Sterically Hindered Triacylglycerol Analogues as Potent Inhibitors of Human Digestive Lipases

Violetta Constantinou-Kokotou,*[a] Victoria Magrioti,[a] and Robert Verger[b]

Abstract: A novel class of inhibitors of human digestive lipases have been developed. Various sterically hindered triacylglycerols based on 2-methyl- and 2-butylglycerol, and/or 2-methyl fatty acids were synthesized. The triacylglycerol analogues 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 human pancreatic and gastric lipases by the sterically hindered triacylglycerol analogues was studied by using the monolayer technique with mixed films of 1,2-dicaprin, which contained variable proportions of each inhibitor. Triolein analogues that contain a butyl group at the 2-position of the glycerol backbone or methyl groups both at the 2-position of glycerol, and the α-position of each oleic acid residue were potent inhibitors; this caused a 50% decrease in HPL activity at 0.003 molar fraction.

Keywords: enzyme inhibitors · enzymes · lipids · monolayers · triacylglycerol analogues

Introduction

Lipids constitute a large part of the biomass of living organisms, and lipolytic enzymes play an essential role in the turnover of this material. In particular, triglycerides (triacylglycerols, TAGs) constitute the major part (95%) of dietary lipids, which are present in the human diet at a quantity of 100–150 g per day in industrially developed countries. Human pancreatic (HPL) and gastric (HGL) lipases are essential enzymes for efficient fat digestion.[1] The hydrolysis of dietary TAGs by these enzymes to form monoacylglycerols and free fatty acids is a necessary step for fat absorption by the enterocytes. Therefore, potent and specific inhibitors of digestive lipases are of interest, because they may find applications as anti-obesity agents. The β-lactone that contains the inhibitor tetrahydrolipstatin is already in clinical use for the treatment of obesity.[2] Phosphonate-type inhibitors have been found to irreversibly inactivate HPL and HGL as well as microbial lipases.[3] Recently a strategy for the rational design of lipase inhibitors was developed, which is based on the incorporation of an activated carbonyl group in a triacylglycerol structure. Thus, lipophilic 2-oxo amides,[4] aldehydes,[5] and trifluoromethylketones,[6] as well as 2-oxo amide and bis(2-oxo)amide triacylglycerol analogues[7] have been studied by the monolayer technique, and have been shown to inhibit HPL and HGL. In this work, we propose a novel approach for the development of lipolytic enzyme inhibitors. We present the synthesis of a new class of sterically hindered triacylglycerol analogues, the study of their surface properties, and their inhibitory effect on HPL and HGL activities; these were measured by the monolayer technique.

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Results and Discussion

Design: To develop novel potent inhibitors of digestive lipases, we decided to maintain the natural substrate backbone, which contains three ester bonds, and to replace a hydrogen atom by incorporating an alkyl group at the carbon atom of the *sn*-2 position or/and at the α-carbon atom of the acyl residue (Scheme 1). The rationale behind the present design was to create structures that closely resembled natural lipase substrates and give steric hindrance around the scissile ester bonds. The replacement of the hydrogen atom of glycerol at the *sn*-2 position by a methyl or a butyl group, and the presence of a methyl group at the carbon atom next to the carbonyl group may reduce the hydrolysis rate of such a triacylglycerol.

Synthesis: 2-Methyl-2-propen-1-ol (**1**) was used as a starting material for the synthesis of triacylglycerol analogues **3a–e**. Dihydroxylation of **1** was performed by treatment with H_2O_2 in H_2O in the presence of a catalytic amount of

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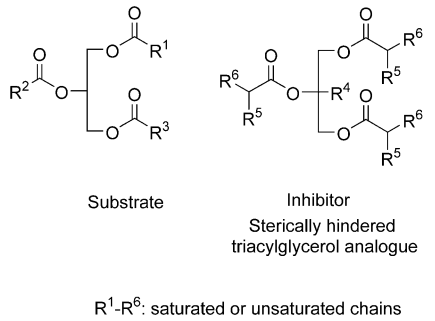
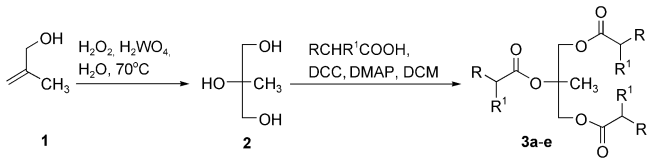


Scheme 1. General structure of the sterically hindered triacylglycerol analogues that were synthesized.

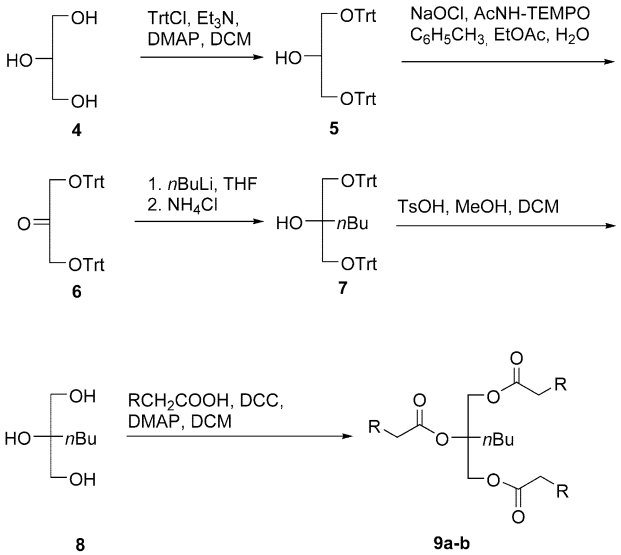
H_2WO_4[8] (Scheme 2). 2-Methylglycerol (**2**) was used as the key intermediate for the synthesis of triesters **3a–e**. Glycerol **4** was used as a starting material for the synthesis of 2-butyl-



3	R	R¹
a	$C_6H_{12}CH{=}CHC_8H_{17}$	H
b	$C_6H_{12}CH{=}CHC_8H_{17}$	CH_3
c	C_8H_{17}	H
d	C_8H_{17}	CH_3
e	$C_{14}H_{29}$	H

Scheme 2. Synthesis of triacylglycerol analogues based on 2-methylglycerol.

glycerol (**8**) (Scheme 3). The two primary hydroxy groups of **4** were protected by the trityl group, and consequently the secondary hydroxy group was oxidized to the corresponding ketone **6** by NaOCl in the presence of 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy free radical (AcNH-TEMPO).[9] Reaction of ketone **6** with *n*BuLi at 0°C under a nitrogen atmosphere produced the protected 2-butylglycerol (**7**), which was deprotected by treatment with $TsOH{\cdot}H_2O$.[10] 2-Butylglycerol (**8**) was used as a starting material for the synthesis of triesters **9a–b**.

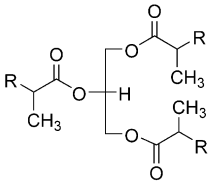


9	R
a	$C_6H_{12}CH{=}CHC_8H_{17}$
b	C_8H_{17}

Scheme 3. Synthesis of triacylglycerol analogues based on 2-butylglycerol.

2-Methyldecanoic acid and 2-methyloleic acid were prepared from decanoic and oleic acid, respectively. The fatty acid was first treated by lithium diisopropylamide (LDA), then 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU), and finally methyl iodide were added to produce the corresponding branched fatty acid.[11]

Triesters **3a–e**, **9a–b**, and **10a–b** were prepared by treatment of the appropriate alcohol, **2**, **8,** and **4**, respectively, with five equivalents of the appropriate acid, and dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine (DMAP).[12] In the case of triacylglycerols **10a–b** (Scheme 4), quantitative esterification was achieved after an



10	R
a	$C_6H_{12}CH{=}CHC_8H_{17}$
b	C_8H_{17}

Scheme 4. Synthesis of triacylglycerol analogues based on glycerol.

overnight treatment. However, in the case of triesters **3a–e** and **9a–b,** stirring for several days was required for esterification of the tertiary hydroxy group.

All intermediates and final products gave satisfactory analytical and spectroscopic data.

Abstract in Greek:

Στην παρούσα εργασία παρουσιάζεται μια νέα τάξη αναστολέων των ανθρώπινων λιπασών της πέψης. Έγινε η σύνθεση μιας σειράς στερεοχημικά παρεμποδισμένων τριακυλογλυκερολών, η δομή των οποίων βασίστηκε στη 2-μεθυλο- και τη 2-βουτυλο-γλυκερόλη και/ή σε 2-μεθυλο-λιπαρά οξέα. Αυτά τα ανάλογα τριακυλογλυκερολών μελετήθηκαν ως προς την ικανότητα τους να σχηματίζουν σταθερά μονομοριακά υμένια στη μεσεπιφάνεια αέρα/νερού με καταγραφή των ισόθερμων καμπυλών επιφανειακής πίεσης/μοριακής επιφάνειας. Η αναστολή που προκαλούν οι ενώσεις αυτές στην ανθρώπινη παγκρεατική και γαστρική λιπάση μελετήθηκε με την τεχνική της μονοστοιβάδας και με τη χρήση μικτών μονομοριακών υμενίων 1,2-δικαπρίνης που περιείχαν τον κάθε αναστολέα σε διάφορες αναλογίες. Το ανάλογο τριολεΐνης που περιέχει μια βουτυλο-ομάδα στη θέση 2- της γλυκερόλης, καθώς και το ανάλογο τριολεΐνης που περιέχει μια μεθυλο-ομάδα στη θέση 2- της γλυκερόλης και στην α-θέση κάθε τμήματος ελαϊκού οξέος, είναι ισχυροί αναστολείς, προκαλώντας 50% ελάττωση στη δραστικότητα της ανθρώπινης παγκρεατικής λιπάσης σε μοριακό κλάσμα 0,003.

Force/area curves of triacylglycerol analogues: The use of the monolayer technique, which is based upon a decrease in surface-pressure due to lipid–film hydrolysis, is advantageous for the study of lipases inhibitors, since with conventional emulsified systems it is not possible to control the "interfacial quality".[13] The kinetic studies of the lipase hydrolysis reactions requires the lipids used as substrates to form a stable monomolecular film at the air/water interface. Alternatively, lipolytic products should desorb rapidly from the interface.[13]

To determine the film stability and the interfacial properties at the air/water interface of the synthesized triacylglycerol analogues, we recorded their force/area curves. The experiments were performed in the reservoir compartment of a "zero-order" trough. A force/area curve was obtained after a small volume of lipid solution, in a volatile solvent, was spread at the air/water or argon/water interface. Moving a mobile barrier at a constant rate progressively reduced the surface of the trough, and the surface pressure was continuously recorded during compression.

Figure 1 shows the molecular area dependency for compounds **3a**, **3b**, **9a**, **10a,** and the natural substrate triolein as

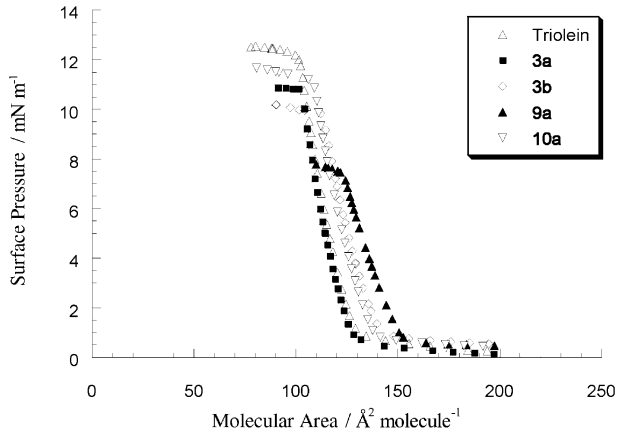


Figure 1. Force/area curves of the triolein analogues. The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The continuous compression experiments were performed in the rectangular reservoir of the zero-order trough.

a function of the surface pressure of a film spread over a buffered subphase at pH 8.0. The surface behavior of these compounds was measured at an argon/water interface to avoid the oxidation of the double bond. By comparing this series of triolein analogues at a given surface pressure value, we observed an increase in the molecular area occupied by these compounds as the size or number of branched groups increased. For example, at its collapse pressure of 7.4 mN m^{-1}, the molecular area of **9a** with a butyl group at the *sn*-2 position is 122 Å^2, whereas the molecular area occupied by **3a** with a methyl group at the *sn*-2 position is 109 Å^2. Also, the molecular area of **3b** is 112 Å^2 in comparison with the molecular area of **10a**, which is 106 Å^2 at the collapse pressure of each compound. As a reference value, under the same experimental conditions, the molecular area of triolein at its collapse pressure was found to be 98 Å^2, in accordance with the literature data (Table 1).[14,15] The collapse pressure of the films of the triolein analogues decreased as the size or number of branched groups increased. The collapse pressure for compound **9a** (7.5 mN m^{-1}) was

Table 1. Collapse pressures and molecular areas of triacylglycerol analogues.

Compound	Collapse Pressure [mN m^{-1}]	Molecular Area at the Collapse Pressure [Å^2 molecule^{-1}]
Triolein	12.2	98
3a	10.8	100
3b	9.9	112
9a	7.5	122
10a	11.2	106
Tricaprin	18.9	78
3c	17.9	71
3d	15.3	96
9b	13.2	106
10b	17.4	88

much lower than the collapse pressure of compound **3a** (10.8 mN m^{-1}) and triolein (13 mN m^{-1}), which was found to be the highest of the series. This behavior was to be expected, since the alkyl substitutions in the triolein analogues increased the hydrophobic character of triolein.

Similar conclusions can also be drawn when comparing the molecular area dependency for compounds **3c**, **3d**, **9b**, **10b**, and tricaprin as a function of the surface pressure (Figure 2). By comparing this series of tricaprin analogues, an increase in the molecular area was also observed with the size and number of branched groups. For example, at 13.3 mN m^{-1}, the molecular area of **9b** is 106 Å^2, whereas the molecular area of **3d** is 102 Å^2.

Accordingly, the collapse pressure decreased as the size or number of branched groups increased. For example, the collapse pressure of compound **9b** (13.2 mN m^{-1}), was much lower compared with the collapse pressure of compound **3c** (17.9 mN m^{-1}). As expected, when the series of trolein ana-

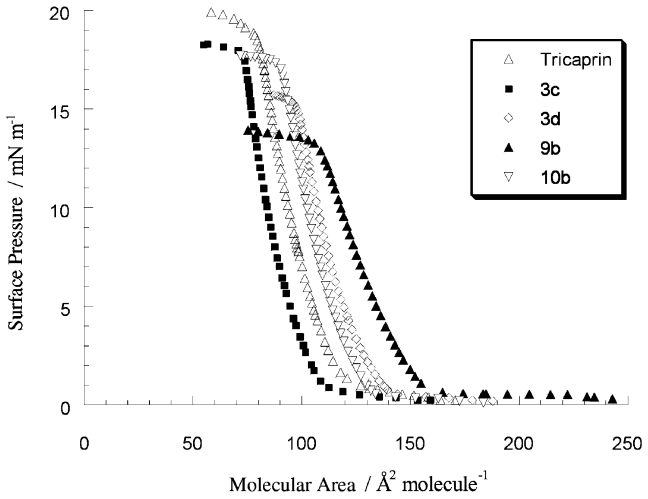


Figure 2. Force/area curves of the tricaprin analogues. The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The continuous compression experiments were performed in the rectangular reservoir of the zero-order trough.

logues and tricaprin analogues were compared, a decrease in molecular area, and an increase in the collapse pressure was observed when the length of the acyl chain was decreased.

Pancreatic and gastric lipase activity on monomolecular films that contain triacylglycerol analogues: The inhibition of HPL and HGL was studied by means of the monomolecular film technique with mixed films of 1,2-dicaprin that contain variable proportions of each synthetic triacylglycerol analogue.[13] The inhibition studies for HPL were performed at a constant surface pressure of 14 mN m^{-1} for the triolein analogues, and 17 mN m^{-1} for the tricaprin analogues. The inhibition studies for HGL were performed at a constant surface pressure of 17 mN m^{-1} for triolein analogues and 20 mN m^{-1} for the tricaprin analogues. At these surface pressure values, HPL and HGL were fully active and linear kinetics were recorded. These surface pressures were chosen to be slightly higher than the collapse pressure of the films for the pure triacylglycerol analogues. It is known that in a mixed film of 1,2-dicaprin that contains a small fraction of inhibitor, the film is stabilized even at a surface pressure higher than the collapse pressure value of the pure inhibitor.

The inhibition of lipolytic enzymes are best quantified in terms of surface molar fraction of inhibitor.[4–7,13] Thus, remaining lipase activity was plotted as a function of the surface molar-fraction (α) of inhibitor. The data that was obtained for HPL, by using the triolein analogues and the tricaprin analogues, are presented in Figure 3 and 4, respectively. A 50% decrease of HPL activity was observed when 0.003 (**9a**), 0.003 (**3b**), and 0.008 (**3a**) molar fractions (α_{50}) of the inhibitors were mixed with a monolayer of 1,2-dicaprin. The α_{50} is defined as the molar fraction of inhibitor which reduces by 50% of the initial rate of lipolysis. It is important to note that when we studied the inhibitory effect of **3a** on HPL at 17 mN m^{-1}, we observed an even higher inhibitory effect ($\alpha_{50}=0.006$). This observation indicates that at high surface pressures, the inhibitory capacity of the sterically hindered triglyceride analogues could be improved. The molar fraction values (α_{50}) for HPL obtained with all the triacylglycerol analogues are summarized in Table 2.

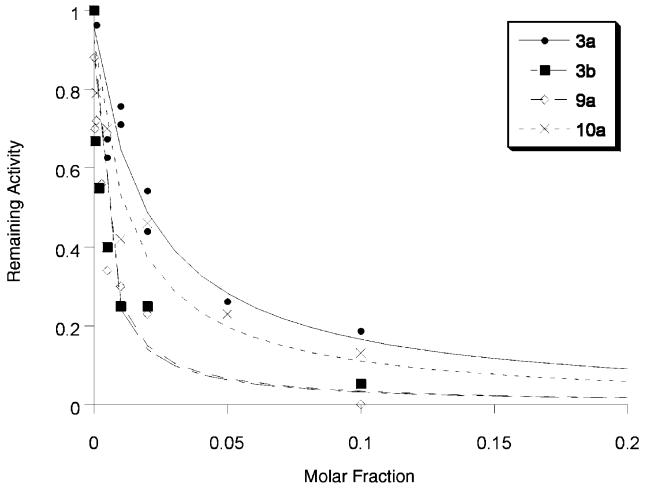


Figure 3. Effect of increasing the concentration of triolein analogues on the remaining activity of HPL on the 1,2-dicaprin monolayer maintained at a constant surface pressure (14 mN m^{-1}). The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of hydrolysis were recorded for 20 min.

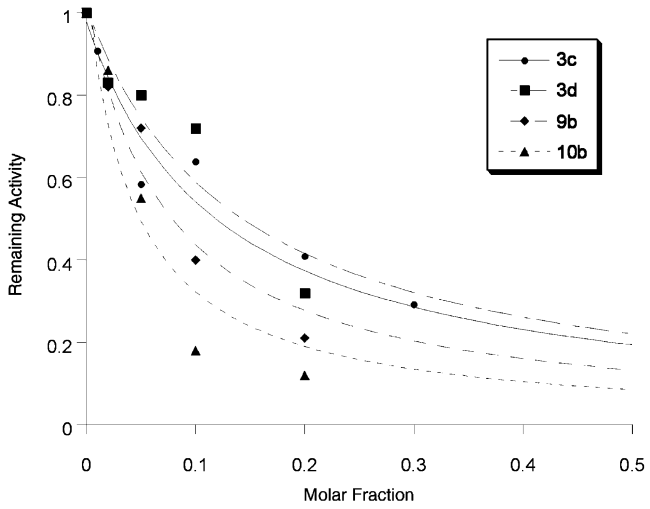


Figure 4. Effect of increasing the concentration of tricaprin analogues on the remaining activity of HPL on the 1,2-dicaprin monolayer maintained at a constant surface pressure (17 mN m^{-1}). The aqueous subphase was composed of Tris/HCl (10 mM, pH 8.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of hydrolysis were recorded for 20 min.

All the compounds were also tested as potential inhibitors of HGL, and the data obtained with triolein and tricaprin analogues, are presented in Figure 5 and 6, respectively. The molar fraction values (α_{50}) for HGL obtained with all the triacylglycerol analogues are summarized in Table 3. A 50% decrease of HGL activity was observed when 0.009 (**9a**) and 0.017 (**3b**) molar fractions of the inhibitors were mixed with a monolayer of 1,2-dicaprin.

Table 2. Inhibition values of triacylglycerol analogues on HPL with the monolayer technique.

Compound [mN m^{-1}]	Surface Pressure	α_{50} [%]
3a	14	0.008
3a	17	0.006
3b	14	0.003
9a	14	0.003
10a	14	0.012
3c	17	0.118
3d	17	0.049
9b	17	0.143
10b	17	0.078

As shown from the data, the length of the acyl chain influences the potency of the inhibition. For both HPL and HGL the presence of oleoyl chains (C18:1) gives rise to a better inhibition capacity relative to the decanoyl chain (C10:0). Comparison of the data obtained for compounds **9a** and **9b** shows that the change from an oleoyl chain to a decanoyl chain may result in up to a 50-fold decrease in the α_{50} value for HPL. Both digestive lipases seem to be better inhibited with the most hindered molecules, such as compounds **9a** (one butyl group at the glycerol C-2), and **3b** (one methyl

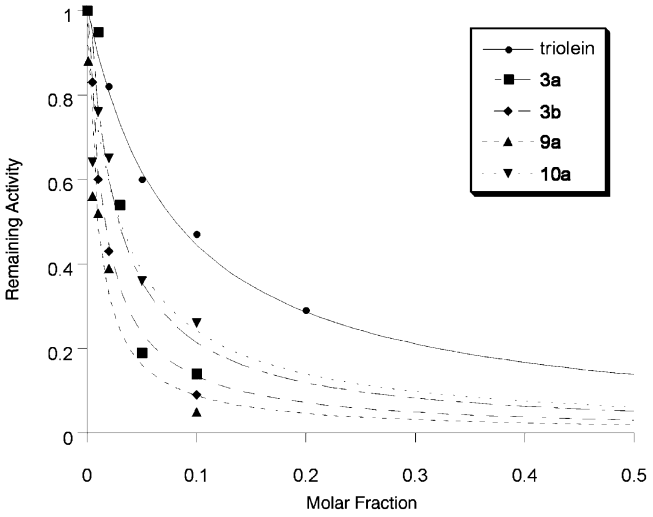


Figure 5. Effect of increasing the concentration of triolein analogues on the remaining activity of HGL on the 1,2-dicaprin monolayer maintained at a constant surface pressure (17 mN m^{-1}). The aqueous subphase was composed of CH_3COONa (10 mM, pH 5.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of hydrolysis were recorded for 20 min.

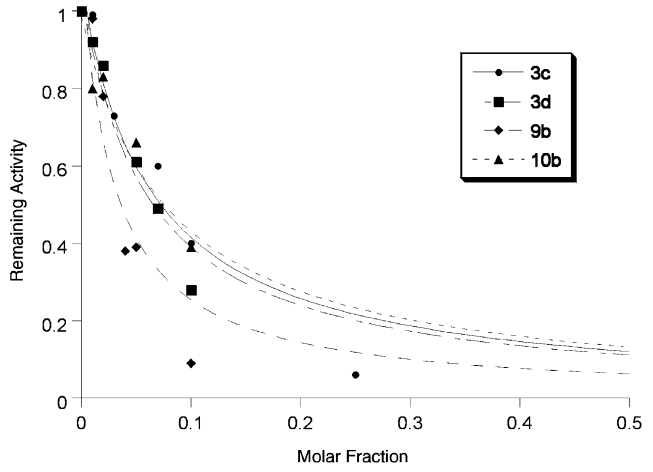


Figure 6. Effect of increasing the concentration of tricaprin analogues on the remaining activity of HGL on the 1,2-dicaprin monolayer maintained at a constant surface pressure (20 mN m^{-1}). The aqueous subphase was composed of CH_3COONa (10 mM, pH 5.0), NaCl (100 mM), $CaCl_2$ (21 mM), and EDTA (1 mM). The kinetics of hydrolysis were recorded for 20 min.

Table 3. Inhibition values of triacylglycerol analogues on HGL with the monolayer technique.

Compound	Surface Pressure [mN m^{-1}]	α_{50} [%]
3a	17	0.029
3b	17	0.017
9a	17	0.009
10a	17	0.028
3c	20	0.071
3d	20	0.055
9b	20	0.036
10b	20	0.078

group at C-2 together with one methyl group at the α-position of each acyl chain).

Among all the compounds tested in this study, the triolein analogue **9a**, bearing a butyl group at the 2-position of the glycerol backbone, was shown to be the most potent inhibitor, which caused a 50% decrease in HPL and HGL activity at 0.003 and 0.009 molar fractions, respectively. The triolein analogue **3b**, bearing methyl groups both at the 2-position of the glycerol, and at the α-position of each oleoyl chain, presented an equal inhibitory activity against HPL, though lower (α_{50} 0.017) against HGL. The triolein analogue **3a** based on 2-methylglycerol, also proved to be a potent inhibitor of HPL and HGL, which caused a 50% decrease in their activity at 0.008 and 0.029 molar fraction, respectively.

On comparing the series of triolein analogues, it seems that the analogue that contains a methyl group at the 2-position of the glycerol, presents a comparable inhibition level against both HPL and HGL as compared to the analogue that contains a methyl group at the α-position of each oleoyl residue. It is clear that the introduction of a methyl substituent, both at the 2-position and α-position, resulted in molecules with enhanced inhibitory potency. However, the presence of a butyl group at the 2-position causes an equal (for HPL) or even better (for HGL) inhibitory effect relative to the methyl substituent.

The tricaprin analogues are relatively weak inhibitors for HPL and showed a better inhibitory capacity against HGL. This observation is in line with the known short and medium chain specificity of HGL. Among the series of tricaprin analogues, compound **9b** that contains 2-butylglycerol backbone exhibited the most potent inhibition capacity (α_{50} 0.036) against HGL.

Up to now, the best synthetic inhibitor of HPL reported in the literature is *O*-hexadecyl-*O*-(p-nitrophenyl) *n*-undecyl phosphonate, with an α_{50} value of 0.003.[16] When we tested this compound by using HPL under our experimental conditions (at 15 mN m^{-1}), it exhibited an α_{50} value of 0.006 molar fraction. In the case of HGL, the highest inhibition was obtained with *O*-undecyl-*O*-(p-nitrophenyl) *n*-decyl phosphonate, which exhibited an α_{50} value of 0.008.[16] From the present study, the triolein analogue **9a**, bearing the butyl group at the 2-position, shows a similar inhibitory effect relative to the most potent synthetic inhibitors reported for HPL and HGL.[16]

During the early seventies it was demonstrated that synthetic triacylglycerols, substituted by a methyl group either at the α-position of the acyl residue or at the C-2 position of the glycerol backbone, presented reduced ability to be hydrolyzed by porcine pancreatic lipase by using a simple monolayer assay.[17] Using the pH-stat titration assay, it was also reported that 2-methylglycerol trioleate was hydrolyzed at a relative rate of 1% relative to triolein.[18] Taking into account the fact that tetrahydrolipstatin bears a lactone ring, it comes as no surprise that poor lipase substrates (that contain a carboxylic ester function) behave as potent competitive lipase inhibitors.[19] For the first time, we demonstrated by using the monolayer technique, that triolein analogues containing a methyl or a butyl group at the *sn*-2 position of the glycerol backbone, or/and at the α-position of the acyl

residue, act as potent inhibitors of human pancreatic and gastric lipases. It is apparent that the development of steric hindrance around the ester bonds of triacylglycerols results in competitive inhibitors of digestive lipases.

Conclusion

By using the monolayer technique we have demonstrated that sterically hindered triacylglycerol analogues are potent human digestive lipase inhibitors. These triglyceride analogues are easily prepared and are chemically similar to the natural substrates of lipases. Our results indicate that an alkyl group at the *sn*-2 position of the glycerol backbone, or/and at the α-position of the acyl residue are important substituents for the design and synthesis of potent inhibitors of digestive lipases. This might be useful for the inhibition of the hydrolysis and absorption of dietary fats in humans, which could be used as agents for the treatment of obesity.

Experimental Section

Materials and methods: Compounds 2-methyl-2-propen-1-ol, decanoic acid, oleic acid, hexadecanoic acid, AcNH-TEMPO, and silica gel (230–400 mesh) 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. HPL, co-lipase, and HGL were purified in the laboratory by using previously described procedures by Verger et al.[13] THF, toluene, and Et_2O were dried by standard procedures, and stored over molecular sieves or Na. Petroleum ether was used and had a b.p. of 40–60 °C. Et_3N was distilled from ninydrin. All other solvents were of reagent grade and used without further purification. Melting points were determined on a Büchi 530 apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR spectroscopy, and COSY (correlation spectroscopy) were obtained in $CDCl_3$, except when otherwise noted, by using a Varian Mercury (200 MHz) spectrometer. FAB mass spectra were obtained on a VG 70-SE mass spectrometer (Manchester, UK). Elemental analyses were performed on a Perkin–Elmer 2400 instrument.

2-Methyl-1,2,3-propenetriol (2): Alcohol **1** (10 g, 139 mmol) and H_2WO_4 (263 mg) were dissolved in twice-distilled H_2O (10 mL) by heating to 70 °C. The temperature was maintained at 70–77 °C by the addition of 30 % H_2O_2 (exothermic). After a consumption of 18.3 g, the reaction mixture was kept for 1 h at 75 °C, and in order to destroy residual H_2O_2, for another 2.5 h at 97 °C. The reaction mixture was cooled to room temperature, filtered, and passed through a Dowex 1-X4 column (27 mL; OH^- form; equilibrated in twice-distilled H_2O) in order to remove H_2WO_4. The eluate was set to pH 6.0 by adding some drops of 30 % H_2SO_4, concentrated, and it was then dried in vacuo to yield **2** (7.3 g, 49 %) as a yellowish opaque oil; ^{1}H NMR (D_2O): $\delta = 3.28$ (s, 4H; CH_2), 0.93 ppm (s, 3H; CH_3); ^{1}H NMR (CD_3OD): $\delta = 4.88$ (b, 1H; OH), 3.40 (s, 4H; CH_2), 1.09 ppm (s, 3H; CH_3); ^{13}C NMR (D_2O): $\delta = 73.2$ (COH), 65.9 (CH_2OH), 19.8 ppm (CH_3); MS (FAB): *m*/*z* (%): 75 (100) [$C_3H_7O_2^+$], 57 (82) [$C_3H_5O^+$], 43 (40) [$C_2H_3O^+$]; elemental analysis calcd (%) for $C_4H_{10}O_3$ (106.1): C 45.27, H 9.50; found: C 45.02, H 9.56.

1,3-Bis(triphenylmethoxy)-2-propanol (5): Trityl chloride (5.0 g, 18 mmol), triethylamine (3.3 mL, 24 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (122 mg, 1.0 mmol) was added to a mixture of glycerol **4** (920 mg, 10 mmol) in dichloromethane (50 mL). The mixture was stirred at room temperature overnight. The organic layer was washed with brine, 10 % citric acid, brine, 5 % $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated in vacuo to give a yellowish solid. The residue was purified by column chromatography by using petroleum ether/EtOAc 9:1 and 8:2 as an eluent to yield **5** (2.85 g, 55 %) as a yellowish solid. M.p. 160–163 °C; ^{1}H NMR: $\delta = 7.20$–7.50 (m, 30H; aromatic CH), 3.98 (m, 1H; C*H*OH), 3.31 (m, 4H; CH_2O), 2.15 ppm (d, $J = 5.6$ Hz, 1H; OH); ^{13}C NMR: $\delta = 143.8$ (aromatic C), 126.8–128.9 (aromatic CH), 86.6 ($C(C_6H_5)_3$), 70.2 (CHOH), 64.5 ppm (CH_2O); elemental analysis calcd (%) for $C_{41}H_{36}O_3$ (576.723): C 85.39, H 6.29; found C 85.23, H 6.38.

1,3-Bis(triphenylmethoxy)-2-propanone (6): A solution of NaBr (590 mg, 5.7 mmol) in water (2.6 mL), and subsequently AcNH-TEMPO (10.6 mg, 0.05 mmol) was added at 0 °C to a solution of compound **5** (3.0 g, 2.6 mmol) in CH_2Cl_2 (15.6 mL). A solution of NaOCl (0.21 g, 2.86 mmol), and $NaHCO_3$ (1.3 g, 15.6 mmol) in H_2O (5 mL) was added dropwise to the resulting biphasic system at 0 °C over a period of 1 h under vigorous stirring. After stirring for 30 min at room temperature, EtOAc (50 mL) and water (20 mL) were added. The organic layer was washed with 10 % aqueous citric acid (25 mL), which contained KI (0.15 g), 10 % aqueous $Na_2S_2O_3$ (25 mL), brine, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography by using petroleum ether/EtOAc 8/2 to yield **6** (2.77 g, 93 %) as a yellowish solid. M.p 183–184 °C; ^{1}H NMR: $\delta = 7.20$–7.40 (m, 30H; aromatic CH), 3.97 ppm (s, 4H; CH_2O); ^{13}C NMR: $\delta =$ 204.6 (CO), 143.1 (aromatic C), 127.5–128.9 (aromatic CH), 87.3 [$C(C_6H_5)_3$], 68.9 ppm (CH_2O); elemental analysis calcd (%) for $C_{41}H_{34}O_3$ (574.7): C 85.69, H 5.96; found C 85.78, H 5.86.

1-(Triphenylmethoxy)-2-[(triphenylmethoxy)methyl]-2-hexanol (7): A solution of *n*BuLi in hexane (2.0 M, 3.0 mL, 6.0 mmol) was added to a solution of compound **6** (2.6 g, 45 mmol) in dry THF (18 mL) at 0 °C under a nitrogen atmosphere. The solution was stirred for an additional 10 min, and then poured into a saturated solution of NH_4Cl. The aqueous layer was washed three times with Et_2O, and then the organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography by using petroleum ether/dichloromethane/EtOAc 85:10:5 to yield **7** (1.8 g, 63 %) as a yellow oil; ^{1}H NMR: $\delta = 7.20$–7.50 (m, 30H; aromatic CH), 3.31 (m, 4H; CH_2O), 2.33 (s, 1H; OH); 1.40–1.60 (m, 2H; CCH_2), 1.10–1.35 (m, 4H; CH_2), 0.89 ppm (m, 3H; CH_3); ^{13}C NMR: $\delta = 143.8$ (aromatic C), 126.9–128.9 (aromatic CH), 86.5 ($C(C_6H_5)_3$), 74.0 (COH), 65.6 (CH_2O), 34.3 (CH_2C), 24.1 (CH_2), 23.2 (CH_2), 14.0 ppm (CH_3); elemental analysis calcd (%) for $C_{45}H_{44}O_3$ (632.8): C 85.41, H 7.01; found C 85.63, H 6.85.

2-Butyl-1,2,3-propanetriol (8): *p*TsOH·H_2O (12 mg, 0.06 mmol) was added to a solution of compound **7** (300 mg, 0.47 mmol) in methanol (4.0 mL), and dichloromethane (10 mL), the solution was then stirred overnight. The solvents were evaporated and water was added. The aqueous layer was washed three times with EtOAc and was concentrated in vacuo to give a yellowish oil. The residue was passed through a short plug af silica gel with chloroform, and then chloroform/methanol 9:1. The solvents were concentrated in vacuo to yield **8** (29 mg, 42 %) as a yellow oil; ^{1}H NMR: $\delta = 3.52$–3.85 (m, 6H; CH_2O, 2 × OH), 2.65 (b, 1H; OH), 1.20–1.52 (m, 6H; 3 × CH_2), 0.92 ppm (d, $J = 6.8$ Hz, 3H; CH_3); ^{13}C NMR: $\delta = 74.2$ (COH), 66.8 (CH_2O), 34.4 (CH_2C), 25.1 (CH_2), 23.3 (CH_2), 14.0 ppm (CH_3); elemental analysis calcd (%) for $C_7H_{16}O_3$ (148.2): C 56.73, H 10.88; found C 56.55, H 10.93.

General procedure for the synthesis of 2-methyl acids: A solution of diisopropylamine (4.12, 29 mmol) in anhydrous THF (25 mL) was charged in a flask under a nitrogen atmosphere. After cooling at 0 °C, *n*-butyllithium (18.1 mL, 29 mmol, 1.6 M in hexane) was slowly added to keep the temperature at 0 °C. After standing at this temperature for an additional 20 min, the appropriate acid (14 mmol) was added dropwise, while maintaining the reaction temperature below 0 °C. A milky-white solution formed, and after 30 min, DMPU (1.7 mL, 14 mmol) was added, the mixture was then stirred at room temperature for 1 h. It was then cooled again at 0 °C, and CH_3I (0.9 mL, 15 mmol) was added rapidly. The reaction was completed by stirring the mixture at room temperature overnight. The product was recovered by neutralization with ice, cooled 10 % HCl, followed by extraction with Et_2O (3 × 30 mL). 444 The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to obtain the corresponding 2-methyl acid.

2-Methyldecanoic acid: Yellowish oil. Yield 2.10 g (80 %); ^{1}H NMR: $\delta =$ 10.50 (b, 1H; COOH), 2.30–2.50 (m, 1H; CH), 1.58–1.80 (m, 2H; CH_2CH), 1.20–1.55 (m, 12H; 6 × CH_2), 1.15–1.20 (m, 3H; CHCH_3), 0.88–1.00 ppm (m, 3H; CH_2CH_3); ^{13}C NMR: $\delta = 183.6$ (COOH), 39.4 (CH), 33.5 (CH_2), 31.9 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 22.6 (CH_2), 16.8 (CHCH_3), 14.1 ppm (CH_2CH_3); elemental

analysis calcd (%) for $C_{11}H_{22}O_2$ (186.3): C 70.92, H 11.90; found: C 71.03, H 11.69.

(*Z*)-2-Methyl-9-octadecenoic acid: Yellow oil. Yield 3.40 g (82%); ^{1}H NMR: δ = 11.50 (b, 1H; COOH), 5.33 (m, 2H; CH=), 2.30–2.50 (m, 1H; CH), 1.95–2.15 (m, 4H; CH_2CH=), 1.58–1.80 (m, 2H; CH_2CH), 1.25–1.55 (m, 20H; 10×CH_2), 1.15–1.20 (m, 3H; CHCH_3), 0.88–1.00 ppm (m, 3CH; CH_2CH_3); ^{13}C NMR: δ = 183.6 (COOH), 130.0 (CH=), 129.7 (CH=), 39.4 (CH), 34.1 (CH_2), 33.5 (CH_2), 31.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 22.7 (CH_2), 16.8 (CHCH_3), 14.1 ppm (CH_2CH_3); elemental analysis calcd (%) for $C_{19}H_{39}O_2$ (296.5): C 76.97, H 12.24; found: C 76.83, H 12.32.

General procedure for the synthesis of triacylglycerol analogues 3a–e, 9a–b, and 10a–b: The appropriate acid (50 mmol) was added in a solution of the appropriate alcohol (10 mmol) in dichloromethane (65 mL), and the solution was cooled to 0°C. Then, a catalytic amount of DMAP was added (122 mg, 1.0 mmol), followed by the addition of a solution of DCC (10.3 g, 50 mmol) in dichloromethane (60 mL). The solution was stirred for 30 min at 0°C for several nights at room temperature. The reaction mixture was filtered through a small pad of celite, and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography by using petroleum ether/Et_2O 95:5, and 9:1 as an eluent.

2-Methyl-2,3-bis{[(*Z*)-1-oxo-8-heptadecenyl]oxy}propyl ester of 9-octadecenoic acid, (*Z*)-(3a): Yellowish oil. Yield 7.01 g (78%); ^{1}H NMR: δ = 5.33 (6H, *m*, 3×CH_2), 4.31 (dd, ^1J = 11.4 Hz, ^2J = 25.4 Hz, 4H; 2×CH_2O), 2.30 (m, 6H; 3×CH_2), 1.99 (m, 12H; 6×CH_2), 1.62 (m, 6H; 3×CH_2), 1.49 (s, 3H; CH_3), 1.27 (s, 72H; 36×CH_2), 0.87 ppm (t, J = 6.2 Hz, 9H; 3×CH_3); ^{13}C NMR: δ = 173.0 (CH), 172.7 (CO), 130.1 (CH), 130.0 (CH), 129.8 (CH), 79.6 (C), 64.7 (CH_2), 35.1 (CH_2), 34.1 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 24.9 (CH_2), 22.6 (CH_2), 18.9 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{58}H_{106}O_6$ (899.5): C 77.45, H 11.88; found: C 77.38, H 12.05.

2-Methyl-2,3-bis{[(*Z*)-2-methyl-1-oxo-8-heptadecenyl]oxy}propyl ester of 9-octadecenoic acid, (*Z*)-(3b): Yellowish oil. Yield 4.52 g (48%); ^{1}H NMR: δ = 5.33 (m, 6H; 3×CH=), 4.33 (m, 4H; 2×CH_2O), 2.30–2.50 (m, 3H; 3×CH), 1.90–2.10 (s, 12H; 6×CH_2CH=), 1.58–1.80 (m, 6H; 3×CH_2CH), 1.25–1.55 (m, 60H; 30×CH_2), 1.15–1.20 (m, 9H; 3×CHCH_3), 0.88–1.00 ppm (m, 9H; 3×$CH_2C H_3$); ^{13}C NMR: δ = 176.0 (CO), 175.6 (CO), 130.0 (CH=), 129.6 (CH=), 79.6 (C), 64.6 (CH_2O), 40.3 (CH), 39.5 (CH), 33.6 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 24.9 (CH_2), 22.7 (CH_2), 18.9 (CH_3), 17.0 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{61}H_{112}O_6$ (941.5): C 77.81, H 11.99; found: C 77.69, H 12.07.

2-Methyl-2,3-bis[(1-oxodecyl)oxy]propyl ester of decanoic acid, (3c): Yellowish oil. Yield 4.15 g (73%); ^{1}H NMR: δ = 4.33 (dd, ^1J = 11.6 Hz, ^2J = 25.2 Hz, 4H; 2×CH_2O), 2.32 (m, 6H; 3×CH_2), 1.61 (m, 6H; 3×CH_2), 1.51 (s, 3H; CH_3), 1.27 (b, 42H; 21×CH_2), 0.88 ppm (t, 9H J = 6.4 Hz; 3×CH_3); ^{13}C NMR: δ = 173.1 (CO), 79.6 (C), 64.9 (CH_2), 64.7 (CH_2), 35.1 (CH_2), 34.1 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 24.1 (CH_2), 22.6 (CH_2), 18.9 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{34}H_{64}O_6$ (568.9): C 71.79, H 11.34; found: C 71.63, H 11.38.

2-Methyl-2,3-bis[(2-methyl-1-oxodecyl)oxy]propyl ester of decanoic acid, (3d): Yellowish oil. Yield 3.48 g (57%); ^{1}H NMR: δ = 4.35 (m, 4H; 2×CH_2O), 2.35–2.55 (m, 3H; 3×CH), 1.58–1.75 (m, 6H; 3×CH_2CH), 1.51 (s, 3H; CH_3), 1.20–1.48 (m, 36H; 18×CH_2), 1.05–1.20 (m, 9H; 3×CHCH_3), 0.88–1.00 ppm (m, 9H; 3×$CH_2C H_3$); ^{13}C NMR: δ = 176.0 (CO), 79.6 (C), 64.6 (CH_2O), 40.3 (CH), 39.5 (CH), 33.7 (CH_2), 31.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.2 (CH_2), 22.6 (CH_2), 18.9 (CH_3), 17.0 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{37}H_{70}O_6$ (610.9): C 72.74, H 11.55; found: C 72.59, H 11.63.

2-{[(*Z*)-1-oxo-8-heptadecenyl]oxy}-2-{{[(*Z*)-1-oxo-8-heptadecenyl]oxy}-methyl}hexyl ester of 9-octadecenoic acid, (*Z*)-(9a): Yellowish oil. Yield 4.22 g (69%); ^{1}H NMR: δ = 5.33 (m, 6H; 3×CH=), 4.35 (dd, ^1J = 11.8 Hz, ^2J = 31.2 Hz, 4H; 2×CH_2O), 2.22–2.50 (m, 6H; 3×CH_2CO), 1.85–2.10 (m, 14H; 6×CH_2CH=, CCH_2), 1.55–1.70 (m, 6H; 3×CH_2), 1.15–1.45 (m, 74H; 37×CH_2), 0.88 ppm (m, 12H; 4×CH_3); ^{13}C NMR: δ = 173.0 (CH), 172.5 (CO), 130.0 (CH), 81.8 (C), 63.0 (CH_2O), 34.2 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 24.9 (CH_2), 24.7 (CH_2), 22.7 (CH_2), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{61}H_{112}O_6$ (941.5): C 77.81, H 11.99; found: C 77.89, H 11.93.

1,1-Bis{[(1-oxodecyl)oxy]methyl}pentyl ester of decanoic acid, (9b): Yellowish oil. Yield 4.22 g (69%); ^{1}H NMR: δ = 4.37 (dd, ^1J = 11.8 Hz, ^2J = 31.2 Hz, 4H; 2×CH_2O), 2.22–2.45 (m, 6H; 3×CH_2CO), 1.85–2.10 (m, 2H; CCH_2), 1.50–1.70 (m, 6H; 3×CH_2), 1.15–1.45 (m, 40H; 20×CH_2), 0.88 ppm (m, 12H; 4×CH_3); ^{13}C NMR: δ = 180.0 (CO), 173.8 (CH), 81.6 (C), 66.5 (CH_2O), 34.5 (CH_2), 34.2 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 24.9 (CH_2), 24.6 (CH_2), 23.1 (CH_2), 22.6 (CH_2), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{37}H_{70}O_6$ (610.9): C 72.74, H 11.55; found: C 72.89, H 11.43.

2-Methyl-2,3-bis{[(*Z*)-2-methyl-1-oxo-8-heptadecenyl]oxy}propyl ester of 9-octadecenoic acid, (*Z*)-(10a): Yellowish oil. Yield 6.68 g (72%); ^{1}H NMR: δ = 5.25–5.45 (m, 7H; 3×CH=, CHO), 4.35 (m, 4H; 2×CH_2O), 2.30–2.55 (m, 3H; 3×CH), 1.90–2.10 (s, 12H; 6×CH_2CH=), 1.58–1.75 (m, 6H; 3×CH_2CH), 1.25–1.55 (m, 60H; 30×CH_2), 1.15–1.20 (m, 9H; 3×CHCH_3), 0.88–1.00 ppm (m, 9H; 3×$CH_2C H_3$); ^{13}C NMR: δ = 176.0 (CO), 130.0 (CH=), 129.7 (CH=), 68.9 (CHO), 62.0 (CH_2O), 39.5 (CH), 39.4 (CH), 33.6 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 22.7 (CH_2), 16.9 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{60}H_{110}O_6$ (927.5): C 77.70, H 11.95; found: C 77.59, H 12.03.

2-Methyl-2,3-bis[(1-oxodecyl)oxy]propyl ester of decanoic acid, (10b): Yellowish oil. Yield 4.78 g (80%); ^{1}H NMR: δ = 5.38 (m, 1H; CHO), 4.35 (m, 4H; 2×CH_2O), 2.35–2.55 (m, 3H; 3×CH), 1.55–1.75 (m, 6H; 3×CH_2CH), 1.20–1.50 (m, 36H; 18×CH_2), 1.10–1.20 (m, 9H; 3×CHCH_3), 0.88–1.00 ppm (m, 9H; 3×$CH_2C H_3$); ^{13}C NMR: δ = 176.2 (CO), 68.8 (CHO), 62.1 (CH_2), 39.5 (CH), 39.4 (CH), 33.6 (CH_2), 31.8 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.2 (CH_2), 22.6 (CH_2), 16.9 (CH_3), 14.1 ppm (CH_3); elemental analysis calcd (%) for $C_{36}H_{68}O_6$ (596.9): C 72.44, H 11.48; found: C 72.59, H 11.35.

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 twice-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), and EDTA (1 mM). The above-mentioned buffer solution was prepared with twice-distilled water, and filtered through a 0.22 μm millipore membrane. Before each utilization, residual surface-active impurities were removed by sweeping and suction of the surface. The force/area curves were automatically recorded upon a continuous compression rate at 0.5 cm min^{-1}.

Enzyme kinetic experiments: The inhibition experiments were performed by using the monolayer technique. The surface pressure of the lipid film was measured by means of the platinum Wilhelmy plate technique coupled with an electromicrobalance. The principle of this method has been described previously by Verger et al.[13]

For the inhibition studies the method of "mixed monomolecular films" was used. This method involved the use of a "zero-order" trough, which consisted of two compartments: a reaction compartment, in which mixed films of substrate and inhibitor are spread, and a reservoir compartment, in which only pure films of substrate are spread. The two compartments are connected to each other by narrow surface channels. HPL (final concentration 8.3 and 4.8 ng mL^{-1} for 14 and 17 mN m^{-1}, respectively), and HGL (final concentration 113 and 56 ng mL^{-1} for 17 and 20 mN m^{-1}, respectively) were injected into the subphase of the reaction compartment, in which 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). Due to the lipolytic action of the enzyme, the surface pressure decreased, and a mobile barrier moved over the reservoir compartment to compress the film, and thus kept the surface pressure constant. The surface pressure was measured on the reservoir compartment. The surface and volume of the reaction compartment was 100 cm^2 and 120 mL, repsectively. 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.

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