Chemo- and Enantioselective Acyl Transfers by Lipases and Acylase I: Preparative Applications in Hydroxymethylpiperidine Chemistry

Katri Lundell, Petka Lehtinen , Liisa T. Kanerva*

Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, Lemminkäisenkatu 2, FIN-20520 Turku, Finland
Fax: (+358)-2-3337955, e-mail: lkanerva@utu.fi

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Abstract: The lipase- and acylase I-catalysed acylation of bifunctional 2- and 3-hydroxymethylpiperidines (**1** and **2**) and the alcoholysis of the corresponding diacylated counterparts **7** and **8** have been studied. Lipase AK from *Pseudomonas fluorescens* allowed the preparative-scale resolution of **7** in neat butanol at 50% conversion whereas the 3-regioisomer **8** reacted with negligible enantioselectivity (E = 7). The lipase- and acylase I-catalysed acylations of **1** and **2** in organic solvents proceeded with low enantioselectivity. On the other hand, more than 90% of **1** and **2** were transformed to amino esters **3** and **4**, respectively, in a highly chemoselective *O*-acylation with 2,2,2-trifluoroethyl butanoate in the presence of *Candida antarctica* lipase A in TBME. The *O*-protected product **3** in the reaction mixture was readily available to another transformation at the piperidine nitrogen although **3** was not separated due to intramolecular *O*→*N* acyl migration. The tendency for acyl migration was much less significant in the case of **4**.

Keywords: acyl migration; chemoselective *O*-acylation; enantioselective alcoholysis; *N*-heterocyclic amino alcohols; lipase catalysis

Introduction

Piperidine alkaloids are typical naturally occurring compounds which exhibit an extensive range of biological activities.[1] Piperidine is the nitrogen analogue of a pyranose ring, and as such its derivatives are potential inhibitors of enzymes working on glycosidic bonds.[2,3] The above cases suffice to emphasise the importance of producing piperidine-based structures for synthetic pharmaceuticals and natural products.

For a compound to be biologically and pharmaceutically active enantiopurity is of pivotal importance. Asymmetric chemical methods to substituted piperidines utilising the chiral pool, chiral chemical catalysts and chiral auxiliaries were previously reviewed.[4] The preparation of small enantiopure building blocks, such as amino alcohols, using enzymatic kinetic resolution or desymmetrisation of a *meso* compound was also described.[2,3,5–15] Accordingly, lipases, pig liver esterase and acylase I were used for the enantioselective *O*-acylation of *N*-protected 2- and 3-hydroxymethylpiperidines (**1** and **2**) and for the hydrolysis of the corresponding *N*-protected amino esters.[5–11] Due to the relatively low enantioselectivities ($E = 23-70$ depending on conditions), kinetic control and repeated resolutions were typically described in order to prepare both enantiomers of 3-hydroxymethylpiperidine **2**.[10,11] The enzymatic preparation of the enantiomers of 2-hydroxymethylpiperidine **1** was less satisfactorily described.[5–9]

In addition to enantioselectivity, chemoselectivity is characteristic to many enzymes and can be highly applicable in synthetic chemistry when polyfunctional molecules are involved. In the case of amino alcohols, chemoselective *O*-acylation is a challenge which, in the case of usual chemical methods calls, for prior protection of the amino group, specific *N*-acylations being more easily controlled. The lipase-catalysed *O*-acylation of ω-amino alcohols [$HO(CH_2)_nNH_2$] was previously well recognised although fast spontaneous *O*→*N* acyl migration was shown to lead to the formation of the *N*-acylated final product with n = 2 and 3.[16,17] On this basis, preferred *O*-acylation can also be suggested for the enantioselective acylation of 2-amino-1-butanol by PPL although only the formation of the *N*-monoacylated and *N,O*-diacylated products were reported.[18] It is common to the above-mentioned *O*→*N* acyl migrations that the amino group situates at the position β or γ to the formed ester function. Accordingly, intramolecular acyl migration by normal addition-elimination mechanism can proceed through five- and six-membered rings, respectively, leading to the formation of the energetically more favourable amide product.

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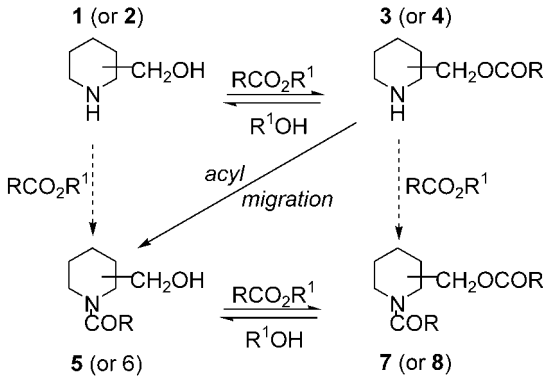


Scheme 1. Compounds **1**, **3**, **5** and **7** represent 2-substituted and **2**, **4**, **6** and **8** 3-substituted piperidines.

In the present work, the enzymatic acylation of piperidine derivatives **1**, **2** and **5** and the alcoholysis of the *N*-acylated amino esters **7** and **8** (R = Me) were studied in the presence of lipases (EC 3.1.1.5) and acylase I (*N*-acylamino acid amidohydrolase, EC 3.5.1.14) under non-aqueous conditions (Scheme 1). The focus has been on developing a method that allows the preparation of the enantiomers of 2-hydroxymethylpiperidine **1** in a single step kinetic resolution. Another focus has been on the studies of enzymatic chemoselectivity as a means to protect the primary alcohol group in **1** and **2**, leaving the secondary amino group free. As the third topic, acyl migration has been considered.

Results and Discussion

Enantio- and Chemoselective Enzymatic Acylation

Highly enantioselective acylation of racemic nucleophiles (alcohols, amines, etc.) with alkyl-activated irreversible acyl donors in the presence of hydrolytic enzymes is one of the most fascinating resolution methods. However, the demand for high enantioselectivity can turn to an insuperable challenge when a primary alcohol acts as a racemic nucleophile. A commonly proposed reason is that the reaction centre is not at the asymmetric centre. In the present work, enzymatic *O*-acylation of **1** and **2** and the following intramolecular *O*→*N* acyl migration in the formation of **5** and **6** provide a possibility for double enzymatic acylation at the primary alcohol function in the formation of **7** and **8** as final products, respectively.

Acylase I, CAL-A, CAL-B, lipase PS, lipase AK and PPL were screened for the chemo- and/or enantioselective acylation of **1** with 2,2,2-trifluoroethyl butanoate (R = Pr) in *tert*-butyl methyl ether (TBME). The number of enzymes in the screening was restricted to those with the most potential on the basis of the previous results for the hydrolysis of *N*-protected 3-hydroxymethylpiperidine and on our experience for the resolutions of 2-amino-1-phenylethanols, 1-phenyl-1,2-ethanediol, and other primary alcohols.[11–13,19–22] The results of the enzyme screening are shown in Table 1. All the possible acylation products **3**, **5** and **7** were observed for CAL-A (traces of **7** are evidently due to the fast acylation of **5**), CAL-B, lipase AK and lipase PS while the diacylated product **7** was not detected for acylase I and PPL in the reaction times of 2 hours. CAL-A and lipase AK as the most interesting enzymes were also screened for the acylation of **2** under the same conditions. The product

Table 2. Enzymatic acylation of **5** (R = Me, 0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M; R = Pr) in TBME at room temperature; reaction time 2 h.

Enzyme	Conversion [%]	*E*
Acylase I	26	2
CAL-A	95	1
CAL-B	78	2
Lipase AK	43	45
Lipase PS	83	3
PPL	20	2

Table 1. Relative amount [%]/ee [%] for the enzymatic acylation of **1** (0.05 M) and **2** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M; R = Pr) in TBME at room temperature.

	Acylation of **1**[a]				Acylation of **2**[b]			
Enzyme	**1**	**3**	**5**	**7**	**2**	**4**	**6**	**8**
Acylase I	88/3	12/22	0.1/28	0	–	–	–	–
CAL-A	4/2	96/0	0	0.3/0	44/14	55/17	1/43	–
CAL-B	21/0	72/0	3/34	4/34	–	–	–	–
Lipase AK	19/43	75/13	3/37	3/89	17/7	75/10	2/6	6/14
Lipase PS	4/0	93/0	1/66	2/54	–	–	–	–
PPL	87/7	12/63	1/54	0	–	–	–	–

[a] Reaction time 2 h.
[b] Reaction time 24 h.

Table 3. Chemoselective *O*-acylation of **1** (0.05 M) with 2,2,2-trifluoroethyl butanoate (0.1 M; R = Pr) in organic solvents by CAL-A preparation (75 mg/mL) at the point where the maximal amount of **3** is observed at room temperature.

				Relative amount [%]			
Row	Solvent	Time [h]	Conv. [%]	**1**	**3**	**5**	**7**
1	THF	8	86	14	81	2	3
2	toluene	2	83	17	80	1	2
3	*t*-amyl alcohol	4	90	10	88	1	1
4	acetonitrile	48	84	16	62	12	10
5	dichloromethane	8	83	17	79	2	2
6	diisopropyl ether	2	96	4	93	1	2
7	TBME	2	97	3	96	0	1
8	TBME[a]	2	83	17	83	0	0
9	TBME[b]	49	92	8	55	8	29

[a] 2,2,2-Trifluoroethyl pentanoate as an acyl donor.
[b] 2,2,2-Trifluoroethyl acetate as an acyl donor.

distribution was similar although the efficiency of enzymatic *O*-acylation was less compared to the acylation of **1**. As a disappointment, enzymatic *N*-acylations of **1** and **2** by CAL-A did not occur although the enzyme smoothly catalysed the highly *S*-enantioselective *N*-acylation of methyl pipecolinate.[23]

In the *O*-acylation of **1** with 2,2,2-trifluoroethyl butanoate (R = Pr) in TBME enzymatic enantioselectivities are generally negligible (Table 1). Lipase AK with $ee_1 = 43\%$ at 81% conversion and with highly enantiopure product **7** ($ee_7 = 89\%$, row 4) shows the highest enantioselectivity. Double enzymatic acylation at the primary alcohol function is now evident, the second acylation step from **5** to **7** being more enantioselective. Encouraged by this and because the formation of product **5** was slow, the present enzymes were screened for the acylation of acetate **5** (R = Me) with 2,2,2-trifluoroethyl butanoate (R = Pr) in TBME (Table 2). Lipase AK as the enantioselective ($E = 45$) lipase smoothly allowed the formation of (*S*)-**7** ($R_{N\text{-acyl}} = Me$ and $R_{O\text{-acyl}} = Pr$; row 4). However, there is a drop in $ee_5 = 71\%$ at 55% conversion (after 24 hours) to $ee_5 = 59\%$ at 59% conversion (after 48 hours) with the simultaneous drop in ee_7 from 91% to 44%. As an evident reason, the enzymatic reaction of the formed (*S*)-**7** with the unreacted enantiomerically enriched primary alcohol (*R*)-**5** (rather than with 2,2,2-trifluoroethanol as R^1OH) to (*S*)-**5** ruins the resolution.

According to the results in Table 1, highly chemoselective *O*-acylations of **1** and **2** with 2,2,2-trifluoroethyl butanoate (R = Pr) in TBME are evident. From the screened enzymes CAL-A as the most effective was chosen for further studies. Accordingly, solvent effects on the CAL-A-catalysed *O*-acylation of **1** with 2,2,2-trifluoroethyl butanoate (R = Pr) were studied. The data at the point where the maximum amounts of the ester product **3** are observed are given in Table 3 (rows 1–7). *O*-Chemoselectivity is excellent in every solvent including acetonitrile where a slow enzymatic reaction allows considerable intramolecular *O*→*N* acyl migration and leads to low maximal yield for **3** (row 4). The reactions proceed smoothly especially in toluene, *t*-amyl alcohol, diisopropyl ether and TBME (rows 2, 3, 6 and 7).

For further optimisation, the effect of an acyl donor was tested for the *O*-acylation of **1** in TBME as the best solvent and CAL-A as a catalyst (Table 3; rows 7–9). The reaction with 2,2,2-trifluoroethyl acetate (R = Me) is slow and leads to low maximal yield (relative amount 55%) for **3** (row 9). The reaction with 2,2,2-trifluoroethyl butanoate (R = Pr) in TBME clearly gives the best result, allowing an almost complete transformation of **1** to **3** in 2 hours (row 7). In the acylation of **2** under the same conditions, the formation of the maximal amount (90%) of **4** takes 48 hours. The extent of acyl migration with time now is relatively low as deduced on the basis of the low content of products **6** and **8**. This deduction is justifiable also on conformational basis because the ester group at the position 3 must be axial for the intramolecular *O*→*N* acyl migration in the formation of **6** through a six-membered ring.

Acylation at the 2-hydroxymethyl group of **1** proceeds to the maximum (97%) in 2 hours and *O*→*N* acyl migration from **3** to **5** and the further acylation to **7** are significant at long reaction times. Due to the acyl migration the efforts to purify butanoate **3** by column chromatography failed, resulting in the product mixture [**1** (7%), **3** (68%) and **5** (25%)] rather than pure **3**. However, the purification of **3** is not necessary in preparative chemistry because the desired transformation at the piperidine nitrogen can be performed immediately in the reaction mixture when **1** has reacted to **3**. In order to show that this proposal is justifiable, the enzymatically produced ester **3** (R = Pr) was quantitatively transformed to diacylated product **9** (corresponds to **7** with $R_{N\text{-acyl}} = Me$ and $R_{O\text{-acyl}} = Pr$) by the chemical *N*-acylation with acetic anhydride immediately after the removal of the enzyme and before product separation

by column chromatography as shown in Experimental Section.

Acyl Migration

It is well documented that acids and bases as well as high temperature enhance acyl migration.[24] In order to study the effect of CAL-A on acyl migration, the reaction of **1** with 2,2,2-trifluoroethyl butanoate (R = Pr) in TBME in the presence of CAL-A was stopped after 2 hours by filtering off the enzyme. After purification on silica a 2,2,2-trifluoroethyl butanoate-free mixture [**1** (7%), **3** (68%) and **5** (25%)] was obtained, containing **3** (R = Pr) as the only possible acyl donor. The mixture was divided into two parts and to one of them a new portion of CAL-A was added. The relative proportions of compounds **1**, **3**, **5** and **7** in the absence (Figure 1a, filled signs) and in the presence of CAL-A (Figure 1b) were then analysed with time by the GC method.

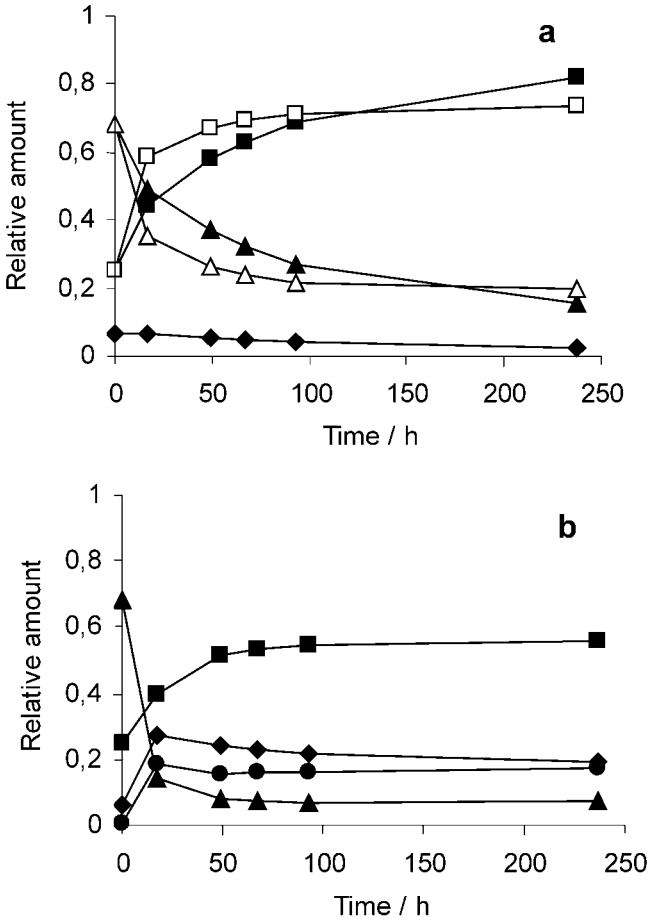
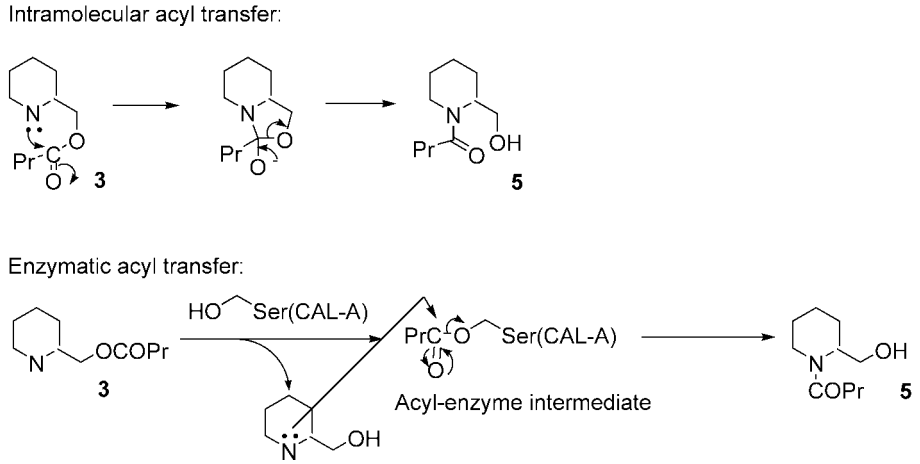


Figure 1. Acyl migration (R = Pr) in TBME at room temperature, **(a)** without CAL-A and **(b)** in the presence of CAL A; **1** (◆),**3** (▲), **5** (■) and **7** (●) as observed and **3** (△), **5** (□) as calculated.

In the presence of CAL-A, the amounts of **1** (◆) and **7** (●) increase in a practically superimposable manner when the initial amount of 7% for **1** is taken into account in the data points with time for curve ◆ (Figure 1b). This leads to an assumption that **1** is formed as the result of the enzymatic hydrolysis of **3** by the water present in the added enzyme preparation. An equal amount of an acyl enzyme intermediate [$PrCO_2CH_2Ser$(CAL-A); Scheme 2] is formed, acylating **5** in the formation of **7**. With this assumption, the sum of curves (◆) and (▲) in Figure 1b gives curve (Δ) in Figure 1a, approximating the disappearance of **3** in the presence of CAL-A for reasons other than enzymatic hydrolysis. With the same reasoning, curve (□; Figure 1a) which equals the sum of curves **5** (■) and **7** (●) in Figure 1b represents the amount of **5** when acyl migration has taken place in the presence of CAL-A. The closely complementary behaviour of curve **3** (Δ) to **5** (□) proposes *O*→*N* acyl migration as the main reason for the faster disappearance of **3** and formation of **5**. The mechanism of this is unclear. As one possibility, some acidic or basic groups of the enzyme molecule are involved. As another possibility the acyl-enzyme intermediate acylates the nitrogen in the liberating **1** (ready at the active site) and leads to the formation of **5** (Scheme 2). The reaction with the alcohol function of **1** only leads back to **3**. The previous results for the *N*-acylation of methyl pipecolinate by CAL-A support this conclusion.[23]

Enantioselective Enzymatic Alcoholysis

After unsuccessful asymmetric acylation, the present enzymes were screened for the alcoholysis of **7** (R = Me) with methanol (0.2 M) in TBME. The results in Table 4 show considerable reactivity except in the case of acylase I. Lipases generally leave the amide bond unreactive and accordingly the formation of **5** (R = Me) as the only new reaction product is expected. However, PPL is an exception, leading to signs of enantiopure **1**. Because of the slow reaction this was not studied more thoroughly. Lipase AK (E = 45) and lipase PS (E = 10) showed highest enantioselectivity and the former was chosen for further consideration. Moreover, enantioselectivities in the cases of acetylated (R = Me; row 4) and butanoylated (R = Pr; row 5) **7** were the same and the work was continued with the acetylated substrate. In order to optimise the resolution conditions various methanol concentrations as well as other alcohols in the place of methanol were studied. The results in Table 5 show decreasing reactivity (conversion reached at a certain time) with increasing alcohol concentration. Butanol as a nucleophile is more favourable than the lower analogues and enantioselectivity increases with increasing butanol concentration (rows 6, 8 and 9) or by decreased temperature (row 7). Finally, the highest enantioselectivity (E = 100, row 9) was



Scheme 2. Intramolecular and enzymatic acyl transfers from **3** to **5**.

Table 4. Enzyme (75 mg/mL) screening for the alcoholysis of **7** (R = Me; 0.05 M) with methanol (0.2 M) in TBME at room temperature; reaction time 24 h.

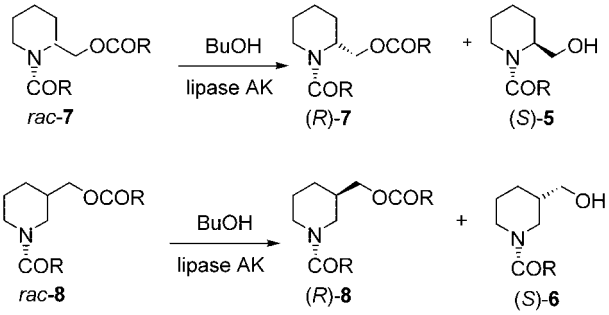
	Relative amount [%]/ee [%]			
Enzyme	**7**	**5**	**1**	*E*
	Acylase I96/2	4/33	–	1
CAL-A	64/16	36/29	–	2
CAL-B	3/3	97/1	–	1
Lipase AK	62/52	38/93	–	45
Lipase AK[a]	65/50	35/95	–	45
Lipase PS	43/84	57/67	–	10
PPL	84/2	14/18	2/100	–

[a] R = Pr.

Table 5. Effect of alcohol concentration for the alcoholysis of **7** (R = Me, 0.05 M) by lipase AK preparation (75 mg/ml) in TBME and in neat butanol at room temperature.

Alcohol	Concentration [M]	Time [h]	Conv. [%]	*E*
MeOH	0.2	24	38	45
MeOH	0.4	24	29	40
MeOH	0.6	24	22	30
PrOH	0.2	24	37	47
PrOH	0.6	24	27	36
BuOH	0.2	24	40	49
BuOH[a]	0.2	24	28	72
BuOH	0.6	24	33	57
BuOH	neat	192	54	100

[a] Temperature 8 °C.



Scheme 3. Lipase AK-catalysed enantioselective alcoholyses of **7** and **8**.

observed when the reaction was performed in neat butanol. When the lipases were screened for the alcoholysis of **8** (R = Pr) the highest enantioselectivity of only $E = 7$ was observed for the lipase AK-catalysed reaction in neat butanol. Accordingly, for the resolution of 3-hydroxymethylpiperidine derivatives the present method is poor compared to the previously published acylation and hydrolysis methods ($E = 23 - 70$).[10,11]

To this end, the usability of alcoholysis for the resolution of **7** (R = Me) was confirmed by performing a gram-scale resolution in neat butanol in the presence of lipase AK as shown in the Experimental Section (Scheme 3). The unreacted (*R*)-**7** and the alcohol product (*S*)-**5** were separated from the resolution mixture after the reaction was stopped at 50% conversion. The absolute configurations of this work are based on the comparison of the measured optical rotations of **5** and **7** to those found in literature.[8] After knowing the absolute configurations in the case of lipase AK catalysis, the peak positions of the enantiomers in the chromatograms can be used to consider which enantiomer reacts faster by various enzymes. This reveals the *S* enantiomer to be more reactive in all lipase-catalysed acylations and alcoholyses of 2-substituted substrates **1**, **5** and **7**, acylase I preferring the reactions of the (*R*)-enantiomer. Interestingly, lipases AK and PS were previously proposed to proceed *S* enantioselectively for the acylation of *N*-Boc protected **2**.[11] Expecting the same enantiopreference for the present reactions of the 3-regioisomer, an opposite

stereochemical structure then is more reactive than in the case of the 2-isomers (Scheme 3).

Conclusion

The present work describes the usefulness of lipase AK for the resolution of racemic diacylated 2-hydroxymethyl piperidine **7** in neat butanol with $E = 100$. The benefit of this method is the simple one-step reaction resulting in the two enantiomers, *N*-acylated amino alcohol (*S*)-**5** with 92% and less reactive (*R*)-**7** with 93% ee, at 50% conversion. The alcoholysis of **7** is favourable over the acylation of *N*-acylated amino alcohol **5** because the reverse enzymatic acylation of **7** by unactivated butyl acetate (another product of enzymatic alcoholysis) is not likely under the reaction conditions whereas the acylation method suffers from the enzymatic alcoholysis of the produced (*S*)-ester **7** with the enantiomerically enriched (*R*)-**5** or with R^1OH (another product of enzymatic acylation), leading to enantiopurity drops with time. The regioisomer **8** in butanol reacts in a less enantioselective manner ($E = 7$).

The present work describes highly chemoselective *O*-acylation of 2- and 3-hydroxymethylpiperidines **1** and **2** in TBME in the presence of lipases. CAL-A as a catalyst and 2,2,2-trifluoroethyl butanoate (R = Pr) as an acyl donor proved to be especially useful in this respect. Enzymatic *O*-acylation competes with intramolecular $O \rightarrow N$ acyl migration. Accordingly, relatively high enzyme contents (75 mg/mL of the enzyme preparation corresponding to 15 mg/mL of commercial enzyme) were used to favour enzymatic acylation. It was shown that $O \rightarrow N$ acyl migration is somewhat faster in the presence of CAL-A than in the absence of the enzyme. By this simple and mild method, 90–98% of **1** (and **2**) can be transformed to amino ester **3** (and **4**), allowing the protection of the primary hydroxy group of the piperidine derivative. Another reaction can then be directed to the free secondary amino group. As an example, the chemical *N*-acylation of **3** by acetic anhydride was performed after the removal of the enzyme.

Experimental Section

General Remarks

Lipases AK (*Pseudomonas fluorecens*) and PS (*Pseudomonas cepacia*) were purchased from Amano Pharmaceuticals and CAL-A (*Candida antarctica* lipase A, Chirazyme L5) and CAL–B (*Candida antarctica* lipase B, Chirazyme L2) from Boehringer-Mannheim. CAL-A and lipases AK and PS were immobilised on Celite in the presence of sucrose as described before,[25] the final preparation containing 20% (w/w) of the lipase. Acylase I from *Aspergillus melleus* (0.49 U/mg) and porcine pancreatic lipase (PPL) were obtained from Sigma. Hydroxymethylpiperidines **1** and **2** were products from Aldrich. Diacylated products were prepared by the reaction of **1** and **2** with acetic (R = Me) or butanoic (R = Pr) anhydrides in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine. The products were purified by eluting with petroleum ether/acetone (gradient 3/1 to 1/3) on silica. *N*-Acylated products **5** and **6** were obtained in the same way but using stoichiometric control. 2,2,2-Trifluoroethyl carboxylates ($RCO_2CH_2CF_3$; R = Me, Pr and Bu) were prepared from the corresponding acid chloride and 2,2,2-trifluoroethanol. Butanol, methanol and the solvents were of the highest analytic grade from Aldrich, Lab Scan Ltd, Riedel-de Haën and J. T. Baker.

The progress of reactions and the ee values of the unreacted substrates and the formed products were followed by taking samples (0.1 mL) at intervals and analysing them by gas chromatography on a Chrompack CP-Chirasil-DEX CB column (25 m). For good baseline separation the unreacted amino/hydroxy group of **1**–**6** in the sample was acetylated with acetic anhydride for enzymatic butanoylation and pentanoylation and with butanoic anhydride for enzymatic acetylation in the presence of pyridine containing DMAP (1%). The determination of E was based on equation $E = \ln[(1 - ee_S)/(1 + ee_S/ee_P)]/\ln[(1 + ee_S)/(1 + ee_S/ee_P)]$ with $c = ee_S/(ee_S + ee_P)$ as based on the original equations of Chen et al.[26] The equation is valid for irreversible acyl transfers when no side reactions of the substrates or products is observed. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Jeol Lambda 400 or a Bruker 200 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were taken on Agilent GC-MS instrument equipped with 5973 network mass selective detector and 6890 N network GC-system. Optical rotations were measured using a Jasco DIP-360 polarimeter.

Enzymatic Reactions

The reactions were typically performed as small-scale experiments at room temperature (23–24 °C) where 2,2,2-trifluoroethyl butanoate (0.1 M) was added in the solution (0.05 M) of **1**, **2** or **5** in an organic solvent. The alcoholysis of **7** and **8** (0.05 M) was performed with an alcohol (0.2–0.6 M) in TBME or in a neat alcohol. The commercial enzyme or enzyme preparation (75 mg/mL) was added in order to start the reaction.

Chemoselective Gram-Scale Preparation of (*rac*)-3

CAL-A preparation (75 mg/mL) was added to **1** (0.60 g, 5.2 mmol) and 2,2,2-trifluoroethyl butanoate (1.57 mL, 10.4 mmol) in TBME (104 mL). After 2 h the enzyme was filtered off at 93% conversion. The isolated product mixture contained **1**, **3**, **5** and **7** (R = Pr) with relative proportions of 6.1, 93, 0.2 and 0.7%, respectively. In order to prevent $O \rightarrow N$ acyl migration the chemical *N*-acylation of the produced **3** (R = Pr) was performed immediately by acetic anhydride (10.4 mmol) in the presence of triethylamine (5.2 mmol) and DMAP. The product ratio remained constant. Purification by column chromatography (ethyl acetate/hexane, 1/1) yielded racemic *N*-acetyl-2-butanoyloxymethylpiperidine (**9**, 0.98 g, 4.3 mmol)

containing 0.6% of **7** (R = Me) and 0.1% of *N*-butanoylacetamide formed from **5**. In GC analysis the retention times for the enantiomers of **9** were 37 and 38 while the corresponding *N*-butanoylacetamide gave the retention times 30 and 31.

(*Rac*)-**9** (corresponds to **7** with $R_{N\text{-acyl}}$ = Me and $R_{O\text{-acyl}}$ = Pr): ^{1}H NMR ($CDCl_3$, 25 °C): δ = 0.9 (t, 3H, CH_3CH_2),1.5 – 1.7 (m, 6H, CH_2), 1.5 (m, 2H, $CH_3CH_2CH_2$), 2.3 (m, 2H, $CH_3CH_2CH_2CO$), 2.1 (s, 3H, $NCOCH_3$), 2.6 (t, 1H, $NCHCH_2O$), 3,2 (t, 2H, NCH_2CH_2), 4.0 – 4.6 (m, 2H, $NCHCH_2O$); ^{13}C NMR ($CDCl_3$, 25 °C): δ = 13.5 (CH_3CH_2), 18.2 ($CH_3CH_2CH_2$), 19.3 ($CH_3CH_2CH_2$), 21.8 (CH_3CO), 24.9 and 25.1 ($CH_2CH_2CH_2$), 26.2 (CH_2CH), 42.4 (CH_2N), 46.3 ($NCHCH_2O$), 61.6 (CH_2OCOPr), 170.0 and 173.2 (CO); MS: M^+ (calcd. for $C_{12}H_{21}NO_3$): 227 (227.30).

Gram-Scale Resolution of 7 (R = Me)

Lipase AK preparation (75 mg/mL) was added to **7** (R = Me; 1.00 g, 5.0 mmol) in butanol (100 mL) to start the reaction. After 382 h the enzyme was filtered off at 50% conversion. Purification by column chromatography (acetone/ petroleum ether 1/1) yielded (*S*)-**5** (0.30 g, 1.9 mmol, ee_5 = 92%, $[\alpha]_D^{20}$: – 54.9 (*c* 1, $CHCl_3$) compared to $[\alpha]_D^{20}$ (lit.)[8]: + 20.5 (*c* 4.0, $CHCl_3$; *R* enantiomer at 45% ee) and (*R*)-**7** (0.48 g, 2.4 mmol, ee_7 = 93%) containing 3% of amide according to the GLC method. (*R*)-**7** was further purified by enzymatic alcoholysis resulting in the product with ee_7 = 97% and $[\alpha]_D^{20}$: + 56.8 (*c* 1, $CHCl_3$) compared to $[\alpha]_D^{20}$ (lit.)[8]: – 18.3 (*c* 9.6, $CHCl_3$) for the *S* enantiomer at 47% ee.

(*S*)-**5** (R = Me): ^{1}H NMR ($CDCl_3$, 25 °C): δ = 1.3 – 1.9 (m, 6H, CH_2), 2.1 (s, 3H, CH_3CO), 2.7 (t, 1H, $NCHCH_2O$), 3.2 (t, 2H,NCH_2CH_2), 3.4 – 4.2 (m, 2H, CH_2OH), 4.8 (O*H*); ^{13} C NMR: δ = 21.8 (CH_3CO), 25.0 and 25.5 ($CH_2CH_2CH_2$), 26.1 (CH_2CH), 50.8 (CH_2N), 55.6 ($NCHCH_2O$), 61.6 (CH_2OH) and 170.0 (CO); MS: M^+ (calcd. for $C_8H_{15}NO_2$): 157 (157.21).

(R)-**7** (R = Me): ^{1}H NMR ($CDCl_3$, 25 °C): δ = 1.3 – 1.7 (m, 6H, CH_2), 2.0 (s, 3H, CH_3CO), 2.1 (s, 3H, $NCOCH_3$), 2.6 (t, 1H, $NCHCH_2O$), 3.1 (t, 2H, NCH_2CH_2), 4.0 – 4.7 (m, 2H, $NCHCH_2O$); ^{13} C NMR ($CDCl_3$, 25 °C): δ = 20.7 ($NCOCH_3$), 21.7 (CH_3CO_2), 25.1 and 25.6 ($CH_2CH_2CH_2$), 26.1 (CH_2CH), 42.4 (CH_2N), 46.4 ($NCHCH_2O$), 61.8 (CH_2OCOMe), 169.9 and 170.8 (CO); MS: M^+ (calcd. for $C_{10}H_{17}NO_3$): 199 (199.25).

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