Novel Trifluoromethyl Ketones as Potent Gastric Lipase Inhibitors

George Kokotos,*[a] Stavroula Kotsovolou,[a] and Robert Verger[b]

Novel inhibitors of human digestive lipases, lipophilic trifluoromethyl ketones, were developed. These analogues of the natural triacylglycerol substrates of lipases were designed to contain the carbonyl group of the trifluoromethyl ketone functionality in place of the carbonyl group of the scissile ester bond at the sn*-1 position. The ester bond at the* sn*-3 position was replaced by an ether bond, while the secondary hydroxy group was either esterified or etherified. The inhibitors were prepared starting from solketal. The inhibition of human pancreatic and gastric lipases by the trifluoromethyl ketones was studied by the monolayer technique. 5,5,5-Trifluoro-1-(dodecyloxymethyl)-4-oxopentyl decanoate is the best synthetic inhibitor of human gastric lipase ever reported (inhibition constant* $\alpha_{50} = 0.003$*).*

KEYWORDS:

enzymes · inhibitors · ketones · lipases · monolayers

Introduction

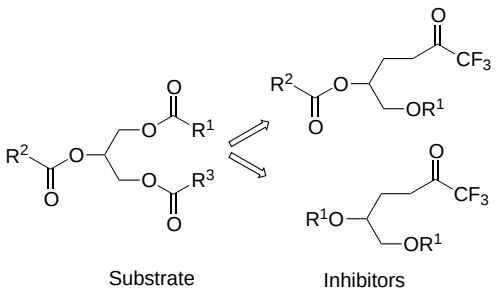
Lipases (triacylglycerol hydrolases, EC 3.1.1.3) constitute a diverse family of enzymes that catalyze the hydrolysis of triacylglycerols (TAGs) and find attractive applications in biotechnology.[1] In humans, gastric and pancreatic lipases play an important role in nutrition processes and are the main enzymes in the digestive tract involved in the hydrolysis of dietary TAGs. The conversion of TAGs into monoacylglycerols and free fatty acids starts in the stomach, where gastric lipase is secreted, and is completed by pancreatic lipase in the small intestine, where absorption of lipolytic products occurs.[2] Therefore, potent and specific inhibitors of digestive lipases are of interest because they may find applications as antiobesity agents. Tetrahydrolipstatin (Orlistat), a β-lactone-containing inhibitor, is now a registered drug for weight reduction.[3]

Synthetic inhibitors of lipases[4] also provide a powerful tool for understanding the molecular mechanisms involved in the catalytic activity of lipases. Both human pancreatic (HPL) and human gastric lipase (HGL) possess a catalytic machinery consisting of a classical catalytic triad (Ser-His-Asp) homologous to that found in serine proteases.[5] A key mechanistic feature of many serine protease inhibitors is the presence of an activated carbonyl group (transition-state isostere) in place of the scissile amide bond.

Rational design of lipase inhibitors requires that the inhibitor consists of two parts: a nucleophilic susceptible function, which may react with the active-site serine residue, and a lipophilic segment containing chemical motifs necessary for specific interactions and proper orientation in the enzyme binding cleft. We have recently demonstrated that lipophilic 2-oxo amides[6] and 2-oxo amide and bis-2-oxo amide triacylglycerol analogues[7] are effective inhibitors of digestive lipases. We now describe the use of the trifluoromethyl ketone group as the reactive functionality of the inhibitor. Peptidyl trifluoromethyl ketones have been reported to inhibit various serine proteases and some of them have entered clinical trials.[8] Various fatty alkyl trifluoromethyl ketones have been reported to inhibit cytosolic phospholipases A_2 (group IVA PLA_2) and calcium-independent phospholipase A_2 (group VI PLA_2),[9] enzymes that contain a Ser residue in their active site.[10]

Results and Discussion

The novel inhibitors were designed taking into consideration the structure of TAGs, which are the natural substrates of lipases (Scheme 1). The ester carbonyl group at the *sn*-1 position of the TAG substrate was replaced by the activated carbonyl group of



Scheme 1. *Structures of inhibitors in comparison with triacylglycerols, the natural substrates of lipases.*

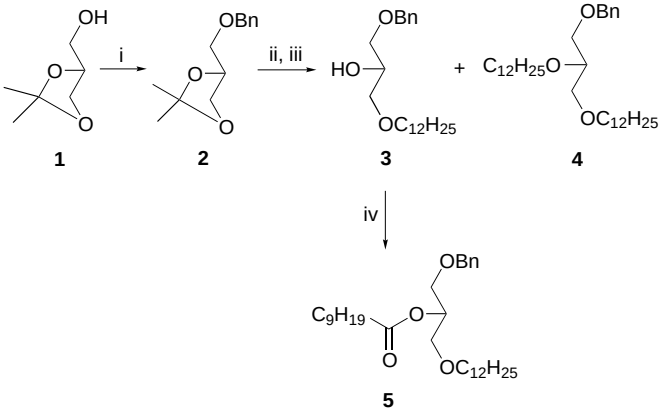
[a] *Prof. G. Kokotos, S. Kotsovolou*
Laboratory of Organic Chemistry
Department of Chemistry, University of Athens
Panepistimiopolis, Athens 15771 (Greece)
Fax: (+30) 210-7274-761
E-mail: gkokotos@cc.uoa.gr

[b] *Dr. R. Verger*
Laboratoire de Lipolyse Enzymatique CNRS-IFR1
UPR 9025, 31 Chemin Joseph-Aiguier
13402 Marseille Cedex 20 (France)

  1439-4227/03/04/01 $ 20.00+.50/0 ChemBioChem **2003**, 4, 90 – 95

the trifluoromethyl ketone functionality. The carboxy ester bond at the *sn*-3 position was replaced by an ether bond, while the carboxy ester bond at the *sn*-2 position was either maintained or replaced by the nonhydrolyzable ether bond. Both digestive lipases show a preference for the hydrolysis of ester bonds at the external positions (*sn*-1 and *sn*-3) of TAGs. Therefore, the carboxy ester bond that involves the secondary hydroxy group is not anticipated to undergo enzymatic hydrolysis.

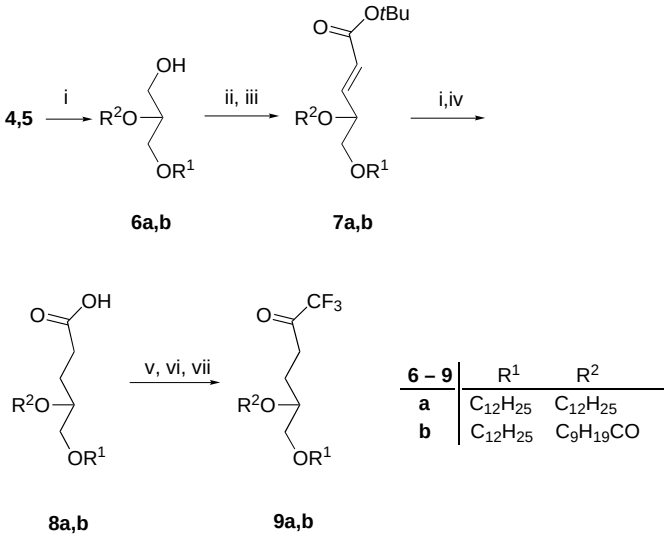
Solketal (**1**) was used as starting material for the synthesis of the target inhibitors. The hydroxy group of **1** was protected by treatment with benzyl bromide and the isopropylidene group was removed (Scheme 2). Etherification with 1-bromododecane



Scheme 2. *i) $C_6H_5CH_2Br$, Bu_4NHSO_4, 50% NaOH/C_6H_6, 45 °C; ii) 4 N HCl/MeOH; iii) $C_{12}H_{25}Br$, Bu_4NHSO_4, 50% NaOH/C_6H_6, 45 °C; iv) $C_9H_{19}COOH$, DCC, DMAP, CH_2Cl_2.*

in a biphasic system of benzene/aqueous sodium hydroxide in the presence of a catalytic amount of Bu_4NHSO_4 produced a mixture of monoalkyl (**3**, 40%) and dialkyl (**4**, 26%) derivatives, which were separated. Compound **3** was then coupled with decanoic acid by using the 1,3-dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) method.[11] The benzyl group of **4** or **5** was removed by catalytic hydrogenation (Scheme 3) and compounds **6a**,**b** were then oxidized to the corresponding aldehydes by NaOCl in the presence of the 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy free radical (AcNH-TEMPO).[12] Wittig olefination of the aldehydes with $Ph_3P{=}CHCOO$*t*Bu produced compounds **7a**,**b**. After catalytic hydrogenation and removal of the *t*Bu group, the carboxylic acids **8a**,**b** were converted into chlorides, which were subsequently converted into trifluoromethyl ketones **9a**,**b** by treatment with $(CF_3CO)_2O$ and C_5H_5N.[13] (*R*)-**9b** was prepared similarly, starting from (*R*)-solketal.

The use of the monolayer technique[14] is advantageous for the study of lipase inhibition since conventional emulsified systems do not allow control of the interfacial quality of the system. However, an accurate kinetic study of the hydrolysis reactions requires that the lipids used form a stable monomolecular film at the air/water interface. Figure 1 shows the molecular area/surface pressure dependency of compounds **9a** and **9b** spread as monomolecular films over a buffered subphase at pH 8.0. The



Scheme 3. *i) H_2, 10% Pd/C; ii) NaOCl, AcNH-TEMPO, NaBr, $PhCH_3$/EtOAc/H_2O, −10 °C; iii) $Ph_3P{=}CHCOO$tBu, THF, 65 °C; iv) 50% CF_3COOH/CH_2Cl_2; v) $(COCl)_2$, DMF, nC_6H_{14}; vi) $(CF_3CO)_2O$, C_5H_5N, 0 °C; vii) H_2O, 0 °C. DMF = dimethylformamide.*

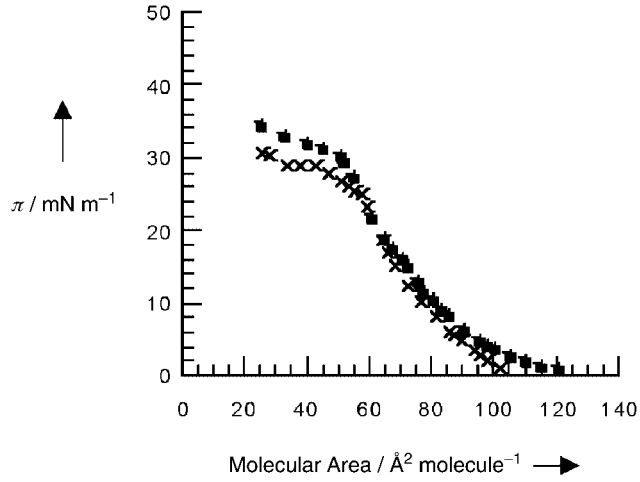


Figure 1. *Molecular area/surface pressure curves for compounds **9a**(×) and **9b**(■). The aqueous subphase was composed of Tris/HCl (10 mM, pH 8), NaCl (150 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The continuous compression experiment was performed in the rectangular reservoir of a "zero order" trough.[14]*

trifluoromethyl ketone derivatives formed stable monomolecular films at the air/water interface.

The inhibition of HPL and HGL was studied by means of the monomolecular film technique[14] with mixed films of 1,2-dicaprin that contained variable proportions of each inhibitor. The inhibition studies were performed at a constant surface pressure of 25 mN m^{-1} for HPL and 27 mN m^{-1} for HGL. Under these experimental conditions, HPL and HGL were active and linear kinetics were recorded.

The remaining lipase activity was plotted as a function of the inhibitor molar fraction (α). The data obtained for HPL and HGL are presented in Figure 2 and Figure 3, respectively. The inhibition constants for all the inhibitors tested, expressed as molar fractions (α_{50}), are summarized in Table 1. The α_{50} value is

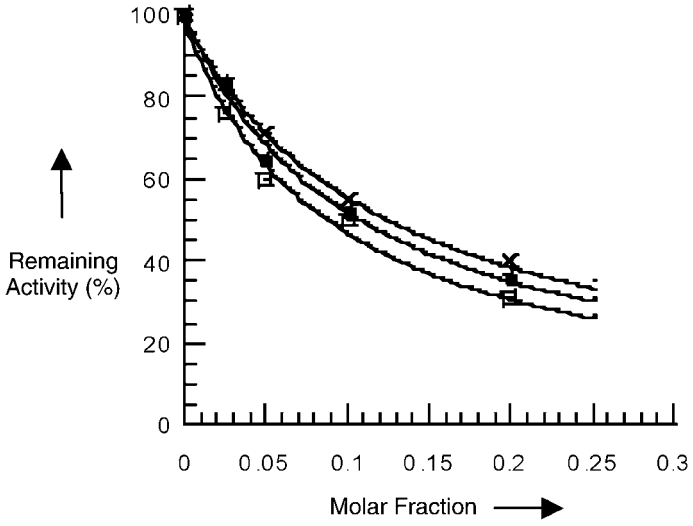


Figure 2. *Effect of increasing concentrations of* ***9a*** *(×),* ***9b*** *(■), and (R)-****9b*** *(□) on the remaining activity of HPL acting upon 1,2-dicaprin monolayers maintained at a constant surface pressure of 25 mN m^{-1}. The aqueous subphase was composed of Tris/HCl (10 mM, pH 8), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of the hydrolysis were recorded over 15 – 20 min.*

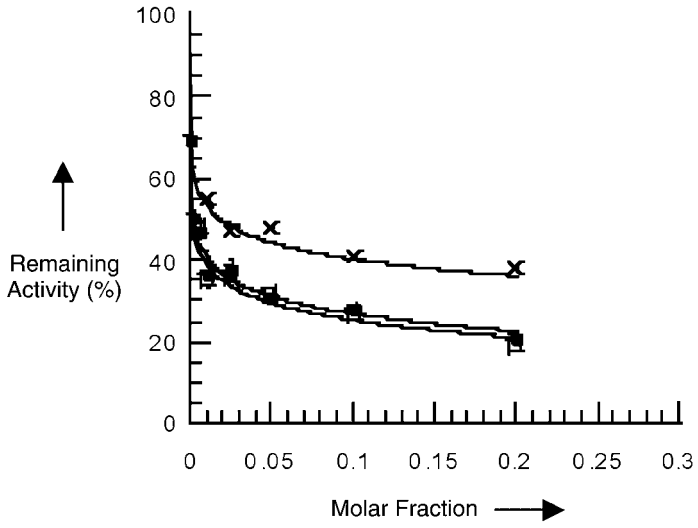


Figure 3. *Effect of increasing concentrations* ***9a*** *(×),* ***9b*** *(■), (R)-****9b*** *(□) on the remaining activity of HGL acting upon 1,2-dicaprin monolayers maintained at a constant surface pressure of 27 mN m^{-1}. The aqueous subphase was composed of CH_3COONa (10 mM, pH 5), NaCl (150 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of the hydrolysis were recorded over 15 – 20 min.*

Table 1. *Inhibition constants (α_{50}) of the trifluoromethylketone inhibitors tested on HPL and HGL with the monolayer technique.*

Compound	α_{50}	
	HPL[a]	HGL[b]
9a	0.111 ± 0.010	0.020 ± 0.004
9b	0.103 ± 0.011	0.003 ± 0.0004
(*R*)-**9b**	0.090 ± 0.007	0.003 ± 0.0004

[a] Surface pressure 25 mN m^{-1}. [b] Surface pressure 27 mN m^{-1}.

defined as the molar fraction of inhibitor that reduces the initial rate of lipolysis by 50%.

As shown by these data, all the trifluoromethyl ketone compounds were found to be potent inhibitors of HGL, whereas they moderately inhibited HPL. Compound **9a** was a fivefold better inhibitor of HGL as compared to HPL, whereas compound **9b** was a 30-fold better inhibitor for HGL. No significant differences in the α_{50} values of the different compounds were observed for HPL. On the contrary, HGL showed a strong preference (sevenfold) for the ester derivatives (**9b** and (*R*)-**9b**) as compared to the ether derivative (**9a**). The observation that a carboxy ester bond involving the secondary hydroxy group (corresponding to the *sn*-2 position of the natural TAG substrates) is important for the inhibition of HGL is in agreement with previous findings on bis-2-oxo amide triacylglycerol analogue inhibitors.[7b] The results obtained with compounds **9b** and (*R*)-**9b** showed that the chirality did not affect their inhibitory effect on either HPL or HGL. It was previously reported[4b] that the best phosphonate inhibitors of HPL and HGL are levorotatory enantiomers and that a highly enantioselective discrimination was observed. However, in this case, the chiral center was at the reactive phosphorus atom, whereas in the present study, the chiral center is the chemically nonreactive asymmetric carbon atom at which the esterified secondary hydroxy group is attached. The trifluoromethyl ketone **9b** is the best synthetic inhibitor of HGL ever reported. It exhibits an α_{50} value equal to that reported for the registered antiobesity drug tetrahydrolipstatin.[15]

In conclusion, we have developed a novel class of potent human gastric lipase inhibitors. The trifluoromethyl keto group is thus a valuable ester bond surrogate for future design and synthesis of powerful inhibitors of lipolytic enzymes.

Experimental Section

General: Solketal, methyl (4*S*)-2,2-dimethyl-1,3-dioxolane-4-carboxylate, and AcNH-TEMPO were purchased from Aldrich. 1,2-Dicaprin was purchased from Sigma. Analytical TLC plates (silica gel 60 F_{254}) and silica gel 60 (70 – 230 mesh) were purchased from Merck. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in ethanol stain. HPL and HGL were purified in the laboratory by using previously described procedures. Et_2O was dried by standard procedures and stored over Na. Et_3N was distilled over ninhydrin. 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. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded in $CDCl_3$ on a Varian Mercury spectrometer operating at 200, 50, 188 MHz, respectively. Chemical shifts for ^{19}F are reported in ppm relative to trifluoroacetic acid (TFA). Mass spectra were obtained on a VG Analytical ZAB-SE instrument. Elemental analyses were performed on a Perkin-Elmer 2400 instrument.

4-[(Benzyloxy)methyl]-2,2-dimethyl-1,3-dioxolan[16] **(2)**: Compound **1** (20 g, 150 mmol) was added to a stirred solution of 50% NaOH (600 mL), benzene (600 mL), Bu_4NHSO_4 (12.7 g, 37.5 mmol), and benzyl bromide (55 mL, 450 mmol). After vigorous stirring for 4 h at 45 – 50 °C, the reaction mixture was allowed to equilibrate to the ambient temperature and EtOAc and water were added. The organic

phase was washed with brine and dried (Na_2SO_4). The product was purified by column chromatography (petroleum ether 40–60 °C, petroleum ether 40–60 °C/EtOAc 8/2). Yield: 20 g (60%); ^{1}H NMR: $\delta = 7.35$ (m, 5H; C_6H_5), 4.60 (s, 2H; $CH_2C_6H_5$), 4.36 (m, 1H; CH), 4.08 (t, $J = 8.5$ Hz, 1H; CHHO), 3.78 (t, $J = 8.5$ Hz, 1H; CHHO), 3.58 (m, 2H; CH_2O), 1.48 (s, 3H; CH_3), 1.42 (s, 3H; CH_3) ppm; ^{13}C NMR: $\delta = 137.7$, 128.2, 127.5, 127.2, 109.0, 74.5, 73.1, 70.7, 66.5, 26.5, 25.1 ppm.

Deprotection and etherification of 2: Compound **2** (20 g, 90 mmol) was treated with 4 N HCl in MeOH (135 mL) for 1 h at room temperature. The solvent and the excess acid were evaporated under reduced pressure and the residue was evaporated twice from Et_2O. The product was added to a stirred solution of 50% NaOH (350 mL), benzene (350 mL), Bu_4NHSO_4 (6.8 g, 20 mmol) and 1-bromododecane (108 mL, 500 mmol). After vigorous stirring for 4 h at 45–50 °C, the reaction mixture was allowed to obtain the ambient temperature and EtOAc and water were added. The organic phase was washed with brine and dried (Na_2SO_4). The products were separated by column chromatography (petroleum ether 40–60 °C, petroleum ether 40–60 °C/EtOAc 12/1, petroleum ether 40–60 °C/EtOAc 7/3).

1-(Benzyloxy)-3-dodecyloxypropan-2-ol[17] (3): Yield: 12 g (40%); ^{1}H NMR: $\delta = 7.35$ (m, 5H; C_6H_5), 4.60 (s, 2H; $CH_2C_6H_5$), 4.00 (m, 1H; CHOH), 3.75–3.38 (m, 6H; $3 \times CH_2O$), 1.62 (m, 2H; CH_2CH_2O), 1.42–1.18 (m, 18H; $9 \times CH_2$), 0.88 (t, $J = 6.2$ Hz, 3H; CH_3) ppm; ^{13}C NMR: $\delta = 137.9$, 128.2, 127.5, 127.2, 73.2, 71.7, 71.5, 71.3, 69.4, 31.8, 29.5, 25.9, 22.5, 14.0 ppm; MS (FAB): *m/z* (%): 373 (100) $[M+Na]^+$, 351 (32) $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{38}O_3$ (350.5): C 75.38, H 10.93; found C 75.14, H 10.99.

Benzyl 2,3-bis(dodecyloxy)propyl ether[18] (4): Yield: 12 g (26%); ^{1}H NMR: $\delta = 7.35$ (m, 5H; C_6H_5), 4.57 (s, 2H; $CH_2C_6H_5$), 3.62–3.41 (m, 9H; CH, $4 \times CH_2O$), 1.58 (m, 4H; $2 \times CH_2CH_2O$), 1.38–1.18 (m, 36H; $18 \times CH_2$), 0.9 (t, $J = 6.8$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 138.4$, 128.2, 127.4, 127.1, 77.8, 73.2, 71.6, 70.6, 70.5, 70.2, 31.9, 30.0, 29.6, 29.5, 29.3, 26.1, 22.6, 14.0 ppm; MS (FAB): *m/z* (%): 519 (22) $[M+H]^+$, 91 (100); elemental analysis calcd (%) for $C_{34}H_{62}O_3$ (518.8): C 78.80, H 12.04; found: C 78.52, H 12.31.

2-(Benzyloxy)-1-(dodecyloxymethyl)ethyl decanoate (5): DCC (8.2 g, 40 mmol) in CH_2Cl_2 (68 mL) was added dropwise to a stirred solution of compound **3** (12 g, 34 mmol), decanoic acid (5.9 g, 34 mmol), and DMAP (1.7 mg, 1.7 mmol) in CH_2Cl_2 (170 mL) at 0 °C. The reaction mixture was kept at 0 °C for 30 min then stirred at room temperature for 24 h, filtered to remove the white precipitate, concentrated under reduced pressure, and purified by column chromatography (petroleum ether 40–60 °C/EtOAc 9/1). Yield: 15 g (89%); ^{1}H NMR: $\delta = 7.35$ (m, 5H; C_6H_5), 5.20 (m, 1H; CHOCO), 4.58 (s, 2H; $CH_2C_6H_5$), 3.64 (m, 4H; $2 \times CH_2O$), 3.41 (m, 2H; CH_2O), 2.35 (t, $J = 7.4$ Hz, 2H; CH_2CO), 1.60 (m, 4H; CH_2CH_2CO, CH_2CH_2O), 1.44–1.18 (m, 30H; $15 \times CH_2$), 0.9 (t, $J = 6.0$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 173.3$, 138.0, 128.2, 127.7, 127.2, 73.1, 71.5, 71.1, 69.1, 68.7, 34.4, 31.8, 29.6, 29.4, 29.2, 29.0, 26.0, 22.6, 14.0 ppm; elemental analysis calcd (%) for $C_{32}H_{56}O_4$ (504.8): C 76.14, H 11.18; found: C 76.48, H 11.25.

General procedure for the removal of the benzyl group: 10% Pd/C catalyst (0.042 g) was added to a solution of compound **4** or **5** (1 mmol) in EtOH (2.5 mL) through which N_2 had been passed for 5 min. The reaction mixture was stirred under H_2 for 5 h at room temperature. The catalyst was removed by filtration through a pad of Celite and the filtrate was evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether 40–60 °C/EtOAc 8/2).

2,3-Bis(dodecyloxy)propan-1-ol[19] (6a): Yield: 0.32 g (75%); m.p.: 37–39 °C; ^{1}H NMR: $\delta = 3.68$–3.37 (m, 9H; $3 \times CH_2O$, CH_2OH, CH), 1.55 (m, 4H; $2 \times CH_2CH_2O$), 1.38–1.15 (m, 36H; $18 \times CH_2$), 0.88 (t, $J = 6.0$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 77.6$, 72.0, 71.0, 70.6, 63.2, 31.9, 29.8, 29.6, 29.5, 29.3, 26.1, 22.6, 14.0 ppm; elemental analysis calcd (%) for $C_{27}H_{56}O_3$ (428.7): C 75.64, H 13.17; found: C 75.99, H 13.51.

2-Hydroxy-1-(dodecyloxymethyl)ethyl decanoate (6b): Yield: 0.24 g (58%); m.p.: 34–36 °C; ^{1}H NMR: $\delta = 4.98$ (m, 1H; CHOCO), 3.79 (d, $J = 6.0$ Hz, 2H; CH_2OH), 3.60 (d, $J = 6{,}0$ Hz, 2H; $CHCH_2O$), 3.4 (t, $J = 6.0$ Hz, 2H; CH_2O), 2.35 (t, $J = 7.4$ Hz, 2H; CH_2CO), 1.58 (m, 4H; CH_2CH_2CO, CH_2CH_2O), 1.45–1.15 (m, 30H; $15 \times CH_2$), 0.88 (t, $J = 6.0$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 173.7$, 72.9, 71.8, 69.9, 62.8, 34.3, 31.8, 29.6, 29.4, 29.3, 29.2, 29.0, 26.0, 25.0, 22.6, 14.1 ppm; elemental analysis calcd (%) for $C_{25}H_{50}O_4$ (414.7): C 72.41, H 12.15; found C 72.79, H 12.52.

General procedure for the preparation of the unsaturated compounds 7a,b: A solution of NaBr (0.12 g, 1.1 mmol) in water (0.5 mL) and subsequently AcNH-TEMPO (2 mg, 0.01 mmol) were added to a solution of compound **6a** or **6b** (1.0 mmol) in a mixture of EtOAc/toluene 1:1 (6 mL) at −10 °C. A solution of NaOCl (0.08 g, 1.1 mmol) and $NaHCO_3$ (0.08 g, 1.0 mmol) in H_2O (0.7 mL) was added dropwise to the resulting biphasic system under vigorous stirring at −10 °C over a period of 15 min. After stirring at −10 °C for 10 min, EtOAc (15 mL) and water (5 mL) were added. The organic layer was washed with 1% aqueous citric acid (10 mL), which contained KI (0.5 g), with 10% aqueous $Na_2S_2O_3$ (10 mL), and with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the aldehyde was used in the next step without any purification. $Ph_3P{=}CHCOOtBu$ (0.37 g, 1.1 mmol) was added to a solution of the aldehyde (0.21 g, 1.0 mmol) in dry THF (5 mL) and the reaction mixture was refluxed for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (petroleum ether 40–60 °C/EtOAc 85/15).

***tert*-Butyl (*E*)-4,5-bis(dodecyloxy)-2-pentenoate (7a)**: Yield: 0.42 g (80%); ^{1}H NMR: $\delta = 6.75$ (dd, $J = 15.8$, $J = 5.8$ Hz, 1H; CH=CHCOO*t*-, 5.97 (dd, $J = 15.8$, $J = 0.8$ Hz, 1H; CH=CHCOO*t*Bu), 4.02 (m, 1H; CH), 3.56–3.35 (m, 6H; $3 \times CH_2O$), 1.60–1.44 (m, 13H, $2 \times CH_2CH_2O$, $C(CH_3)_3$), 1.36–1.13 (m, 36H, $18 \times CH_2$), 0.88 (t, $J = 6.6$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 165.5$, 142.6, 124.2, 80.4, 78.2, 72.9, 71.7, 70.1, 31.9, 29.8, 29.6, 29.4, 29.3, 28.1, 28.0, 26.0, 22.6, 14.0 ppm; MS (FAB): *m/z* (%): 547(5) $[M+Na]^+$, 57 (100); elemental analysis calcd (%) for $C_{33}H_{64}O_4$ (524.9): C 75.52, H 12.29; found: C, 75.78, H, 12.01.

***tert*-Butyl (*E*)-4-(decanoyloxy)-5-(dodecyloxy)-2-pentenoate (7b)**: Yield: 0.29 g (56%); ^{1}H NMR: $\delta = 6.76$ (dd, $J = 15.4$, $J = 5.9$ Hz, 1H; CH=CHCOO*t*Bu), 5.91 (dd, $J = 15.4$, $J = 1.2$ Hz, 1H; CH=CHCOO*t*Bu), 5.58 (m, 1H; CHOCO), 3.59–3.38 (m, 4H; $2 \times CH_2O$), 2.37 (t, $J = 7.4$ Hz, 2H; CH_2CO), 1.76–1.38 (m, 13H; $C(CH_3)_3$, $2 \times CH_2$), 1.37–1.17 (m, 30H; $15 \times CH_2$), 0.88 (t, $J = 6.0$ Hz, 6H; $2 \times CH_3$) ppm; ^{13}C NMR: $\delta = 172.8$, 165.0, 141.6, 124.4, 80.6, 71.6, 71.3, 70.8, 34.3, 31.8, 29.6, 29.4, 29.2, 28.1, 28.0, 26.0, 24.8, 22.6, 14.1 ppm; MS (FAB): *m/z* (%): 533 (15) $[M+Na]^+$, 57 (100); elemental analysis calcd (%) for $C_{31}H_{58}O_5$ (510.8): C 72.89, H 11.45; found: C 72.63, H 11.74.

General procedure for the preparation of the carboxylic acids 8a,b: 10% Pd/C catalyst (0.04 g) was added to a solution of compound **7a** or **7b** (1 mmol) in EtOH (2.5 mL) through which N_2 had been passed for 5 min. The reaction mixture was stirred under H_2 for 24 h at room temperature. The catalyst was removed by filtration through a pad of Celite and the filtrate was evaporated under reduced pressure. The product was purified by column chromatography (petroleum ether 40–60 °C/EtOAc 9/1) and then treated with TFA (3.5 mL) in CH_2Cl_2 (3.5 mL) for 1 h at room temperature. The solvent and the excess acid were evaporated under reduced pressure and the residue was crystallized from Et_2O.

4,5-Bis(dodecyloxy)pentanoic acid (8a): Yield: 0.42 g (90%); m.p.: 39–40 °C; ^{1}H NMR: $\delta = 3.62$ (m, 1H; CH), 3.43 (m, 6H; 3 × CH_2O), 2.48 (t, $J = 7.2$ Hz, 2H; CH_2COOH), 1.85 (m, 2H; CH_2CH_2COOH), 1.55 (m, 4H; 2 × CH_2CH_2O), 1.40–1.09 (m, 36H; 18 × CH_2), 0.88 (t, $J = 6.0$ Hz, 6H; 2 × CH_3) ppm; ^{13}C NMR: $\delta = 179.5$, 77.4, 73.0, 71.6, 70.4, 31.9, 30.0, 29.6, 29.5, 29.3, 28.1, 28.0, 26.1, 22.7, 14.0 ppm; MS (FAB): *m/z* (%): 493 (30) $[M+Na]^+$, 471 (25) $[M+H]^+$; elemental analysis calcd (%) for $C_{29}H_{58}O_4$ (470.8): C 73.99, H 12.42; found: C 74.11, H 12.69.

4-(Decanoyloxy)-5-(dodecyloxy)pentanoic acid (8b): Yield: 0.41 g (90%); m.p.: 42–43 °C; ^{1}H NMR: $\delta = 5.03$ (m, 1H; CHOCO), 3.41 (m, 4H; 2 × CH_2O), 2.37 (m, 4H; 2 × CH_2CO), 1.98 (m, 2H; CH_2CH_2COOH), 1.54 (m, 4H; CH_2CH_2COO, CH_2CH_2O), 1.40–1.02 (m, 30H; 15 × CH_2), 0.88 (t, $J = 6.0$ Hz, 6H; 2 × CH_3) ppm; ^{13}C NMR: $\delta = 176.4$, 173.2, 71.3, 71.0, 65.6, 34.2, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0, 25.6, 24.8, 22.5, 14.9 ppm; MS (FAB): *m/z* (%): 479 (30) $[M+Na]^+$, 457 (35) $[M+H]^+$; elemental analysis calcd (%) for $C_{27}H_{52}O_5 \cdot 0.5\,H_2O$ (465.7): C 69.63, H 11.47; found: C 69.55, H 11.68.

General procedure for the synthesis of the trifluoromethyl ketones 9a,b: DMF (40 μL) and oxalyl chloride (0.42 mL, 4.8 mmol) were added to a solution of carboxylic acid **8a** or **8b** (1.0 mmol) in hexane (40 mL). The solution was stirred for 1 h at room temperature. After filtration of the white solid and evaporation a colorless oil was produced (acid chloride), which was solubilized in CH_2Cl_2 (10 mL). TFAA (0.84 mL, 6.0 mmol) and dry pyridine (0.62 mL, 8.0 mmol) were added dropwise to this solution at 0 °C consecutively. The solution was stirred vigorously for 30 min and then H_2O (2 mL) was added. The solution remained for 30 min at 20 °C and was then washed with a solution of brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product was purified by column chromatography (CH_2Cl_2).

1,1,1-Trifluoro-5,6-bis(dodecyloxy)-2-hexanone (9a): Yield: 0.29 g (55%); ^{1}H NMR: $\delta = 3.58–3.32$ (m, 7H; CH, 3 × CH_2O), 2.85 (t, $J = 7.0$ Hz, 2H; CH_2CO), 1.89 (m, 2H; CH_2CH_2CO), 1.54 (m, 4H; 2 × CH_2CH_2O), 1.41–1.12 (m, 36H; 18 × CH_2), 0.88 (t, $J = 6.0$ Hz, 6H; 2 × CH_3) ppm; ^{13}C NMR: $\delta = 191.0$, 115.3, 77.4, 72.6, 71.7, 70.2, 34.3, 31.9, 29.6, 29.5, 29.3, 26.1, 22.7, 14.1 ppm; ^{19}F NMR: $\delta = -1.4$ (CF_3); MS (FAB): *m/z* (%): 523 (12) $[M+H]^+$, 337 (8), 169 (9); elemental analysis calcd (%) for $C_{30}H_{57}F_3O_3$ (522.8): C 68.93, H 10.99; found: C 69.22, H 11.27.

5,5,5-Trifluoro-1-(dodecyloxymethyl)-4-oxopentyl decanoate (9b): Yield: 0.20 g (40%); ^{1}H NMR: $\delta = 5.04$ (m, 1H; CHOCO), 3.51–3.37 (m, 4H; 2 × CH_2O), 2.78 (t, $J = 7.2$ Hz, 2H; CH_2COCF_3), 2.30 (t, $J = 7.1$ Hz, 2H; CH_2COO), 2.02 (m, 2H; $CH_2CH_2COCF_3$), 1.67–1.44 (m, 4H; CH_2CH_2COO, CH_2CH_2O), 1.42–1.12 (m, 30H; 15 × CH_2), 0.88 (t, $J = 6.0$ Hz, 6H; 2 × CH_3) ppm; ^{13}C NMR: $\delta = 191.2$, 173.4, 115.3, 71.7, 71.4, 70.7, 34.3, 32.3, 31.9, 29.6, 29.4, 29.2, 26.0, 25.0, 22.7, 14.1 ppm; ^{19}F NMR: $\delta = -1.4$ (CF_3). MS (FAB): *m/z* (%): 509 (6) $[M+H]^+$, 323 (20), 169 (13); elemental analysis calcd (%) for $C_{28}H_{51}F_3O_4$ (508.7): C 66.11, H 10.11; found: C 66.12, H 10.47.

(*R*)-9b: Spectroscopic data identical to those obtained for compound **9b**. $[\alpha]_D = 6.3$ ($c = 1.5$ in EtOAc).

Monomolecular film experiments: Surface pressure–area curves were measured in the rectangular reservoir compartment of a "zero order" trough (14.8 cm wide and 24.9 cm long). Before each experiment the trough was 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 double-distilled water. The lipidic film was spread with a Hamilton syringe as a solution in $CHCl_3$ (approximately 1 mg mL^{-1}) over an aqueous subphase of tris(hydroxymethyl)aminomethane (Tris)/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and ethylenediaminetetraacetate (EDTA; 1 mM). The above buffer solution was prepared with double-distilled water and filtered through a 0.22-μm Millipore membrane. Before each utilization, residual surface-active impurities were removed by sweeping and suction. The force/area curves were automatically recorded at a continuous compression rate of 4.8 cm min^{-1}.

Enzyme kinetics experiments: The inhibition experiments were performed by using the monolayer technique. The surface pressure of the lipid film was measured by the platinum Wilhelmy plate technique coupled with an electromicrobalance. The principle of this method has been described previously by Verger et al.[14a]

The method of "mixed monomolecular films" was used for the inhibition studies. This method involves the use of a "zero-order" trough that consists 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. HPL (final concentration 7.4 ng mL^{-1}) and HGL (final concentration 81 ng mL^{-1}) were injected into the subphase of the reaction compartment, where efficient stirring was applied. In the case of HPL the aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), ($CaCl_2$ 21 mM), and (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), and EDTA (1 mM). When the surface pressure decreased as a result of the lipolytic action of the enzyme, a mobile barrier moved over the reservoir compartment to compress the film and thus keep the surface pressure constant. The surface pressure was measured in 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. Linear kinetics were obtained in all cases. Each experiment was duplicated.

This work was supported by the Greek Secretariat for Research and Technology and the French Foreign Ministry.

[1] a) R. D. Schmid, R. Verger, *Angew. Chem.* **1998**, *112*, 1694–1720; *Angew. Chem. Int. Ed.* **1998**, *37*, 1608–1633; b) U. T. Bornsheurer, R. J. Kazlauskas, *Hydrolases in Organic Synthesis*, Wiley-VCH, Weinheim, **1999**; c) M. T. Reetz, S. Wilensek, D. Zha, K.-E. Jaeger, *Angew. Chem.* **2001**, *113*, 3701–3703; *Angew. Chem. Int. Ed.* **2001**, *40*, 3589–3591.

[2] a) F. Carrière, J. A. Barrowman, R. Verger, R. Laugier, *Gastroenterology* **1993**, *105*, 876–888; b) F. Carrière, C. Renou, V. Lopez, J. De Caro, F. Ferrato, H. Lengsfeld, A. De Caro, R. Laugier, R. Verger, *Gastroenterology* **2000**, *119*, 949–960.

[3] a) J. B. Hauptman, F. S. Jeunet, D. Hartmann, *Am. J. Clin. Nutr.* **1992**, *55*, 309–313; b) N. Finer, W. P. T. James, P. G. Kopelman, M. E. J. Lean, G. Williams, *Int. J. Obes.* **2000**, *24*, 306–313.

[4] For reviews, see: a) Y. Gargouri, S. Ransac, R. Verger, *Biochim. Biophys. Acta* **1997**, *1344*, 6–37; b) J.-F. Cavalier, G. Buono, R. Verger, *Acc. Chem. Res.* **2000**, *33*, 579–589.

[5] a) M.-P. Egloff, F. Marguet, G. Buono, R. Verger, C. Cambillau, H. van Tilbeurgh, *Biochemistry* **1995**, *34*, 2751–2762; b) A. Roussel, S. Canaan, M.-P. Egloff, M. Riviere, L. Dupuis, R. Verger, C. Cambillau, *J. Biol. Chem.* **1999**, *274*, 16995–17002.

[6] a) A. Chiou, T. Markidis, V. Constantinou-Kokotou, R. Verger, G. Kokotos, *Org. Lett.* **2000**, *2*, 347–350; b) A. Chiou, R. Verger, G. Kokotos, *Lipids* **2001**, *36*, 535–542.

[7] a) G. Kokotos, R. Verger, A. Chiou, *Chem. Eur. J.* **2000**, *6*, 4211–4217; b) S. Kotsovolou, A. Chiou, R. Verger, G. Kokotos, *J. Org. Chem.* **2001**, *66*, 962–967.

[8] For a review, see: D. Leung, G. Abbenante, D. P. Fairlie, *J. Med. Chem.* **2000**, *43*, 305–341.

[9] D. A. Six, E. A. Dennis, *Biochim. Biophys. Acta* **2000**, *1488*, 1 – 19.
[10] a) E. J. Ackermann, K. Conde-Frieboes, E. A. Dennis, *J. Biol. Chem.* **1995**, *270*, 445 – 450; b) F. Ghomashchi, R. Loo, J. Balsinde, F. Bartoli, R. Apitz-Castro, J. D. Clark, E. A. Dennis, M. H. Gelb, *Biochim. Biophys. Acta* **1999**, *1420*, 45 – 56.
[11] B. Neises, W. Steglich, *Angew. Chem.* **1978**, *17*, 556 – 557; *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 522 – 524.
[12] M. R. Leanna, T. J. Sowin, H. E. Morton, *Tetrahedron Lett.* **1992**, *33*, 5029 – 5032.
[13] J. Boivin, L. El Kaim, S. Z. Zard, *Tetrahedron* **1995**, *51*, 2573 – 2584.
[14] a) R. Verger, G. H. de Haas, *Chem. Phys. Lipids* **1973**, *10*, 127 – 136; b) S. Ransac, M. G. Ivanova, R. Verger, I. Panaitov, *Methods Enzymol.* **1997**, *286*, 263 – 292.
[15] S. Ransac, Y. Gargouri, H. Moreau, R. Verger, *Eur. J. Biochem.* **1991**, *202*, 395 – 400.
[16] R. J. Howe, T. Malkin, *J. Chem. Soc.* **1951**, 2663 – 2667.
[17] F. Bauer, K.-P. Ruess, M. Lieflaender, *Liebigs Ann. Chem.* **1991**, 765 – 768.
[18] H. C. Berk, K. E. Zwickelmaier, J. E. Franz, *Synth. Commun.* **1985**, *15*, 57 – 60.
[19] F. Paltauf, F. Spencer, *Chem. Phys. Lipids* **1968**, *2*, 168 – 172.

Received: July 8, 2002 [F 451]