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Highly Selective Enzymatic Kinetic Resolution of Primary Amines at 80 °C: A Comparative Study of Carboxylic Acids and Their Ethyl Esters as Acyl Donors

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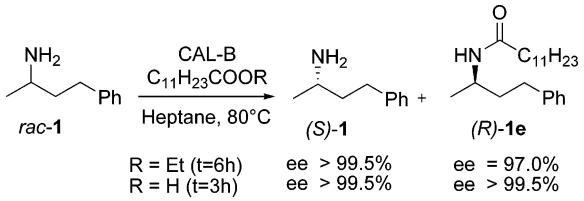

Optimization of the kinetic resolution of 2-amino-4-phenyl-butane was achieved at 80 °C using CAL-B-catalyzed aminolysis of carboxylic acids and their ethyl esters. The reactions carried out with long chain esters and the corresponding acids as acyl donors proceeded with remarkably high enantioselectivity. The use of carboxylic acids as acylating agents led to a marked acceleration of the reaction rate compared to their ester counterparts. Lauric acid led to enantiomeric excesses superior to 99.5% for both the remaining amine and the corresponding lauramide at 50% conversion (reached in 3 h). These optimized conditions were applied to the resolution of a series of aliphatic and benzylic amines.

Introduction

Optically pure amines are building blocks of major importance to industrial chemistry, since they are used for the asymmetric synthesis of agrochemicals and pharmaceuticals products.[1] Biotransformations are becoming more and more important in industrial processes.[2] Dynamic kinetic resolution (DKR),[3−5] and deracemization[3,6] involving biocatalysts offer alternatives to purely chemical asymmetric catalysis which generally requires expensive chiral catalysts.[7]

We have recently reported that the combination of enzymatic resolution with thiyl radical-mediated racemization led to optically pure aliphatic amides from the corresponding racemic aliphatic amines in good yield and high enantiomeric excess.[5]

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The racemization process involves reversible H-abstraction at the chiral center in position α relative to the NH_2 group.[8] The rate constant for the limiting step, that is, bimolecular hydrogen abstraction by alkylthiyl radical from aliphatic amines was estimated to be 10^4 M^{-1} s^{-1} at 80 °C.[8] The thermally initiated racemization carried out in the presence of AIBN proceeds through a chain mechanism. To be efficient, the reaction has to be performed at a temperature close to 80 °C. Thereby, the performance of a highly selective enzymatic resolution at 80 °C was of paramount importance to optimize the DKR process.

We report in this article the detailed study of kinetic resolution optimization that enabled the performance of the above-cited dynamic kinetic resolutions of aliphatic amines.[5] The screening of selected acyl donors was achieved with the purpose to improve the kinetic resolution of primary aliphatic amines using the immobilized CAL-B catalyst (Novozym 435 from Novo Nordisk A/S, Denmark) at 80 °C. 2-Amino-4-phenyl-butane (**1**) was chosen as model for this study, the best conditions were then applied to a series of aliphatic and benzylic amines.

Results and Discussion

The thermostable lipase B from *Candida antartica* (CAL-B) has proven to be the most effective biocatalyst for aminolysis reactions in organic solvents.[9] Numerous studies of enzymatic aminolysis have been performed by Gotor,[10] Sheldon,[11] and other groups.[12−14] Improving the lipase catalytic activity and selectivity can be made by changing the solvent,[14] or the acyl donor,[15] or both. The lipase-catalyzed resolution of racemic amines is reputedly successful in organic solvent of low polarity.[14] Since the final goal behind this study was to carry out DKR reactions under optimal conditions,[5] the enzymatic resolution was optimized in heptane (one of the best solvents for the radical-mediated racemization) despite the rather poor results reported for CAL-B-mediated acylations in hexane[16] or cyclohexane.[14c,15c]

The selection of appropriate acyl donors is crucial for the kinetic resolution of amines.[2b,15,16] Although they are highly reactive irreversible acyl donors, enol ethers are not suitable for this purpose, since they release carbonyl compounds that may react with the aminogroup to form imines. Furthermore, since amines are good nucleophiles, highly reactive acyl donors might lead to amides through spontaneous nonenzymatic nucleophilic displacement by the racemic substrate, which would lower the enantiomeric excess of the acylated product. Carbonates have been used as acyl donors for the resolution of amines, since they lead to carbamates from which amines are easily recovered. A preliminary trial was carried out in the presence of 1 equiv of diallylcarbonate. At 80 °C, 64% conversion was reached in 7.5 h, and the reaction led to rather low enantiomeric excesses for both the remaining amine (*S*)-**1** (46% ee_S, 39% yield) and the corresponding (*R*)-carbamate (26% ee_R, 71% yield). Even though at 80 °C spontaneous aminolysis was shown not to interfere with the enzymatic reaction, this family of acyl donors was discarded, and further assays were limited to ethyl esters and corresponding acids.

Comparative results obtained with racemic amine (**1**) are given in Table 1.

We first used simple esters, the most common one being ethyl acetate (entry 1). At 80 °C, ethyl acetate led to 50% conversion in 7 h, but the enantiomeric excesses did not exceed 91% for (*R*)-amide **1a** and 92% for the remaining (*S*)-amine **1**.[17] The enantioselectivity was the lowest in the series.

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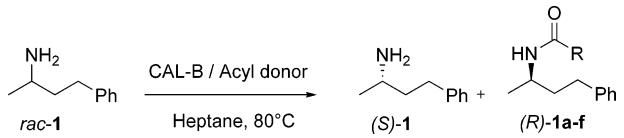
TABLE 1. Influence of the Acyl Donor on the Kinetic Resolution of Amine (1) with CAL-B at 80 °C in Heptane



				(*S*)-**1**		(*R*)-amide				
entry	acyl donor[a]	time (h)	*T* (°C)	ee$_s$ (%)[b]	yield % (NMR)[c]	amide	ee$_R$ (%)[d]	yield %[e] (NMR)[c]	*c* (%)[f]	*E*[g]
1	$MeCO_2Et$	7	80	92.0	(43)	**1a**	91.0	23 (46)	50.3	69
2	$MeOCH_2CO_2Et$	1.5	80	>99.5	(40)	**1b**	77.4	39 (53)	56.4	100
3	$C_3H_7CO_2Et$	9	80	97.6	(47)	**1c**	95.5	− (47)	50.5	>200
4	$C_7H_{15}CO_2Et$	6	80	>99.5	(47)	**1d**	98.0	42 (48)	50.5	≫500
5	$C_{11}H_{23}CO_2Et$[k]	6	80	>99.5	(47)	**1e**	97.0	50 (42)	50.8	≫500
6	$C_{15}H_{31}CO_2Et$[k]	5	80	>99.5	(40)	**1f**	99.2	− (46)	50.2	≫500
7	$C_3H_7CO_2H$	4	80	99.0	(41)	**1c**	97.1	− (44)	50.5	≫200
8	$C_7H_{15}CO_2H$	3	80	>99.5	(48)	**1d**	98.6	48 (49)	51.5	≫500
9	$C_{11}H_{23}CO_2H$	3	80	>99.5	(39)	**1e**	>99.5	− (45)	50.0	≫500
10	$C_{11}H_{23}CO_2H$[h]	3	80	>99.5	(39)	**1e**	>99.5	− (46)	50.0	≫500
11	$C_{11}H_{23}CO_2H$	4.5	70	>99.5	(41)	**1e**	>99.5	− (44)	50.0	≫500
12	$C_{11}H_{23}CO_2H$	5.3	60	>99.5	(50)	**1e**	>99.5	− (46)	50.0	≫500
13	$C_{11}H_{23}CO_2H$	10	50	>99.5	(49)	**1e**	>99.5	− (52)	50.0	≫500
14	$C_{11}H_{23}CO_2H$[i]	5	80	>99.5	(50)	**1e**	>99.5	− (50)	50.0	≫500
15	$C_{11}H_{23}CO_2H$[j]	6	80	>99.5	(48)	**1e**	>99.5	− (48)	50.0	≫500
16	$C_{15}H_{31}CO_2H$	3	80	>99.5	(49)	**1f**	>99.5	49 (50)	50.2	≫500

[a] General conditions unless otherwise stated: **1** (1 mmol); acyl donor (1 mmol); CAL-B, Novozym 435 (200 mg); heptane (10 mL), 80 °C. [b] Determined by chiral GC after derivatization in trifluoroacetamide. [c] Pentamethylbenzene was used as internal standard. [d] Determined by chiral HPLC. [e] Isolated yield. [f] Calculated according to $c = ee_{amine}/(ee_{amine} + ee_{amide})$. [g] Enantioselectivity factors were calculated according to $E = \ln[(1 - c)(1 - ee_{amine})]/\ln[(1 - c)(1 + ee_{amine})]$.[18] Owing to sensitivity to experimental errors, *E* values calculated in the range 200−300 are reported as >200, values in the range 300−500 are given ≫200, and values in the range 900−1000 are given ≫500. [h] Lauric acid (0.6 equiv). [i] CAL-B (100 mg). [j] CAL-B (50 mg). [k] $C_{11}H_{23}CO_2Et$ (ethyl laurate), $C_{15}H_{31}CO_2Et$ (ethyl palmitate).

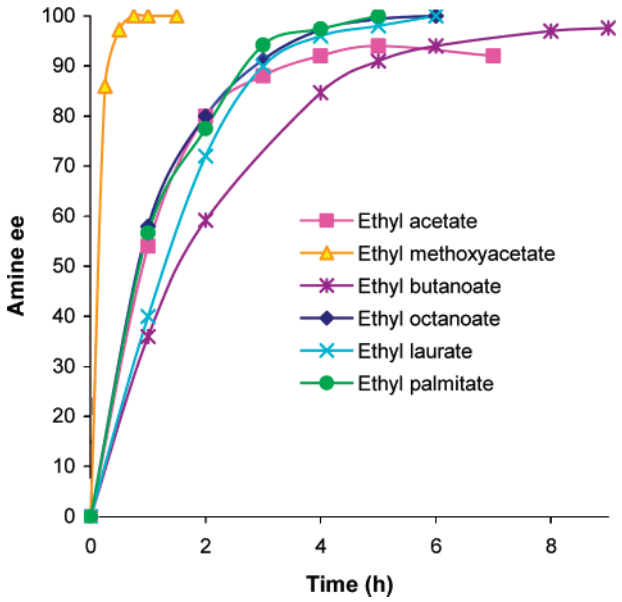


FIGURE 1. Plot of amine **1** enantiomeric excess versus time for the aminolysis of ethyl esters.

Increasing the acyl moiety chain length improved the enantioselectivity. The enantiomeric excess of the amide varied from 95.5% for ethyl butyrate (**1c**) to 97 and 98% for ethyl laurate (**1e**) and ethyl octanoate (**1d**), respectively, and became superior to 99% for ethyl palmitate (**1f**) for conversions close to 50% (entries 3−6). Concomitantly, the enantiomeric excess of the remaining amine varied from 97.6% for ethyl butyrate to >99.5% for ethyl octanoate, ethyl laurate, and ethyl palmitate. Figure 1 shows the plot for the variation of the amine enantiomeric excess versus time for the various esters.

The time necessary to reach 50% conversion increased, going from 7 h for ethyl acetate to 9 h for ethyl butyrate. Further increase of the acyl group chain length resulted in the acceleration of the reaction, since the same conversion was reached in 6 h for ethyl octanoate and ethyl laurate and in 5 h for ethyl palmitate. Simultaneously, the enantioselectivity reached remarkably high values, the rate constant for the conversion of the (*R*)-enantiomer being more than 2 orders of magnitude higher than the rate constant for the conversion of the (*S*)-enantiomer.

A similar effect of the acyl group chain-length has been reported for the enzymatic resolution of *sec*-butylamine; the highest enantioselectivities were obtained with ethyl esters of long chain fatty acids, although the rate of conversion at 25 °C decreased, while the carbon chain length of the acid moiety increased.[15c] It must be noted that the influence of the number of carbons in the acyl moiety on CAL-B-catalyzed aminolysis of esters should by no means be considered as general trend for lipase catalysis. Contradictory reports about the influence of the chain length of the acylating agent are known for transesterifications catalyzed by lipase PS.[19] In the enzymatic acylation of alcohols using *Pseudomonas sp*. lipase and acid anhydrides as acyl donors, increasing the chain length resulted in the acceleration of the conversion rate, but with concomitant lowering of the enantioselectivity.[20] The lipase's affinity toward long-chain acyl groups might be responsible for the accelaration, and according to CAL-B structure, the acyl moiety lies in a large hydrophobic pocket.[10c,21,22]

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Because of the electron-withdrawing effect of the methoxy-group, ethyl 2-methoxyacetate deserves a separate comment (Table 1, entry 2). The α-methoxy group activates the reactivity at the carbonyl group. Ethyl 2-methoxyacetate was more reactive than ethyl acetate and all the other esters in the series. This ester has been used in other lipase-mediated resolution of amines.[10e,15d,23] It is reputed to be the optimum acyl donor for industrial amine resolution processes.[1b,c] Park has suggested that hydrogen bonding between the *β*-oxygen atom in methoxyacetate and the proton of the amine in the transition state might be a key factor in the rate enhancement.[15d] In our case, the rate of the enzymatic reaction was very fast, and 56.4% conversion was reached in only 1.5 h. Within less than 1 h, the amide enantiomeric excess reached a maximum and started decreasing. The enantioselectivity was high ($E = 100$), but it was far lower than those that were registered with long chain esters (the low enantiomeric excess of amide **1b** reflects the partial acylation of (*S*)-**1** due to the 56.4% conversion).

As briefly mentioned previously, a leaving group that is too good is not appropriate for enzymatic acylation of good nucleophiles like amines. The influence of the nature of the leaving group on the resolution step itself is not clear-cut. According to the mechanism generally accepted for lipases,[21,24] the leaving group is not present in the enantioselectivity determining step. We have focused our attention on the use of carboxylic acids as acylating agents.[25] The latter have been scarcely used as acyl donors in enzymatic aminolysis because one would predict that the formation of an unreactive amine salt would prevent the formation of the acyl−enzyme intermediate due to the low reactivity of the carboxylate. However, the formation of the salt is an equilibrium, and the small amount of free acid available to the enzyme is sufficient for the aminolysis to proceed, making prior activation of the acid unnecessary.

The enzymatic reaction in organic solvent was slow when using oleic acid and lauramine to prepare the corresponding amide (60% conversion in 12 days at room temperature, 80% in 15 days).[25a] It was further demonstrated that the amides derived from 1-phenylethylamine and dodecanoic acid, and other aliphatic acids, could be synthesized in high enantiomeric excess, under reduced pressure in non-solvent system, and in ionic liquids at temperature ranging from 30 to 55 °C.[25b] The rate of amonolysis of octanoic acid in ionic liquids was found comparable or even faster than the ones observed in organic solvents.[25d]

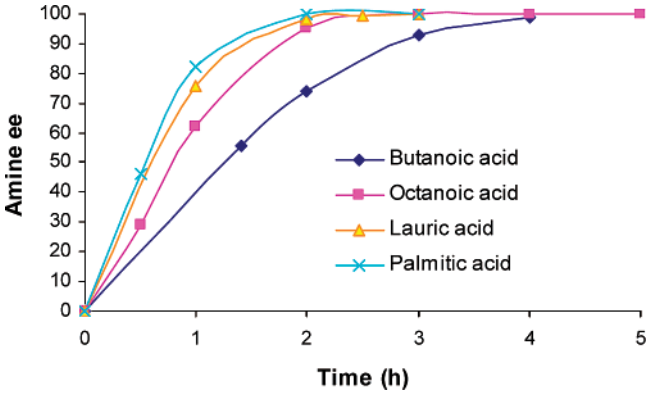

FIGURE 2. Plot of amine **1** enantiomeric excess versus time for carboxylic acids acyl donors.

The data given in Table 1 (entries 7−16) demonstrate that with an acid/amine ratio of 1,[26] contrary to the prediction of a slower rate of conversion, 50% conversion was obtained in reaction times ranging from 3 to 6 h depending on the acid. It can be noted that lowering the amount of acid to 0.6 equiv (entries 9 and 10) had nearly no effect on the reaction rate.

At 80 °C, the time needed to reach 50% conversion was roughly half the time needed when using the corresponding ethyl ester (entries 3 and 7; 4 and 8; 5 and 9; 6 and 16).[27] It has been suggested that the negative effect produced on the reaction rate resulting from the formation of the amine salt might be compensated by the higher specificity of the enzyme for the acid than for the ester.[25c] The incidence of the chain length on the reaction rate paralleled that observed with ethyl esters as acyl donors (Figures 1 and 2).[28]

Amides **1e** and **1f** were prepared in very high enantiomeric excesses. The enantioselectivity was not significantly affected by the nature of the leaving group. This is consistent with the mechanism, since the selectivity is determined in the acyl−enzyme complex cleavage step.

Acetic acid and 2-methoxyacetic acid are exceptions. The former did not give any conversion. In the case of the latter, in 5 h, the conversion reached 43.9%; the enantiomeric excess of the remaining amine and the amide were 76% and 97%, respectively. The reaction was shown not to be reversible.

The influence of the temperature on the reaction rate was examined (entries 9 and 11−13). Lowering the temperature from 80 to 50 °C resulted in slowing down the rate of the enzymatic

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(26) The rate of CAL-B-catalyzed amidation of oleic acid with *N*-methylglucamine in organic solvents was reported to decrease at acid/amine ratios higher than 1, see: Maugard, T.; Remaud-Simeon, M.; Monsan, P. *Biochim. Biophys. Acta* **1998**, *16*, 181−204.

(27) Upon request of a referee, attempts to perform the reaction in acetonitrile (HPLC grade, <0.01% (v/v) H_2O), a non-hydrogen bonding solvent, were made. This solvent was selected for its polar character ($\epsilon = 32$), quite different from heptane ($\epsilon = 1.92$). It led to very poor results, no conversion after 24 h in the presence of lauric acid, and amine ee was only 21% after 6 h in the presence of ethyl laurate. In this case, owing to the slow rate of conversion, ethyl methoxy acetate would be a better acylating agent than ethyl laurate (87% amine ee, 98% amide ee; 47% conversion ($E = 270$, after 24 h at 80°C).

(28) The rate of enzymatic acylation of chiral alcohols with fatty acids is also influenced by the number of carbons in the acyl chain. The acyl binding site of Chirazyme L2 (CAL-B) can accomodate acyl groups up to the carbon number 16, see: (a) Suan, C.; Sarmidi, M. R. *J. Mol. Catal. B: Enzym.* **2004**, *28*, 111−119. For related observations in the acylation of flavonoids, see: (b) Ardhaoui, M.; Falcimaigne, A.; Ognier, S.; Engasser, J. M.; Moussou, P.; Pauly, G.; Ghoul, M. *J. Biotechnol.* **2004**, *110*, 265−271.

TABLE 2. Influence of Enzyme Recycling on the Aminolysis of Lauric Acid with Amine 1 in 5h at 80 °C

	(*S*)-**1**		(*R*)-**1e**	
cycle[a]	ee[b]	yield (%)[c]	ee[d]	yield (%)[c]
1 (fresh)	>99.5	46	99.2	42
2	>99.5	42	99.3	41
3	99.4	46	99.2	42
4	97.9	43	99.1	41
5	92.6	43	99.0	41

[a] General conditions: **1** (1 mmol); lauric acid (1 mmol); CAL-B, Novozym 435 (100 mg); heptane (10 mL), 80 °C. The resin was filtered off, rinsed with ether, and then dried under vacuum for 2 h before being recycled. [b] Determined by chiral GC after derivatization in trifluoroacetamide. [c] NMR yield with pentamethylbenzene as internal standard. [d] Determined by chiral HPLC.

reaction roughly by a 2.7 factor. The initial rate was 2.15 μmol h^{-1} mg^{-1} at 80 °C, while it was 0.79 μmol h^{-1} mg^{-1} at 50 °C. The enantioselectivity ratio did not change significantly due to its high range.

Dividing the amount of CAL-B relative to the amine by 4 resulted in slowing down the reaction rate approximately by a factor of 2 (entries 9, 14, and 15).

The higher reactivity, associated to high enantiomeric ratios, that was observed with carboxylic acids might be due to the release of a water molecule in the stereospecificity pocket. The role of water on the enzyme activity is fundamental.[14a] Enzyme activity correlates to water activity, and less water is necessary in hydrophobic solvents. It has recently been reported that the presence of a water molecule enhances the enantioselectivity of CAL-B with respect to the acylation of 2-pentanol. Molecular modeling suggested that the binding of a water molecule in the active site could obstruct the binding of the slowly reacting enantiomer.[29]

It has also been pointed out by several authors that the reactions of amines are often slower than the reactions of alcohols in spite of their higher nucleophilicity.[10c,12c,25g] In redesigning the commonly accepted serine-mediated mechanism for the aminolysis of esters, Gotor[10c] has suggested that unassisted attack of the amine onto the carbonyl group was plausible and that a water molecule might be necessary to facilitate the proton transfer from the resulting ammonium group to the histidine residue. This is consistent with the rationale proposed by Hult,[12c] according to which the proton transfer from the acyl acceptor to the histidine residue would be slower in the case of an amine than in the case of an alcohol.

Attention was paid to the recycling of the enzyme. The NMR yields and enantiomeric excesses determined after four consecutive recycling cycles of the enzyme are given in Table 2.

The thermal stability of the enzyme was evaluated in the absence of substrate and acyl donor in heptane at 80 °C and 200 rpm. No change in lipase activity was recorded after 5 h at 80 °C; a 72% residual activity was registered after heating for 22 h. The stability of Novozym 435 was also examined by determining its residual activity after incubation under the reaction conditions.[30] Under the aminolysis conditions, 100−95% residual activity remained after 2−5 h of reaction. The residual activity was 95−70% after 6−9 h of reaction. Thereby, it can be concluded that most of the loss of activity originated from thermal degradation.

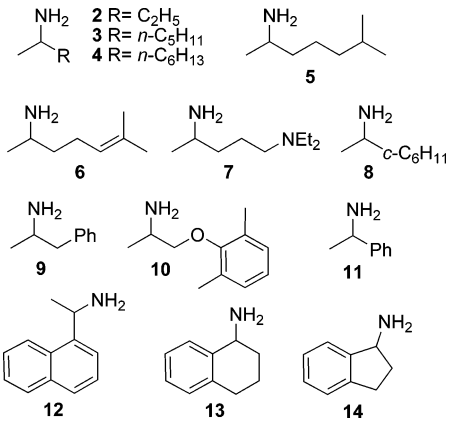

FIGURE 3. Structures of amines **2**−**14**.[31]

The optimal conditions determined for the resolution of amine **1** with lauric acid as the acyl donor were then applied to amines **2**−**14** (Figure 3). The data are collected in Table 3.

The small difference in the steric bulk of ethyl group compared to methyl group explains the low factor of discrimination between the two enantiomers of amine **2**. The enantioselectivity factor was 4 times higher than that reported by Gotor.[10g]

Aliphatic amines structurally close to **1**, that is, **3**, **4**, **5**, **6**, and **7**, were very efficiently resolved at 80 °C. The time necessary to reach 50% conversion ranged from 6 to 9.5 h. For all these amines, the enantioselectivity factor was far greater than 500. Ramification with a methyl group at a remote position (**5**) slightly slowed down the reaction, more than the trisubstituted-double bond in **6** (entries 4 and 5). The terminal diethylamino group in **7** slowed down the reaction some more, possibly because the additional basic function contributes to decrease the amount of free acid in the reaction medium.

It must be noted that the isolated yield of the remaining (*S*)-amine was low in several instances. Amine volatility resulted in loss during the isolation. This is the case for amine **2** which is the most volatile in the series and for which the yield of the recovered substrate was not determined.[32]

The enzymatic reaction was much slower with amine **8**, since 14 h were needed to reach 50% conversion, presumably because of the steric bulk of the cyclohexyl substituent. Surprizingly, the acylation was slower for amine **9**, bearing a phenyl group in β-position relative to the amino group, than for amine **10** bearing a much hindered aryloxy group. Although lower, the *E factor* remained very high.

Benzylic amines **11**−**14** gave *E* factors as high as aliphatic amines with the exception of amine **12**. It must be noted that the enzymatic reaction was extremely slow, even at 80 °C for amines **12** and **13**, whereas the times necessary to reach 50%

(29) Léonard, V.; Fransson, L.; Lamare, S.; Hult, K.; Graber, M. *ChemBioChem* **2007**, *8*, 662−667.

(30) The activity was measured using a standard protocol, i.e., by incubating 10−20 mg of lipase in 10 mL of heptane containing 0.1 M lauric acid, 0.1 M 1-propanol, and 0.05 M *n*-hexadecane as internal standard for quantitative GC analysis, at 40°C and 200 rpm for 2−10 min.

(31) For comparative data for the resolution of amine **1**, see refs 10f, 12b,d; for amine **2**, see refs 10g, 12c, 13b, 15c; for amine **3**, see refs 10g, 13b; for amine **4**, see refs 12c, 13b,c; for amine **8**, see ref 15b; for amine **9**, see ref 13g; for amine **10**, see ref 10f; for amine **11**, see refs 10g, 12a,d, 13b,c,g, 14c, 15b,d; for amine **12**, see refs 10g, 13a,e,g, 14c; for amine **13**, see refs 12c, 13a,e; for amine **14**, see refs 12b,c,d, 15b.

(32) Boiling points: **2** (63°C), **2** (142°C), **5** (154°C), heptane (102°C).

TABLE 3. Kinetic Resolution of Amines 2−14 with CALB at 80 °C in Heptane with Lauric Acid as the Acyl Donor[a]

			(*S*)-remaining amine		(*R*)-amide				
entry	amine	time (h)	ee$_s$ (%)[b]	yield % isolated (NMR)[c]	amide	ee$_R$ (%)[d]	yield % isolated (NMR)[c]	*C* (%)[e]	*E*[f]
1	**2**	6	95.0	Nd	**2e**	65.0	55	59.4	16
2	**3**	8	>99.5	10 (41)	**3e**	96.9	45 (49)	50.8	≫500
3	**4**	6	>99.5	41 (43)	**4e**	>99.5	45 (41)	50	≫500
4	**5**	8.5	>99.5	17 (41)	**5e**	>98.0	38 (43)	50.5	≫500
5	**6**	7.5	>99.5	28	**6e**	99.5	42	50.1	≫500
6	**7**	9.5	>99.5	31 (43)	**7e**	99.8	44 (44)	50	≫500
7	**8**	14	>99.5	48	**8e**	99.0	49.5	50.2	≫500
8	**9**	9	98.0	27[g]	**9e**	98.0	46	50	≫200
9	**10**	7	>99.5	27 (44)	**10e**	>98.0	39 (41)	50.5	≫500
10	**11**	4	>99.5	45	**11e**	99.0	46	50.2	≫500
11	**12**	25	97.9	42	**12e**	94.0	48	51	>100
12	**13**	26	>99.5	34	**13e**	96.7	42	50.8	≫500
13	**14**	2	>99.5	44	**14e**	>99.5	47	50	≫500

[a] General conditions unless otherwise stated: substrate (1 mmol); lauric acid (1 mmol); CAL-B, Novozym 435 (100 mg); heptane (10 mL), 80 °C. [b] Determined by chiral GC after derivatization in trifluoroacetamide. [c] Pentamethylbenzene was used as internal standard. [d] Determined by chiral HPLC. [e] Calculated according to $c = ee_{amine}/(ee_{amine} + ee_{amide})$. [f] Calculated according to $E = \ln[(1 - c)(1 - ee_{amine})]/\ln[(1 - c)(1 + ee_{amine})]$.[18] Owing to sensitivity to experimental errors, *E* values calculated in the range 100−200 are reported as >100, values calculated in the range 200−300 are reported as >200, values in the range 300−500 are given ≫200, and values in the range 900−1000 are given ≫500. [g] Remaining **9** was isolated in 38% yield when starting from 5mmol of substrate.

conversion were the shortest in all the series for amine **11** and particularly for amine **14**.

Very low selectivity factors (5 and 17, respectively) were reported for the CAL-B-mediated resolutions of amines **12** and **13** performed at 60 °C in isopropyl ether, in the presence of 4−5 equiv of ethyl acetate.[12a] A plausible correlation was made with the pK_a of the amines (pK_a = 9.5 for **12** and pK_a = 9.7 for **13**). Since a higher pK_a value might result in a higher reactivity of both enantiomers of the amine, one might expect a decrease in selectivity. In our case, there was no significant effect on the selectivity, but the pK_a value should exert two opposite influences. A more basic amine should slow down the rate of conversion by decreasing the amount of free acid in the medium, and at the same time, it should increase the rate of nucleophilic attack on the acyl−enzyme complex. It is thereby very difficult to evaluate the relative contribution of steric hindrance and basicity upon the rate of the enzymatic conversion and its enantioselectivity.

1-Aminoindane (**14**) and, to a lesser extent, 1-phenethyl amine (**11**) reacted like amine **1** much faster than the other amines in the series.[33] This cannot be ascribed to its basicity (pK_a = 9.53). The aromatic substituents might be favorable in this case for reactivity. As suggested by Sheldon,[9c] nonsteric interactions also contribute to the enantiorecognition.

Conclusion

The kinetic resolution of 2-amino-4-phenyl-butane was optimized at 80 °C using CAL-B-catalyzed aminolysis of carboxylic acids and their ethyl esters. The reactions carried out with long chain esters and the corresponding acids as acyl donors all proceeded with remarkably high enantioselectivity. The use of carboxylic acids as acylating agents led to a marked acceleration of the reaction rate compared to their ethyl ester counterparts. Lauric acid led to enantiomeric excesses superior to 99.5% for both the remaining amine and the corresponding lauramide at 50% conversion (reached in 3 h). Excellent results were similarly recorded for a series of aliphatic and benzylic amines. Finally, it must be recalled that this optimization of enzymatic resolution with CAL-B at 80 °C found its justification in the performance of DKR experiments associating enzymatic resolution with CAL-B and thiyl radical-mediated racemization at 80 °C that were successful with both ethyl laurate and lauric acid as acyl donors.[5,34]

Experimental Section

General Procedure for Kinetic Resolutions. A solution of amine (1 mmol), acyl donor (1 mmol), and *C. antartica* lipase B (CAL-B, Novozym 435) (200 mg) in heptane (10 mL) was heated for 1.5 to 26 h at 80 °C (with a magnetic agitation at 250 rpm). The reaction was monitored by GC. When the analysis showed the completion of the reaction, the mixture was cooled down to room temperature, and the resin was filtered through a glass sinter and rinsed with Et_2O. The filtrate was concentrated *in vacuo*. The residue was dissolved in $CDCl_3$, and pentamethylbenzene (0.25 mmol) was added as an NMR internal standard to afford the crude yields for amine and amide. The crude was dissolved in CH_2Cl_2, and K_2CO_3 was added. The solution was filtered and concentrated *in vacuo*. The residue was purified by column chromatography on basic alumina (neat Et_2O to Et_2O/MeOH 90:10) to give amide as a white solid and amine as a colorless oil.

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Supporting Information Available: Experimental procedures and NMR spectra for **1a**−**f**, **2e**, **3e**, **4e**, **5e**, **6**, **6e**, **7e**, **8e**, **9**, **9e**, **10e**, **11e**, **12e**, **13e**, and **14e**. This material is available free of charge via the Internet at http//pubs.acs.org.

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(33) (a) For comparative resolution of these amines with aminoacylase I from *Aspergillus melleus* in the presence of 2-methoxyacetate, see ref 12b. (b) For their resolution with penicillin acylase from *Alcaligenes faecalis* in aqueous solvent in the presence of phenylacetamide, see ref 12d.

(34) In the DKR experiments reported in ref 5, the use of lauric acid as acylating agent led to transform *rac*-**1** into (*R*)-**1e** (71% yield and ee > 99%); the use of ethyl laurate led to the same amide in 70% isolated yield and 99% ee.