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Synthesis and Study of a Lipophilic α-Keto Amide Inhibitor of Pancreatic Lipase

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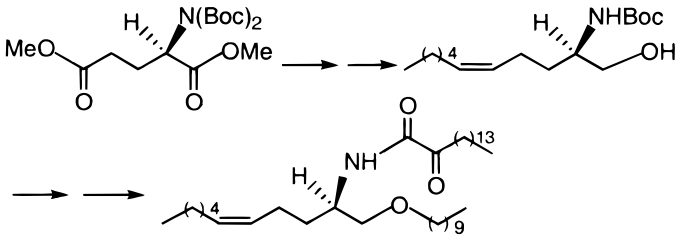
ABSTRACT



A lipophilic α-keto amide, inhibitor of pancreatic lipase, was synthesized using a lipidic 2-amino alcohol as backbone. The chiral key intermediate 2-(*tert*-butyloxycarbonylamino)-D-undecen-5-ol was synthesized starting from D-glutamic acid. The inhibitor formed a stable monomolecular film at the air/water interface as shown by a force/area curve. Inhibition studies using the monomolecular film technique with mixed films of 1,2-dicaprin containing variable proportions of the inhibitor showed a 50% decrease in lipase activity at a 0.14 molar fraction.

Lipases are versatile tools for biotechnology and have been employed by organic chemists for a long time to catalyze chemo-, regio-, and stereoselective transformations.[1,2] In humans, pancreatic and gastric lipases are essential enzymes for efficient fat digestion.[3] The hydrolysis of dietary triacylglycerols by these enzymes is a necessary step for fat absorption by the enterocytes. Potent and specific inhibitors of these enzymes are of interest because they may find application as anti-obesity agents.[4] Furthermore, inhibitors of various lipases of animal and microbial origin may contribute to a better understanding the mechanisms of lipase action.[5]

The active site of pancreatic lipase contains Ser-His-Asp, a triad resembling the catalytic triad of serine proteases, as it has been proven by chemical modification,[6] site-directed mutagenesis,[7] and crystallographic data.[8]

Many inhibitors of serine proteases consist of a substrate-

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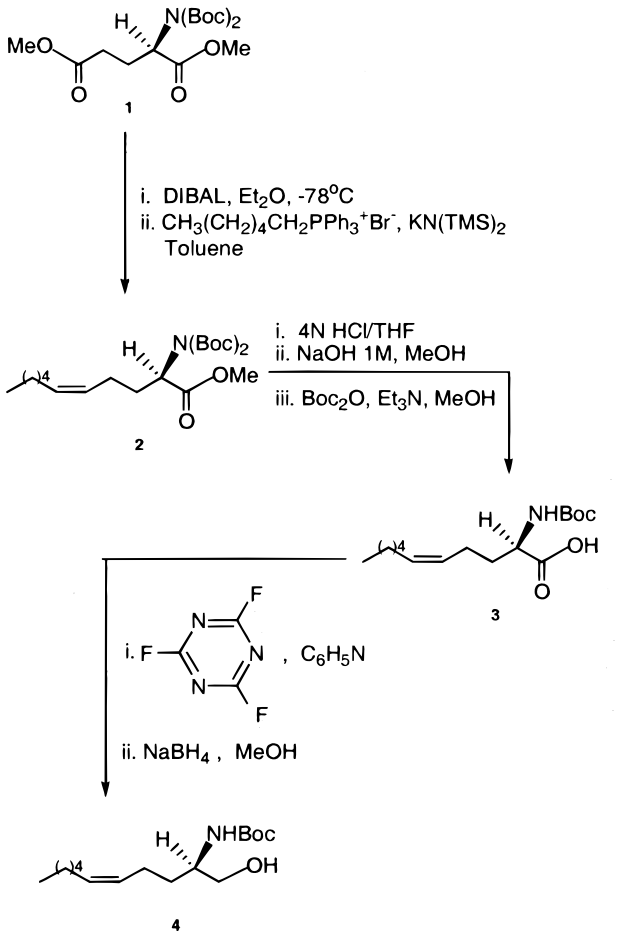
like structure incorporating a reactive carbonyl group at the site of the scissile amide bond, capable of reacting with the active site of the enzyme. A number of reactive carbonyl groups, such as fluorinated ketones,[9] α-keto esters,[10] α-keto amides,[11] and 1,2-diketones,[12] have been successfully used in the design of protease inhibitors. In such cases the mechanism of action involves most likely a nucleophilic attack by the hydroxyl group of the active site serine onto the electrophilic carbonyl group of the inhibitors, followed by the formation of a hemiacetal adduct, which mimics the tetrahedral intermediate involved in the enzymatic cleavage. Furthermore, fatty alkyl trifluoromethyl ketones have been reported to inhibit phospholipases A_2.[13] Most recently α-keto amide triglyceride analogues as inhibitors of *Staphylococcus hyicus* lipase have been reported.[14]

To develop novel inhibitors of lipases we incorporated the α-keto amide function into a lipidic amino alcohol backbone, ensuring the lipophilicity of the final product. In this paper we thus report the synthesis of such a typical molecule, the study of its surface properties, and its inhibitory effects on pancreatic lipase activity studied by the monomolecular film technique.

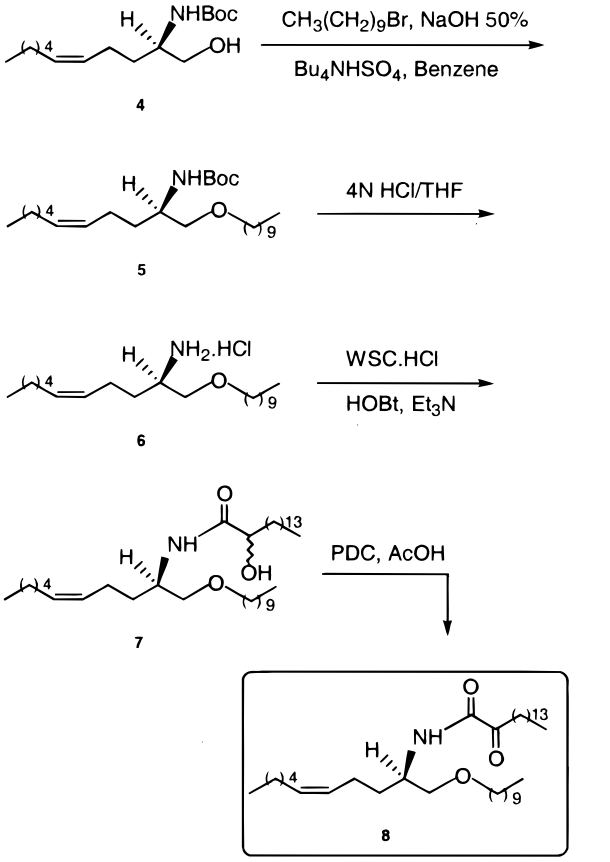
The lipidic amino alcohol **4**, used as backbone for the α-keto amide inhibitor, was synthesized starting from D-glutamic acid, as described in Scheme 1. Dimethyl *N*,*N*-di-Boc-glutamate (**1**) was reduced using DIBAL under controlled conditions,[15] and the resulting aldehyde was submitted to Wittig reaction with the suitable ylide to produce the fully protected unsaturated lipidic amino acid **2**. The Boc-protected amino acid **3**, obtained by the appropriate deprotection−protection procedure,[15] was converted into the corresponding fluoride and reduced in situ by treatment with sodium borohydride and dropwise addition of methanol[16] to produce the amino alcohol **4**.[17]

The etherification procedure took place under phase transfer conditions. The hydroxy component **4** was treated with *n*-decyl bromide in a biphasic system of benzene/aqueous sodium hydroxide in the presence of a catalytic amount of Bu_4NHSO_4 and afforded the ether derivative **5** (Scheme 2) in satisfactory yield. Removal of the Boc group using HCl/THF led to the corresponding free amino compound **6**, which was coupled with 2-hydroxyhexadecanoic acid using 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide

Scheme 1



Scheme 2



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(WSC·HCl)[18] as a condensing agent in the presence of 1-hydroxybenzotriazole (HOBt).

The racemic 2-hydroxy fatty acid was prepared by deamination of the corresponding 2-aminohexadecanoic acid[19] using $NaNO_2$ under acidic conditions. The α-hydroxy amide **7** was converted to the corresponding α-keto amide **8**[20] using pyridinium dichromate (PDC) in acetic acid, which proved to be an effective agent for the oxidation affording the desired product in good yield.

The use of the monolayer technique, which is based upon surface pressure decrease due to the film hydrolysis, is advantageous for the study of lipase inhibition since with conventional emulsified systems it is not possible to control their "interfacial quality".[21] The kinetic studies of the lipase hydrolysis reactions requires that the lipids used as substrates form a rather stable monomolecular film at the air/water interface.[22]

To determine the film stability and the interfacial properties of the α-keto amide derivative **8**, we have recorded its force/area curve at the air/water interface. The experiment was performed in the reservoir compartment of a "zero-order" trough. Figure 1 gives the surface pressure dependency as a

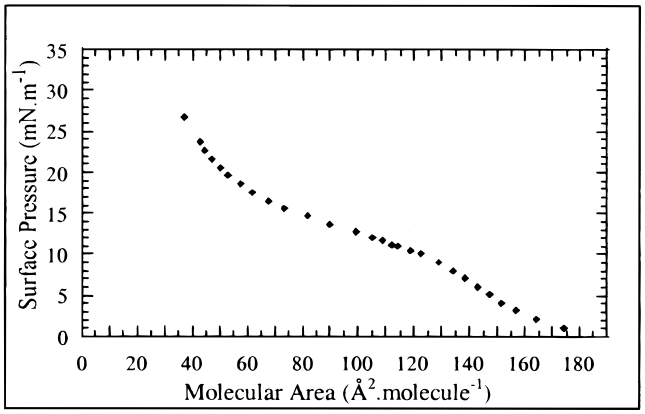

Figure 1. Force/area curve of compound **8**. The aqueous subphase was composed of Tris/HCl 10 mM, pH 8, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The continuous compression experiment was performed in the rectangular reservoir of the "zero order" trough.[23]

function of the molecular area of a film spread over a buffered subphase at pH 8.0. The large molecular area of the film formed by this compound may be attributed to the presence of the double bond as well as the two alkyl chains.

The inhibition of pancreatic lipase was studied using the monomolecular film technique[22,23] with mixed films of 1,2-dicaprin containing variable proportions of compound **8**. For the kinetic studies a surface pressure of 20 mN·m^{-1} was selected. At this value of surface pressure PPL is highly active and characterized by linear kinetics.

Remaining lipase activity was measured as a function of the inhibitor molar fraction (α) (Figure 2). Lipase hydrolysis

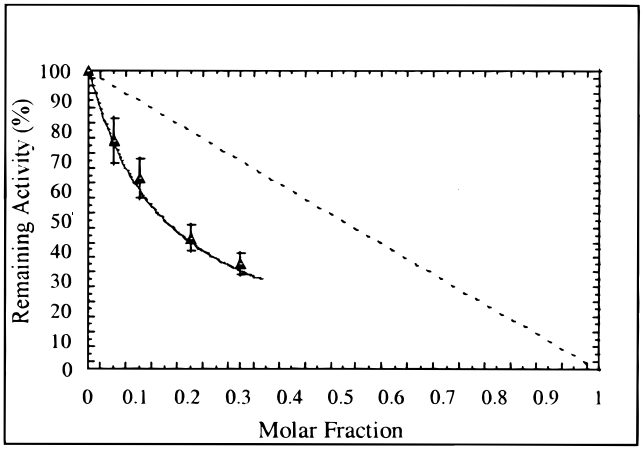

Figure 2. Effect of increasing concentrations of **8** on the hydrolysis rate by PPL of 1,2-dicaprin monolayer maintained at a constant surface pressure of 20 mN·m^{-1}. The aqueous subphase was composed of Tris/HCl 10 mM, pH 8, NaCl 100 mM, $CaCl_2$ 21 mM, EDTA 1 mM. The kinetics of hydrolysis were recorded during 15−20 min.

rates of 1,2-dicaprin decreased as the molar fraction of the inhibitor increased. The dotted line corresponds to the surface dilution phenomena, which reflects the decrease in lipase activity that would be observed if a nonsubstrate, noninhibitor compound, i.e., so-called "surface dilutor", were present in the monomolecular film. A 50% decrease in lipase activity was observed when 14.1 ± 3.5% of 1,2-dicaprin had been substituted by the inhibitor compound **8**. Although this value corresponds to a rather weak inhibition of pancreatic lipase,

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(17) Yield 84%; oil; $[\alpha]_D$ +3.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.4 (m, 2H), 4.6 (br, 1H), 3.7−3.5 (m, 3H), 2.2−1.9 (m, 4H), 1.6−1.4 (m, 11H), 1.3 (m, 6H), 0.9 (t, J = 7 Hz, 3H); MS (FAB) *m/z* (%) 308 (12) [M + Na$^+$], 286 (9) [M + H$^+$], 230 (68), 186 (100). Anal. Calcd for $C_{16}H_{31}NO_3$: C, 67.33; H, 10.95; N, 4.91. Found: C, 67.09; H, 11.24; N, 4.82. Enantiomeric excess >95% was indicated by ^{1}H NMR and ^{19}F NMR analysis of the corresponding Mosher ester.

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(20) Yield 57%; oil; $[\alpha]_D$ +3.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.1 (d, J = 8 Hz, 1H), 5.4 (m, 2H), 4.0 (m, 1H), 3.4 (m, 4H), 2.9 (t, J = 7 Hz, 2H), 2.1−1.9 (m, 4H), 1.8−1.5 (m, 6H), 1.4−1.1 (m, 42H), 0.9 (m, 9H); ^{13}C NMR (200 MHz, $CDCl_3$) δ 199.4, 159.7, 130.9, 128.2, 71.5, 49.0, 36.8, 31.9−22.3, 14.1−14.0; MS (FAB) *m/z* (%) 579 (24) [M + H$^+$], 578 (100) [M$^+$], 352 (12), 225 (18). Anal. Calcd for $C_{37}H_{71}NO_3$: C, 76.89; H, 12.38; N, 2.42. Found: C, 76.62; H, 12.56; N, 2.34.

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it is comparable to the α_{50} values recently reported for chiral acylglycerol analogues belonging to the phosphonate type inhibitors.[24] However, more potent alkylphosphonate inhibitors have been synthesized recently by Cavalier et al.[25]

In conclusion, the α-keto amide group seems to be a promising approach for the development of novel lipase inhibitors after its incorporation into an optimized lipophilic amino alcohol backbone. The methology developed in this paper allows for the synthesis of chiral inhibitors bearing various saturated or unsaturated alkyl chains.

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Supporting Information Available: Characterization data for compounds **2**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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