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Enantioselective lipase-catalyzed reactions of methyl pipecolinate: transesterification and *N*-acylation

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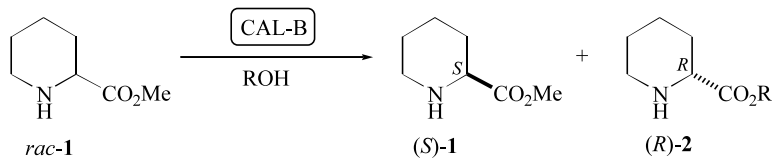
Abstract—The present research introduces the highly (*S*)-selective (*E*>>100) acylation at the secondary ring nitrogen of methyl pipecolinate as a novel resolution method with *Candida antarctica* lipase A. Catalysis by lipase B leads to reactions at the methyl ester function of the substrate in an almost non-enantioselective manner.

(*S*)-Pipecolic acid (piperidine-2-carboxylic acid) as a lysine metabolite occurs widely in nature as a free nonproteinogenic amino acid and as a constituent of natural products,[1] and hence both enantiomers are of commercial value as building blocks for many biologically and pharmaceutically active compounds.[2] (*S*)-Lysine serves as a typical chiral pool reagent for the preparation of (*S*)-pipecolic acid.[3–5] Chemical and chemoenzymatic asymmetric routes to pipecolic acid enantiomers have also been reported.[6–10] Resolution methods include fractional crystallization[11,12] and enzymatic pathways.[2,13,14] Economical and environmental benefits of enzymatic kinetic resolution over traditional chemical methods arise from the fact that only catalytic amounts of a reusable enzyme are needed for asymmetric induction under benign reaction conditions.

In our ongoing work on the synthetic applications of lipases, we have examined the very different behaviors of lipases A (CAL-A) and B (CAL-B) from *Candida antarctica* toward polyfunctional compounds in nonaqueous media.[15–20] Thus, excellent chemoselectivity and from good to excellent enantioselectivity of CAL-A were observed for the *N*-acylation of numerous β-substituted β-amino esters as primary amine substrates.[15,16] CAL-A also catalyzed the acylation of the secondary alcohol group of 1-phenyl-1,2-ethanediol in the presence of the primary alcohol function in a highly enantioselective manner.[20] This indicates exceptional behavior in that the lipase preferred to act at sterically hindered positions. On the other hand, the CAL-B-catalyzed reactions of the β-amino esters with achiral esters proceeded with relatively low chemoselectivity, enantioselective transesterification at the ester function rather than *N*-acylation being favored.[17,18] Moreover, the importance of the sterically nonhindered position as the reaction site for CAL-B became evident.[19,20] The previous results also convinced us that highly enantioselective ester alcoholyses would be possible using CAL-B.[17–20]

In this paper we report a novel and highly effective lipase-catalyzed method for the resolution of *N*-heterocyclic amino esters using methyl pipecolinate **1** as a model compound. For that purpose, the chemo- and enantioselective alcoholysis and transesterification reactions of **1** in the presence of CAL-B and *N*-acylations in the presence of CAL-A were studied (Schemes 1 and 2).[21]



rac-**1** (*S*)-**1** (*R*)-**2**

Scheme 1.

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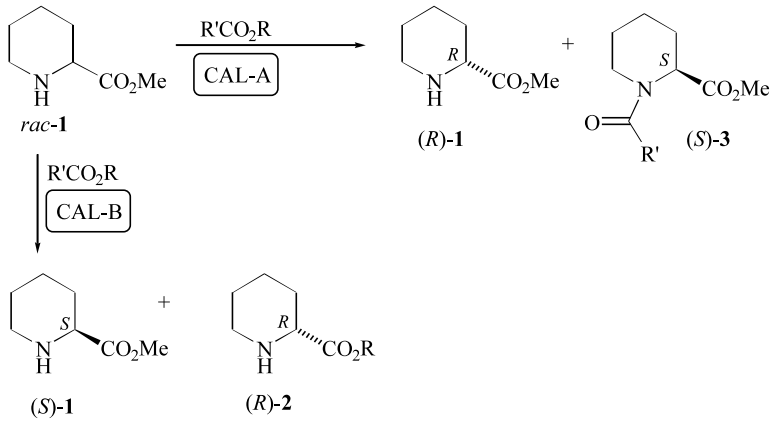

Scheme 2.

*Reaction of **1** as an ester*. Our high expectations for the CAL-B-catalyzed alcoholysis of **1** were disappointed when low enantioselectivities were found in terms of the observed *E* (enantiomer ratio) values[22] (Scheme 1; Table 1, rows 1–4). The reaction in 2-propanol (R = ^{i}Pr) with $E = 15$ at 8°C was the most promising (row 4). Reactions at the ester function of the substrate proceeded smoothly in the formation of enantiomerically enriched (*R*)-**2** in neat alcohols as shown by high conversions [*C*(II)/%]. Chemical ester alcoholysis in 1-butanol clearly competed with the enzymatic one although its proportion was insignificant at short reaction times as shown by the conversion values C(I)/% for the chemical reaction compared to the C(II)/% values in the presence of CAL-B after 40 minutes (rows 1 and 2). This allows the calculation of the *E* values for enzymatic reactions. On the other hand, the CAL-A-catalyzed alcoholysis of **1** gave racemic **2** (R = Bu) at 20% total conversion after 24 h, the proportion of the simultaneous chemical reaction then being already approximately 10% as determined separately by monitoring the reaction, under otherwise identical conditions, but in the absence of the enzyme.

As was observed for the lipase-catalyzed resolutions of various piperidine derivatives[23–25] and in our previous work for those of amino acid esters,[17–19] *N*-protection was expected to make **1** a better substrate for CAL-B catalysis. However, the CAL-B-catalyzed reaction of the *N*-acetylated substrate (*rac*-**3**; Scheme 2, R′ = Me) with neat butanol was extremely slow (conversion <5% after 8 days) and the reaction was mostly chemical in nature. The increased steric size of the *N*-acetylated substrate could explain why CAL-B does not work. It is worth emphasizing that lipases typically leave amide bonds untouched.

Previously it was proposed that secondary amines are not substrates for lipases.[25] In accordance with this, chemical or CAL-B-catalyzed *N*-acylation was not detected in the case of the studied acyl donors shown in Table 1 (rows 5–7). On the other hand, chemoselective transesterification by CAL-B catalysis proceeded smoothly at the ester function of **1** with butyl acetate and butyl butanoate, leading to the formation of slightly enantiomerically enriched butyl esters (*R*)-**2** (Scheme 2, lower part, R = Bu; Table 1, rows 5 and 6). 2,2,2-Trifluoroethyl butanoate as an acyl donor gave enzymatic transesterification product **2** (R = CH_2CF_3, row 7) in an extremely slow reaction. Chemical reaction was observed only in the case of butyl acetate as an acyl donor (row 5). Practically no transesterification by CAL-B was observed with sterically hindered *rac*-**3** (R′ = Me) in butyl butanoate (conversion <5% after 5 days).

*Reactions of **1** as a secondary amine*. At this point with unsuccessful trials in directing enantioselective enzymatic reactions to the ester function of **1** it was time to reconsider our experience with lipases. Thus, we came to consider CAL-A and its above-mentioned exceptional behaviors as a lipase.[15,16,20] In contrast to CAL-

Table 1. Reactions of methyl pipecolinate (0.1 M) in neat alcohol and ester: *C*(**I**) (%) is conversion in the absence of the enzyme and *C*(**II**) (%) in the presence of CAL-B after 40 minutes at room temperature (25°C).

	Solvent	CAL-B (mg/ml)	*C*(**I**) (%)	*C*(**II**) (%)	*E*(**II**)
1	BuOH	10	0.3	82	3
2	BuOH[a]	10	0.08	55	4
3	^{i}PrOH	10	0.01	22	9
4	^{i}PrOH[a]	10	–	11	15
5	AcOBu	5	0.2	28	2
6	$PrCO_2Bu$	5	–	55	3
7	$PrCO_2CH_2CF_3$[b]	10	–	2	1

[a] Temperature 8°C.
[b] 0.2 M in TBME.

B, the CAL-A-catalyzed reaction of **1** with 2,2,2-trifluoroethyl butanoate in organic solvents proceeded exclusively at the secondary ring nitrogen, leading to the formation of (*S*)-**3** (Scheme 2, upper part, R′ = Pr; Table 2). Chemical transesterification/alcoholysis at the ester function (Table 1, row 7) or *N*-acylation was not observed. *tert*-Butyl methyl ether (TBME) was chosen as the best solvent due to high enantioselectivity (*E*> 100) and short reaction time needed to reach 50% conversion (Table 2, row 10). There was no reaction in dichloromethane, chloroform or DMF as a solvent. This is the first time that lipase-catalyzed highly enantioselective acylation at a secondary nitrogen has been described. Thus, e.g. for the acylation of hydroxymethyl piperidines with various hydrolytic enzymes, the observed *N*-acylated product was proposed to be the result from a fast *O*→*N* acyl migration in the initially formed *O*-acyl derivative.[25]

The reactions of **1** with various achiral acyl donors in TBME were tested and the results are shown in Table 3. 2,2,2-Trifluoroethyl butanoate, 3-butenoate and 4-pentenoate (rows 2–4) are the most suitable for resolution purposes. Only in the case of 2,2,2-trifluoroethyl chloroacetate (row 5) as an acyl donor, did chemical *N*-acylation (10% in 96 h) compete with the enzymatic one and led to somewhat lowered enantiopurity (ee = 92%) for (*S*)-**3** (R′ = CH_2Cl). The use of diallyl carbonate and ethyl butanoate as acyl donors resulted in the enzymatic formation of allyl (8% from the observed 24% conversion after 24 h) and ethyl esters (traces after 24 h), respectively, in addition to the *N*-acylation products (*S*)-**3** (R′ = CH_2=$CHCH_2O$ and R′ = Pr, respectively).

To this end, the gram-scale resolution of **1** in TBME was performed at room temperature. For that purpose **1** (1.50 g, 10.5 mmol) and 2,2,2-trifluoroethyl butanoate (21 mmol) were dissolved in TBME (105 ml) and CAL-A (7.9 g containing 20% (w/w) of the enzyme on Celite) was added. At 48% conversion the enzyme was filtered off. (*R*)-**1** (0.72 g, 4.02 mmol, ee 90%, $[\alpha]_D^{24}$ +7.09 (*c* 1.00, MeOH)) was precipitated as the hydrochloride salt. (*S*)-**3** (R′ = Pr, 0.88 g, 4.12 mmol; ee 98%, $[\alpha]_D^{24}$ −77.7 (*c* 1.01, MeOH)) was purified by column chromatography on silica (elution with acetone:petroleum ether 1:1).

In conclusion, we have reported the application of CAL-A as a highly enantio- and chemoselective catalyst for the acylation of the secondary amine **1** under conditions where CAL-B leads chemoselectively to reaction at the ester function. Noteworthy are the opposite enantioselectivities of CAL-A for *N*-acylation and CAL-B for transesterification and alcoholysis as shown in Schemes 1 and 2. Absolute configurations were determined using commercially available (*S*)-pipecolic acid as a reference.

Table 2. CAL-A-catalyzed (75 mg/ml) acylation of methyl pipecolinate (0.1 M) with trifluoroethyl butanoate (0.2 M) in different solvents at room temperature (25°C).

	Solvent	Time (h)	ee$^{(R)\text{-}1}$ (%)	ee$^{(S)\text{-}3}$ (%)	*C* (%)	*E*
1	CH_3CN	22	62	99	39	>100
2	CH_2Cl_2	24	1	>99	1	–
3	$CHCl_3$	24	2	>99	1	–
4	DMF	24	2	>99	2	–
5	Hexane	24	24	>99	20	>100
6	Toluene	72	16	>99	13	>100
7	THF	72	22	>99	18	>100
8	Et_2O	72	84	>99	46	>100
9	$^iPr_2O$	4	39	99	28	>100
10	TBME	9	95	99	49	>100

Table 3. Effect of an acyl donor (0.2 M) for the CAL-A-catalyzed (75 mg/ml) acylation of methyl pipecolinate (0.1 M) in TBME at room temperature (25°C)

	Acyl donor	Time (h)	ee$^{(R)\text{-}1}$ (%)	ee$^{(S)\text{-}3}$ (%)	*C* (%)	*E*
1	2,2,2-Trifluoroethyl acetate	24	40	>99	29	>100
2	2,2,2-Trifluoroethyl butanoate	9	95	99	49	>100
3	2,2,2-Trifluoroethyl 3-butenoate	43	96	95	50	>100
4	2,2,2-Trifluoroethyl 4-pentenoate	6	94	96	49	>100
5	2,2,2-Trifluoroethyl chloroacetate	24	18	92	17	–[a]
6	Diallyl carbonate	24	18	>99	24	–[b]
7	Ethyl butanoate	24	12	97	11	–[b]
8	Ethyl butanoate[c]	24	20	98	17	–[b]

[a] Chemical *N*-acylation significant.
[b] Enzymatic reaction at the ester function observed parallel with the *N*-acylation as the main reaction.
[c] Ethyl butanoate as the solvent.

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References

1. Zabriskie, T. M.; Kelly, W. L.; Liang, X. *J. Am. Chem. Soc.* **1997**, *119*, 6446–6447.
2. Petersen, M.; Sauter, M. *Chimia* **1999**, *53*, 608–612.
3. Fujii, T.; Miyoshi, M. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1341–1342.
4. Ohtani, B.; Tsuru, S.; Nishimoto, S.; Kagiya, T. *J. Org. Chem.* **1990**, *55*, 5551–5553.
5. Kisfaludy, L.; Korenczki, F.; Katho, A. *Synthesis* **1982**, 163.
6. Berrien, J.-F.; Royer, J.; Husson, H.-P. *J. Org. Chem.* **1994**, *59*, 3769–3774.
7. Fernández-García, C.; McKervey, M. A. *Tetrahedron: Asymmetry* **1995**, *6*, 2905–2906.
8. Myers, A. G.; Gleason, J. L.; Yoon, T. *J. Am. Chem. Soc.* **1995**, *117*, 8488–8489.
9. Sánchez-Sancho, F.; Herradón, B. *Tetrahedron: Asymmetry* **1998**, *9*, 1951–1965.
10. Nazabadioko, S.; Perez, R. J.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 1597–1604.
11. Shiraiwa, T.; Shinjo, K.; Kurokawa, H. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3251–3255.
12. Hockless, D. C. R.; Mayadunne, R. C.; Wild, S. B. *Tetrahedron: Asymmetry* **1995**, *6*, 3031–3037.
13. Ng-Youn-Chen, M. C.; Serreqi, A. N.; Huang, Q.; Kazlauskas, R. J. *J. Org. Chem.* **1994**, *59*, 2075–2081.
14. Eichhorn, E.; Roduit, J.-P.; Shaw, N.; Heinzmann, K.; Kiener, A. *Tetrahedron: Asymmetry* **1997**, *8*, 2533–2536.
15. Kanerva, L. T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1705–1716.
16. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
17. Gedey, S.; Liljeblad, A.; Fülöp, F.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 2573–2581.
18. Gedey, S.; Liljeblad, A.; Lázár, L.; Fülöp, F.; Kanerva, L. T. *Can. J. Chem.* **2002**, in press.
19. Liljeblad, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **1999**, *10*, 4405–4415.
20. Virsu, P.; Liljeblad, A.; Kanerva, A.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2001**, *12*, 2447–2455.
21. CAL-A (Chirazyme L5) and CAL-B (Chirazyme L2) were from Boehringer-Mannheim. CAL-A 20 was adsorbed on Celite before use by dissolving the enzyme (5 g) and sucrose (3 g) in Tris·HCl buffer (120 ml, 20 mM, pH 7.8). The addition of celite (17 g) was followed by evaporation to dryness. Methyl pipecolinate hydrochloride was purchased from Aldrich; the hydrochloride was removed by ammonia in chloroform. The reactions were followed by GLC on Chrompack CP-Chirasil-DEX CB column; from 92°C (25 min) to 140°C with 40°C/min and retention times 20.9 and 22.1 min for (*R*)- and (*S*)-**3** when R′=Me and 43.9 and 45.0 min when R′=Pr, respectively.
22. $E=\ln[(1-ee_1)/(1-ee_1/ee_P)]/\ln[(1-ee_1)/(1+ee_1/ee_P)]$ with $c=ee_1/(ee_1+ee_P)$ as derived from the original equations in: Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695–707.
23. Danieli, B.; Lesma, G.; Passarella, D.; Silvani, A. *Tetrahedron: Asymmetry* **1996**, *7*, 345–348.
24. Chênevert, R.; Ziaarani, G. M.; Morin, M. P.; Dasser, M. *Tetrahedron: Asymmetry* **1999**, *10*, 3117–3122.
25. Asensio, G.; Andreu, C.; Marco, J. A. *Tetrahedron Lett.* **1991**, *32*, 4197–4198.