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Lipase catalysed synthesis of diacyl hydrazines: an indirect method for kinetic resolution of chiral acids

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Abstract

The acylation of hydrazine, to afford the *N,N′*-diacyl derivatives, was catalysed by a number of lipases. The rates of the first and second steps depended on the lipase and the type of solvent used. Water, up to 0.4 M, had no detrimental effect on the yield and complete conversion to the *N,N′*-diacyl derivative was accomplished with some lipases. The hydrazide of 2-(4-isobutylphenyl)propanoic acid (ibuprofen), prepared by non-enzymatic reaction of ibuprofen methyl ester with hydrazine, acted as a nucleophile towards several lipases that do not accept ibuprofen derivatives as the acyl donor, but the enantiomer differentiation was inefficient in most cases. The best result was obtained with *Pseudomonas* lipoprotein lipase on EP 100 which formed the (*R*) enantiomer of the product (*N*-octanoyl-*N′*-2-(4-isobutylphenyl)propanoylhydrazine) with an enantiomeric ratio *E* of 26. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Lipase; *N*-Acylhydrazine; Hydrazide; *N,N′*-Diacylhydrazine; Ibuprofen

1. Introduction

It is now well established that in organic media lipases (EC 3.1.1.3) readily catalyze a variety of acylation reactions which they do not perform in nature [1]. Numerous examples of the successful use of alkyl amines, ammonia and hydrogen peroxide as acyl acceptors have been published since the late 1980s. It is remarkable that the stable hetero-substituted nitrogen nucleophiles hydroxylamine and hydrazine and their derivatives have received rather less attention [2–8].

In the course of our investigation of (hetero) nitrogen nucleophiles we found that the reaction of octanoic acid with hydrazine [9] gave rise to a small amount of an apolar byproduct that was detected by HPLC. This byproduct was subsequently shown to be *N,N′*-dioctanoyl hydrazine (**2**), which results from further acylation of the initially formed octanohydrazide (**1**) (Fig. 1).

The formation of *N,N′*-diacylhydrazines from acid hydrazides is known. Hydrazides of amino acids, for example, are used as carboxyl protecting agents in peptide chemistry [10]. They can act as an acyl donor but also as a nucleophile via the terminal nitrogen atom of the hydrazide moiety [11]. Few authors mention the resulting

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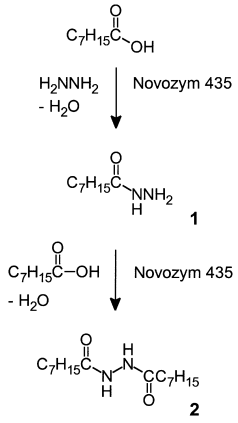

Fig. 1. Lipase catalysed acylation of hydrazine.

diacyl hydrazines, however. Diacyl hydrazines of amino acids can be synthesised from activated esters, such as *Z*-amino acid esters upon treatment with hydrazine and a protease [12]. Low yields of diacylhydrazines (relative to the amount of enzyme used) have been reported by Gotor et al. [4,13,14].

The enzyme catalysed acylation of alkanohydrazides could be a useful technique for the indirect resolution of carboxylic acids via their hydrazide derivative, synthesised either by enzyme catalysis or chemically. The latter methodology could be used for resolving carboxylic acids that are, e.g., for steric reasons, too unreactive for a conventional enzyme-catalysed kinetic resolution.

Activated carboxylic esters, which have commonly been used as acyl donors in aminolysis reactions, have the disadvantage of a considerable uncatalysed background reaction with hydrazine which is, due to the α-effect [15], a very reactive nucleophile. Carboxylic acids have never been used as acyl donors, although they would be attractive reagents because they do not suffer from a background reaction with hydrazine.

In this paper we report the lipase-catalysed acylation of hydrazine and alkanohydrazides by alkanoic acids in a one-pot procedure. The influence of the lipase and the reaction medium on the course of the reaction has been explored. We have also investigated the enantioselective acylation of the hydrazide derived from (*R*,*S*)-ibuprofen as an example of an indirect kinetic resolution.

2. Experimental

2.1. Chemicals

All solvents were of analytical purity and were dried over activated Uetikon CaA zeolite prior to use. Accurel EP100 (a macroporous polypropylene) was kindly donated by Akzo Nobel Faser. All other reagents were purchased from Aldrich or Acros and used as received unless they had to be synthesised.

2.2. Enzymes

Novozym 435 (*Candida antarctica* lipase on Lewatit E), SP 525 (pure *C. antarctica* lipase B), SP 526 (*C. antarctica* lipase A), SP 523 (*Thermomyces lanuginosus* lipase [2]) and Lipozym IM 20 (*Rhizomucor miehei* lipase on Duolite A 568) were kindly donated by Novo Nordisk. *Pseudomonas alcaligenes* lipase was obtained from Gist-brocades as a gift. *Pseudomonas* lipoprotein lipase was donated by Roche Diagnostics. CLEC-CR (cross-linked crystals of *C. rugosa* lipase) and CLEC-PC (cross-linked crystals of *P. cepacia* lipase) were received from Altus Biologics as a gift. Immobilised enzymes (Novozym 435, Lipozym IM 20, CLEC-CR and CLEC-PC) were used as received; SP 525, SP 526, SP523, *P. alcaligenes* lipase and *Pseudomonas* lipoprotein lipase were immobilised on Accurel EP 100 according to a published procedure [16]. A slightly different procedure was followed in the immobilization of the lipases from *C. antarctica* B and *P. alcaligenes.*

Accurel EP 100 (1 g) was pretreated as described by Pedersen and Eigtved [16] and added to a solution of *C. antarctica* lipase B (Novo SP525, 0.2 g, 35 kLU) in 25 ml 0.01 M phos-

[2] *Thermomyces lanuginosus* was formerly named *Humicola lanuginosa*.

phate buffer pH 9 (1 LU will liberate 1 μmol of butyric acid from tributyrin per minute). After a 20-h shaking at room temperature, 8.3 kLU of lipase activity was still found in the supernatant. The solid material was removed by filtration, washed with 30 ml buffer and dried in vacuo for 16 h at 40°C. The activity in a tributyrin assay was 5.1 kLU/g.

P. alcaligenes lipase (0.3 g, 425 kLU) was dissolved in 25 ml water (pH 9 due to buffer salts present in the preparation). After removal of the solid material by centrifugation, 380 kLU of lipase activity was recovered in the supernatant. After addition of Accurel EP 100 (2 g) no residual lipase activity could be detected in the supernatant. After filtration, washing and drying as described above the activity in a tributyrin assay was 4.7 kLU/g.

2.3. Analysis and equipment

The progress of the reactions of octanoic acid and ibuprofen derivatives was monitored with HPLC on a Waters 8 × 100 mm 4μ Novapak C_{18} reversed phase RCM column with a Waters 510 pump, a Shimadzu SPD-6A UV detector, a Shodex RI SE-61 RI detector and a Spectra-Physics SP 4270 integrator. The eluent was methanol–water (65:35, v/v) containing 0.05 M acetate buffer pH 4.3 at a flow of 1.0 ml/min.

Chiral HPLC of *N*-octanoyl-*N*′-2-(4-isobutylphenyl)-propanoylhydrazine was performed on a Baker 4.6 × 250 mm 5μ Chiralcel OD column with a Waters 510 pump and a Waters 486 UV detector operating at 254 nm, eluent isopropyl alcohol–hexane (98:2, v/v) at a flow rate of 0.5 ml/min.

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-400S spectrometer. Mass spectra were recorded on a VG 70 SE spectrometer with the EI method.

2.4. Synthesis of starting and reference compounds

Octanohydrazide (**1**) was synthesised from 10 ml ethyloctanoate (8.78 g, 51 mmol) and 10 ml hydrazine hydrate (206 mmol) in 25 ml methanol. After 48 h at 40°C, the solvent was evaporated in vacuo and the residue was recrystallised from hexane/isopropyl alcohol (20:1, v/v); yield 6.53 g (81%). ^{1}H NMR: δ 6.80 (1H, N*H*, s), δ 3.60 (2H, NH_2, s), (δ 2.15 (2H, CH_2-2, t), δ 1.64 (2H, CH_2-3, qi), δ 1.29 (8H, C$H_2$4–7, m), δ 0.88 (3H, CH_3, t). ^{13}C NMR: δ 174.01 (C═O), δ 34.62 (CH_2-2), δ 31.66 (CH_2-3), δ 29.24, 28.96 (CH_2-4,5), δ 25.51 (CH_2-6), δ 22.59 (CH_2-7), δ 14.05 (CH_2-8). MS: m/z = 158 (14%), 127 (54%), 109 (10%), 83 (6%), 74 (33%), 57 (100%). mp. 87°C.

N,*N*′-Dioctanoyl hydrazine (**2**) was synthesised by slowly adding 1.63 g (10 mmol) octanoylchloride in 10 ml diethyl ether to a vigorously stirred ice-cold solution of 1.58 g (**1**) (10 mmol) and 1 ml pyridine in 25 ml diethyl ether. After stirring for 1 h, 35 ml water was added. The heterogeneous reaction mixture was filtered; the residue was washed with a little ether and recrystallised from isopropyl alcohol. Yield 1.99 g (70%). ^{1}H NMR: δ 8.25 (2H, N*H*, s), δ 2.23 (4H, CH_2-2, t), δ 1.65 (4H, CH_2-3, qi), δ 1.28 (16H, C$H_2$4–7, m), δ 0.88 (6H, CH_3, t). ^{13}C NMR: δ 170.91 (C═O), δ 33.04 (CH_2-2), δ 31.06 (CH_2-3), δ 28.39, 28.32 (CH_2-4,5), δ 24.94 (CH_2-6), δ 21.94 (CH_2-7), δ 13.84 (CH_2-8). MS: m/z = 284 (3%), 158 (71%), 127 (66%), 74 (29%), 57 (100%). mp. 154°C.

Racemic- and (*S*)-ibuprofen hydrazide 2-(4-isobutylphenyl)propanohydrazide) (**3**) was synthesised from 10 g racemic or (*S*)-ibuprofen methyl ester (45.5 mmol) and 10 ml hydrazine (206 mmol) hydrate in 25 ml methanol. After 48 h at 40°C the solvent was evaporated in vacuo and the residue was recrystallised from hexane/isopropyl alcohol (20:1 v/v). Yield 7.31 g (73%). 2-(4-Isobutylphenyl) (**3**), ^{1}H NMR (DMSO-d_6): (propanohydrazide moiety) δ 9.98 (1H, N*H*,d), 3.60 (1H, C*H*, q), δ 3.37 (2H, NH_2, br.s), δ 1.32 (3H, CH_3, d), (ring) δ 7.3–7.0 (4H, C*H*2, 3, 5, 6, m), (isobutyl moiety) δ 2.39 (2H, CH_2, d) δ 1.78 (1H, C*H*, m), δ 0.93 (6H, CH_3, d). ^{13}C NMR: (propanoyl moiety) δ 172.067 (C═O),δ 44.140 (C2), δ

22.077 (C3), (ring) δ 139.261 (C1), δ 138.686 (C4),δ 128.852, 128.627, 126.960 (C2, C3, C5, C6), (isobutyl moiety) δ 42.429 (C1), δ 29.509(C2), δ 18.205, 18.103 (C3, C4). MS: *m/z* 220 (27%), 188 (23%), 161 (100%), 145 (9%) 119 (30%), 105 (11%), 91 (21%), 57 (25%). Racemate mp. 75°C, (*S*)-ibuprofen hydrazide mp. 92°C.

Racemic- and *N*-octanoyl-*N′*-(2,*S*)-2-(4-isobutylphenyl)propanoylhydrazine (**4**) were synthesised as reference compounds from 1.0 g racemic or (2,*S*)-2-(4-isobutylphenyl)propionyl chloride (4.45 mmol), 750 mg octanohydrazide (4.75 mmol) and 1 ml pyridine in 50 ml ice-cold *tert*-butyl methyl ether. After washing three times with 50 ml 0.1 M HCl, three times with 50 ml 0.1 M $NaHCO_3$ and three times with 50 ml water, the organic layer was dried on Na_2SO_4 and the solvent was evaporated in vacuo. The residue was recrystallised from hexane; yield 1.31 g (85%). ^{1}H NMR ($CDCl_3$): (propanohydrazide moiety) δ 9.02 (2H, N*H*,d), 3.66 (1H, C*H*, q), δ 1.57 (3H, CH_3, d), (ring) δ 7.21, 7.08 (4H, C*H*2,3,5,6, dd), (isobutyl moiety) δ 2.42 (2H, CH_2, d), δ 1.83 (1H, C*H*, m), δ 0.88 (6H, CH_3, m), (octanoyl moiety) δ 2.21 (2H, CH_2, t), δ 1.60 (2H, CH_2, t), δ 1.13 (8H, CH_2, m), δ 0.88 (3H, CH_3, s). ^{13}C NMR: δ 170.765, 169.586 (C═O), (propanoyl moiety), δ 45.042 (C2), δ 22.390 (C3), (ring) δ 140.980 (C1), δ 137.304 (C4),δ 129.603, 127.288 (C2, C3, C5, C6), (isobutyl moiety) δ 44.409 (C1), δ 29.174(C2), δ 18.350 (C3, C4), (octanoyl moiety) δ 34.051, 31.635, 30.150, 28.970, 25.331, 22.594 (C2–C7), δ 14.041 (C8). MS: 328 (6%), 285 (11%), 244 (12%), 220 (13%), 161 (100%), 149 (17%), 119 (26%), 91 (21%), 57 (43%). mp. 115°C (racemate), 138°C ((*S*)-enantiomer).

2.5. Reactions

Reactions were performed in 30 ml glass vessels with a Teflon-coated cap in a stirred thermostated oil bath at 40°C or at room temperature. Initial rate measurements with octanoic acid except for the acylation of **3** were performed with 4 mmol of substrate, 2 mmol of nucleophile and 10 mg immobilised enzyme in 10 ml solvent. The initial rate was calculated by fitting a line through the data points at $< 10\%$ conversion. Other reactions with ethyl octanoate or octanoic acid were performed with 2 mmol of acyl donor and 1 mmol hydrazine monohydrate and 50 mg of immobilised lipase at 40°C. The enantioselective reactions were performed with 110 mg (1 mmol) **3**, 72 mg octanoic acid (1 mmol) and 50 mg immobilised lipase or 10 mg CLEC in 5 ml isooctane at 40°C.

Samples of 50 μl were taken at regular intervals with a cut-off pipette from the stirred reaction mixtures, diluted with 200 μl isopropyl alcohol and analysed by HPLC. In some cases, when heavy precipitation prevented unbiased sampling, separate reactions were performed for each data point.

2.6. Isolation of the products

For analytical purposes **1** and **2** were isolated. Compound **1** was isolated from the reaction mixture of the reaction with Novozym 435 in isooctane after 1 h. The solvent was evaporated in vacuo and the residue taken up in *tert*-butyl methyl ether and filtered to remove any traces of **2**. The organic layer was washed with 0.1 M $NaHCO_3$, dried over Na_2SO_4 and concentrated by evaporation in vacuo. Recrystallization of the residue from petroleum ether (bp 40–60°C) afforded the pure product, which was identical with the product of chemical synthesis.

Compound **2** could be isolated from the reaction mixture with *Pseudomonas* lipoprotein lipase by simple filtration. Recrystallization of crude **2** from isopropyl alcohol afforded an analytical sample. The same procedure was used for the isolation of **4** from the reaction mixture of the reaction with *Pseudomonas* lipoprotein lipase after prolonged reaction time. In this case, the product was recrystallised from hexane.

3. Results and discussion

3.1. Acylation of hydrazine with ethyloctanoate and octanoic acid

In an initial experiment we investigated the lipase-catalysed reaction of hydrazine with a twofold excess of ethyl octanoate. Immobilised *C. antarctica* lipase B (Novozym 435) was used as catalyst because it had emerged from related work as the catalyst of choice. The reaction was carried out in *tert*-butyl alcohol because it is inert and dissolved the reactants and products well. The course of the reaction is depicted in Fig. 2A. The first step, the acylation of hydrazine to octanohydrazide (**1**), was rapid and hydrolysis of the donor was negligible [9] despite the presence of 0.2 M water (from the hydrazine hydrate). After 1 h, when all hydrazine had been consumed, enzymatic hydrolysis of ethyl octanoate became predominant, only one out of every ten turnovers resulting in acyl transfer to **1** and formation of **2** at this stage of the reaction. The formation of octanoic acid soon abated, however, because it was similarly converted into **2**.

This observation prompted us to start from octanoic acid; the course of its conversion into **1** and subsequently into **2** is depicted in Fig. 2B. Compared with ethyl octanoate, the first step was slower by almost an order of magnitude. This would be expected because carboxylic acids are in general less active acyl donors than their esters. Moreover, part of the octanoic acid is present as its unreactive hydrazinium salt. Surprisingly, ethyl octanoate and octanoic acid acylated **1** at the same rate. Hence, it would seem that in the second step the acylation of the lipase is not rate-limiting. The enzymatic acylation of **1** continued unabated and reached 30% conversion after 2 days (Fig. 2B). Although the reaction was rather slow, its progress did not seem to suffer from the accumulating water in the reaction mixture. With other enzymes and other solvents quantitative yields of **2** could be obtained (vide infra). We tentatively conclude that **2** is hydrolytically stable under the reaction conditions, which is in agreement with the literature [17].

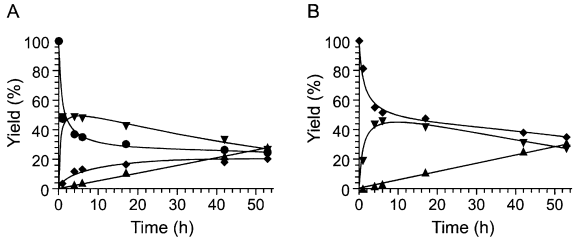

Fig. 2. Formation of **1** and **2** from hydrazine and ethyl octanoate (A) or octanoic acid (B). ●, Ethyl octanoate; ◆, octanoic acid; ▼, **1**; ▲, **2**. Conditions: 1 mmol hydrazine hydrate, 2 mmol octanoic acid or ethyl octanoate and 25 mg Novozym 435 in 5 ml *tert*-butyl alcohol at 40°C. 100% Yield of **2** equals 1 mmol.

We decided to focus on the unprecedented lipase-catalysed acylation of **1**. Octanoic acid was used as acylating agent because the procedure is simple and has not been described before; moreover, it has the advantage of not being accompanied by a background reaction.

3.2. Solvent effects

The initial rates of the acylation of hydrazine as well as of **1** by octanoic acid were measured in a number of solvents, ranging from hydrophilic to non-polar (Table 1). Novozym 435 was again used as catalyst. Typically hydrazine was approximately an order of magnitude more reactive than **1** (Table 1) but the reactions were influenced in different ways by the solvent. The acylation of hydrazine was more than twice as fast in the medium-polarity solvents *tert*-butyl methyl ether and benzene compared with reaction in a hydrophilic solvent, such as *tert*-butyl alcohol. It would seem that the inhibition of lipase by *tert*-butyl alcohol, which has been noted previously [18], is also apparent in this case. In contrast, the acylation of **1** was only moderately affected by the solvent. The suggested change in rate-limiting step noted above may cause this difference in solvent effect.

The synthesis of **2** from **1** and octanoic acid in *tert*-butyl alcohol (Table 1) was faster by

Table 1
Synthesis of **1** and **2** catalysed by Novozym 435 in different solvents

Solvent	Initial synthesis rate (μmol g^{-1} min^{-1})	
	1[a]	**2**[b]
Hexane	700	56
2,2,3-Trimethylpentane	610	54
Benzene	1070	55
tert-Butyl methyl ether	1090	50
1,2-Dimethoxyethane	400	36
Dioxane	320	40
tert-Butyl alcohol	430	35

[a]Reaction conditions: 576 mg (4 mmol) octanoic acid, 100 mg (2 mmol) hydrazine monohydrate and 10 mg Novozym 435 in 10 ml of solvent at 40°C.
[b]Reaction conditions: 576 mg (4 mmol) octanoic acid, 316 mg (2 mmol) **1** and 10 mg Novozym 435 in 10 ml of solvent at 40°C.

almost an order of magnitude than the corresponding reaction in Fig. 2B, although the reaction conditions were very similar. We ascribe this effect to the presence of 0.4 M water in the latter case, which interferes as a competing nucleophile, causing non-productive hydrolysis of the acyl-enzyme intermediate. Assuming that the water does not influence the rate of acylation of the lipase, the observed rate effect would indicate that, in the presence of 0.4 M water, only 1 out of every 10 turnovers of the acyl-enzyme intermediate results in transfer of the acyl group to **1**. We note that in the corresponding reaction starting from ethyl octanoate a similar ratio was observed with 0.2 M water present (Fig. 1A, vide supra).

3.3. Effects of the lipase

A number of microbial lipases were compared as catalyst in the consecutive reactions of octanoic acid with hydrazine and **1**. Isooctane was chosen as the solvent because it had performed well in the second step (Table 1) and because it dissolves the product only sparingly. Moreover, the relatively high water activity in this solvent is beneficial for lipases that do not perform well at low water activity, such as, e.g., *T. lanuginosus* lipase [19].

The course of the reactions is depicted in Fig. 3. The activity of the lipases diverged widely, as also becomes apparent from the yields of **2** which ranged from 12% with *P. alcaligenes* lipase to quantitative conversion (*T. lanuginosus* lipase) after 450 min. The water that was present in the reaction mixture — 0.2 M at the outset, increasing to 0.4 M at complete conversion — seemingly had no detrimental effect on the yield.

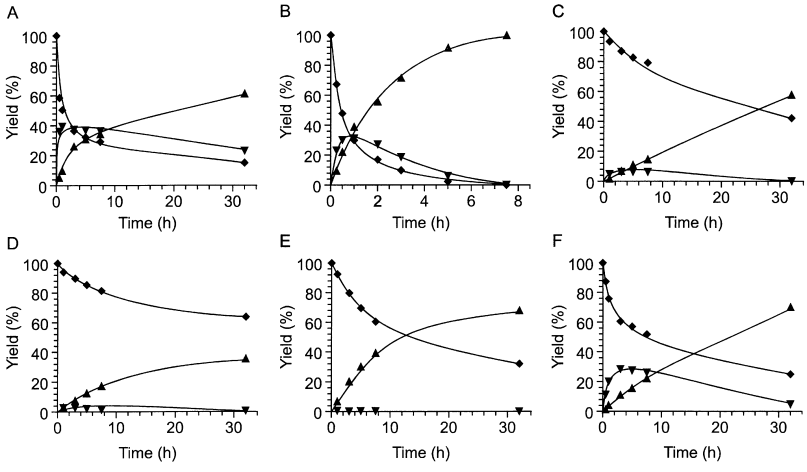

Fig. 3. Time-course of the formation of **1** and **2** from hydrazine and octanoic acid in isooctane. Lipase: A, *C. antarctica* B; B, *T. lanuginosus*; C, *C. antarctica* A; D, *P. alcaligenes*; E, *Ps.* lipoprotein; F, Lipozym IM 20. ◆, Octanoic acid; ▼, **1**; ▲, **2**. Conditions: 1 mmol hydrazine hydrate, 2 mmol octanoic acid and 50 mg immobilised lipase in 5 ml isooctane at 40°C. 100% Yield of **2** equals 1 mmol.

The amounts of the intermediate product **1** that accumulated in the steady state also varied widely, reflecting the preference of the lipases for reaction with either hydrazine or **1**. The amounts of **1** in the reaction mixtures of *Pseudomonas* lipoprotein lipase, *P. alcaligenes* lipase and *C. antarctica* lipase A were too low to calculate initial rates for the first step. Hence, these lipases converted **1** faster than hydrazine when both nucleophiles were present in the solution.

Compound **1** was also acylated in separate experiments, using the same enzymes. The initial reaction rates are presented in Table 2.

3.4. Kinetic resolution of a chiral hydrazide

The hydrazide derived from ibuprofen (2-(4-isobutylphenyl)-propano-hydrazide, **3**) was selected as a suitable model reactant for exploring the resolution of chiral hydrazides. Ibuprofen and ibuprofen esters [20–23] have been the focus of a considerable effort aimed at kinetic resolution and the chiral preferences of the lipases that accept these compounds as acyl donor are known: *C. antarctica* lipase B preferentially converts the (*R*)-enantiomer [21,23] while *C. rugosa* lipase is (*S*)-specific [20,22].

Conversion of ibuprofen to **3** makes it a nucleophile and hence it will bind in a different position in the enzyme. For their natural reaction lipases need more space in the nucleophile (diacylglycerol) binding subsite than in the fatty acid binding subsite. Hence, lipases generally accommodate quite bulky nucleophiles in the acceptor subsite, whereas the acyl moiety is subject to much more stringent steric restrictions [1].

All lipases tested accepted **3** as a nucleophile and, although the reaction rates varied widely (Tables 3 and 4), the reactions were relatively fast and several lipases accomplished 100% conversion within one day. This contrasts with the sluggish reactions when the ibuprofen moiety is used as the acyl donor. For example, Novozym 435 typically converts these donors at rates of 0.1–1 μmol g^{-1} min^{-1} [2]. The specific rates of the two cross-linked enzyme crystal (CLEC) preparations were high, but it should be considered that CLECs consist of pure protein, contrary to the adsorbed enzymes. From these, the high activity of *C. antarctica* A, *P. alcaligenes* and *Pseudomonas* lipoprotein lipases is notable. The difference in steric restriction of the acyl and nucleophile subsites is underscored by the extremely slow acylation of

Table 2
Acylation of octanohydrazide catalysed by different lipases[a]

Lipase source	Initial rate (μmol g^{-1} min^{-1})
C. antarctica A (SP526)	40
C. antarctica B (SP525)	54
C. antarctica B (Novozym 435)[b]	54
P. alcaligenes	64
T. lanuginosus (SP523)	89
Pseudomonas lipoprotein	115
Rh. miehei (Lipozym IM 20)[c]	44

[a]Reaction conditions: 576 mg (4 mmol) octanoic acid, 316 mg (2 mmol) **1** and 10 mg immobilised enzyme (on Accurel EP100 unless indicated otherwise) in 10 ml isooctane 40°C.
[b]Commercial preparation on Lewatit E.
[c]Commercial preparation on Duolite A 568.

Table 3
Acylation of ibuprofen hydrazide with octanoic acid

Lipase	Initial rate[a] (μmol g^{-1} min^{-1})	*E*	Preferred enantiomer
C. antarctica A (SP526)	17	6	*R*
C. antarctica B (SP525)	3.0	2	*S*
C. antarctica B (Novozym 435)[b]	1.6	2	*S*
P. alcaligenes (on EP 100)	12	2	*R*
T. lanuginosus (SP523)	6.0	2	*R*
Pseudomonas lipoprotein	22	8	*R*
Rh. miehei (Lipozym IM 20)[c]	1.9	2	*S*
C. rugosa (CLEC-CR)	54	2	*S*
P. cepacia CLEC-PC	35	2	*R*

[a]Reaction conditions: 110 mg **3** (0.5 mmol), 72 mg octanoic acid (0.5 mmol) and 50 mg immobilised enzyme (on Accurel EP100 unless noted otherwise) or 10 mg CLEC in 5 ml isooctane at 40°C.
[b]Commercial preparation on Lewatit E.
[c]Commercial preparation on Duolite A 568.

Table 4
Acylation of ibuprofen hydrazide with octanoic acid at room temperature

Lipase	Initial rate[a] (μmol g^{-1} min^{-1})	Time (h)	Conv. (%)	*ee* (% *R*)	*E*
C. antarctica A (SP526)	8.3	30	43	74	11
Pseudomonas lipoprotein	9.3	18	50	82	26

[a]Reaction conditions: 110 mg **3** (0.5 mmol), 72 mg octanoic acid (0.5 mmol) and 50 mg immobilised enzyme (on Accurel EP100) in 5 ml isooctane.

1 by ibuprofen which yields the same product (Fig. 4). Novozym 435 catalysed this reaction at an initial rate of only 0.06 μmol g^{-1} min^{-1}.

The enantiomeric differentiation of most lipases was disappointingly low in all cases. *Pseudomonas* lipoprotein lipase and *C. antarctica* lipase A displayed the highest *E* values (8 and 6, respectively) and both enzymes were (*R*)-specific. The low *E* values are probably caused by the relatively large distance between the nucleophilic nitrogen and the stereogenic center. Besides, it should be noted that **3** is rather small compared to cholesterol, which is the natural leaving group for *Pseudomonas* lipoprotein lipase. It is to be expected that the fit of **3** in the nucleophile subsite of this enzyme is insufficient for a good discrimination.

Lowering the reaction temperature to room temperature produced an increase in enantioselectivity. The three enzymes with the highest initial rate at 40°C (except the two CLECs) were tested. With *Pseudomonas* lipoprotein lipase the *E* value increased from 8 to 26, but for *C. antarctica* lipase A and *P. alcaligenes* lipase the increase was smaller. In all three cases the reaction rate was reduced as would be expected.

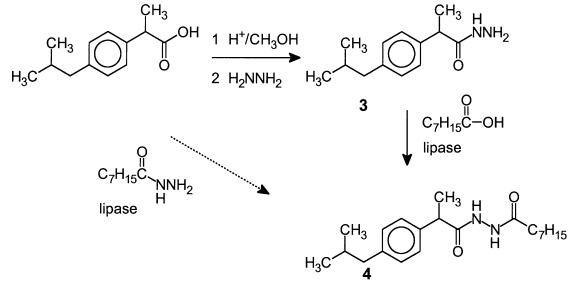

Fig. 4. Indirect kinetic resolution of ibuprofen with lipases that do not accept it as an acyl donor. Chemical conversion to the racemic hydrazide converts it to a nucleophile.

4. Conclusions

The acylation of hydrazine to the *N*,*N*′-diacyl derivative is catalysed by a variety of lipases. Octanoic acid is an efficient acyl donor, particularly in the second step. Several lipases mediate the acylation of the intermediate *N*-acyl hydrazine faster than that of hydrazine itself.

The chiral hydrazide derived from ibuprofen (*N*-octanoyl-*N*′-2-(4-isobutylphenyl)propanoylhydrazine) was smoothly acylated, compared to reactions with ibuprofen as acyl donor, by several lipases but the enantioselectivity was generally low. *C. antarctica* lipase A and *Pseudomonas* lipoprotein lipase gave the best results. They were both (*R*)-specific and the latter catalysed the reaction with an enantiomeric ratio of 26 when the reaction was performed at room temperature.

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