Triacylglycerols Based on 2-(*N*-*tert*-Butoxycarbonylamino)oleic Acid Are Potent Inhibitors of Pancreatic Lipase

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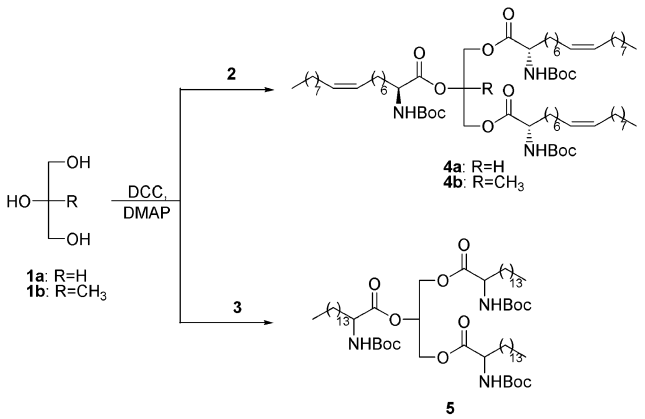
Abstract: A novel class of potent human pancreatic lipase (HPL) inhibitors was developed. Triacylglycerol analogues containing 2-(*N*-*tert*-butoxycarbonylamino) fatty acids were synthesized, and their ability to form stable films at the air/water interface was studied. The inhibition of human digestive lipases by the compounds synthesized was studied by the monolayer technique, and the triesters of glycerol and 2-methylglycerol with 2-(*N*-*tert*-butoxycarbonylamino)oleic acid were found to be potent inhibitors of HPL.

Introduction. In humans, pancreatic (HPL) and gastric (HGL) lipases play an important role in nutrition processes and are the main enzymes in the digestive tract involved in the hydrolysis of dietary triacylglycerols (TAGs). The conversion of TAGs into diacylglycerols, 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 the absorption of lipolytic products occurs.[1] Therefore, potent and specific inhibitors of digestive lipases are of interest because they may find applications as antiobesity agents. The β-lactone-containing lipase inhibitor tetrahydrolipstatin (Orlistat) is now a registered drug for weight reduction.[2] Synthetic phosphonate-type inhibitors of digestive lipases, though not suitable for medical use, provide a powerful tool for understanding the molecular mechanisms involved in the catalytic activity of lipases.[3] In the past few years a number of electrophilic inhibitors of digestive lipases have been reported. The activity of various triacylglycerol analogues containing activated carbonyl groups, such as 2-oxoamide,[4−7] aldehyde,[8] trifluoromethyl ketone[9] against HPL and HGL has been studied by the monolayer technique.

In the present communication we demonstrate that triacylglycerols containing (*N*-*tert*-butoxycarbonylamino)oleic acid are potent inhibitors of pancreatic lipase. The rationale behind the design of these inhibitors was to maintain the backbone of the natural substrate (triolein) of lipases increasing the steric hindrance around the scissile ester bonds. The introduction of a *tert*-butoxycarbonylamino group at the α-carbon atom of the oleic acid residue results in a highly increased steric hindrance around the ester group, which is attacked by the nucleophilic hydroxyl of the lipase active site serine. The synthesis of the novel inhibitors, the study of their interfacial properties, and the inhibitory activities against HPL and HGL, measured by the monolayer technique, are presented in this work.

Scheme 1



2: (2*S*,9*Z*)-$CH_3(CH_2)_7CH{=}CH(CH_2)_6CH(NHBoc)COOH$;
3: $CH_3(CH_2)_{13}CH(NHBoc)COOH$

Chemistry. The synthesis of (*S*)-2-aminooleic acid starting from either methyl (2*S*)-2-[bis(*tert*-butoxycarbonylamino)]-5-oxopentanoate or *tert*-butyl (2*S*)-2-[bis-(*tert*-butoxycarbonylamino)]-5-oxopentanoate has been recently reported.[10,11] (2*S*,9*Z*)-2-(*tert*-Butoxycarbonylamino)octadec-9-enoic acid (**2**) was prepared by treatment of (*S*)-2-aminooleic acid with di-*tert*-butyl dicarbonate.[12] Glycerol (**1a**) and 2-methyl-glycerol (**1b**)[13] were esterified with **2** using dicyclohexylcarbodiimide (DCC) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (Scheme 1).[14] Triacylglycerol **5** was similarly synthesized using racemic 2-(*tert*-butyloxycarbonylamino)hexadecanoic acid (**3**). The products were fully characterized by ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, and elemental analysis.[15]

Force/Area Curves of 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 lipases inhibitors since with conventional emulsified systems it is not possible to control the "interfacial quality".[16] The kinetic studies of the lipase hydrolysis reactions require that the lipids used form a stable monomolecular film at the air/water interface.

To determine the film stability and the interfacial properties at the air/water 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 increase was continuously recorded during compression.

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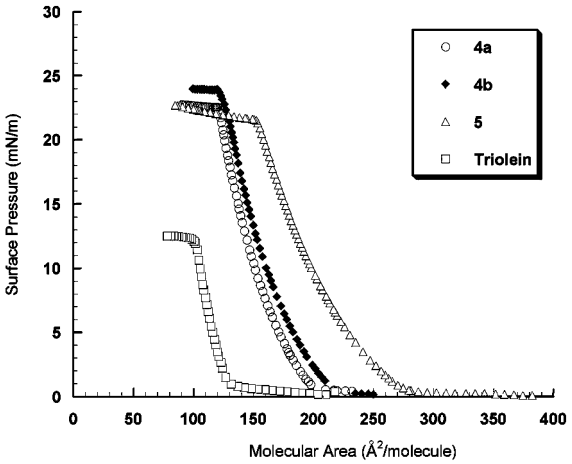
Chart 1. Force/Area Curves of the Triacylglycerol Analogues **4a**, **4b**, **5**, and Triolein[a]



[a] 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.

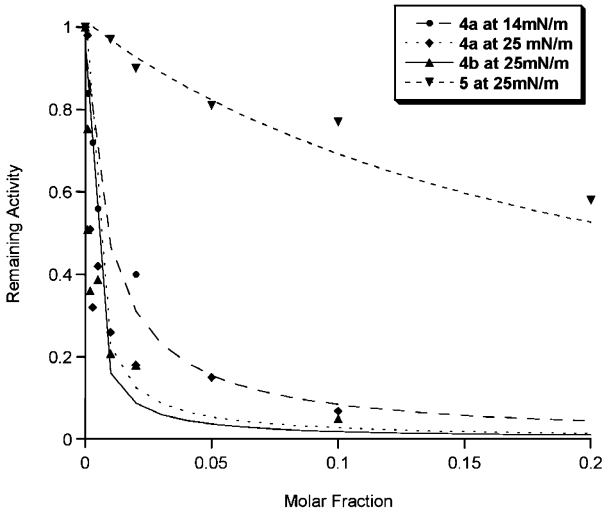
Chart 1 shows the molecular area dependency for compounds **4a**, **4b**, **5**, and the natural substrate triolein as a function of the surface pressure of a film spread over a buffered subphase at pH 8.0. The surface behavior of these compounds was measured at the argon/water interface to avoid the oxidation of the double bond. Comparing the novel analogues **4a** and **4b** with the natural substrate, a significant increase in the collapse pressure was observed. For example, the collapse pressure increased from 12.2 mN/m (triolein) to 22.5 mN/m (**4a**). Triacylglycerol analogue **5** also formed a stable film with a collapse pressure value of 24.5 mN/m. It is clear that the Boc-protected amino group at position 2 of the acyl chain significantly stabilizes the monomolecular films of triacylglycerols analogues **4a**, **4b**, and **5**. Furthermore, the incorporation of the Boc-protected amino group resulted in an increase in the molecular area occupied by these analogues. For example, at 11 mN/m, the molecular area of **4a** is 148 Å, whereas the molecular area occupied by triolein is 104 Å. The comparison of force/area curves of the triolein analogues (**4a** and **4b**) and the tripalmitin analogue (**5**) showed an increase in the area per molecule as the carbon chain from a C18:1 changed to a C16:0 carbon chain.

Pancreatic and Gastric Lipase Activity on Monomolecular Films Containing Triacylglycerol Analogues 4a and 4b. The inhibition of HPL and HGL was studied by means of the monomolecular film technique with mixed films of 1,2-dicaprin containing variable proportions of triacylglycerol analogues **4a** and **4b**.[16] For HPL, the inhibition studies were performed at a constant surface pressure of 14 mN/m or 25 mN/m for compound **4a** and 25 mN/m for compound **4b.** For HGL, we used a constant surface pressure of 17 mN/m or 27 mN/m for compound **4a** and 27 mN/m for compound **4b**. At these surface pressure values, HPL and HGL were active and linear kinetics were recorded.

Inhibition of lipolytic enzymes are best reported in terms of surface molar fraction of inhibitor.[16,17] Remaining lipase activity was plotted as a function of the surface molar fraction (α) of inhibitor. The data obtained for compounds **4a**, **4b**, and **5** on the HPL activity are presented in Chart 2. In the case of **4a** and **4b** lipase hydrolysis rates of 1,2-dicaprin decreased sharply as the molar fraction of inhibitor increased. A 50% decrease of HPL activity was observed when 0.003 or 0.002 molar fraction of inhibitor **4a** or **4b**, respectively, was mixed with a monolayer of 1,2-dicaprin at 25 mN/m, used as lipase substrate. At 14 mN/m, the inhibitory effect of **4a** on HPL was lower, indicating that a better inhibition was achieved at a surface pressure value (25 mN/m) where HPL activity was maximum. No significant inhibitory effect on HPL was observed when the triacylglycerol analogue **5** was used.

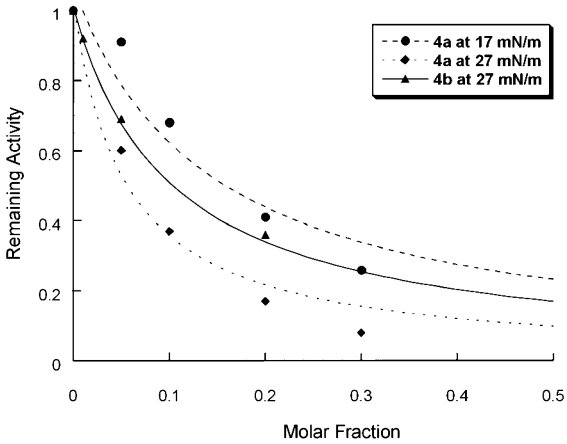
Compounds **4a** and **4b** were also tested as potential inhibitors of HGL, and the data obtained from these tests are presented in Chart 3. A 50% decrease of HGL activity was observed at 27 mN/m surface pressure

Chart 2. Effect of Increasing Concentration of Triolein Analogues **4a**, **4b**, and **5** on the Remaining Activity of HPL on the 1,2-Dicaprin Monolayer Maintained at a Constant Surface Pressure (25 or 14 mN/m)[a]



[a] 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.

Chart 3. Effect of Increasing Concentration of Triolein Analogues **4a** and **4b** on the Remaining Activity of HGL on the 1,2-Dicaprin Monolayer Maintained at a Constant Surface Pressure (27 or 17 mN/m)[a]



[a] 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 1. Inhibition Constants of Compounds **4a** and **4b** on Digestive Lipases

	HPL		HGL	
compd	surface pressure (mN/m)	α_{50}	surface pressure (mN/m)	α_{50}
4a	14	0.009 ± 0.002	17	0.150 ± 0.025
4a	25	0.003 ± 0.001	27	0.057 ± 0.015
4b	25	0.002 ± 0.001	27	0.104 ± 0.020

when 0.057 or 0.104 molar fraction of compound **4a** or **4b**, respectively, was mixed with a monolayer of 1,2-dicaprin, used as lipase substrate. The inhibitory effect of **4a** on HGL activity was also tested at 17 mN/m. As previously described in the case of HPL, the inhibition of HGL was also higher at the surface pressure of 27 mN/m where HGL displayed its highest catalytic activity.

The inhibitory data are summarized in Table 1. The α_{50} was previously defined as the molar fraction of inhibitor which reduced by 50% the initial rate of lipolysis. As shown from these data, the triolein analogues **4a** and **4b** are potent inhibitors of HPL and exhibit a weaker inhibition on HGL activity. For example, compound **4a** shows a 19-fold better inhibitory effect against HPL in comparison to HGL. Up to now, the best reported synthetic inhibitor of HPL is *O*-hexadecyl-*O*-(*p*-nitrophenyl) *n*-undecyl phosphonate, with an α_{50} value of 0.003.[18] Thus, we described in the present report two triacylglycerol analogues which exhibit a similar, or an even better, inhibitory effect when compared to the most potent synthetic inhibitor of HPL. However, it is worth noticing that the phosphonate inhibitors were reported, from X-ray diffraction analysis, to form a covalent phosphorus−oxygen bonding with the active site serine of lipases. In contrast, the triacylglycerol analogues reported presently behave probably such as noncovalent inhibitors.

During the early 1970s, it was been reported that synthetic triacylglycerols substituted by a methyl group either at the α-position of the acyl residue or at the 2-position of the glycerol backbone presented a reduced ability to be hydrolyzed by porcine pancreatic lipase.[19] Makriyannis et al. has recently developed metabolically stable anandamide analogues by sterically hindering the scissile amide bond through the introduction of α-methyl groups.[20] Here for the first time, we demonstrated that two triolein analogues containing the sterically hindered *tert*-butoxycarbonylamino group at the α-position of the oleic acid residue act as potent inhibitors of human pancreatic lipase.

In conclusion, using the monomolecular film technique, we have demonstrated that triacylglycerols based on (*N*-α-*tert*-butoxycarbonylamino)oleic acid are potent human pancreatic lipase inhibitors. From a chemical point of view, such analogues are similar to natural lipase substrates. Our results indicate that the Boc-protected amino group at the α-position of the acyl chain is an interesting substituent for the design and synthesis of potent inhibitors of lipolytic enzymes.

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Supporting Information Available: Details of experimental procedures for the synthesis of compounds, analytical characterization data, and monomolecular film experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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