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Lipase-Catalysed Enantioselective Hydrolysis: Interpretation of the Kinetic Results in Terms of Frontier Orbital Localisation

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Abstract: The initial velocities of the enantioselective hydrolysis of the esters derived from mono- and bicyclic alcohols and hemiacetals catalysed by Lipolase™ in water have been determined. The differences in hydrolysis rates within groups of sterically similar substrates have been interpreted in terms of frontier orbital localisation.

INTRODUCTION

Up to now, the enzymatic kinetic resolution of enantiomers of a number of chiral alcohols and acids upon the lipase-catalysed hydrolysis of their esters in water has been described.[1-3] Although this method is now less favoured in comparison with lipase-catalysed enantioselective transesterification in organic solvents, it retains the importance for the regioselective deacylation of polyol esters[1] (for instance, in carbohydrate chemistry) as well as provides the opportunity to perform deacylation under mild conditions. A significant advantage of using aqueous medium arises from the possibility to monitor the conversion rate on a pH-stat. Thus, the hydrolysis of the esters catalysed by lipases in water appears to be a field of continuing interest. Consequently, the prognosis of the hydrolysis rates, the enantio- and regiopreference as well as the stability of putative substrates in water is of major significance.

In this paper an attempt is made to apply the frontier orbital localisation parameters calculated by using a semiempirical quantum chemical method MNDO to the interpretation of significant differences in the hydrolysis rates of sterically similar substrates.

The hydrolysis of three following types of substrates,

1) the esters of cyclic hemiacetals,

2) the esters of α-substituted carbomonocyclic alcohols,

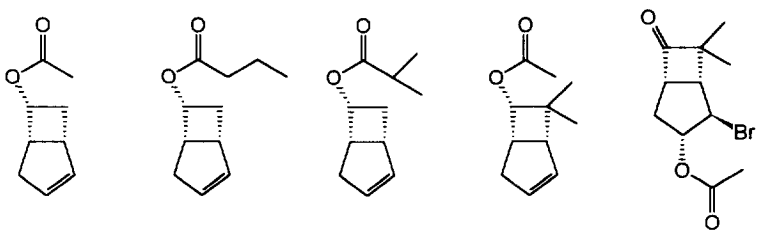
3) the esters of bicyclo[3.2.0]heptane derivatives,

catalysed by Lipolase in water has been investigated in this study.

Cyclic 3-substituted hemiacetals[4] have been found to be valuable chiral auxiliaries.[5] Up to now, only few studies on the enzymatic optical resolution of hemiacetals have been published.[6,7] The enantioselective lipase-catalysed hydrolysis of the alkyl acyl acetal moiety has been successfully used for the optical resolution of chloral methyl acetyl acetal that is highly stabilised by chlorine atoms.[6] However, the application of the enzymatic hydrolysis of the hemiacetal esters is limited due to their insufficient stability in water (see **S5**, Table1).

trans-α-Substituted carbomonocyclic alcohols have been used as chiral "building blocks" for natural product synthesis.[8] Their enzymatic optical resolution via hydrolysis of the corresponding esters catalysed by lipases from *Candida cylindracea* (CCL) and *Pseudomonas sp.* has been studied.[9] All the esters except the protected α-amino derivatives were hydrolysed smoothly yielding products of e.e. exceeding 90%.

The hydrolysis of the third group of esters, bicyclo[3.2.0]heptane derivatives, has been extensively studied.[1-3] Various commercially available enzymes, CCL and lipases from *Mucor miehei* as well as from porcine pancreas (PPL) were effective catalysts for the enantioselective hydrolysis of the acetate **1**.[10] The ester **1**



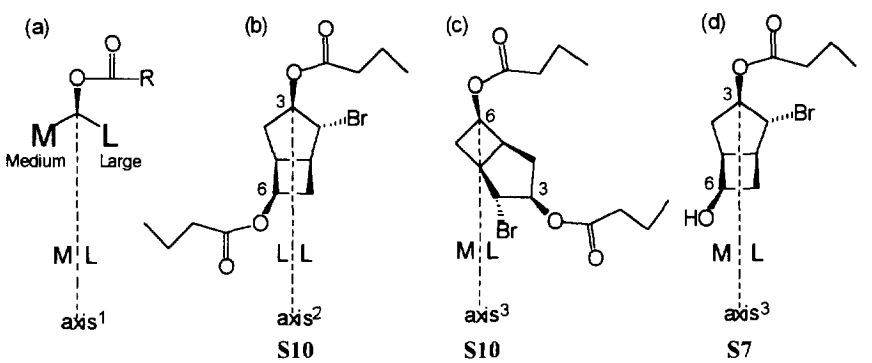
1 **2** **3** **4** **5**

is hydrolysed at a high rate and with a very high enantioselectivity also by *Pseudomonas fluorescens* lipase.[11]

The dependence of the hydrolysis rate on the structure of the acyl group has been established[12] for the lipases Amano AK, Amano SAM-II and for the *Pseudomonas sp.* lipase. The initial velocity of the hydrolysis was found to decrease in the order: $\mathbf{1} \xrightarrow{4\text{-}7X} \mathbf{2} \xrightarrow{2\text{-}3X} \mathbf{3}$ in the proportion shown above the arrows. The change in the hydrolysis rate for the substrates **1** and **2** appears to be opposite when using a highly purified PPL as a catalyst.[13] It should be emphasized that the butyric ester **2** having a rigid bicyclic framework was hydrolysed by PPL at an exceptionally high rate being comparable to those corresponding to natural triglyceride substrates. The rate of the PPL-catalysed hydrolysis of the acetate **1** was found to be more than two orders of magnitude lower than that measured for the butyrate **2**.[13]

Besides the structure of the acyl group (chain length, branching), the degree of a steric fit of the substrate molecule derived from an alcohol having a rigid cyclic framework into the enzyme active site crucially affects the hydrolysis rate. Molecular modelling studies aimed at prognosticating the enantioselectivity of the reaction have been carried out.[1]

The low hydrolytic reactivity of some ester substrates has been explained in terms of a steric shielding of the reaction centre by neighbouring parts of the molecule, usually by α-substituents.[14] A good example illustrating the shielding of the reaction centre is the ester **4**, "being only marginally accepted by lipases due to the location of the ester moiety on the inaccessible concave face of the rigid bicyclic framework" (ref.14). This simple model of the steric shielding of the reaction centre[14a] can provide a valuable preliminary prognosis of the enantio- and regiopreference of the hydrolysis as well as the hydrolytic inactivity of a substrate if used in connection with the general enantiopreference rule for lipases.[1] For instance, the regiospecific order of the lipase-catalysed hydrolysis of the ester groups of **S10**[15] (Table 3) (as well as the lipase-catalysed desymmetrisation of some meso-compounds[16]) could be explained in terms of steric shielding (Fig.1). In the case depicted in Fig. 1(b), the C_6-acyloxy group is expected to shield another potential reaction centre, the C_3-acyloxy moiety.[17] The reverse shielding does not take place (see Fig. 1(c)) in accordance with the general enantiopreference rule for lipases (Fig. 1(a))[1]. After the enantioselective cleavage of the C_6-acyloxy group, the C_3-butanoyloxy group was readily hydrolysed[15] by Lipolase probably since it became sterically accessible to the enzyme (Fig. 1(d)).



1: the ester (indicated by the axis) hydrolysed preferably by lipases[1];

2: the ester (indicated by the axis) not hydrolysed by Lipolase;

3: the ester (indicated by the axis) hydrolysed by Lipolase;

Figure 1. The regiospecific order of hydrolysis of the ester groups of the diester **S10** explained in terms of steric shielding in accordance with the general enantiopreference rule for lipases.[1]

It can be concluded that the hydrolysis rate for artificial ester substrates can change from zero up to the rates corresponding to those of the best natural triglyceride substrates due to steric factors only. Thus, the steric effects on the rate of hydrolysis should be taken into account prior to looking for other interpretations of the substrate reactivity.

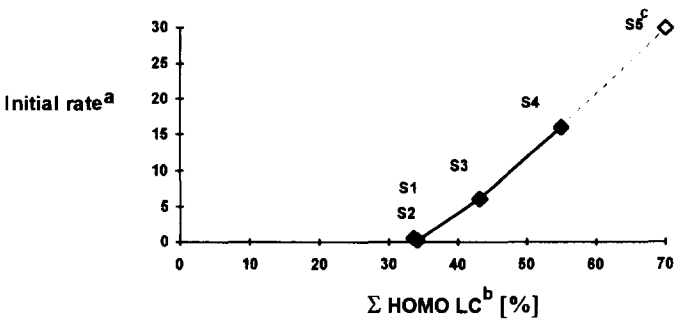
RESULTS AND DISCUSSION

The initial velocities[18] of the hydrolysis[19] of cyclic artificial substrates **S1-S14**[15,20,21] catalysed by Lipolase[22] were measured; the products were separated and their optical purity as well as the absolute configuration were established.[15,21,23]

The initial rates[24] were analysed within groups and pairs of substrates of high steric similarity. In order to interpret the substantial differences in the hydrolytic reactivity of sterically similar substrates, the semiempirical quantum chemical calculations using the program MOPAC6[25] were carried out. For the determination of possible conformations of the substrates their heats of formation were calculated with geometry optimisation by the MNDO[26] method. The most favourable conformations were found to be similar within the following groups: **S1-S4** (Table 1), **S6-S9** (Table 2) and **S10-S14** (Table 3). The frontier orbital (HOMO, LUMO) localisation coefficients (LC) and charge distribution within the molecule were calculated for the most favoured conformation of the substrate. The results of quantum chemical calculations and kinetic measurements are listed in the tables. The conversion rates of the enzymatic hydrolysis of **S6-S9** in supercritical carbon dioxide ($SCCO_2$)[21] are listed in Table 2 as well.

A. The Lipolase-catalysed hydrolysis of the esters of monocyclic hydroxycompounds (Table 1)

The initial rates of the Lipolase-catalysed hydrolysis of sterically similar substrates **S1,S2** and **S3,S4** differed by more than one order of magnitude. A comparison of these results with those of quantum chemical calculations shows that the initial rates of hydrolysis correlate with the integrated HOMO LC of the O–C=O



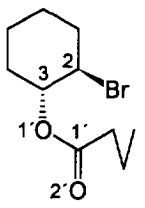
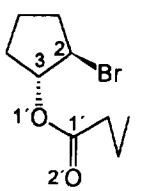
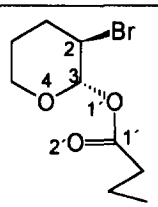
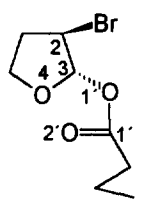
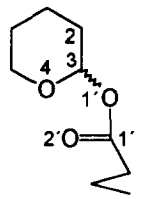
a: Values listed in Table 1;

b: Σ HOMO LC for O–C=O atoms: **S1** – 33.6%,**S2** – 34.2%, **S3** – 43.1%, **S4** – 55%, **S5** – 70%.

c: **S5** decomposed spontaneously (Table 1).

Figure 2. Dependence of the initial rate of the Lipolase-catalysed hydrolysis of substrates **S1-S4** on the integrated HOMO LC for the O–C=O atoms forming the ester moiety.

Table 1. Esters of monocyclic hydroxy compounds.[20]

Localisation of H: HOMO (%) and L: LUMO (%) Distribution of C: CHARGE [A.U.]		 S1	 S2	 S3	 S4	 S5
Br	H	34.2	37.5	6.5	7.1	
	L	40.8	41.5	42.0	42.6	
	C	-0.137	-0.123	-0.116	-0.102	
C_2	H	6.0	6.7	2.1	1.4	4.8
	L	53.2	53.2	52.7	52.4	0.07
	C	0.009	-0.005	-0.024	-0.033	-0.031
C_3	H	8.7	8.1	1.9	1.7	2.7
	L	1.0	0.6	0.9	0.7	4.2
	C	0.163	0.156	0.333	0.312	0.329
O_4	H			22.6	16.6	2.1
	L			0.1	0.3	0.5
	C			-0.345	-0.330	-0.362
$O_{1'(C3)}$	H	16.5	15.6	6.8	7.8	17.3
	L	0.9	0.2	1.0	0.6	2.4
	C	-0.345	-0.334	-0.372	-0.360	-0.378
$C_{1'(C3)}$	H	0.5	0.6	1.8	2.1	2.6
	L	0.6	0.2	0.6	0.4	56.3
	C	0.360	0.360	0.368	0.368	0.365
$O_{2'(C3)}$	H	16.6	18.0	34.5	45.1	50.1
	L	0.1	0.04	0.1	0.1	31.1
	C	-0.352	-0.353	-0.340	-0.339	-0.300
INITIAL RATE [a,b]		0.60	0.25	6.12	16.0	c
LUMO [eV]		0.253	0.347	0.011	0.018	0.933
HOMO [eV]		-11.294	-11.262	-11.230	-11.312	-10.996

a: μmole/min per 1.0 ml of Lipolase;

b: E>52 and E>22 were estimated for the hydrolysis of **S1**, **S2** and **S3**, **S4**, respectively;

c: spontaneous decomposition of the ester **S5** was observed in water at pH 7.

atoms forming the ester moiety (Fig. 2). This relationship leads us to the conclusion that the protonation of the ester moiety may be the rate-limiting process for the lipase-catalysed hydrolysis of these esters.

Spontaneous decomposition of the ester **S5** in water at pH 7 was observed. The instability of **S5** could be explained by the very high degree of localisation of HOMO on the ester group: (HOMO, 70%, as well as LUMO, 89.8% - were localised on the O–C=O atoms). The stabilisation of bromoesters **S3** and **S4** towards water in comparison with the unsubstituted **S5** could be characterised by a significant enhancement (10-fold) in the localisation of the HOMO on the oxygen atom involved in the heterocycle (probably by the localisation of the LUMO mainly on the C–Br atoms as well).

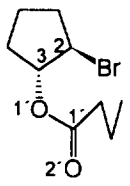
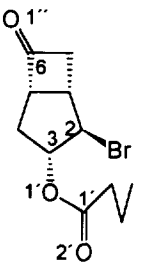
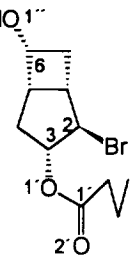
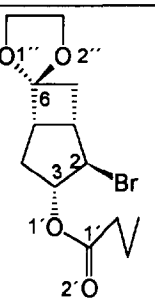
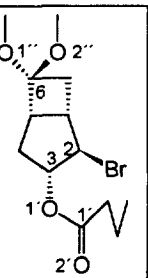
B. The hydrolytic reactivity of trans-α-bromocyclopentanol butyrates ***S2, S6-S9*** *(Table 2)*

The hydrolysis rates and enantioselectivities of the majority of artificial substrates of different types[1-3] as well as the resistance of some esters towards lipases can be explained in terms of purely steric effects. At the same time it seems noteworthy that a group of esters with "abnormal" stability towards lipase-catalysed hydrolysis has been observed. Some of the "abnormal" inactive ester substrates are sterically very similar to reactive esters. However, they appear to be completely resistant to lipases in water or can be cleaved by lipases only with a marginal reaction rate and enantioselectivity, that could be assigned to non-specific catalysis whereas it could be assumed that lipases are not able to recognise the interface between water and the substrate in a proper manner. The formation of suspension in H_2O by "abnormal" substrates, for instance by **S6**, **S8** and **5**[14], has been expected.[15] It should be mentioned here that lipases hydrolyse only substrate emulsions in water. The attempts to use cosolvents[14,15] have not given a satisfactory result.[7] Herein we should propose an approach to distinguishing these unreactive esters.

It has been reported[15] that the ester **S6** (Table 2) with the 6-oxo group shows a drastically lower hydrolytic reactivity than its reduced counterpart, 6-*endo*-hydroxy-ester **S7**. The ester **S6** was hydrolysed in water by Lipolase at a very low rate and with a poor enantioselectivity only in the presence of ethyl ether as a cosolvent while the hydroxyester **S7** was cleaved smoothly and with a high enantioselectivity without the assistance of the cosolvent. The ester **S9** was hydrolysed at a very low rate whereas the ester **S8** was completely resistant to Lipolase, either in the presence or absence of the cosolvent. In the case of the latter pair of substrates, the steric shielding of the reaction centre by the ketal group could be expected to cause the substrate inactivity. Contrary to this supposition our trials on the Lipolase-catalysed hydrolysis in $SCCO_2$ demonstrate (Table 2) that the steric shielding (evidently affecting the hydrolysis of **S9**) is not the crucial factor[21] causing the resistance of **S8** to Lipolase in water.

The hydrolytic reactivity of the substrates **S2** and **S6-S9** appears to be in a certain relationship with their HOMO LC, whereas the LUMO localisation was found to be very similar for all these substrates. HOMO of the "abnormal" inactive esters, **S6** and **S8,** is localised in a restricted part of the molecule apart from the reaction centre: the most prominent HOMO LC correspond to the oxygen atoms attached to C_6, whereas HOMO LC of the atoms forming the ester group are close to zero. For hydrolytically reactive esters **S2**, **S7** and **S9,** the HOMO LC for the oxygen atoms of the ester group were found to be essentially different from zero. Very large differences in HOMO localisation were also observed for the bromo-substituent and the carbon atom C_2 attached to the bromine: in the case of inactive **S6** and **S8** the corresponding HOMO LC were approximately equal to zero, whereas in the case of reactive esters, **S2**, **S7** and **S9,** the HOMO localisation on the C_2 and Br atoms is significant. The charge distribution calculated for the substrates **S2** and **S6-S9** seems to be irregular with respect to their reactivity.

Table 2. trans-α-Bromocyclopentanol butyrates.

Localisation of H: HOMO (%) and L: LUMO (%) Distribution of C: CHARGE [A.U.]		 **S2** [20]	 **S6**[15,21]	 **S7**[15]	 **S8**[21]	 **S9**[21]	H_2O
Br	H	37.5	0.1	13.5	0.05	18.7	
	L	41.5	41.4	41.1	41.2	41.3	
	C	-0.123	-0.119	-0.129	-0.127	-0.126	
C_2	H	6.7	0.1	2.1	0.1	3.0	
	L	53.2	52.4	53.1	53.0	53.0	
	C	-0.005	0.015	0.023	0.021	0.020	
C_3	H	8.1	0.6	3.7	0.6	3.6	
	L	0.6	0.7	0.7	0.7	0.7	
	C	0.156	0.155	0.157	0.157	0.156	
$O_{1'(C3)}$	H	15.6	0.3	6.2	0.4	6.8	
	L	0.2	0.3	0.3	0.3	0.3	
	C	-0.334	-0.337	-0.332	-0.332	-0.333	
$C_{1'(C3)}$	H	0.6	0.01	0.04	0.01	0.2	
	L	0.2	0.2	0.2	0.2	0.2	
	C	0.3600	0.360	0.360	0.360	0.360	
$O_{2'(C3)}$	H	18.0	0.1	2.8	0.2	5.3	
	L	0.04	0.04	0.04	0.04	0.04	
	C	-0.353	-0.350	-0.355	-0.355	-0.353	
C_6	H		7.8	9.7	11.6	2.6	
	L		0.3	0.1	0.1	0.1	
	C		0.226	0.114	0.240	0.282	
$O_{1''(C6)}$	H		48.0	26.5	20.8	12.5	
	L		0.1	0.01	0.01	0.01	
	C		-0.246	-0.302	-0.319	-0.364	
$O_{2''(C6)}$	H				21.9	19.8	
	L				0.003	0.04	
	C				-0.320	-0.365	
INITIAL RATE[a] (H_2O)		0.25	0[b]	1.36	0[b]	very slow[c]	
CONV. RATE ($SCCO_2$)[d]		not determined	35-40%	35-40%	3-5%	3-5%	
LUMO [eV]		0.347	0.110	0.357	0.356	0.287	4.4
HOMO [eV]		-11.263	-10.742	-11.075	-10.689	-11.183	-12.0

a: μmole/min per 1.0 ml of the Lipolase preparation;

b: no products detected;

c: product (bromohydrin) was detected, but the reaction rate was insufficient to be measured on a pH-stat;

d: conversion rate of the hydrolysis catalysed by Lipolase in supercritical carbon dioxide.[1]

It could be concluded that the different hydrolytic reactivity of **S6** and **S8**, on the one hand, and that of **S2**, **S7** and **S9**, on the other arises from the substrate/H_2O interaction since the esters **S6** and **S8** gave optically active material upon Lipolase-catalysed hydrolysis in $SCCO_2$ medium. The complete hydrolytic inactivity of **S6** and **S8** in water is evidently related to a very high degree of HOMO localisation in the restricted part of the molecule apart from the ester group that could provide a source of energy for interaction with water (hydrogen bond formation) leading to the formation of a system inaccessible to the enzyme. This conclusion is supported by the differences in HOMO energetic levels corresponding to **S6**, **S8** and **S2**, **S7**, **S9** if compared with that of LUMO of H_2O, too.

*C. The hydrolysis of the cyclobutanol ester substrates **S10-S14** (Table 3)*

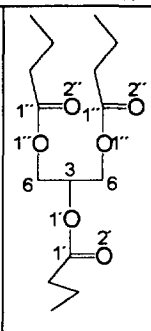
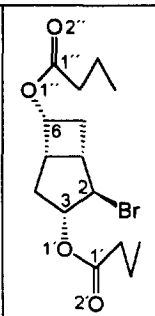
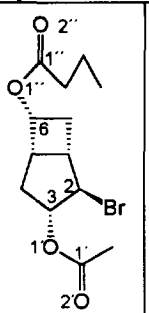
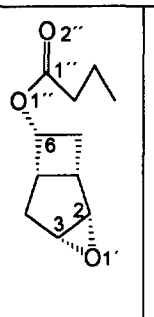
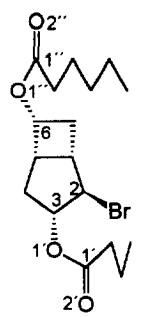
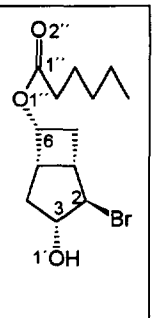
In the substrates **S10** and **S13,** the Lipolase-catalysed hydrolysis of the C_3-butanoyloxy group occurred after the cleavage of the 6-acyloxy moiety.[15] In the case of **S10**, **S13** and **S14** the occurrence of spontaneous dehydrobromination consequent to ester hydrolysis was observed[15]. Taking into account the small effects of these side reactions on the observed reaction rates, the hydrolysis rates of **S10** and **S12** as well as those of another pair, **S11** and **S13**[28], can be considered to be equal, respectively (in a satisfactory approximation also **S14** belongs to the latter pair). The reaction rates of **S11** and **S13** were found to be about one order of magnitude lower than those measured for **S10** and **S12**. The nearby constant difference in the hydrolysis rates of the bipolar substrates **S10-S14** leads us to the conclusion that the difference could be arising from the same origin, perhaps from the possibility of two opposite orientations of the bipolar molecules on the interface.

A comparison of the substrates **S10** and **S11** shows that the steric shielding of the reaction centre by the C_3-acyloxy group is excluded indeed as it was expected (Fig.1), since the substrate **S10** bearing a more bulky butanoyloxy group at C_3 reacted faster than **S11** with a sterically less demanding acetoxy substituent.

Examination of the frontier orbital LC shows that the sterically similar substrates **S10**, **S11** and **S13** have close HOMO and LUMO localisation features. The HOMO localisation on the reaction centre (the ester group) of **S12** and **S14** is very close as well. We can also see that there is no such a great localisation of HOMO as was observed for **S6** and **S8,** whereas in the substrates **S10-S14** the HOMO localisation on the atoms forming the reaction centre is moderate (comparable to **S7**, **S9**; Table 2). Thus, results of the calculations show that the esters **S10-S14** should have a moderate hydrolytic reactivity that was verified by the experiment. The nearly constant difference in the reactivity of the substrates **S10**, **S12** vs. **S11**, **S13** is really very complicated to be explained in terms other than the different hydrophobicity (prevailing, evidently, over the factors reflected by the HOMO localisation features) of the two opposite parts of the molecule competing in interface formation. It is evident because for the esters **S10** and **S11** all the other features are identical.

In order to study the reactivity of tributyrin as well as differences between its primary and secondary butanoyloxy groups that are cleaved in a 1,3-specific manner by the majority of lipases the semiempirical

Table 3. Cyclobutanol ester substrates and tributyrin.

Localisation of H: HOMO (%) and L: LUMO (%) Distribution of C: CHARGE [A.U.]			 **S10**[20]	 **S11**[15]	 **S12**[20]	 **S13**[15]	 **S14**[21]
Br	H		30.0	29.6		28.9	28.1
	L		41.2	41.1		41.1	40.9
	C		-0.126	-0.128		-0.128	-0.137
C_2	H		4.6	3.9	14.1	3.9	4.6
	L		52.9	52.8	0.01	52.8	53.8
	C		0.021	0.022	0.036	0.022	0.030
C_3	H	6.6	6.3	5.5	12.8	5.4	8.3
	L	0.03	0.7	0.8	0.001	0.8	0.6
	C	0.144	0.155	0.156	0.015	0.155	0.123
$O_{1'(C3)}$	H	15.1	12.0	10.6	33.0	10.6	24.0
	L	0.03	0.3	0.6	0.000	0.6	0.4
	C	-0.328	-0.334	-0.337	-0.258	-0.335	-0.314
$C_{1'(C3)}$	H	0.1	0.2	0.1		0.1	
	L	0.2	0.2	0.3		0.4	
	C	0.362	0.359	0.351		0.358	
$O_{2'(C3)}$	H	7.4	8.4	6.4		6.6	
	L	0.1	0.04	0.04		0.04	
	C	-0.357	-0.352	-0.351		-0.351	
C_6	H	6.0 / 3.7	4.1	4.9	3.8	4.8	3.2
	L	0.4 / 0.02	0.1	0.1	0.5	0.07	0.1
	C	0.174/0.193	0.145	0.145	0.148	0.145	0.145
$O_{1''(C6)}$	H	5.7 / 5.7	6.4	8.3	4.3	8.3	4.7
	L	5.0 / 0.1	0.01	0.01	5.2	0.01	0.01
	C	-0.336/-0.342	-0.321	-0.321	-0.318	-0.321	-0.318
$C_{1''(C6)}$	H	0.9 / 1.1	0.2	0.2	0.1	0.3	0.1
	L	58.0./ 0.9	0.04	0.03	59.0	0.03	0.04
	C	0.358/0.360	0.355	0.355	0.358	0.354	0.355
$O_{2''(C6)}$	H	16.4 / 20.8	4.4	5.9	2.6	6.1	2.6
	L	28.4 / 0.4	0.02	0.02	28.8	0.01	0.02
	C	-0.353/-0.356	-0.352	-0.352	-0.358	-0.352	-0.355
INITIAL RATE [a,b]		10^4	3.67	0.36	3.32	0.42	0.85
LUMO [eV]		0.839	0.278	0.250	1.001	0.254	0.374
HOMO [eV]		-11.109	-11.236	-11.266	-10.907	-11.252	-11.100

a: μmole/min per 1.0 ml of Lipolase ,

b: E>90 was estimated[15] for the hydrolysis of **S10-S14**.

quantum chemical calculations were performed for this highly reactive substrate, too. The HOMO localisation was found to be 2-3 times higher on the carbonyl oxygen atom of the primary butanoyloxy groups than that of the secondary one. This feature could be related to the factors governing the interface formation in the case of identical hydrophobic acyl chains and is probably leading together with steric reasons to 1,3-regiopreference observed for the majority of lipases. On the other hand, the very high rate of Lipolase-catalysed hydrolysis of tributyrin is evidently related to its conformational flexibility.

Conclusions:

1. The initial velocities of the Lipolase-catalysed hydrolysis of three groups of esters in water have been recorded. The substrates were classified into groups by steric similarity. The wide diversity in the hydrolysis rates corresponding to sterically similar substrates has been interpreted in terms of HOMO localisation as well as different hydrophobicity of those parts of the substrate molecule competing in interface formation.
2. The rates of the enzymatic hydrolysis of sterically similar monocyclic substrates **S1-S4** were found to correlate with the integrated HOMO LC for the O–C=O atoms forming the ester group. This relationship shows that the protonation of the ester moiety can be the rate-limiting process for the lipase-catalysed hydrolysis of these substrates.
3. The resistance of some esters to Lipolase has been found to be related to the HOMO localisation apart from the reaction centre on a restricted part of the substrate molecule that leads to the formation of a system inaccessible to the enzyme.
4. The moderate hydrolytic reactivity of the ester substrates **S10-S14** was predicted by examination of the HOMO localisation parameters. The difference in the initial rates of the hydrolysis of two groups of substrates (**S10**, **S12** vs. **S11**, **S13**, **S14**) was approximately one order of magnitude. This was assumed to arise from two possible orientations of the bipolar substrate molecules on the interface between water and the substrate. The initial rate was higher for the substrates bearing the reaction centre in the less hydrophobic part of the molecule which could be therefore oriented to water.
5. No certain relationship was found to occur between the hydrolytic reactivity of the artificial substrates and the LUMO localisation as well as charge distribution features of their molecules.

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17. Examination of molecular framework models proved that the linearly remote substituents present in a bicyclic molecule can be located rather close to each other in space (for instance, the ester groups of **S10**).
18. The initial velocities were calculated from the slope of NaOH consumption vs. time plot during 5-25% conversion.
19. General procedure. Hydrolysis was carried out on a pH-stat ("Radiometer", Denmark) under vigorous stirring in 15 ml of water at pH 7 containing 0.15 M NaCl, 2 mM $CaCl_2$; 0.4-0.6 mmol (100 µl) of the substrate and 0.25-1.0 ml of Lipolase.[22] The forming acid was titrated using NaOH (0.175 M). The reaction vessel was thermostated at 20°C. The product was extracted with EtOAc, purified over silica and the e.e. was determined by HPLC of the corresponding Mosher esters or correlation of the optical rotation values with those measured for the optically pure standards. The absolute configuration of the substrates preferred by the enzyme were proved (by comparison with standards synthesized starting from the compounds of known configuration[21,23]) to correspond to those presented in the tables.
20. Characterisation of compounds. (1S,2S)-**S1**: ^{13}C NMR see ref. 9; TLC - R_f=0.47 (C_6H_6); $[\alpha]^{20}_{546}$+44 (c 1.0, C_6H_6; e.e.>94%[20a]); (1S,2S)-**S2**: ^{13}C NMR ($C_{1\text{-}5}$; $C_{1(1\text{-}4)}$) - 81.8, 53.0, 34.5, 21.7, 29.5, 172.6, 36.2, 18.5, 13.6; TLC - R_f=0.49 (C_6H_6); $[\alpha]^{20}_{546}$+88 (c 1.0, C_6H_6; e.e. >94%[20a]); (2R,3S)-**S3**: ^{13}C NMR ($C_{2\text{-}6}$; $C_{2(1\text{-}4)}$) - 94.2, 47.4, 30.5, 23.5, 64.4; 171.6, 36.1, 18.3, 13.6; TLC - R_f=0.30 (C_6H_6); $[\alpha]^{20}_{546}$ +54 (c 1.2, C_6H_6; e.e. >94%[20a]); (2R,3S)-**S4**: ^{13}C NMR ($C_{2\text{-}5}$; $C_{(2)1\text{-}4}$) - 102.6, 49.0, 33.3, 68.2; 172.1, 36.2, 18.2, 13,6; TLC - R_f=0.34 (C_6H_6); $[\alpha]^{20}_{546}$ - 96 (c 1.0, C_6H_6, e.e.>94%[20a]); **S5**: IR - 2960, 2890, 1750, 1470, 1460,

1370 cm^{-1}; TLC - R_f=0.16 (C_6H_6). **S10**: ^{13}C NMR ($C_{1\text{-}7}$; $C_{(3)1\text{-}4}$; $C_{(6)1\text{-}4}$) - 44.4, 56.5, 84.7, 29.5, 42.8, 64.7, 32.9; 172.2, 36.5, 18.5, 13.8; 172.7, 36.1, 18.5, 13.7; IR - 2970, 2880, 1730, 1460, 1430, 1370, 1350, 1300, 1250, 1170, 1100, 1030, 1000, 700 cm^{-1}; TLC - R_f=0.11 (C_6H_6); **S12**: ^{13}C NMR ($C_{1\text{-}7}$; $C_{(6)1\text{-}4}$) - 34.2, 66.7, 61.9, 27.8, 45.5, 65.3, 28.1; 173.3, 36.2, 18.6, 13.7; IR - 3010, 2960, 2870, 1720, 1460, 1420, 1375, 1340, 1250, 1170, 1090, 1050, 970, 840, 680 cm^{-1}; TLC - R_f=0.07 (C_6H_6).

20a. The optical rotation value corresponds to the hydrolytically less active enantiomer separated from the hydrolysis product over silica and being estimated to the pure enantiomer by NMR using chiral shift reagent.

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24. Concerning the comparability of the initial rates measured for different substrates we examined the influence of the following conditions which were varied for different reasons: 1) the molar concentration of the substrate was varied since the constant volume of the substrate was added[19] in order to keep the substrate/H_2O v/v ratio constant; 2) the amount of the enzyme preparation was varied because of widely different reaction rates. All the other parameters were kept strictly constant. The variation of these two parameters in the range indicated[19] was estimated to affect the initial rate not more than 10%. It should be emphasized that the differences in initial rates discussed are mainly in the range of one order of magnitude or more.

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27. The optical resolution of the parent alcohol of some hydrolytically inactive esters have been performed very successfully by lipase-catalysed transesterification in organic solvent media.[4,21]

28. The rates of the Lipolase-catalysed hydrolysis of triacylglycerols of C_3-C_9 acyl chain length (Table 4) were recorded (pH 8.0; t° 25°; 0.15 M NaCl; 1.0 mM $CaCl_2$; reaction volume was 10 ml; 4 mg of the Lipolase preparation was used; 100 µl of triacylglycerol was hydrolysed) in order to get guidelines for evaluation of the influence of the acyl chain length on the hydrolytic reactivity of the ester substrates. The following relationship was found: $V^{C_4}_{max} = 0.78\, V^{C_6}_{max}$.

Table 4.

Chain length of the acyl group	C_3	C_4	C_5	C_6	C_7	C_8	C_9
V_{max} [µmol/min]	9.1	32.4	56.2	41.7	70.8	64.6	60.3

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