on 1,2-dicaprin monolayer maintained at a constant surface pressure (15 mN m^{-1}). The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of hydrolysis were recorded for 20 min.

dotted line corresponds to surface-dilution phenomena, which reflects the decrease of lipase activity that would be observed if a nonsubstrate, noninhibitor compound, that is, a so-called surface dilutor, were present in the monomolecular film. A 50% decrease of lipase activity was observed when 5.0 ± 0.9% and 6.2 ± 0.8% (expressed in molar fraction) of the inhibitors (*S*)-**6b** and (*R*)-**6b**, were mixed with monolayers of 1,2-dicaprin, respectively. The inhibitor molar fractions α_{50} obtained for all the 2-oxo amide triacylglycerol analogues are summarized in Table 1. The α_{50} is defined as the molar fraction of inhibitor which reduces by 50 per cent the initial rate of

Table 1. Inhibition values of 2-oxo amide triacylglycerol analogues on porcine pancreatic lipase with the monolayer technique.

Compound	Surface pressure [mN m^{-1}]	α_{50} [%]
(*S*)-**6a**	15	5.2 ± 0.9
(*S*)-**6b**	15	5.0 ± 0.9
(*S*)-**6c**	10	4.6 ± 0.5
(*S*)-**6d**	15	5.6 ± 0.7
(*S*)-**6e**	10	5.0 ± 0.7
(*S*)-**6f**	15	4.5 ± 0.4
(*R*)-**6b**	15	6.2 ± 0.8
(*R*)-**6c**	10	7.0 ± 0.8
(*R*)-**6d**	15	5.3 ± 0.5
(*R*)-**6e**	10	4.5 ± 0.4
(*R*)-**6f**	15	4.4 ± 0.6

lipolysis. As shown from these data, no significant differences in the α_{50} values were observed when the chain length of either the ether or the 2-oxo amide moieties was varied. In the case of PPL, the chirality at the *sn*-2 carbon did not affect the α_{50} value. This behaviour may be attributed to the low stereopreference of PPL,[27] in agreement with the data obtained with HPL against triacylglycerols and triacylglycerol analogues.[9, 27, 28]

Compounds **6b**, **d**, **f** were also studied as inhibitors of HGL and the α_{50} values obtained are summarized in Table 2. The inhibition studies were performed at a constant surface pressure of 15 mN m^{-1}. At this value of surface pressure

Table 2. Inhibition values of 2-oxo amide triacylglycerol analogues on human gastric lipase with the monolayer technique.

Compound	Surface pressure [mN m^{-1}]	α_{50} [%]
(*R*)-**6b**	15	7.9 ± 1.8
(*S*)-**6b**	15	15.9 ± 5.8
(*R*)-**6d**	15	5.6 ± 0.8
(*S*)-**6d**	15	10.8 ± 3.2
(*R*)-**6f**	15	6.1 ± 1.5
(*S*)-**6f**	15	14.1 ± 5.1

HGL was active, and linear kinetics were recorded (data not shown). Contrary to the results obtained with PPL, in the case of HGL the three pairs of enantiomers of 2-oxo amide triacylglycerol analogues tested, (*R*)- and (*S*)-**6b**, **d**, **f**, displayed a differential inhibitory effect (Table 2). The inhibition depends on the chirality at the *sn*-2 carbon of the glycerol backbone. The enantiomers with the (*R*)-configuration proved to be better (two-fold) inhibitors than the corresponding structures with the (*S*)-configuration. Furthermore, the results obtained indicate a dependency of the α_{50} value upon the length of the alkoxy chain. A minimum of 5.6 ± 0.8% was observed for the α_{50} value for compound (*R*)-**6d**, with a chain length of fourteen carbon atoms.

0947-6539/00/0622-4214 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 22

Up to now the best phosphonate inhibitor of HPL reported in the literature is the *O*-hexadecyl-*O*-(*p*-nitrophenyl) *n*-undecyl phosphonate, with an α_{50} value of 0.3%.[10] This compound was tested with PPL under our experimental conditions and exhibited an α_{50} value of 0.6% at a surface pressure of 15 mN m^{-1}. The α_{50} values reported for a series of chiral organophosphorus acylglycerol analogues,[9] in which one carbonyl was replaced by a phosphonate group, varied from 13–20%. One can conclude that the 2-oxo amide triacylglycerol analogues reported here exhibit a much stronger inhibitory effect as compared with the acylglycerol phosphonate inhibitors. Our results indicate that the 2-oxo amide group is a valuable substituent for future design and synthesis of powerful inhibitors of lipolytic enzymes.

Experimental Section

Materials and methods: Compounds (*R*)- and (*S*)-3-aminopropane-1,2-diol, (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid and AcNH/TEMPO were purchased from Aldrich. 1,2-Dicaprin was purchased from Sigma. Analytical TLC plates (silica gel60 F_{254}) and silica gel60 (70–230 mesh) were purchased from Merck. PPL[29] and HGL[30] were purified at the laboratory using previously described procedures. Et_3N was distilled from ninydrin. All other solvents and chemicals were of reagent grade and used without further purification. Melting points were determined on a Buchi 530 apparatus and are uncorrected. Specific rotations were measured on a Perkin–Elmer 141 Polarimeter using a 10 cm cell. ^{1}H NMR, ^{13}C NMR, DEPT and COSY spectra were obtained in $CDCl_3$ using a Varian Mercury (200 MHz) spectrometer. NMR spectra were recorded for both the enantiomers of each compound prepared and were identical. To avoid repetition these data are presented in the experimental section only for one enantiomer of each pair. Mass spectra were obtained on a VG Analytical ZAB-SE instrument. Elemental analyses were performed on a Perkin–Elmer 2400 instrument.

Synthesis

3-*tert*-Butoxycarbonylaminopropane-1,2-diol (2): Et_3N (34.3 mL, 0.245 mol) and subsequently di-*tert*-butyl dicarbonate (11.46 g, 52.5 mmol) were added in portions to a stirred solution of 3-aminopropane-1,2-diol (3.19 g, 35 mmol) in MeOH (350 mL). The reaction mixture was stirred for 5–10 min at 40–50 °C and for 30 min at room temperature. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel with $CHCl_3$/MeOH (9:1).

(*R*)-3-*tert*-Butoxycarbonylaminopropane-1,2-diol ((*R*)-2): Yield: 6.69 g (100%); m.p. 53–55 °C; $[\alpha]_D = -6.6$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 5.3$ (br, 1H; OCONH), 3.9 (br, 2H; 2 × OH), 3.8 (m, 1H; C*H*OH), 3.5 (m, 2H; CH_2OH), 3.2 (m, 2H; CH_2NH), 1.4 (s, 9H; $(CH_3)_3$C); ^{13}C NMR: $\delta = 157.3$ (OCONH), 80.0 ($(CH_3)_3C$), 71.3 (CHOH), 63.6 (CH_2OH), 42.7 (CH_2NH), 28.3 ($(CH_3)_3$C); $C_8H_{17}NO_4$ (191.2) (%): calcd: C 50.25, H 8.96, N 7.32; found: C 49.93, H 9.24, N 7.11.

(*S*)-3-*tert*-Butoxycarbonylaminopropane-1,2-diol ((*S*)-2): Yield 6.68 g (99%); m.p. 51–52 °C; $[\alpha]_D = +6.7$ ($c = 0.5$ in $CHCl_3$); $C_8H_{17}NO_4$ (191.2) (%): calcd: C 50.25, H 8.96, N 7.32; found: C 50.02, H 9.15, N 7.01.

General procedure for the synthesis of *N*-*tert*-butoxycarbonyl-2,3-bis(alkoxy)propanamines (3a–c): Aqueous NaOH (50%, 3 mL) and Bu_4NHSO_4 (1.02 g, 3 mmol) were added at room temperature to a stirred solution of compound **2** (1.15 g, 6 mmol) and the appropriate alkyl bromide (36 mmol) in benzene (3 mL). After vigorous stirring for 6 h at 50–60 °C, the reaction mixture was allowed to obtain ambient temperature, and EtOAc and water were added. The organic phase was washed with brine and dried (Na_2SO_4). The residue was purified by column chromatography using petroleum ether 40–60 °C/EtOAc 9:1 as eluent.

(*R*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(decyloxy)propanamine ((*R*)-3a): Yield 1.59 g (56%); oil; $[\alpha]_D = +11.3$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 4.9$ (br, 1H; OCONH), 3.7–3.1 (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.6 (m, 4H; 2 × OCH$_2$CH_2), 1.5 (s, 9H; $(CH_3)_3$C), 1.3 (m, 28H; 14 × CH_2), 0.9 (m, 6H; 2 × CH_3); ^{13}C NMR: $\delta = 156.0$ (OCONH), 79.5 ($(CH_3)_3C$), 77.0 (CHO), 71.7, 71.3 and 70.2 (CH_2OCH_2, CHOCH_2), 41.9 (CH_2NH), 31.9–22.7 (lipidic chain), 28.4 ($(CH_3)_3$C), 14.1 (CH_3); $C_{28}H_{57}NO_4$ (471.8) (%): calcd: C 71.29, H 12.18, N 2.97; found: C 71.06, H 12.42, N 2.81.

(*S*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(decyloxy)propanamine ((*S*)-3a): Yield 1.70 g (60%); oil; $[\alpha]_D = -11.3$ ($c = 0.5$ in $CHCl_3$); $C_{28}H_{57}NO_4$ (471.8) (%): calcd: C 71.29, H 12.18, N 2.97; found: C 70.94, H 12.51, N 2.73.

(*R*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(tetradecyloxy)propanamine ((*R*)-3b): Yield 1.80 g (51%); oil; $[\alpha]_D = +8.9$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 4.9$ (br, 1H; OCONH), 3.7–3.1 (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.6 (m, 4H; 2 × OCH$_2$CH_2), 1.5 (s, 9H; $(CH_3)_3$C), 1.3 (m, 44H; 22 × CH_2), 0.9 (m, 6H; 2 × CH_3); ^{13}C NMR: $\delta = 156.0$ (OCONH), 79.5 ($(CH_3)_3C$), 77.0 (CHO), 71.6, 71.7 and 70.2 (CH_2OCH_2, CHOCH_2), 41.9 (CH_2NH), 31.9–22.7 (lipidic chain), 28.4 ($(CH_3)_3$C), 14.1 (CH_3); $C_{36}H_{73}NO_4$ (584.0) (%): calcd: C 74.04, H 12.60, N 2.40; found: C 73.76, H 12.93, N 2.19.

(*S*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(tetradecyloxy)propanamine ((*S*)-3b): Yield 1.91 g (54%); oil; $[\alpha]_D = -8.9$ ($c = 0.5$ in $CHCl_3$); $C_{36}H_{73}NO_4$ (584.0) (%): calcd: C 74.04, H 12.60, N 2.40; found: C 74.30, H 12.88, N 2.54.

(*R*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(hexadecyloxy)propanamine ((*R*)-3c): Yield 1.42 g (37%); m.p. 42–43 °C; $[\alpha]_D = +6.7$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 4.9$ (br, 1H; OCONH), 3.7–3.1 (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.6 (m, 4H; 2 × OCH$_2$CH_2), 1.5 (s, 9H; $(CH_3)_3$C), 1.3 (m, 52H; 26 × CH_2), 0.9 (m, 6H; 2 × CH_3); $C_{40}H_{81}NO_4$ (640.1) (%): calcd: C 75.06, H 12.76, N 2.19; found: C 74.80, H 13.07, N 1.95.

(*S*)-*N*-*tert*-Butoxycarbonyl-2,3-bis(hexadecyloxy)propanamine ((*S*)-3c): Yield 1.30 g (34%); m.p. 42–43 °C; $[\alpha]_D = -6.5$ ($c = 0.5$ in $CHCl_3$); $C_{40}H_{81}NO_4$ (640.1) (%): calcd: C 75.06, H 12.76, N 2.19; found: C 74.72, H 13.02, N 1.89.

General procedure for the synthesis of 2,3-bis(alkoxy)propanamine hydrochlorides (4a–c): Compound **3** (2.4 mmol) was treated with HCl (4N) in THF (30 mL) for 1 h at room temperature. The solvent and the excess acid were evaporated under reduced pressure, and the residue was recovered from reevaporation twice from THF and Et_2O.

(*R*)-2,3-Bis(decyloxy)propanamine hydrochloride ((*R*)-4a): Yield 0.98 g (100%); oil; $[\alpha]_D = +14.1$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 3.7–3.3$ (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.7–1.4 (m, 4H; 2 × OCH$_2$CH_2), 1.3 (m, 28H; 14 × CH_2), 0.9 (m, 6H; 2 × CH_3).

(*S*)-2,3-Bis(decyloxy)propanamine hydrochloride ((*S*)-4a): Yield 0.98 g (100%); oil; $[\alpha]_D = -14.2$ ($c = 0.5$ in $CHCl_3$).

(*R*)-2,3-Bis(tetradecyloxy)propanamine hydrochloride ((*R*)-4b): Yield 1.24 g (99%); m.p. 57–58.5 °C; $[\alpha]_D = +11.2$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 3.7–3.3$ (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.7–1.4 (m, 4H; 2 × OCH$_2$CH_2), 1.3 (m, 44H; 22 × CH_2), 0.9 (m, 6H; 2 × CH_3).

(*S*)-2,3-Bis(tetradecyloxy)propanamine hydrochloride ((*S*)-4b): Yield 1.25 g (100%); m.p. 58–60 °C; $[\alpha]_D = -11.4$ ($c = 0.5$ in $CHCl_3$).

(*R*)-2,3-Bis(hexadecyloxy)propanamine hydrochloride ((*R*)-4c): Yield 1.38 g (100%); m.p. 67–70 °C; $[\alpha]_D = +11.0$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR: $\delta = 3.7–3.4$ (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH), 1.7–1.4 (m, 4H; 2 × OCH$_2$CH_2), 1.3 (m, 52H; 26 × CH_2), 0.9 (m, 6H; 2 × CH_3).

(*S*)-2,3-Bis(hexadecyloxy)propanamine hydrochloride ((*S*)-4c): Yield 1.38 g (100%); m.p. 68–71 °C; $[\alpha]_D = -11.1$ ($c = 0.5$ in $CHCl_3$).

General procedure for the synthesis of *N*-[2,3-bis(alkyloxy)propyl]-2-hydroxyalkanamides (5a–f): Et_3N (0.59 mL, 4.2 mmol) and subsequently WSCI (0.42 g, 2.2 mmol) and HOBt (0.27 g, 2 mmol) were added at 0 °C to a stirred solution of the appropriate 2-hydroxy acid (2 mmol) and compound **4** (2 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The organic layer was washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by column chromatography using petroleum ether 40–60 °C/EtOAc 3:2 as eluent.

(*S*)-*N*-[2,3-Bis(decyloxy)propyl]-2-hydroxydodecanamide ((*S*)-5a): Yield 0.93 g (82%); m.p. 45–46.5 °C; ^{1}H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (m, 1H; C*H*OH), 3.7–3.2 (m, 9H; CH_2OCH_2, CHOCH_2, CH_2NH), 1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH$_2$CH_2), 1.3 (m, 44H; 22 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 174.1$ (CONH), 76.6 (CHO), 72.0 (CHOH), 71.8, 71.5 and 70.2 (CH_2OCH_2, CHOCH_2), 40.4 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.6 (lipidic chain), 14.1 (CH_3); $C_{35}H_{71}NO_4$ (570.0) (%): calcd: C 73.76, H 12.56, N 2.46; found: C 73.44, H 12.81, N 2.14.

(*R*)-*N*-[2,3-Bis(decyloxy)propyl]-2-hydroxyhexadecanamide ((*R*)-5b): Yield 1.01 g (81%); m.p. 52.5–53.5 °C; ^{1}H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (m, 1H; C*H*OH), 3.7–3.2 (m, 9H; CH_2OCH_2, C*H*OCH_2, CH_2NH),

1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH_2CH_2), 1.3 (m, 52H; 26 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 174.2$ (CONH), 76.6 (CHO), 72.0 (CHOH), 71.8, 71.5 and 70.2 (CH_2OCH_2, $CHOCH_2$), 40.3 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.6 (lipidic chain), 14.1 (CH_3); $C_{39}H_{79}NO_4$ (626.1) (%): calcd: C 74.82, H 12.72, N 2.24; found: C 74.51, H 12.98, N 2.07.

(*S*)-*N*-[2,3-Bis(decyloxy)propyl]-2-hydroxyhexadecanamide ((*S*)-5b): Yield 1.08 g (86%); m.p. 52–54 °C; $C_{39}H_{79}NO_4$ (626.1) (%): calcd: C 74.82, H 12.72, N 2.24; found: C 74.55, H 12.97, N 2.00.

(*R*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-hydroxydodecanamide ((*R*)-5c): Yield 1.08 g (79%); m.p. 59.5–60 °C; ^1H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (br, 1H; $CHOH$), 3.7–3.2 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH_2CH_2), 1.3 (m, 60H; 30 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 174.0$ (CONH), 76.6 (CHO), 72.0 (CHOH), 71.8, 71.5 and 70.2 (CH_2OCH_2, $CHOCH_2$), 40.5 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.6 (lipidic chain), 14.1 (CH_3); $C_{43}H_{87}NO_4$ (682.2) (%): calcd C 75.71, H 12.85, N 2.05; found C 75.43, H 13.08, N 1.83.

(*S*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-hydroxydodecanamide ((*S*)-5c): Yield 1.00 g (73%); m.p. 55–56 °C; $C_{43}H_{87}NO_4$ (682.2) (%): calcd: C 75.71, H 12.85, N 2.05; found: C 75.34, H 13.03, N 1.84.

(*R*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-hydroxyhexadecanamide ((*R*)-5d): Yield 1.05 g (71%); m.p. 65–67 °C; ^1H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (m, 1H; $CHOH$), 3.7–3.2 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH_2CH_2), 1.3 (m, 68H; 34 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 174.0$ (CONH), 76.6 (CHO), 71.9 (CHOH), 71.8, 71.4 and 70.2 (CH_2OCH_2, $CHOCH_2$), 40.4 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.7 (lipidic chain), 14.1 (CH_3); $C_{47}H_{95}NO_4$ (738.3) (%): calcd: C 76.46, H 12.97, N 1.90; found: C 74.12, H 13.26, N 2.06.

(*S*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-hydroxyhexadecanamide ((*S*)-5d): Yield 1.12 g (76%); m.p. 69–70.5 °C; $C_{47}H_{95}NO_4$ (738.3)(%): calcd: C 76.46, H 12.97, N 1.90; found: C 76.57, H 13.31, N 1.67.

(*R*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-hydroxydodecanamide ((*R*)-5e): Yield 0.99 g (67%); m.p. 64.5–65 °C; ^1H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (m, 1H; $CHOH$), 3.7–3.2 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH_2CH_2), 1.3 (m, 68H; 34 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 173.9$ (CONH), 76.6 (CHO), 72.0 (CHOH), 71.8, 71.5 and 70.2 (CH_2OCH_2, $CHOCH_2$), 40.3 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.6 (lipidic chain), 14.1 (CH_3); $C_{47}H_{95}NO_4$ (738.3) (%): calcd: C 76.46, H 12.97, N 1.90; found: C 76.21, H 12.66, N 1.72.

(*S*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-hydroxydodecanamide ((*S*)-5e): Yield 1.04 g (68%); m.p. 63–65 °C; $C_{47}H_{95}NO_4$ (738.3) (%): calcd: C 76.46, H 12.97, N 1.90; found: C 76.19, H 13.28, N 1.74.

(*R*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-hydroxyhexadecanamide ((*R*)-5f): Yield 1.46 g (91%); m.p. 67–70 °C; ^1H NMR: $\delta = 6.9$ (br, 1H; CONH), 4.1 (m, 1H; $CHOH$), 3.7–3.2 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 1.9–1.5 (m, 6H; CH_2CHOH, 2 × OCH_2CH_2), 1.3 (m, 76H; 38 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 173.8$ (CONH), 76.6 (CHO), 72.0 (CHOH), 71.6, 71.9 and 70.2 (CH_2OCH_2, $CHOCH_2$), 40.5 (CH_2NH), 35.0 (CH_2CHOH), 31.9–22.7 (lipidic chain), 14.1 (CH_3); $C_{51}H_{103}NO_4$ (794.4) (%): calcd: C 77.11, H 13.07, N 1.76; found: C 76.82, H 13.35, N 1.66.

(*S*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-hydroxyhexadecanamide ((*S*)-5f): Yield 1.41 g (89%); m.p. 69–71 °C; $C_{51}H_{103}NO_4$ (794.4) (%): calcd: C 77.11, H 13.07, N 1.76; found: C 77.23, H 13.26, N 1.83.

General procedures for the synthesis of *N*-[2,3-bis(alkyloxy)propyl]-2-oxoalkanamides (6a–f)

Procedure A: oxidation of compounds 5 using pyridinium dichromate: Pyridinium dichromate (PDC) (1.35 g, 3.6 mmol) was added to a solution of compound **5** (1.2 mmol) in glacial acetic acid (6 mL). After stirring for 2 h at room temperature the mixture was neutralised with aqueous $NaHCO_3$ (5%) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using petroleum ether 40–60 °C/EtOAc 9:1 as eluent.

Procedure B: oxidation of compounds 5 using NaOCl/AcNH/TEMPO: A solution of NaBr (136 mg, 1.32 mmol) in water (0.6 mL) and subsequently AcNH-TEMPO (2.6 mg, 0.012 mmol) were added at 0 °C to a stirred solution of compound **5** (1.2 mmol) in a mixture of EtOAc/toluene 1:1 (7.2 mL). To the resulting biphasic system was added under vigorous stirring a solution of NaOCl (98 mg, 1.32 mmol) and $NaHCO_3$ (302 mg, 3.6 mmol) in H_2O (3.8 mL) dropwise at 0 °C over a period of 1 h. After stirring for 15 min at room temperature, EtOAc (15 mL) and water (5 mL) were added. The organic layer was washed with 10% aqueous $NaHSO_3$ (10 mL) which contained KI (60 mg), 10% aqueous $Na_2S_2O_3$ (10 mL), brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography.

(*S*)-*N*-[2,3-Bis(decyloxy)propyl]-2-oxododecanamide ((*S*)-6a): Procedure A: yield 0.51 g (75%); oil; overall yield 37%; $[\alpha]_D = -13.8$ ($c = 0.5$ in $CHCl_3$); ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz, 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 × OCH_2CH_2), 1.3 (m, 42H; 21 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 199.0$ ($COCONH$), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3 and 70.3 (CH_2OCH_2, $CHOCH_2$), 40.1 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 568 (100) $[M^+]$; $C_{35}H_{69}NO_4$ (567.9) (%): calcd: C 74.02, H 12.25, N 2.47; found: C 74.25, H 12.49, N 2.65.

(*R*)-*N*-[2,3-Bis(decyloxy)propyl]-2-oxohexadecanamide ((*R*)-6b): Procedure A: yield 0.57 g (76%); overall yield 34%; $[\alpha]_D = +11.7$ ($c = 0.5$ in $CHCl_3$); m.p. 35–37 °C; ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz, 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 × OCH_2CH_2), 1.3 (m, 50H; 25 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 199.0$ ($COCONH$), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3 and 70.3 (CH_2OCH_2, $CHOCH_2$), 40.7 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 624 (100) $[M^+]$; $C_{39}H_{77}NO_4$ (624.0) (%): calcd: C 75.06, H 12.44, N 2.24; found: C 74.81, H 12.76, N 2.11.

(*S*)-*N*-[2,3-Bis(decyloxy)propyl]-2-oxohexadecanamide ((*S*)-6b): Procedure A: yield 0.54 g (72%), Procedure B: yield 0.70 g (94%); overall yield 48%; m.p. 35–37 °C; $[\alpha]_D = -11.9$ ($c = 0.5$ in $CHCl_3$); $C_{39}H_{77}NO_4$ (624.0) (%): calcd: C 75.06, H 12.44, N 2.24; found: C 74.75, H 12.63, N 2.02.

(*R*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-oxododecanamide ((*R*)-6c): Procedure A: yield 0.68 g (83%); overall yield 33%; m.p. 46–47 °C; $[\alpha]_D = +9.8$ ($c = 0.5$ in $CHCl_3$); ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz, 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 × OCH_2CH_2), 1.3 (m, 58H; 29 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 199.0$ ($COCONH$), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3 and 70.3 (CH_2OCH_2, $CHOCH_2$), 40.7 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 680 (100) $[M^+]$; $C_{43}H_{85}NO_4$ (680.1) (%): calcd: C 75.93, H 12.60, N 2.06; found: C 75.70, H 12.84, N 1.93.

(*S*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-oxododecanamide ((*S*)-6c): Procedure A: yield 0.64 g (79%); overall yield 31%; m.p. 45–46 °C; $[\alpha]_D = -9.6$ ($c = 0.5$ in $CHCl_3$); $C_{43}H_{85}NO_4$ (680.1) (%): calcd: C 75.93, H 12.60, N 2.06; found: C 75.65, H 12.91, N 2.14.

(*R*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-oxohexadecanamide ((*R*)-6d): Procedure A: yield 0.58 g (66%), Procedure B: Yield 0.80 g (91%); overall yield 24%; m.p. 51–52.5 °C; $[\alpha]_D = +8.9$ ($c = 0.5$ in $CHCl_3$); ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz, 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 × OCH_2CH_2), 1.3 (m, 66H; 33 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 199.0$ ($COCONH$), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3, 70.3 (CH_2OCH_2, $CHOCH_2$), 40.7 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 736 (100) $[M^+]$; $C_{47}H_{93}NO_4$ (736.3) (%): calcd: C 76.67, H 12.73, N 1.90; found: C 76.34, H 12.95, N 2.02.

(*S*)-*N*-[2,3-Bis(tetradecyloxy)propyl]-2-oxohexadecanamide ((*S*)-6d): Procedure A: yield 0.61 g (69%); overall yield 31%; m.p. 48–50 °C; $[\alpha]_D = -8.7$ ($c = 0.5$ in $CHCl_3$); $C_{47}H_{93}NO_4$ (736.3) (%): calcd: C 76.67, H 12.73, N 1.90; found: C 76.36, H 13.00, N 1.67.

(*R*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-oxododecanamide ((*R*)-6e): Procedure B: yield 0.87 g (99%); overall yield 24%; m.p. 53.5–54.5 °C; $[\alpha]_D = +9.2$ ($c = 0.5$ in $CHCl_3$); ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz; 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 × OCH_2CH_2), 1.3 (m, 66H; 33 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: $\delta = 199.0$ ($COCONH$), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3 and 70.3 (CH_2OCH_2, $CHOCH_2$), 40.7 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 736 (100) $[M^+]$; $C_{47}H_{93}NO_4$ (736.3) (%): calcd: C 76.67, H 12.73, N 1.90; found: C 76.82, H 12.99, N 1.72.

(*S*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-oxododecanamide ((*S*)-6e): Procedure B: yield 0.87 g (98%); overall yield 22%; m.p. 54–55.5 °C; $[\alpha]_D = -9.2$ ($c = 0.5$ in $CHCl_3$); $C_{47}H_{93}NO_4$ (736.3) (%): calcd: C 76.67, H 12.73, N 1.90; found: C 76.32, H 13.06, N 1.67.

(*R*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-oxohexadecanamide ((*R*)-6f): Procedure A: Yield 0.30 g (32%), Procedure B: yield 0.80 g (84%); overall yield 28%; m.p. 59–60.5 °C; $[\alpha]_D = +7.9$ ($c = 0.5$ in $CHCl_3$); ^1H NMR: $\delta = 7.4$ (br, 1H; CONH), 3.7–3.3 (m, 9H; CH_2OCH_2, $CHOCH_2$, CH_2NH), 2.9 (t, $J = 7$ Hz, 2H; CH_2CO), 1.6 (m, 6H; CH_2CH_2CO, 2 ×

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OCH_2CH_2), 1.3 (m, 74H; 37 × CH_2), 0.9 (m, 9H; 3 × CH_3); ^{13}C NMR: δ = 199.0 (*C*OCONH), 160.3 (CONH), 76.3 (CHO), 71.9, 71.3, 70.3 (CH_2OCH_2, $CHOCH_2$), 40.7 (CH_2NH), 36.8 (CH_2CO), 31.9–22.7 (lipidic chain), 14.1 (CH_3); MS: *m/z* (%): 792 (100) [M^+]; $C_{51}H_{101}NO_4$ (792.4) (%): calcd: C 77.31, H 12.85, N 1.77; found: C 76.98, H 13.13, N 1.68.

(*S*)-*N*-[2,3-Bis(hexadecyloxy)propyl]-2-oxohexadecanamide ((*S*)-6 f): Procedure B: yield 0.79 g (83%); overall yield 25%; m.p. 57–59 °C; $[\alpha]_D$ = −7.9 (*c* = 0.5 in $CHCl_3$); $C_{51}H_{101}NO_4$ (792.3) (%): calcd: C 77.32, H 12.85, N 1.77; found: C 77.04, 13.10, N 1.56.

Monomolecular film experiments

Force/area curves: Surface pressure/area curves were measured in the rectangular reservoir compartment of the zero order trough (14.8 cm wide and 24.9 cm long). Before each experiment the trough was at first washed with tap water, then gently brushed in the presence of distilled ethanol, washed again with plenty of tap water and finally rinsed with doubly distilled water. The lipidic film as a solution in $CHCl_3$ (approximately 1 mg mL^{-1}), was spread with a Hamilton syringe over an aqueous subphase of Tris/HCl (10 mM), pH 8.0, NaCl (100 mM), $CaCl_2$ (21 mM), EDTA (1 mM). The above buffer solution was prepared with doubly distilled water and filtered through a 0.22 μm Millipore membrane. Before each utilisation, residual surface-active impurities were removed by sweeping and suction of the surface.[26] The force/area curves were automatically recorded upon a continuous compression rate at 4.8 cm min^{-1}.

Enzymes kinetics experiments: The inhibition experiments were performed by using the monolayer technique. The surface pressure of the lipid film was measured using the platinum Wilhelmy plate technique coupled with an electromicrobalance. The principle of this method has been described previously by Verger et al.[26]

For the inhibition studies we used the method of mixed monomolecular films. This method involves the use of a zero-order trough, consisting of two compartments: a reaction compartment, where mixed films of substrate and inhibitor are spread, and a reservoir compartment, where only pure films of substrate are spread. The two compartments are connected to each other by narrow surface channels. PPL (final concentration 3.9 ng mL^{-1}) and HGL (final concentration 185 ng mL^{-1}) were injected into the subphase of the reaction compartment, where efficient stirring was applied. In the case of PPL the aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), EDTA (1 mM). In the case of HGL the aqueous subphase was composed of CH_3COONa/HCl (10 mM), pH 5.0, NaCl (100 mM), $CaCl_2$ (21 mM), EDTA (1 mM). When, owing to the lipolytic action of the enzyme, the surface pressure decreased a mobile barrier was moved over the reservoir compartment to compress the film and thus keep the surface pressure constant. The surface pressure was measured on the reservoir compartment. The surface of the reaction compartment was 100 cm^2 and its volume 120 mL. The reservoir compartment was 14.8 cm wide and 24.9 cm long. The lipidic films were spread from a chloroform solution (approximately 1 mg mL^{-1}). The kinetics were recorded for 20 min. In all cases linear kinetics were obtained. Each experiment was duplicated.

Acknowledgement

This work was supported by the European Community (BIO2-CT943041), the Greek Secretariat for Research and Technology and the French Foreign Minister (PLATON program 98054). A.C. thanks the French Embassy in Athens and the French Government for a fellowship.

[1] a) A. M. Klibanov, *Trends Biotechnol.* **1997**, *15*, 97–101; b) T. Anthonsen, J. A. Jongejan, *Methods Enzymol.* **1997**, *286*, 473–495; c) R. D. Schmid, R. Verger, *Angew. Chem.* **1998**, *110*, 1694–1720; *Angew. Chem. Int. Ed.* **1998**, *37*, 1608–1633; d) M. T. Reetz, K. E. Jaeger, *Top. Curr. Chem.* **1999**, *200*, 31–57; e) Y. L. Khmelnitsky, J. O. Rich, *Curr. Opin. Chem. Biol.* **1999**, *3*, 47–53.

[2] F. K. Winkler, A. D′Arcy, W. Hunziker, *Nature (London)* **1990**, *343*, 771–774.

[3] D. Blow, *Nature (London)* **1991**, *351*, 444–445.

[4] A. Roussel, S. Canaan, M.-P. Egloff, M. Riviere, L. Dupuis, R. Verger, C. Cambillau, *J. Biol. Chem.* **1999**, *274*, 16995–17002.

[5] R. Verger, G. H. de Haas, *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 77–117.

[6] E. A. Dennis, *Drug Dev. Res.* **1987**, *10*, 205–220.

[7] a) S. Patkar, F. Björkling in *Lipases. Their Structure, Biochemistry and Application* (Eds.: P. Wooley, S. B. Petersen), Cambridge University Press, Cambridge, **1994**, pp. 207–224; b) S. Ransac, Y. Gargouri, F. Marguet, G. Buono, C. Beglinger, P. Hildebrand, H. Lengsfeld, P. Hadvary, R. Verger, *Methods Enzymol.* **1997**, *286*, 191–231.

[8] a) F. Marguet, C. Cudrey, R. Verger, G. Buono, *Biochim. Biophys. Acta* **1994**, *1210*, 157–166; b) J.-F. Cavalier, S. Ransac, R. Verger, G. Buono, *Biochemistry* **1995**, *34*, 2751–2762; c) F. Marguet, J.-F. Cavalier, R. Verger, G. Buono, *Eur. J. Org. Chem.* **1999**, *7*, 1671–1678.

[9] F. Marguet, I. Douchet, J.-F. Cavalier, G. Buono, R. Verger, *Colloids Surf., B* **1999**, *13*, 37–45.

[10] J.-F. Cavalier, S. Ransac, R. Verger, G. Buono, *Chem. Phys. Lipids* **1999**, *100*, 3–31.

[11] a) M. L. M. Mannesse, J. W. P. Boots, R. Dijkman, A. J. Slotboom, H. T. W. M. Van der Hijden, M. R. Egmond, H. M. de Haas, G. H. Verheij, *Biochim. Biophys. Acta* **1995**, *1259*, 56–64; b) P. Stadler, G. Zandonella, L. Haalck, F. Spener, A. Hermetter, F. Paltauf, *Biochim. Biophys. Acta* **1996**, *1304*, 229–244.

[12] a) M. L. Drent, E. A. Vanderveen, *Int. J. Obesity* **1993**, *17*, 241–244; b) C. Güzelhan, J. Odink, J. J. N. Jansenzuidema, D. Hartmann, *J. Int. Med. Res.* **1994**, *22*, 255–265; c) M. L. Drent, E. A. Vanderveen, *Obes. Res.* **1995**, *3*, S623-S625; d) A. B. R. Thomson, A. De Pover, M. Keelan, E. Jarocka-Kyrta, M. T. Clandinin, *Methods Enzymol.*, **1997**, *286*, 3–44; e) M. Leonhardt, B. Hrupka, W. Langhans, *Eur. J. Nutrition* **1999**, *38*, 1–13.

[13] a) B. Imperiali, R. H. Abeles, *Biochemistry* **1986**, *25*, 3760–3767; b) J.-P. Beugue, D. Bonnet-Delpon, *Tetrahedron* **1991**, *47*, 3207–3253.

[14] Z. Li, G. S. Patil, Z. E. Colubski, H. Hori, K. Tehrani, J. E. Foreman, D. D. Eveleth, R. T. Bartus, J. C. Powers, *J. Med. Chem.* **1993**, *36*, 3472–3480.

[15] Z. Li, A.-C. Ortega-Vilain, G. S. Patil, D.-L. Chu, J. E. Foreman, D. D. Eveleth, J. C. Powers, *J. Med. Chem.* **1996**, *39*, 4089–4098.

[16] S. Mehdi, M. R. Angelastro, J. P. Burkhart, J. R. Kehl, N. P. Peet, P. Bey, *Biochem. Biophys. Res. Commun.* **1990**, *166*, 595–600.

[17] S. Tsutsumi, T. Okonogi, S. Shibahara, S. Ohuchi, E. Hatsushiba, A. A. Patchett, G. Christensen, *J. Med. Chem.* **1994**, *37*, 3492–3502.

[18] a) P. D. Edwards, E. F. Meyer, J. J. Vijayalakshmi, P. A. Tuthill, D. A. Andisik, G. Gomes, A. Strimpler, *J. Am. Chem. Soc.* **1992**, *114*, 1854–1863; b) A. Y. Lee, M. Hagihara, R. Karmacharya, M. W. Albers, S. L. Schreiber, J. Clardy, *J. Am. Chem. Soc.*, **1993**, *115*, 12619–12620; c) K. Hakansson, A. Tulinsky, M. M. Abelman, T. A. Miller, G. P. Vlasuk, P. W. Bergum, M. S. L. Lim-Wilby, T. K. Brunck, *Bioorg. Med. Chem.* **1995**, *3*, 1009–1017.

[19] T.-C. Liang, R. H. Abeles, *Biochemistry*, **1987**, *26*, 7603–7608.

[20] E. Ponnusamy, U. Fotadar, A. Spisni, D. Flat, *Synthesis* **1986**, 48–49.

[21] J. C. Sheehan, P. A. Cruickshank, G. L. Boshart, *J. Org. Chem.* **1961**, *26*, 2525–2528.

[22] G. Kokotos, V. Martin, V. Constantinou-Kokotou, W. A Gibbons, *Amino Acids* **1996**, *11*, 329–343.

[23] a) M. R. Leanna, T. J. Sowin, H. E. Morton, *Tetrahedron Lett.* **1992**, *33*, 5029–5032; b) Z. Ma, J. M. Bobbitt, *J. Org. Chem.* **1991**, *56*, 6110–6114.

[24] G. A. Dale, D. L. Dull, H. S. Mosher, *J. Org. Chem.*, **1969**, *34*, 2543–2549.

[25] Y. Gargouri, S. Ransac, R. Verger, *Biochim. Biophys. Acta* **1997**, *1344*, 6–37.

[26] R. Verger, G. H. de Haas, *Chem. Phys. Lipids* **1973**, *10*, 127–136.

[27] E. Rogalska, C. Cudrey, F. Ferrato, R. Verger, *Chirality* **1993**, *5*, 24–30.

[28] S. Ransac, E. Rogalska, Y. Gargouri, A. M. T. J. Deveer, F. Paltauf, G. H. de Haas, R. Verger, *J. Biol. Chem.* **1990**, *265*, 20263–20270.

[29] R. Verger, G. H. de Haas, L. Sarda, P. Desnuelle, *Biochim. Biophys. Acta* **1969**, *188*, 272–282.

[30] Y. Gargouri, H. Moreau, R. Verger, *Biochim. Biophys. Acta* **1989**, *1006*, 255–271.

Received: January 24, 2000 [F2258]