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Carba Analogues of Triglycerides—Isosteric Mimics for Natural Lipids. Novel Substrates for the Determination of Regio- and Enantioselectivities Displayed by Lipases

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Abstract—The novel carba analogues **1a,b** and **2** were synthesized and demonstrated to be isosteric identical mimics for natural triglycerides. They can be used for the system independent determination of regio- and enantioselectivities displayed by lipases. They are, moreover, attractive starting materials for novel carba analogues of phospholipids, PAF analogues and PAF antagonists.

Introduction

Triacylglycerol hydrolases [Lipases, E.C. 3.1.1.3] and phospholipases [E.C. 3.1.1.4] are known to hydrolyze their natural substrates—triglycerides and phospholipids—with varying but frequently high selectivities regarding both the positions of the fatty acids on the glycerol backbone and also their chain length as well as their degree of unsaturation. In recent years several analytical methods for the determination of these parameters have been described in the literature by a number of research groups[1] including ours.[2] While it thus seems possible to determine the regioselectivities and substrate tolerances of esterhydrolases with reasonable accuracy the situation is quite different regarding the enantioselectivities which these enzymes could potentially display for the following reasons (Scheme I).

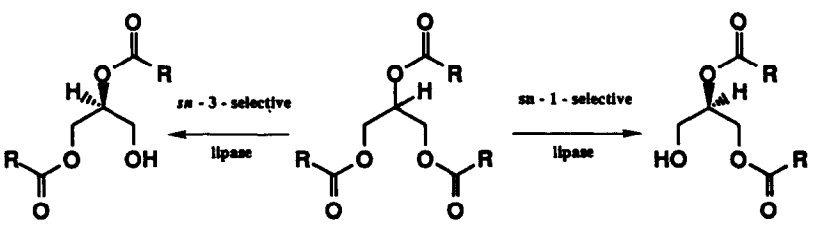
Problems arise both from the rapid acyl group migrations which are notorious for partially hydrolyzed glycerides and—concomitantly—from the instability of the initially formed, enantiomerically pure or enriched *sn*-1,2- or *sn*-2,3-diglycerides (Scheme I).

Thus, prerequisites for the successful determination of the enantioselectivities which are—as nicely demonstrated by Verger *et al.*[3] in fact displayed by numerous lipases—extremely small conversions (< 2–6 %) and rapid derivatizations of the primary reaction products prior to analysis.

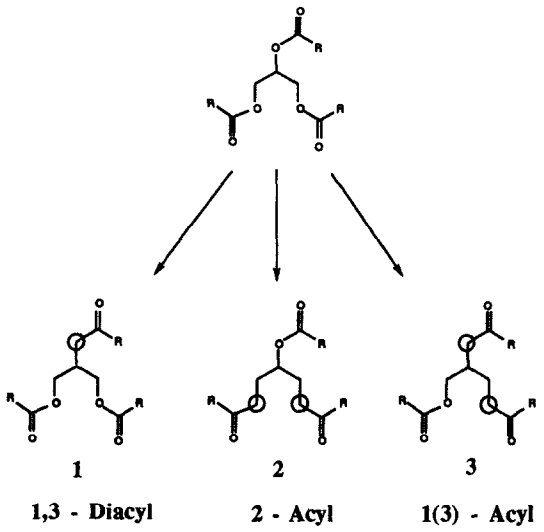
However, (a) enantioselective transformations of achiral triglycerides (Scheme I)—by differentiation of the enantiotopic acyl groups in *sn*-1- or 3- position—can be strongly conversion dependent;[4] (b) there are possibilities for experimental variability in the derivatisations. Both can be sources for error. Moreover, the method is only of analytical value, the resulting products are not suitable for further synthetic use.

In view of this situation we felt that isosteric mimics of natural lipids would be attractive alternatives for a system and medium independent determination of all important enzyme paramenters such as regio- and enantioselectivities, as well as for the synthesis of novel carba analogues of lipids and phospholipids.

Using classical concepts of medicinal chemistry and drug design—and also based on earlier experiments with lipases[5]—one could argue that the replacement of an sp^3-oxygen by an sp^3-carbon atom in triglycerides would lead to such mimics **1–3** of very high similiarity with natural lipids by a minimal deviation of bond angles and bond distances (Scheme II).



Scheme I. Enantioselective, lipase catalyzed transformations of achiral triglycerides.



Scheme II. Carba analogues of triglycerides as mimics for natural lipids.

In view of the fact that 1,3-acyl group migrations are of much less importance as compared to 1,2-acyl group migrations, products resulting from e.g. **1** would also meet the essential criterion of high stability.

Competitive hydrolyses of **1** and **2** would also allow a system and medium independent determination of regioselectivities displayed by lipases.

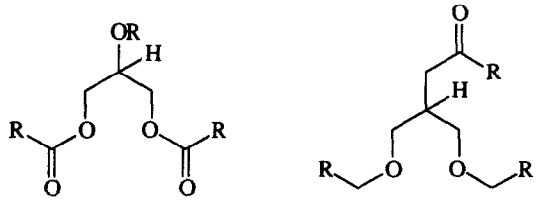
Moreover, if successful, this approach would also allow the synthesis of numerous novel carba analogues of a wide variety of enantiomerically pure glycerides, phospholipids and derivatives thereof with numerous potentially interesting biological activities like lipase and phospholipase inhibitors, PAF (platelet aggregation factor) analogues and antagonists.

The success of the whole concept, however, would entirely depend on our ability to demonstrate that the above mimics, i.e. carba-analogues of triglycerides (a) could be synthesized; and (b) would bind to and (c) be converted by the above enzymes.

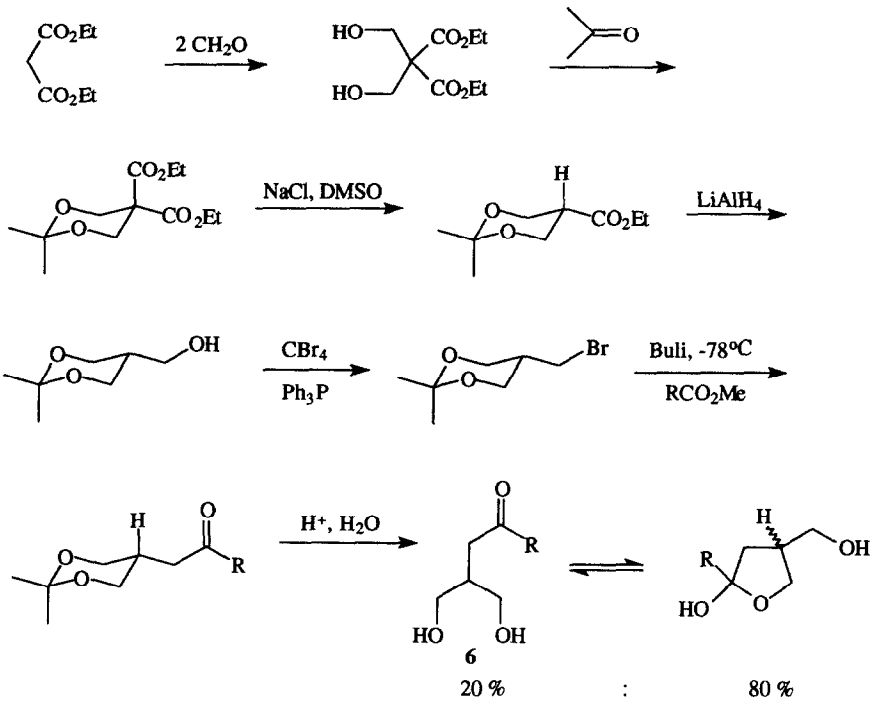
Carba Analogues of Triglycerides—Syntheses

Mimics for 1,3-diacylglycerols ***1a,b***

Several structural analogues of triglycerides have been prepared in the past, notably the so-called etherlipids like **4** and **5**[6a,b] and selected phospholipids.[6c,d]



Unfortunately, however, we did not find any evidence in the literature for a previous synthesis of the required carba-analogues **1** and **2**. The possible reason for this became evident to us following our experiences with the synthetic strategy outlined in Scheme III.



Scheme III. Attempted synthesis of carba analogues **1a,b** (route 1).

While all steps of the synthetic route [condensation of diethylmalonate with formaldehyde, ketalisation of the thus obtained diol, decarboxylation of the malonate moiety, reduction, bromination, and condensation with the carboxylic ester] proceeded as anticipated, we were unable to isolate the diol **6** in reasonable yield. The ease of intramolecular lactol formation leads to an equilibrium mixture with only 20 % of **6** and thus precluded further synthetic efforts along this route.[7]

Clearly, in order to prevent this obtrusive lactol formation we had to employ an intermediate with a 'masked' carbonyl group from which this functionality could be regenerated at the end of the synthetic sequence. This strategy, outlined in Scheme IV was indeed successful and allowed the synthesis of the first carba-analogues **1a,b** in sufficient quantities and acceptable yields.

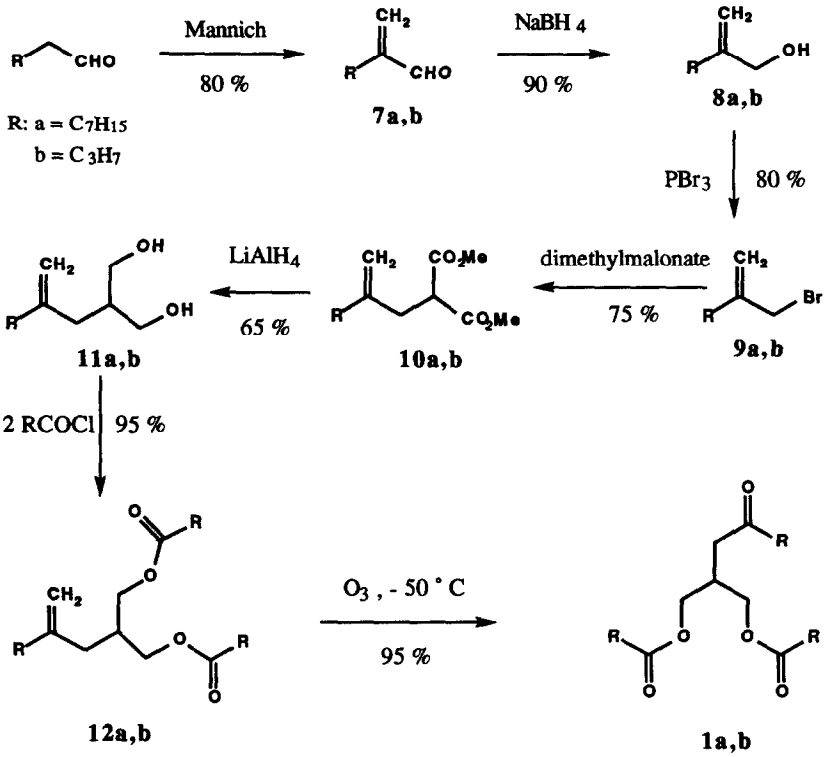
Mannich reaction [$Me_2NH{\cdot}HCl$, $CH_2{=}O$, 70–80 %] of an aldehyde with the desired carbon chain length [R = C_7H_{15}, C_3H_7] allows the convenient introduction of the essential *exo*- methylene function. Reduction of the resulting acroleins **7a,b** [$NaBH_4$, 70–80 %] results in the corresponding allylic alcohols **8a,b** which, after bromination [PBr3, pyridine, 70–80 %] to **9a,b**, condensation with dimethylmalonate [$CH_2(CO_2Me)_2$, KO*t*Bu, 62–86 %] to **10 a,b** and reduction [$LiAlH_4$, 50–74 %] leads to the desired diols **11a,b**, structural analogues of **6** with a masked carbonyl group.

Acylation of the two primary hydroxy groups in **11a,b** [RCOCl, pyridine, 68–75 %] leading to **12 a,b**, followed by ozonolysis [O_3, –78 °C, CH_2Cl_2, HOAc, Me_2S, 75–93 %] of the *exo*-methylene groups finally leads to the desired carba analogues **1a,b**. In spite of the relatively long synthetic sequence involving a total of eight steps, the overall yields of 11–26 % are quite acceptable, indicating yields of 60–80 % in most steps.

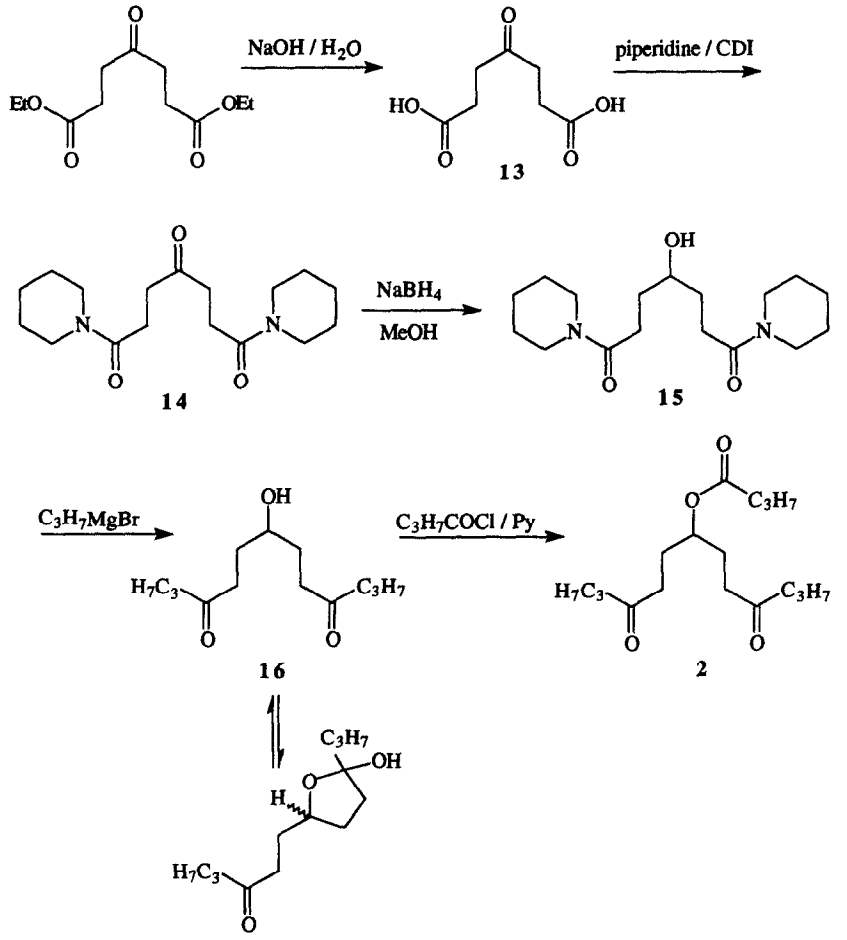
Mimics for 2-acylglycerols 2

In order to provide carba analogues of 2-acyl glycerols which are needed for the system independent determination of regioselectivities we decided to synthesize also the corresponding diketone **2** (Scheme V).

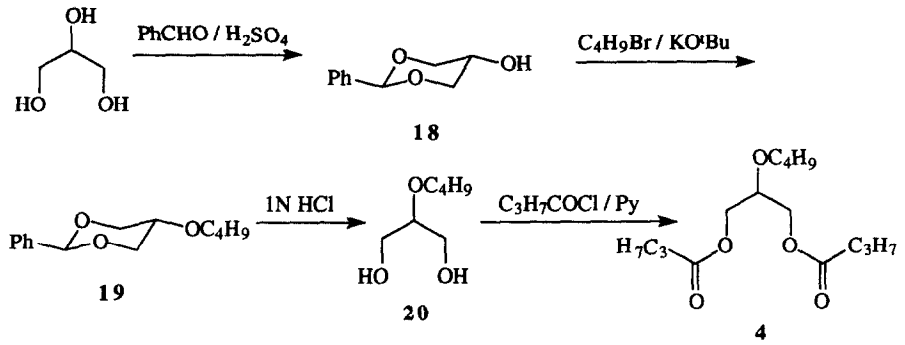
Conversion of 4-oxopimelic acid **13** into the corresponding dipiperidine derivative **14** [piperidine, *N,N'*-carbonyldiimidazole, 88 %], and reduction of **14** [$NaBH_4$, MeOH, 93 %] achieved the 4-hydroxypiperidide **15** in overall yield of 72 %. In theory, reaction of **15** with two equivalents of *n*-propylmagnesium bromide should directly lead to the desired diketone **16** in good yield. Unfortunately, however, this is not the case. Obtained is a mixture of products from which the desired diketone **16** was isolated by repeated chromatography in 12 % yield. The low yield could in part be caused by the existence of an equilibrium between **16** and the corresponding ketal. There are precedences described in the literature with structurally related compounds.[6d] Attempts to further optimize the yield, e.g. by introduction of protection groups in the 2-position have been unsuccessful so far. Nevertheless, by conversion of **16** into **2** [C_3H_7COCl, pyridine, 81 %] we were able to isolate sufficient material in order to develop a system independent method for the determination of regioselectivities displayed by lipases (see below).



Scheme IV. Synthesis of carba analogues **1a,b** (route 2).



Scheme V. Synthesis of carba analogue **2**.



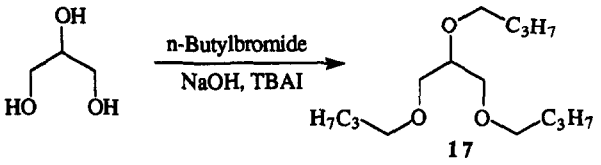
Scheme VI. Synthesis of etherlipid **4**.

Etherlipids 4 and 17

As outlined above, certain etherlipids have been employed previously as models for triglycerides.[6a,b] In order to provide material for the comparison of results from binding studies (see below) or enzymatic transformations we decided to synthesize the corresponding 2-butyloxy-1,3-*sn*-dibutyrin **4** and glyceroltributylether **17**.

For the synthesis of **4** as outlined in Scheme VI it was of course necessary to protect the primary hydroxy groups of the glycerol moiety [PhCH=O, H_2SO_4, 72 %] prior to the introduction of the ether bond [*n*-BuBr, KOtBu, 80 %]. Deprotection [1*N* HCl, 45 %] leads to the corresponding diol (**20**) in moderate yield (water soluble !) which can then be conveniently esterified [C_3H_7COCl, pyridine 76 %] leading to the desired model compound **4**.

Compound **17** is conveniently prepared by phase transfer catalyzed reaction of glycerol with *n*-butylbromide (Scheme VII).



17

Scheme VII. Synthesis of etherlipid **17**.

Carba Analogues of Triglycerides — Binding Studies with Porcine Pancreatic Lipase (PPL)

As outlined already in the introduction, it was essential for the whole concept to establish that the above synthesized mimics of triglycerides are indeed accepted by the enzymes as substrates. In order to compare the behaviour of triglycerides with both etherlipids and our carba analogues we decided to embark on binding studies with a whole series of compounds, i.e. tributyrin, tricaprylin, their structural analogues **1a,b**, **12b**, **2** and the etherlipids **4** and **17**. These molecules display a considerable structural variety but are identical regarding the structural backbone and are comparable regarding the number of carbon atoms in the side chains.

The method employed is based on a procedure described earlier by Borgstrom and Donner[8] allowing a semiquantitative (relative) determination of lipase (PPL) affinities towards structurally different substrates by competitive binding of the lipase in a medium containing sodium taurodeoxycholate with a concentration above the critical micelle concentration of 4 mM and NaCl.

Following the described procedure, 0.5 mL of each the above substrates were incubated in 10 mL of citrate buffer (pH 6) containing 4 mM sodium taurodeoxycholate, 0.1 M NaCl and 10 mg of the purified lipase from porcine pancreas. The mixtures were incubated for 5 min while being connected to the automatic burette of an autotitrator. The fatty acids produced during this incubation time were continuously titrated with 0.1 N NaOH thus providing the total activity of the PPL employed.

After 5 min the mixtures were centrifuged and the phases separated. The relative activities of the lipase in the the aqueous and organic phases are a direct measurement of their concentrations and thus provide evidence for the lipase affinities to the employed substrates. Thus, 0.5 mL of tributyrin was added to the aqueous phase and the remaining lipase activity determined by means of the autotitrator.

For the determination of the lipase activity in the organic phase, fresh buffer was added together with 0.5 mL tributyrin and the liberated fatty acids were again determined by autotitration. The determined activities from all experiments (averages of three measurements) are summarized in Table 1.
Clearly, considerable differences between the employed substrates can be detected immediately. A high lipase activity in the separated organic phase is an indication for a high affinity of the lipase to the corresponding substrate surface while a high activity in the aqueous phase indicates only low affinity to the substrate surface.

In agreement with the earlier results by Borgstrom and Donner[8] the lipase from porcine pancreas exhibits the highest affinity to triglycerides—here tributyrin and tricaprylin. In these cases the highest proportion of the activities are found in the organic phase. On the other hand, practically all of the activity is found in the aqueous phase if the ether lipids **4** and **17** are employed. This observation clearly supports earlier assumptions by Paltauf[9] that the 2-acyl groups on the glycerol backbone are indeed essential for the binding to the lipase. It further indicates that etherlipids are not necessarily preferred models for triglycerides. The relative affinity can be expressed by **A** according to equation 1.

$$\mathbf{A} = \frac{\text{Uorg.} \times 100}{(\text{Uaq.} + \text{Uorg.})} \qquad \mathbf{A} = \text{rel. affinity}, \ \mathbf{U} = \text{activity} \quad (1)$$

Based on the highest affinity value for tributyrin (**A** = 100) the situation becomes very obvious from the graphical representation shown in Figure 1. As hoped for, the keto analogues **1a,b**, **2** proved to be excellent model compounds for tributyrin and tricaprylin, respectively with very similiar affinities.

Surprisingly—and in view of further synthetic applications highly desirable—the methylene analogue **12b** (carrying an *exo*-methylene group) proved to be of considerable similiarity to the triglycerides, probably mainly for steric reasons.

Table 1. Binding studies—affinities of PPL towards various lipids

Substance	Total activity (μmol/h)[1)]	Activity in the aqueous phase (μmol/h)[1)]	Activity in the organic phase (μmol/h)[1)]
Tributyrin	83	10	73
Tricaprylin	67	17	50
Ketone **1a**	65	20	45
Ketone **1b**	76	16	60
Ketone **2**	83	23	60
Methylene deriv.**12b**	65	27	38
Triether **17**	69	66	3
Monoether **4**	74	70	4

[1)]Activity of PPL was measured during the first five minutes of conversion; conditions of the binding studies (pH 6).

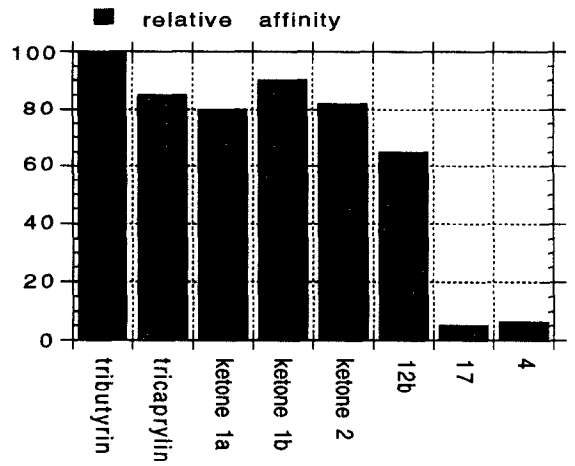


Figure 1. Relative affinities of PPL towards various lipids (A = 100 for tributyrin).

In summary, it can be stated that—at least regarding the relative affinities towards porcine pancreatic lipase—the synthesized carba analogues **1a,b** and **2** are excellent mimics for triglycerides. The high importance of steric factors is indicated by the considerable affinity of the keto analogue **12b** towards PPL. Replacement of the 2-acyl group by an ether function leads to a total breakdown of affinity.

It remains now to be shown that the above described mimics of triglycerides are indeed converted by lipases.

Carba Analogues of Triglycerides—Enzymatic Hydrolysis and Alcoholysis in Presence of the Lipases from Porcine Pancreas (PPL) and *Pseudomonas fluorescens* (PFL).

Introduction

If the above synthesized mimics of triglycerides are indeed accepted as substrates by lipases, similiar or identical kinetic parameters should be observed as compared to the natural triglycerides. For this purpose we studied the competitive hydrolysis and methanolysis of 2-(2-Oxononyl)-1,3-propanediol dicaprylate (**1a**) and tricaprylin under otherwise identical conditions with the two different, largely 1,3-selective lipases from porcine pancreas (PPL) and *Pseudomonas fluorescens* (PFL) (Scheme VIII).

The relative rates of transformation can be defined by the competition factor α according to equation 2.

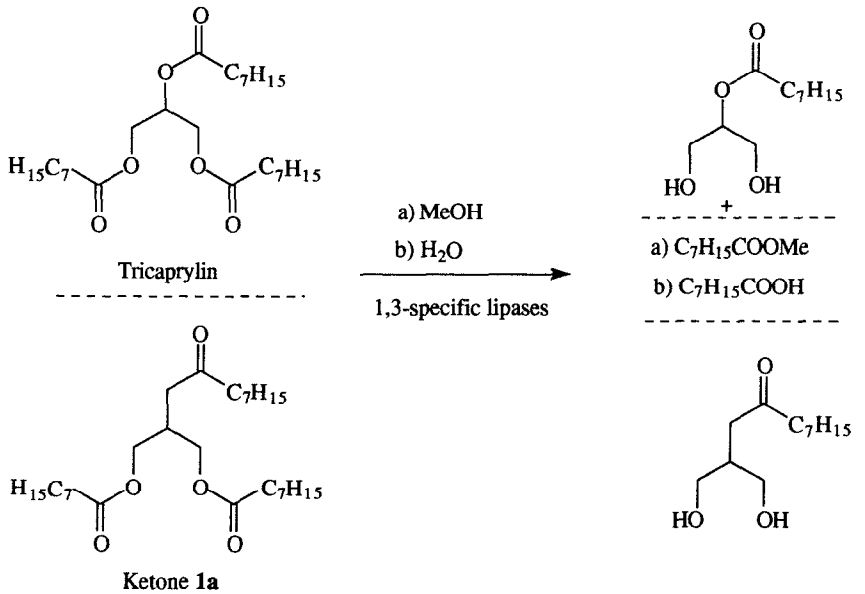
$$\alpha_1 \cdot \log\frac{(S_1)}{(S_1)_0} = \alpha_2 \cdot \log\frac{(S_2)}{(S_2)_0} = \ldots = \alpha_N \cdot \log\frac{(S_N)}{(S_N)_0} \qquad (2)$$

$(S_{1,2,\ldots,N})_0$ are the initial concentrations of the triglycerides and models 1,2,...,n ; α_1 reference factor for tricaprylin = 1

Provided the concentrations of tricaprylin and **1a** are identical at the beginning of the transformations, α describes the ratio of initial rates for both enzymatic transformations. A α_N value close to 1.0 would indicate identical behaviour of tricaprylin with the model substance N. α Thus becomes a relative measure for the similarities between the natural substrate tricaprylin and **1a**.

Competitive enzymatic alcoholyses of tricaprylin and 1a

For this an equimolar mixture of tricaprylin and **1a** were incubated at room temperature with a tenfold molar excess of MeOH in *t*-BuOMe in presence of 10 mg of the corresponding lipase (PPL, PFL both lyophilized). Every 30 min samples were drawn from the reaction mixtures and the degree of substrate conversion determined by GC (± 5–10 %). The time dependence for the alcoholysis experiment in presence of PPL is shown in Figure 2. The practically identical rates of transformation for tricaprylin and the corresponding mimic **1a** are evident.



Scheme VIII. Competitive alcoholyses and hydrolyses of tricaprylin and its mimic **1a**.

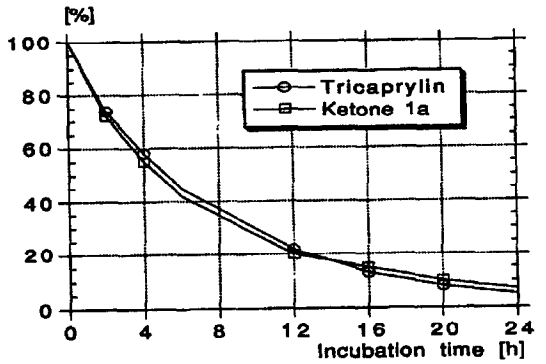


Figure 2. Competitive alcoholysis of tricaprylin and **1a** in presence of PPL.

Enzymatic hydrolyses of tricaprylin and 1a

Similarily, the hydrolytic activities of the two lipases towards tricaprylin and **1a** were determined. For this tricaprylin and **1a** were incubated separately at 30 °C in phosphate buffer (pH 7.0) with 10 mg each of the corresponding lipase. The rates of transformations were determined by continuous titration of the liberated fatty acids with 0.1 N NaOH solution using an autotitrator. As shown in Figure 3, the rates of transformation for both tricaprylin and the mimic **1a** are practically identical.

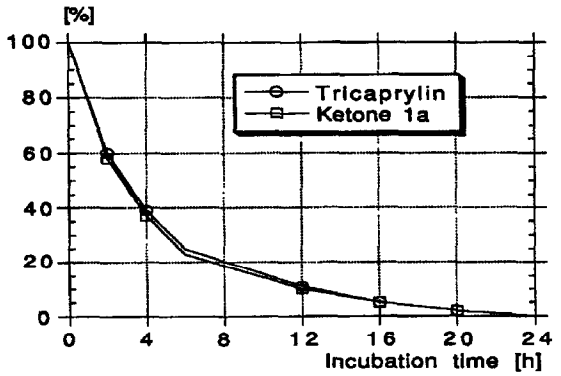


Figure 3. Enzymatic hydrolyses of tricaprylin and ketone **1a** in presence of PPL.

Discussion

From the initial rates the competition factors α have been determined in all cases using equation 2. The obtained values are summarized in Table 2.

From both Figures 2 and 3 and Table 2, it is obvious that the kinetic parameters in both the alcoholyses and hydrolyses are identical for the natural triglyceride and the mimic **1a** within the limits of experimental error.

In summary, it can be stated that—based both on affinity studies and enzymatic transformations—the above synthesized carba analogues of triglycerides **1a,b** and **2** are highly suitable mimics for natural lipids. They are indeed the first totally system independent models for triglycerides and are therefore ideally suited for the determination of regio- and enantioselectivities displayed by these lipases.

Carba Analogues of Triglycerides—Quantitative and System Independent Determination of Regioselectivities Displayed by Lipases

Introduction

As already outlined in the introduction it is well known that lipases, next to showing different degrees of substrate tolerance (fatty acid chain length and degree of unsaturation) these enzymes generally (but not always !) display distinct preferences regarding the positions of the acyl groups on the glyceride moiety. In accordance to this observation they are frequently qualitatively classified with the terms 1,3-specific or unspecific.

Since no quantitative assignments for these properties existed in the literature until recently,[2a,c] we have developed a method for a quantitative determination of the regioselectivities displayed by lipases.[2a] The so-called RE-values, determined by competition experiments are a quantitative measure of such regioselectivities regarding the 1,3- and 2-positions, respectively. A high RE-value indicates high 1,3-specificity, while a low value indicates lacking specificity. This method is, however, only applicable for the behaviour of lipases in organic solvents.

Based on the affinity studies and the enzyme catalyzed transformations described above we felt that these mimics of triglycerides, i.e. **1b** and **2**, would allow the *system- and medium independent* determination of regioselectivities displayed by lipases.

Determination of regioselectivities displayed by lipases using 1b and 2

Using the above described principle of competitive hydrolyses (equation 2), the competition factor γ could be determined from the concomitant hydrolyses of the triglyceride mimics **1b** and **2** (Scheme IX, equation 3).

Table 2. Competitive enzymatic alcoholyses and hydrolyses of tricaprylin and its mimic **1a**

Reaction	Lipase	Competition factor α	
		tricaprylin	ketone **1a**
Transesterifications	*Porcine pancreas*	1	0.96
	Pseudomonas fluorescens	1	0.94
Hydrolyses	*Porcine pancreas*	1	0.97
	Pseudomonas fluorescens	1	0.98

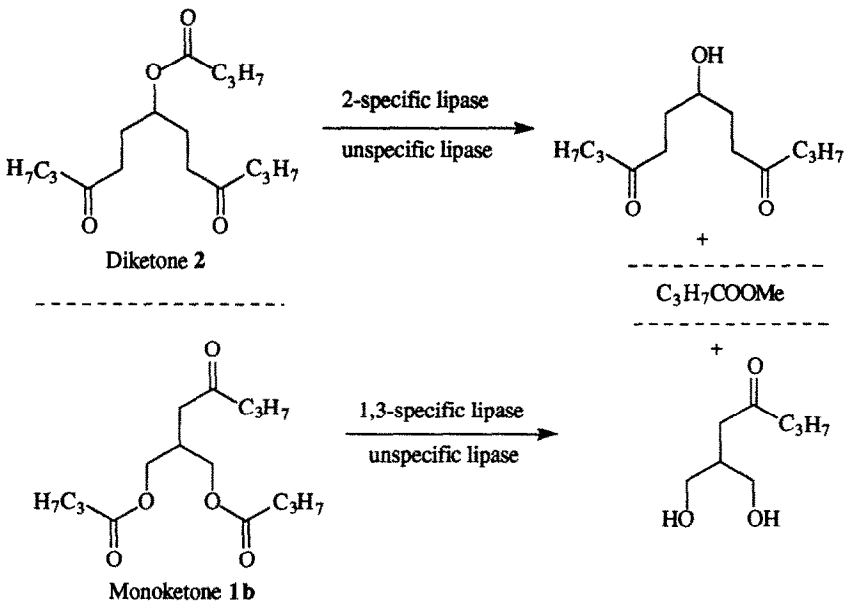
$$\gamma_1 \cdot \log \frac{(S_1)}{(S_1)_0} = \gamma_2 \cdot \log \frac{(S_2)}{(S_2)_0} = \ldots = \gamma_N \cdot \log \frac{(S_N)}{(S_N)_0} \quad (3)$$

$(S_{1,2,\ldots,N})_0$ are the initial concentrations of the triglyceride models 1,2,...,n;

γ_1 reference factor for **1b** = 1; γ_2 factor for **2**.

Clearly, 1,3-specific lipases will only hydrolyze **1b**, while 2-specific lipases would exclusively convert **2**. Unspecific lipases would, of course, hydrolyze both substrates. The competition factor γ describes the ratio of the initial rates of transformation for the two substrates. Since there are two primary acyl groups in **1b**, a stoichiometric correction is required and $\beta = 0.5\ \gamma$ is used for the comparison of the obtained values. $\beta = 1$ would thus denominate an unselective lipase, $\beta >> 1$ would describe a highly 1,3-selective lipase and $\beta << 1$ a 2-specific lipase. These β-values were determined for the competitive alcoholysis of **1b** and **2** in presence of ten different lipases. For this, an equimolar mixture of 2-(2-oxopentyl)-1,3-propanediol dibutyrate (**1b**) and 7-butyroxitridecane-4,10-dione (**2**) were incubated at room temperature with a 10 molar excess of MeOH in *n*-hexane together with 10 mg of the corresponding lipase. In 30 min intervals samples were drawn from the reaction mixtures and analyzed by GC (estimated error ± 5–10 %). The time dependence of substrate consumption is shown in Figures 4 and 5 for two different lipases with clearly different behaviour.

While PPL almost exclusively transforms **1b**, the lipase from *Penicillium camembertii* converts both substrates. From the initial rates of transformations the competition values γ were determined according to equation 3 and listed as so-called selectivity factors β in Table 3. In Table 4 the regioselectivities of the same lipases, expressed as RE^{2a}- and β-values (this paper) from both methods ofdeterminations are compared. The results are rather identical with only minor deviations in selected cases.



Scheme IX. Competitive alcoholyses of **1b** and **2** in presence of lipases.

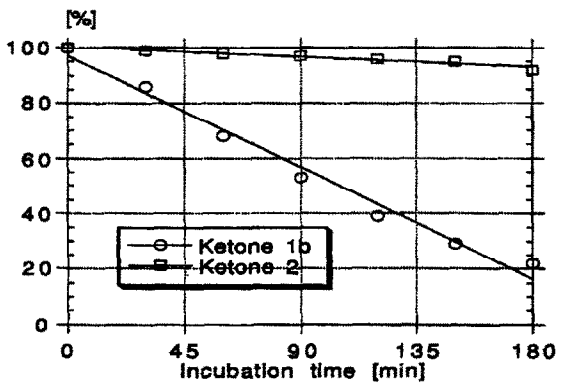


Figure 4. Regioselectivities of lipases. Competitive alcoholyses of **1b** and **2** in presence of PPL.

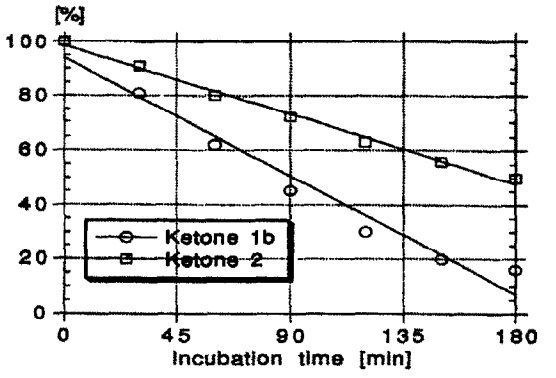


Figure 5. Regioselectivities of lipases. Competitive alcoholyses of **1b** and **2** in presence of the lipase from *Penicillium camembertii*.

Table 3. Regioselectivities of various lipases

Lipase from	γ	β[a)]	Selectivity
Porcine pancreas	32,7	16,3	1,3-specific
Rhizomucor miehei (Lipozyme)	50,9	25,5	1,3-specific
Penicillium camembertii	2,3	1,1	unspecific
Rhizopus arrhizus	12,0	6,0	1,3-selective
Rhizopus delemar	58,7	29,4	1,3-specific
Chromobacterium viscosum	24,2	12,1	1,3-specific
Geotrichum candidum	2,5	1,3	unspecific
Candida rugosa	10,6	5,3	1,3-selective
Pseudomonas fluorescens	17,2	8,6	1,3-selective
Pseudomonas sp.	2,1	1,1	unspecific

[a)] β > 10 = 1,3-specific, 10 > β > 5 = 1,3-selective, β < 5 = unspecific.

Table 4. Regioselectivities of lipases determined by two different methods

Lipase from	RE[a)]	Selectivity	β	Selectivity
Porcine pancreas	95,9	1,3-specific	16,3	1,3-specific
Rhizomucor miehei (Lipozyme)	83,7	1,3-selective	25,5	1,3-specific
Penicillium camembertii	16,3	unspecific	1,1	unspecific
Rhizopus arrhizus	88,0	1,3-selective	6,0	1,3-selective
Rhizopus delemar	98,1	1,3-specific	29,4	1,3-specific
Chromobacterium viscosum	92,7	1,3-specific	12,1	1,3-specific
Geotrichum candidum	59,1	unspecific	1,3	unspecific
Candida rugosa	79,5	1,3-selective	5,3	1,3-selective
Pseudomonas fluorescens	79,0	1,3-selective	8,6	1,3-selective
Pseudomonas sp.	21,5	unspecific	1,6	unspecific

[a)] RE > 90 = 1,3-specific, 90 > RE > 70 = 1,3-selective, RE < 70 = unspecific; Lit.[2a]

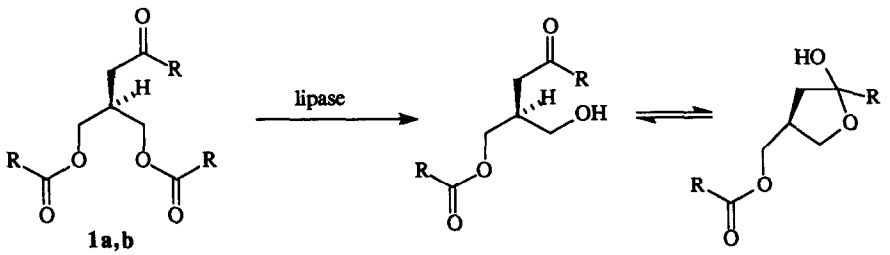
It should be noted that the data obtained for the first time are totally independent of spontaneous or enzyme catalyzed acyl group migrations and thus describe exclusively the selectivity of lipases towards the primary and secondary hydroxyl groups in glycerides.

Summary and Outlook

With the above described experiments we have been able to show that carba analogues of triglycerides, obtained by formal replacement of sp^3 oxygen by sp^3 carbon atoms are indeed ideal isosteric mimics for natural lipids. They bind to lipases with almost identical affinities and are transformed by these enzymes with practically identical rates as compared to triglycerides. They are therefore ideally suited as substrates for the system independent determination of regioselectivities displayed by lipases. We are now also in the position to use these molecules for the system independent determination of enantioselectivities. For this, however, a number of analytical problems have to be solved first, in particular those resulting from the notorious lactol formation in all monoacylated ketone analogues, e.g. the products resulting from the hydrolysis or alcoholysis of **1a,b** (Scheme X).

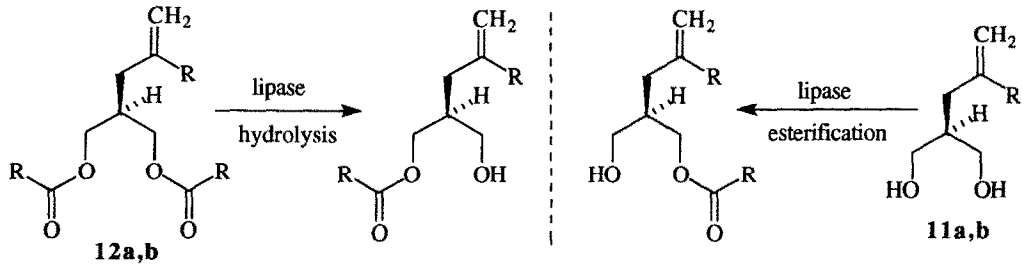
Recently, we were able to demonstrate—in analogy to earlier experiments by K. Mori[10] and ourselves[11] that the corresponding products resulting from **12a,b** can be obtained enantiomerically pure by enzyme catalyzed transformations (Scheme XI).[12]

What remains to be done is the unequivocal determination of absolute configuration and the interconversion of the products resulting from both transformations. Experiments of this nature are underway together with the synthesis of carba analogous glycerides and phospholipids.



1a,b

Scheme X. Products resulting from enantioselective transformations of **1a,b**.



Scheme XI. Enantioselective transformations of carba analogues **11a,b** and **12a,b**.

Table 5. Sources of enzymes

Porcine pancreas	Fluka lipase 62300
Rhizomucor miehei (Lipozyme)	Novo
Penicillium camembertii	Amano G
Rhizopus arrhizus	Amano
Rhizopus delemar	Amano D10
Chromobacterium viscosum	CV-lipase Toyo Jozo Chemical Co., Ltd, Japan
Geotrichum candidum	Amano GC5
Candida rugosa	Amano AY30
Pseudomonas fluorescens	Amano SAMI
Pseudomonas sp.	Amano SAMII

Experimental

All boiling points and melting points are uncorrected. Melting points were taken using a Büchi 510 apparatus. ^1H NMR spectra were obtained using a Bruker WM 250 spectrometer 250 MHz in $CDCl_3$ with TMS as an internal standard. ^{13}C NMR spectra were obtained using a Bruker at 62,896 MHz in $CDCl_3$ with TMS as an internal standard and were long range decoupled. IR spectra were measured on Perkin Elmer spectrophotometer 1420, solid samples as KBr pellets, liquid samples as films.

Gas chromatographic analyses were carried out with Carlo Erba Fractovap 2250 series gas-chromatograph. Conditions: FID-detector, split-splitless-injection 1:20, fused silica capillary column, stationary phase: methyl silicon (SE 30, Macherey-Nagel, Düren), 0.15 mm, 25 m, carrier He, 0.6 bar. Integrator HP 3390 and Shimadzu 14-Afs gas chromatograph. Conditions: FID-detector, on column injection, autosampler Shimadzu-14 for 12 samples, fused silica gel capillary column, phase: methylsilicon (SE 30, Macherey–Nagel, Düren 0.15 mm, 25 m, carrier H_2, 1.8 bar. Integrator Shimadzu GC-5. Silica gel 60 (70–230 mesh Fa. Merck) and analytical TLC plates 60-F254 were purchased from Merck. Ozonizations were carried out with ozone generator Fischer AG, 3 g ozone/h. For the purification and drying of solvents and reagents standard methods were used. All reagents unless specially mentioned are commercially available. Enzymatic hydrolyses were carried out using an autotitrator supplied by Radiometer, Copenhagen. All enzymes used were (if not otherwise mentioned) crude preparations which were commercially available.They were obtained as lyophilizates and stored at 4 °C (Table 5).

Syntheses

2-Methylene-nonanal 7a. Aqueous formaldehyde solution (58.3 mL, 0.78 mol, 37 %) was mixed with *n*-nonanal (100 ml, 0.78 mol). After addition of dimethylamine hydrochloride (59.0 g, 0.73 mol) the solution was refluxed for 48 h at 70 °C. Then water (600 mL) was added and the mixture steam distilled. The distillate was extracted with diethylether (3 × 200 mL) and the whole organic phases were washed with saturated NaCl solution and dried ($MgSO_4$). The solvent was evaporated and the residue fractionated under vacuum (bp_{12} 86–87 °C). **7a** was obtained as a yellowish liquid (73 g, 87 %).

IR (Film): 3360, 3040, 2950–2850, 1680, 1620, 1450, 1320, 1230, 1120, 940, 725; ^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.8 (t, 3H, CH_3, J = 6.5 Hz), 1.3 (m, 10H, CH_2-chain), 2.1 (t, 2H, $CH_2C{=}CH_2$, J = 7.2 Hz), 5.9, 6.2 (m, 2H, $C{=}CH_2$), 9.5 ppm (s, 1H, CHO); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9 (CH_3), 22.5 – 31.6 (6 signals, CH_2-carbon chain), 133.7 ($CH_2{=}C$), 150.8 ($CH_2{=}C$), 194.6 ppm (CHO).

2-Methylene-nonan-1-ol 8a. 2-methylenenonanal (**7a**) (73 g, 0.47 mol) was dissolved in ethanol (400 mL). To this solution $NaBH_4$ (8.7 g, 0.24 mol) was carefully added under ice cooling, keeping the temperature below 30 °C. The mixture was stirred for 12 h at room temperature, the solvent was evaporated and the residue poured into water (200 mL). After extraction with diethylether (3 × 200 mL) the collected organic layers were washed with saturated NaCl solution, dried ($MgSO_4$) and concentrated. The residue was fractionated under vacuum ($bp_{0.1}$ 67–69 °C); **8a** was obtained as a colourless oil (53 g, 72 %).

IR (Film): 3600–2800, 3080, 2950–2910, 1640, 1440, 1220, 1020, 890, 725; ^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (t, 3H, CH_3, J = 6.7 Hz), 1.3 (m, 10H, CH_2-chain), 2.0 (t, 2H, $CH_2C{=}CH_2$, J = 7.5 Hz), 2.4 (br, 1H, OH), 4.0 (s, 2H, CH_2OH), 4.8, 5.0 ppm (2H,

C=CH_2); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9 (*C*H_3), 22.5–32.8 (6 signals, *C*H_2-carbon chain), 65.5 (*C*H_2OH), 108.6 (*C*H_2=C), 149.1 ppm (CH_2=*C*).

2-Methylene-1-bromononane ***9a***. 2-Methylene-nonan-1-ol (**8a**) (53.0 g, 0.34 mol) and dry pyridine (5 mL) were dissolved under dry nitrogen in anhydrous diethylether (200 mL) and the solution cooled down to –5 °C. Then PBr_3 (13.2 mL, 0.14 mol), dissolved in diethylether (100 mL) was added dropwise, keeping the temperature below 0 °C. The mixture was then heated under reflux for 2 h and stirred for another 12 h at room temperature. After that the mixture was washed with 1 N HCl (2 × 100 mL), saturated $NaHCO_3$ solution and dried ($MgSO_4$). The solvent was evaporated and the residue fractionated under vacuum (bp_{15} 105–106 °C); **9a** was obtained as a colourless oil (56 g, 75 %).

IR (Film): 3080, 3000–2910, 1640, 1430, 1200, 1020, 900, 720; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (t, 3H, C*H*$_3$, *J* = 6.6 Hz), 1.3 (m, 10H, C*H*$_2$-chain), 2.2 (t, 2H, C*H*$_2$C=CH_2, *J* = 7.4 Hz), 3.95 (s, 2H, C*H*$_2$Br), 4.95, 5.15 ppm (2H, C=C*H*$_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9 (*C*H_3), 22.5–33.2 (6 signals, *C*H_2 carbon chain), 36.7 (*C*H_2Br), 114.6 (*C*H_2=C), 145.5 ppm (CH_2=*C*).

(2-Methylenenonyl)-diethylmalonate ***10a***. KO^{t}Bu (13.4 g, 0.12 mol) was suspended in dry diethylether (150 mL). Under ice cooling diethylmalonate (44 g, 0.275 mol), dissolved in diethylether (200 mL) was added to this mixture within 10 min. After dropwise addition of 2-methylene-1-bromononane (**9a**) (25 g, 0.11 mol), dissolved in dry diethylether (100 mL) the mixture was stirred under reflux for 12 h. Water (400 mL) was added to the cooled mixture and the product was extracted with diethylether (3 × 200 mL). The collected organic layers were washed with saturated NaCl solution, dried ($MgSO_4$) and concentrated. Then the excess of diethylmalonate was removed under vacuum at 15 torr and the remaining oil was fractionated under vacuum ($bp_{0.01}$ 108–110 °C); **10a** was obtained as a colourless viscous oil (28.2 g, 86 %).

IR (Film): 3080, 2980–2860, 1730, 1640, 1430, 1360, 1230, 1140, 1030, 880, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (t, 3H, C*H*$_3$, *J* = 6.6 Hz), 1.3 (m, 10H, C*H*$_2$-chain), 2.0 (t, 2H, C*H*$_2$C=CH_2, *J* = 7.5 Hz), 2.6 (d, 2H, C=C-C*H*$_2$-CH(COOR)$_2$, *J* = 7.8 Hz), 3.6 (t, 1H, C=C-CH$_2$-C*H*(COOR)$_2$, *J* = 7.8 Hz), 3.7 (s, 6H, C*H*$_3$OCO), 4.7, 4.75 ppm (2H, C=C*H*$_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9 (*C*H_3), 22.5–34.6 (6 signals, *C*H$_2$-carbon chain), 35.8 (C=C-*C*H$_2$-CH(COOR)$_2$), 50.1 (C=C-CH$_2$-*C*H(COOR)$_2$), 52.3 (OCO*C*H$_3$), 110.7 (*C*H_2=C), 145.6 (CH_2=*C*), 169.3 ppm (*C*OOR).

2-(2-Methylenenonyl)-1,3-propanediol ***11a***. $LiAlH_4$ (4.3 g, 0.11 mol) was suspended in dry diethylether (200 mL) under nitrogen and under ice cooling (2-methylenenonyl)-diethylmalonate (**10a**) (28.0 g, 0.1 mol), dissolved in diethylether (100 mL) was slowly added, thereby maintaining a weak reflux. After the completed addition the mixture was stirred for another 12 h at room temperature. Ethylacetate (20 mL) and then water (10 mL) were carefully added. The precipitate was separated by filtration and the organic phase washed with saturated NaCl solution (2×), dried ($MgSO_4$) and concentrated. The remaining oil was fractionated under vacuum leading to crude **11a** (13.2 g, 66 %, $bp_{0.1}$ 135–140 °C). Further purification by chromatography on silica gel (eluent hexane/diethylether 1:1), produced pure 2-(2-methylenenonyl)-1,3-propanediol **11a** (10.0 g, 50 %).

IR (Film) : 3600–2600, 3080, 2980–2840, 1650, 1470, 1390, 1090, 1040, 960, 890, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.7 (t, 3H, C*H*$_3$, *J* = 6.2 Hz), 1.25 (m, 10H, C*H*$_2$- chain), 2.0 (m, 5H, C*H*$_2$C=CH_2, C=C-C*H*$_2$C*H*), 3.5 (m, 4H, C*H*$_2$OH), 3.7 (br, 2H, O*H*), 4.7, 4.75 ppm (2H, C=C*H*$_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9 (*C*H_3), 22.5–35.5 (7 signals, *C*H_2-carbon chain), 39.5 (C=C-CH$_2$-*C*H), 65.5 (*C*H_2OH), 110.7 (*C*H_2=C), 147.2 ppm (CH_2=*C*).

2-(2-Methylenenonyl)-1,3-propanediol dicaprylate ***12a***. 2-(2-Methylenenonyl)-1,3-propanediol (**11a**) (3.7 g, 15 mmol) and dry pyridine (2.7 g, 37 mmol) were dissolved under an atmosphere of dry nitrogen in anhydrous CCl_4 (30 mL) and the solution cooled to 0 °C. Freshly distilled caprylic acid chloride (5.0 g, 34 mmol), dissolved in CH_2Cl_2 (20 mL) was added slowly. After the completed addition the solution was stirred for 24 h at 40 °C and then washed with 1N HCl (50 mL), saturated $NaHCO_3$ solution and saturated NaCl solution (50 mL). The separated organic layer was dried ($MgSO_4$) and concentrated. The remaining oil was purified by chromatography on silica gel (eluent *n*-hexane/diethylether 6:1). Obtained was 2-(2-methylenenonyl)-1,3-propanediol dicaprylate **12a** as a colourless oil (4.7 g, 68 %, mp 0 °C).

IR (Film): 3080, 3000–2840, 1740, 1640, 1450, 1390, 1230, 1190, 1090, 990, 860, 720; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (m, 9H, (C*H*$_3$), 1.3 (m, 26H, C*H*$_2$-chain), 1.6 (m, 4H, C*H*$_2$C*H*$_2$COOR), 2.0 (t, 2H, C*H*$_2$C=CH_2, *J* = 7.8 Hz), 2.05 (d, 2H, C=C-C*H*$_2$CH, *J* = 7.4 Hz), 2.2 (m, 1H, C*H*), 2.3 (t, 4H, C*H*$_2$COOR, *J* = 7.2 Hz), 4.05 (m, 4H, C*H*$_2$OCO), 4.7, 4.8 ppm (2H, C=C*H*$_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.9, 13.95 (*C*H_3), 22.5–35.3 (14 signals, *C*H_2-carbon chain, C=C-CH$_2$-*C*H), 63.7 (*C*H_2OCO), 111.5 (*C*H_2=C), 146.0 (CH_2=*C*), 173.6 ppm (*C*OOR).

2-(2-Oxononyl)-1,3-propanediol dicaprylate ***1a***. 2-(2-Methylenenonyl)-1,3-propanediol dicaprylate (**12a**) (4.6 g, 10 mmol) was dissolved in CH_2Cl_2 and glacial acetic acid (9:1 v/v, 100 mL) and the obtained solution was cooled down to –78 °C. At this temperature ozone was introduced until the solution became blue. After this the solution was purged with nitrogen at –78 °C for 5 min and Me_2S (20 mL) was added in one portion. Under continuous stirring the solution was allowed to warm up slowly to room temperature and stirring was continued for another 2 h. The

organic phase was washed with saturated $NaHCO_3$ solution (2 × 50 mL), saturated NaCl solution (50 mL), dried ($MgSO_4$) and concentrated. The remaining oil was recrystallized from methanol. 2-(2-Oxononyl)-1,3-propanediol dicaprylate (**1a**) was obtained (4.3 g, 93 %) as colourless needles (mp 32–33 °C).

IR (Film): 3000–2840, 1740, 1710, 1470, 1380, 1250, 1230, 1190, 890, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (m, 9H, CH_3), 1.25 (m, 24H, CH_2-chain), 1.6 (m, 6H, CH_2CH_2COR, ester and ketone), 2.25 (t, 4H, CH_2COOR, J = 7.3 Hz), 2.35 (t, 2H, $CH_2C{=}O$, J = 7.5 Hz), 2.45 (d, 2H, O=C-CH_2CH, J = 6.6 Hz), 2.6 (m, 1H, CH), 4.05 ppm (m, 4H, CH_2OCO); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.8 (2 signals, CH_3), 22.4–31.4 (9 signals, CH_2-carbon chain), 32.8 (CH), 33.9 (CH_2COOR), 40.8 (CH_2COR), 43.1 ($ROCCH_2CH$), 63.5 (CH_2OCO), 173.4 ($COOR$), 208.4 ppm (CO, ketone).

2-Methylenepentanal ***7b***. In analogy to the preparation of **7a**, using pentanal (86.1 g, 1 mol), 37 % aqueous formaldehyde solution (100 mL, 1.2 mol) and dimethylamine hydrochloride (100 g, 1.2 mol) **7b** was obtained as a yellowish liquid (70.7 g, 72 %, bp_{12} 115–117 °C).

IR (Film): 3040, 2950–2850, 1670, 1620, 1470, 1320, 1250, 1190, 950, 720; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 1.0 (t, 3H, CH_3, J = 7.3 Hz), 1.4 (m, 4H, CH_2-chain), 2.1 (t, 2H, $CH_2C{=}CH_2$, J = 7.5 Hz), 6.0, 6.3 (m, 2H, $C{=}CH_2$), 9.6 ppm (s, 1H, CHO).

2-Methylene-pentan-1-ol ***8b***. In analogy to the preparation of **8a**, using methylenepentanal **7b** (44.1 g, 0.45 mol), $NaBH_4$ (9.5g 0.25 mol) **8b** was obtained as a colourless oil (33.5 g, 75 %, bp_{15} 55–56 °C).

IR (Film): 3600–2800, 3070, 2960–2900, 1635, 1450, 1230, 1180, 1020, 950, 720; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (t, 3H, CH_3, J = 7.2 Hz), 1.4 (m, 2H, CH_2-chain), 2.0 (t, 2H, $CH_2C{=}CH_2$, J = 7.1 Hz), 2.45 (br, 1H, OH), 4.0 (s, 2H, CH_2OH), 4.8, 5.0 ppm (2H, $C{=}CH_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.6 (CH_3), 20.7, 34.0 (CH_2-carbon chain), 65.4 (CH_2OH), 108.7 ($CH_2{=}C$), 148.7 ppm ($CH_2{=}C$).

2-Methylene-1-bromopentane ***9b***. In analogy to the preparation of **9a**, using methylene-pentan-1-ol (**8b**), pyridine (5 mL) and PBr_3 (11.8 mL, 0.13 mol) **9b** was obtained a as colourless liquid (39.1 g, 72 %, bp 145–147 °C).

IR (Film): 3080, 2980–2910, 1640, 1450, 1250, 1110, 950, 890, 720; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (t, 3H, (CH_3, J = 7.3 Hz), 1.5 (m, 2H, CH_2-chain), 2.2 (t, 2H, $CH_2C{=}CH_2$, J = 7.6 Hz), 4.0 (s, 2H, CH_2Br), 5.0, 5.15 ppm (2H, $C{=}CH_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.6 (CH_3), 20.4, 35.3 (CH_2-carbon chain), 36.7 (CH_2Br), 114.8 ($CH_2{=}C$), 145.3 ppm ($CH_2{=}C$).

(2-Methylenepentyl)-diethylmalonate ***10b***. In analogy to **10a**, using 2-methylene-1-bromopropane (**9b**) (67.1 g, 0.5 mol) and KO^{t}Bu (28.0 g, 0.25 mol) **10b** was obtained as a colourless, viscous oil (29.8 g, 62 %, bp_{12} 117–119 °C).

IR (Film): 3080, 2980–2860, 1735, 1635, 1450, 1390, 1320, 1230, 1090, 1030, 890, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (t, 3H, CH_3, J = 7.3 Hz), 1.4 (m, 2H, CH_2-chain), 1.9 (t, 2H, $CH_2C{=}CH_2$, J = 7.7 Hz), 2.6 (d, 2H, C=C-CH_2-$CH(COOR)_2$, J = 7.8 Hz), 3.6 (t, 1H, C=C-CH_2-$CH(COOR)_2$, J = 7.8 Hz), 3.7 (s, 6H, CH_3OCO), 4.7 ppm (2H, $C{=}CH_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.4 (CH_3), 20.4, 34.5 (CH_2-carbon chain), 37.8 (C=C-CH_2-$CH(COOR)_2$), 50.1 (C=C-CH_2-$CH(COOR)_2$), 52.2 ($OCOCH_3$), 110.8 ($CH_2{=}C$), 145.2 ($CH_2{=}C$), 169.3 ppm ($COOR$).

2-(2-Methylenepentyl)-1,3-propanediol ***11b***. In analogy to **11a**, using (2-methylenepentyl)-diethylmalonate (**10b**) and $LiAlH_4$ (7.0 g, 0.18 mol) **11b** was obtained as a colourless, highly viscous oil (15.5 g, 70 %).

IR (Film): 3600–2600, 3075, 2960–2860, 1640, 1430, 1320, 1190, 1050, 990, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.85 (t, 3H, CH_3, J = 7.3 Hz), 1.35 (m, 2H, CH_2-chain), 1.9 (m, 5H, $CH_2C{=}CH_2$, C=C-CH_2CH), 3.2 (br, 2H, OH), 3.65 (m, 4H, CH_2OH), 4.75 ppm (2H, $C{=}CH_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.6 (CH_3), 22.5, 34.5, 37.5 (3 signals, CH_2-carbon chain), 41.8 (C=C-CH_2-CH), 65.6 (CH_2OH), 110.9 ($CH_2{=}C$), 146.9 ppm ($CH_2{=}C$).

2-(2-Methylenepentyl)-1,3-propanediol dibutyrate ***12b***. In analogy to **12a**, using (2-methylenepentyl)-1,3-propanediol (**11b**), (2.3 g, 15 mmol), pyridine (2.5 g, 34 mmol) and butyric acid chloride (3.3 g, 34 mmol) **12b** was obtained as a colourless, highly viscous oil (3.6 g, 74 %).

IR (Film): 3080, 2960–2860, 1740, 1635, 1470, 1320, 1250, 1090, 990, 890, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (m, 9H, CH_3), 1.4 (m, 2H, CH_2-chain), 1.65 (m, 4H, CH_2CH_2COOR), 1.95 (t, 2H, $CH_2C{=}CH_2$, J = 7.8 Hz), 2.05 (d, 2H, C=C-CH_2CH, J = 7.4 Hz), 2.15 (m, 1H, CH), 2.25 (t, 4H, CH_2COOR, J = 7.4 Hz), 4.0 (m, 4H, CH_2OCO), 4.75 ppm (2H, $C{=}CH_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.5, 13.6 (CH_3), 18.3, 20.5, 34.8, 35.0, 36.0, 36.1 (6 signals, CH_2-carbon chain, C=C-CH_2-CH), 63.7 (CH_2OCO), 111.7 ($CH_2{=}C$), 145.7 ($CH_2{=}C$), 173.4 ppm ($COOR$).

2-(2-Oxopentyl)-1,3-propanediol dibutyrate ***1b***. In analogy to **1a** using (2-methylenepentyl)-1,3-propanediol dibutyrate (**12b**) (3.0 g, 10 mmol) **1b** (2.6 g, 86 %) was obtained as a colourless, highly viscous oil after fractionation under vacuum ($bp_{0.1}$ 140 °C).

IR (Film): 2980–2840, 1735, 1710, 1450, 1390, 1230, 1190, 1110, 890, 720; ^{1}H NMR (250 MHz, $CDCl_3$,

TMS) δ: 0.9 (m, 9H, CH_3), 1.55 (m, 6H, CH_2CH_2COR, ester and ketone), 2.2 (t, 4H, CH_2COOR, J = 7.3 Hz), 2.35 (t, 2H, $CH_2C{=}O$, J = 7.8 Hz), 2.4 (d, 2H, O=C-CH_2CH, J = 7.3 Hz), 2.6 (m, 1H, CH), 4.0 ppm (m, 4H, CH_2OCO); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.45 (2 signals, CH_3), 17.0, 18.1 (2 signals, CH_2-carbon chain), 32.8 (CH), 35.8 (CH_2COOR), 40.9 (CH_2COR), 44.9 ($ROCCH_2CH$), 63.4 (CH_2OCO), 173.2 (COOR), 208.3 ppm (CO, ketone).

4-Oxoheptane-1,7-dicarboxylic acid 13. 4-Oxoheptane-1,7-diethyldicarboxylate (107 mL, 0.5 mol) was added to a solution of NaOH (44 g, 1.1 mol) in water (500 mL). The resulting emulsion was stirred at room temperature for 15 h until a clear solution was obtained. This solution was extracted once with *n*-hexane (100 mL). Under ice cooling the pH-value was adjusted pH 1–2 with concentrated hydrochloric acid. After 2 h at 4 °C the resulting precipate was collected by filtration. 4-oxoheptane-1,7-dicarboxylic acid (**13**) (75.5 g, 87 %) was obtained as colourless, crystalline leaves (mp 142–143 °C).

^{13}C NMR (62.896 MHz, DMSO-d_6) δ: 27.7 (CH_2COOH), 36.7 ($COCH_2$), 173.8 (COOR), 207.8 ppm (CO, ketone).

4-Oxoheptane-1,7-dicarboxylic acid dipiperidide 14. To a solution of 4-oxoheptane-1,7-dicarboxylic acid (**13**) (14.3 g, 82 mmol) in anhydrous THF (60 mL) a solution of *N,N'*-carbonyl diimidazole (CDI) (26.5 g, 165 mmol) in THF (60 mL) was slowly added under ice cooling. After the completed addition the mixture was stirred at room temperature for another 2 h. Piperidine (24.6 mL, 246 mmol) was added and the mixture stirred at room temperature for another 12 h. Then the solution was concentrated on a rotavapor, 2 M sulfuric acid (100 mL) was added to the solution which was stirred for *ca* 10 min. The aqueous phase was extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution (2 × 50 mL), dried ($MgSO_4$) and concentrated; **14** was obtained as a yellowish liquid (22.3 g, 88 %) without any additional purification.

^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 1.5 (m, 12H, piperidine ring), 2.6 (t, 4H, CH_2CO, J = 6.4 Hz), 2.7 (t, 4H, CO-CH_2CH_2, J = 6.4 Hz), 3.4 ppm (m, 8H, $CON(CH_2)_2$); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 24.3 ($CH_2(CH_2)_2NR$), 25.3, 26.1 (CH_2CH_2NR), 26.8 (CH_2CONR), 37.3 (CO-CH_2), 42.6, 46.2 ($CON(CH_2)_2$, piperidine), 169.6 (CONR), 209.2 ppm (CO, ketone).

4-Hydroxyheptane-1,7-dicarboxylic acid dipiperidide 15. To a cooled solution of 4-oxoheptane-1,7-dicarboxylic acid dipiperidide (**14**) (22.2 g, 72 mmol) in methanol (100 mL) $NaBH_4$ (1.40 g, 36 mmol) was carefully added, keeping the temperature below 30 °C. After the completed addition the mixture was stirred at room temperature for another 12 h. The solvent was evaporated, 200 mL water was added and the mixture extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with saturated NaCl solution (100 mL), dried ($MgSO_4$) and concentrated. The yellowish residue was recrystallized from *t*-BuOMe; **15** (21.1 g, 93 %) was obtained as a colourless, crystalline substance, mp 87–88 °C.

^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 1.5 (m, 12H, piperidine ring), 2.35 (t, 4H, CH_2CHOH, J = 7.3 Hz), 2.55 (t, 4H, CH_2CH_2CONR, J = 7.3 Hz), 3.4 (m, 8H, $CON(CH_2)_2$), 3.5 (m, 1H, CHOH), 4.0 ppm (br, 1H, OH); ^{13}C-NMR (62.896 MHz, $CDCl_3$) δ: 24.0 ($CH_2(CH_2)_2NR$), 25.2, 26.0 (CH_2CH_2NR), 28.9 (CH_2CONR), 32.6 (CO-CH_2), 41.8, 45.9 ($CON(CH_2)_2$, piperidine), 68.9 (CHOH), 170.4 ppm (CONR).

7-Hydroxytridecane-4,10-dione 16. To a suspension of magnesium shavings (6.0 g, 245 mmol) in anhydrous THF (200 mL) *n*-propylbromide (22 mL, 240 mmol) was slowly added keeping the mixture at a gentle reflux. After 2 h refluxing most of the magnesium was dissolved and the solution was filtered under a nitrogen atmosphere over an inverse glass sinter filterplate and then added dropwise to a solution of **15** (7.5 g, 24 mmol) in anhydrous THF (300 mL). After the completed addition the mixture was stirred at room temperature for another 15 h. The solvent was evaporated and 3 N hydrochloric acid (70 mL) was added under ice cooling. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic layers washed with saturated NaCl solution (100 mL), dried ($MgSO_4$) and concentrated. The residue was purified by chromatography on silica gel (eluent diethylether), followed by recrystallization from *n*-hexane; **16** (0.69 g, 12 %) was obtained as colourless crystals (mp 37–39 °C).

^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (t, 6H, CH_3, J = 6.9 Hz), 1.6 (m, 4H, CH_2CH_3), 1.75 (m, 4H, HOCH-CH_2), 2.4 (m, 8H, CH_2COR), 3.1 (br, 1H, OH), 3.65 ppm (m, 1H, CHOH); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.5 (CH_3), 17.1 (CH_2CH_3), 27.8 (HOCH-CH_2), 38.1, 43.2 (CH_2COR), 68.8 (CHOH), 206.2 ppm (COR).

7-Butyroxytridecane-4,10-dione 2. **16** (0.60 g, 2.6 mmol) and anhydrous pyridine (0.5 g, 6.8 mmol) were dissolved under nitrogen in dry CCl_4 (10 mL) and the mixture cooled to 0 °C. To this solution freshly distilled butanoylchloride (0.3 g, 3 mmol), dissolved in CCl_4 (5 mL) was slowly added. The solution was stirred at room temperature for 24 h. A colourless precipitate was formed. The solution was then washed with 1 N hydrochloric acid (2 × 5 mL), saturated $NaHCO_3$ solution (2 × 5 mL), saturated NaCl solution (2 × 5 mL), dried ($MgSO_4$) and concentrated. Crude **2** was obtained as a yellowish oil, which was further purified by chromatography on silica gel (eluent *n*-hexane/diethylether 1:1); **2** was obtained as a colourless, viscous oil (0.63 g, 81 %).

IR (Film): 2980–2840, 1735, 1710, 1470, 1380, 1230, 1110, 1090, 850, 725; ^1H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (m, 9H, CH_3), 1.6 (m, 6H, CH_2CH_2COR, ester and ketone), 1.8 (m, 4H, OCOCH-CH_2), 2.2 (t, 2H, CH_2COOR, J = 7.5 Hz), 2.35 (m, 8H, $CH_2C{=}O$), 4.8 ppm (m, 1H, CHOCO); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.55 (2 signals, CH_3), 17.1, 18.4 (2 signals, CH_2-

carbon chain), 27.8 (HOCH-CH_2), 36.2 (CH_2COOR), 38.2, 44.6 (CH_2COR), 72.4 (CHOCO), 173.3 (COOR), 204.6 ppm (CO, ketone).

Glyceroltributylether 17. NaOH (40 g, 1 mol) was dissolved in water (200 mL). To this solution glycerol (12.0 g, 0.11 mol) and tetrabutylammonium iodide (12.0 g) were added and the mixture heated to 80 °C until a clear solution was obtained. At this temperature 1-bromobutane (70.0 g, 0.5 mol) was added dropwise and the mixture stirred at this temperature for another 24 h. The solution was cooled and extracted with diethylether (3 × 100 mL). The collected organic layers were washed with saturated NaCl solution (100 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue fractionated under vacuum; **17** was obtained as a colourless oil (22.3 g, 78 %, bp_{15} 135 °C).

IR (Film): 2980–2840, 1735, 1710, 1470, 1380, 1230, 1110, 1090, 850, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (m, 9H, (CH_3), 1.35 (m, 6H), 1.55 (m, 6H, CH_2-chain), 3.5 (m, 11H, O-CH, O-CH_2); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.7 (2 signals, CH_3), 19.1, 19.15 (CH_2-carbon chain), 31.6, 32.0 ($CH_2CH_2CH_3$), 70.1, 70.7, 71.2 (CH_2-O), 77.7 ppm (CH-O).

5-Hydroxy-2-phenyl-1,3-dioxane 18. Freshly distilled benzaldehyde (50.0 g, 0.47 mol), glycerol (55.0 g, 0.60 mol) and 10 drops of concentrated H_2SO_4 were mixed in a three neck flask fitted with a gas inlet and a water separator. After the addition of toluene (300 mL) the suspension was stirred under reflux, while a continous stream of CO_2 was introduced to the solution. The produced water (8.5 mL) was collected in the separator. After cooling to 0 °C for 2 days **18** was obtained as a solid material which was further purified by recrystallization (diethylether/*n*-hexane 1:1); **17** was obtained as a mixture of *cis/trans* isomers (60.7 g, 72 %, mp 60–71 °C).

^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 3.6 (br, 1H, OH), 4.2 (m, 5H, glycerol skeleton), 5.6 (s, 1H, OCHO), 7.4 ppm (m, 5H, Ph-H); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 63.7 (HOCH), 72.0 (CH_2OR), 101.3 (OCHO), 125.7, 128.0, 128.9, 137.6 ppm (phenyl).

2-Butyloxy-5-phenyl-1,3-dioxane 19. To a suspension of KO^{t}Bu (3.4 g, 30 mmol) in THF (100 mL) **18** (4.5 g, 25 mmol) was added and the mixture stirred at room temperature for 1 h. 1-Bromobutane (4.0 g, 29 mmol), dissolved in THF (50 mL) was added dropwise to the red mixture. After 24 h stirring at room temperature the reaction mixture was filtered and the filtrate concentrated on a rotavapor. The residue was dissolved in diethylether (150 mL), washed with 1N HCl (2 × 40 mL), saturated $NaHCO_3$ solution (2 × 40 mL), saturated NaCl solution (2 × 30 mL) and dried ($MgSO_4$). The solvent was evaporated and the resulting yellow oil recrystallized (diethylether/*n*-hexane 1:1); **19** was obtained as colourless crystals (4.7 g 80 %, mp 30–33 °C).

^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.95 (t, 3H, CH_3, *J* = 7.2 Hz), 1.4 (m, 2H, CH_2CH_3), 1.7 ($CH_2CH_2CH_3$), 3.25 (m, 1H, CHOR), 3.55 (t, 2H, $OCH_2CH_2CH_2CH_3$, *J* = 7.4 Hz), 4.0–4.4 (m, 4H, glycerol skeleton), 5.55 (s, 1H, OCHO), 7.45 ppm (m, 5H, Ph-H); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.7 (CH_3), 19.2, 31.7 (CH_2-carbon chain), 68.5, 68.9 (CH_2OR), 70.4 (ROCH), 101.1 (OCHO), 126.0, 127.9, 128.7, 138.0 ppm (phenyl).

2-O-Butylglycerol 20. **19** (2.36 g, 10 mmol) was suspended in 1 N HCl (25 mL) and the mixture heated under reflux for 3 h. The organic layer was separated and the aqueous phase was washed with *n*-hexane (2 × 20 mL) and then continuously extracted with diethylether for 24 h. The combined organic phases were dried ($MgSO_4$) and concentrated, **20** was obtained as an colourless oil (0.67 g, 45 %).

^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.95 (t, 3H, CH_3, *J* = 7.3 Hz), 1.4 (m, 2H, CH_2CH_3), 1.7 ($CH_2CH_2CH_3$), 3.6 ppm (m, 7H, OCHR); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.7 (CH_3), 19.4, 31.6 (CH_2-carbon chain), 61.3 (CH_2OH), 68.2 (CH_2OR), 71.1 ppm (ROCH).

2-O-Butyl-1,3-dibutyrin 4. **20** (0.60 g, 4 mmol) and anhydrous pyridine (1.0 g, 13.6 mmol) were dissolved under nitrogen in dry CCl_4 (10 mL) and the solution cooled to 0 °C. Freshly distilled butanoyl chloride, dissolved in dry CCl_4 (10 mL) was added dropwise to this mixture, which was stirred for another 24 h at room temperature. The mixture was washed with 1 N HCl (2 × 5 mL) dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica gel to give **4** (0.87 g, 78 %) as a colourless oil.

IR (Film): 2980–2840, 1735, 1470, 1390, 1230, 1190, 1080, 990, 890, 725; ^{1}H NMR (250 MHz, $CDCl_3$, TMS) δ: 0.9 (m, 9H, CH_3), 1.5 (m, 6H, CH_2CH_3 ester and ether), 1.7 ($CH_2CH_2CH_3$, ether), 2.2 (t, 4H, CH_2COOR, J = 7.3 Hz), 3.6 ppm (m, 3H, OCHR), 3.9 ppm (m, 4H, CH_2OCO); ^{13}C NMR (62.896 MHz, $CDCl_3$) δ: 13.4, 13.7 (2 signals, CH_3), 17.8, 19.1, 29.8 (3 signals, CH_2-carbon chain), 35.8 (CH_2COOR), 63.4 (CH_2OCO), 68.2 (CH_2OR), 71.1 (ROCH), 173.2 ppm (COOR).

Studies

Determination of lipase affinities. Purified lipase from porcine pancreas (Sigma Chemie) (10 mg) was dissolved in 20 mL of an aqueous buffer (citrate/NaOH; pH 6.0 containing 4 mM sodium taurodesoxycholate and 0.1 M NaCl). To this mixture 0.5 mL of the corresponding lipid sample (tricaprylin, tributyrin, the carba analogues **1a**, **1b**, **2** and the ether lipids **4** and **17**) was added and the mixture incubated for 5 min at the autotitrator, where the released fatty acids were continuously titrated with 0.1 N NaOH. From this measurement the total activity of the lipase was determined. After 5 min the organic phases were separated by centrifugation (6000 rpm, 10 min).

For the determination of the lipase activity in the *aqueous layer*, tributyrin (0.5 mL) was added and the resulting emulsion incubated again while the liberated fatty acids were again continuously titrated with 1 N NaOH.

For the determination of the lipase activity in the *organic layer*, buffer and tributyrin (0.5 mL) were added and the released fatty acids again titrated. The affinity of the lipase to the corresponding lipid surface was estimated from the ratio of activities in both (aqueous and organic) phases.

Competitive alcoholyses of tricaprylin and the ketone **1a**. Tricaprylin (1.0 g, 2.1 mmol) and 2-(2-oxononyl)-1,3-propanediol dicaprylate (**1a**) (1.0 g, 2.1 mmol) were dissolved in *n*-hexane (20 mL). Methanol (5 mL) and triacontane (0.1 %, w/v) as internal standard were added. To this emulsion the investigated lipase preparation (PPL, *Pseudomonas fluorescens*) (10 mg) was added and the resulting mixtures stirred at room temperature. At the beginning of the reaction, samples (0.5 mL) were taken every 30 min and after 4 h at 2 h intervals. All solid materials were removed by filtration and the dissolved glycerides were silylated and analyzed by GC as described previously.[2a]

GC-analysis—sample preparation. For the gas chromatographic analysis of glycerides a sample containing *ca* 1 mg material was, after removal of all solvents by purging with dry nitrogen at 40 °C, treated with 0.2 mL of a mixture of bis(trimethylsilyl)-trifluoracetamide and trimethylchlorosilane (10:1, w/w) and the mixture heated in a closed vial for 3 h at 80 °C. After cooling to room temperature the sample was diluted to a 0.1 % solution by adding cyclohexane and analysed by gas chromatography .

GC-analyses—conditions. A thus prepared sample (0.1 μL) was injected in the high temperature gas chromatograph from Carlo Erba under the following conditions:

Injector temperature programmed: 120–360 °C/50 °C/min, detector temperature 360 °C.

Temperature programme: 120 °C (1 min isothermal) to 340 °C (25°C/min, 1 min isothermal); 340–360 °C (1°C/min). Under those conditions all educts, products and internal standards are well separated as proven by co-injection experiments. All reported data are average values of three experiments with a relative deviation of 5–10 %. This error is in the normal range of gas chromatographic investigations.

Competitive hydrolyses of tricaprylin and ketone **1a**. Tricaprylin (1.0 g, 2.1 mmol) or 2-(2-oxononyl)-1,3-propanediol dicaprylate (**1a**) (1.0 g, 2.1 mmol) were suspended in phosphate buffer (20 mL, pH 7) at 30 °C. The corresponding lipase preparation was added and the produced suspension was stirred at room temperature. The released caproic acid was again titrated continuously with 0.1 N NaOH using the autotitrator. In addition samples were taken every 4 h, extracted with diethylether, dried ($MgSO_4$) and concentrated. After the silylation of the free hydroxy functions as described above, the samples were analyzed by GC. Both experiments indicated a complete conversion of the educts after 24 h.

Regioselectivity of lipases—determination. 7-Butyroxytridecane-4,10-dione (**2**) (50 mg) and 2-(2-oxopentyl)-1,3-propanediol dibutyrate (**1b**) (50 mg) were dissolved in *n*-hexane (1 mL). Methanol (50 μL) and as internal standard triacontane (0.1 %, w/v) were added. The corresponding lipase preparation (10 mg) was added to the resulting emulsion and the mixtures shaken at room temperature. Every 30 min samples (0.5 mL) were taken, solid materials removed and the components determined after silylation as described above.

Regioselectivities of lipases—GC-analyses. A thus prepared sample (0.1 μL) was injected in the gas chromatograph (Shimadzu) under the following conditions: injector temperature programmed: 120–350 °C (50 °C/min); detector temperature 350 °C. Temperature programme: 120 °C (1 min isothermal) to 320 °C (5 °C/min, 1 min isothermal). Under these conditions all educts, products and internal standards are well separated as proven by co-injection experiments. All reported data are average values of three experiments with a relative deviation of 5–10 %. This error is in the normal range of gas chromatographic investigations. From the time dependence of the educt consumptions the competition values α were determined and thus the regioselectivities of numerous lipases.

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