LIPASE-CATALYZED ENANTIOSELECTIVE HYDROLYSIS OF 4-ALKYL-1,4-DIHYDROPYRIDINE DERIVATIVES: SYNTHESIS OF (+)- AND (-)-METHYL 2-(PHENYLTHIO)ETHYL 1,4-DIHYDRO-2,4,6-TRIMETHYL-3,5-PYRIDINEDICARBOXYLATE (PCA 4248)

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Asymmetric synthesis of (+)- and (-)-PCA 4248 (PAF antagonist) was achieved using lipase-catalyzed enantioselective hydrolysis of the acyloxymethyl esters. The enzymatic reaction proceeded under the mild conditions in an organic solvent.

KEYWORDS 1,4-dihydropyridine; lipase; enantioselective hydrolysis; acyloxymethyl ester; PAF antagonist

Since 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates were found to be highly effective calcium antagonists about twenty years ago,[1] many dihydropyridines were investigated, and have been studied and developed as clinically useful against cardiovascular diseases or hypertension.[2] In the case of analogs possessing an asymmetric carbon at 4-position, the two enantiomers were reported to show quite different biological activities,[3] and we reported asymmetric syntheses of their derivatives from prochiral substrates (bisacyloxylmethyl 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates) using lipase-catalyzed enantioselective hydrolysis.[4]

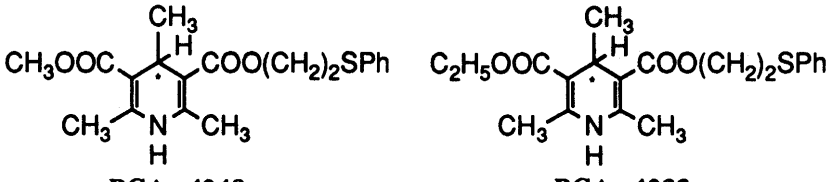


Chart 1

On the other hand, 1,4-dihydropyridine derivatives were recently reported to have anticancer activity[5] or to interfere with platelet aggregation,[6] but lacked any cardiovascular effects (Chart 1). PCA 4248 and PCA 4233 are new types of PAF antagonists, developed in a series of 1,4-dihydropyridines, but they have been synthesized as racemates. Differences in biological activities between enatiomers of PCA 4248, and PCA 4233 should be made clear, because some enantiomers of chiral drugs produced considerable side effects. Taking into account the importances of their clinical use, we planned a synthesis of the optically pure derivatives.

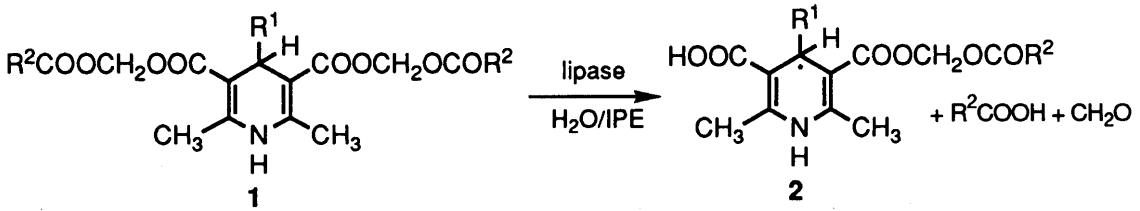
We describe in this paper the asymmetric synthesis of 4-alkyl-1,4-dihydropyridine derivatives, and synthesis of optically active (+)- and (-)-PCA 4248 using lipase-catalyzed enantioselective hydrolysis of bispivaloyloxymethyl 1,4-dihydro-2,4,6-

trimethyl-3,5-pyridinedicarboxylate (**1a**) in an organic solvent. The preliminary screening tests of various lipases revealed that lipase B (from *Pseudomonas fragi*) was effective for hydrolysis of the pivaloyloxymethyl ester (**1a**), which was prepared from chloromethyl pivalate with dihydropyridinedicarboxylic acid.[7,8] The enzymatic reaction was carried out by stirring a mixture of the substrate (1 mmol) and a crude lipase B (100 mg:20000 U) in diisopropyl ether (IPE) saturated with water.

The hydrolysis proceeded smoothly to give (+)-1,4-dihydro-2,4,6-trimethyl-5-pivaloyloxymethoxycarbonyl-3-pyridinecarboxylic acid (**2a**),[9] chiral building block for PCA 4248, and PCA 4233, in 91% optical yield with lipase B.[10] The results of lipase-catalyzed enantioselective hydrolysis of 4-alkyl-1,4-dihydropyridines are shown in Table I. Though lipase B was well suited for hydrolysis of 4-methyl derivative (**1a**), enantioselectivity of lipase P for hydrolysis of 4-benzyl derivatives (**1c**) was better than that of lipase B (entries 3,4). In cyclohexyl derivatives, steric hindrance of the cyclohexyl group seemed to interfere with the hydrolysis of acyloxymethyl groups (entries 5-7). These results showed that 4-substituent groups affected the reactivities and enatioselectiveities of lipases.

The (+)-monoester (**2a**) obtained was converted to (+)-PCA 4248[11] via the intermediates **3** and **4** by successive treatment with diazomethane, alkaline, thionylchloride, and 2-(phenylthio)ethanol (Chart 2). On the other hand, (-)-PCA 4248 was obtained as shown in Chart 3.

Table I. Lipase-Catalyzed Asymmetric Synthesis of 4-Substituted-1,4-dihydropyridines[a]

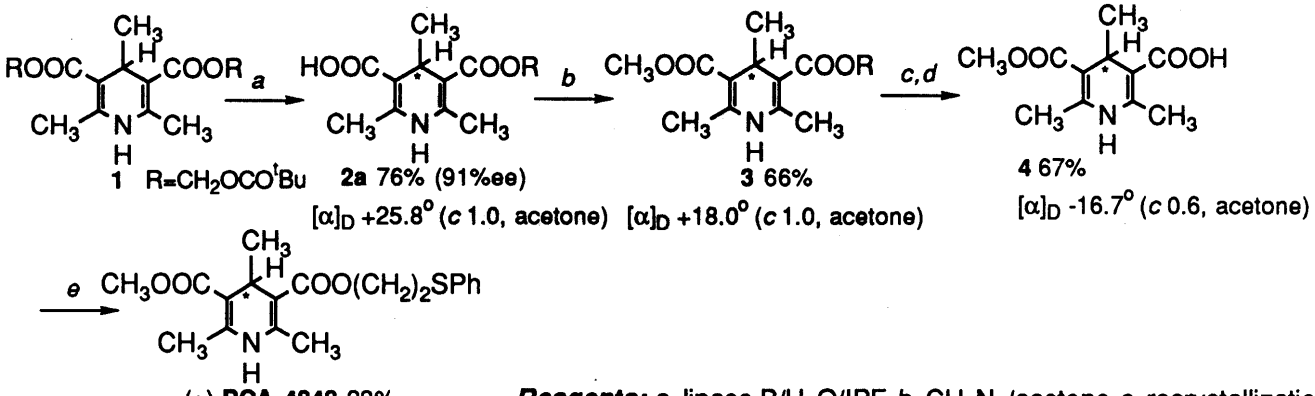


	Substrate					Product			
Entry	No.	R^1	R^2	Lipase (/mmol)	Time (h)	No.	C.Y. (%)[b,c]	O.Y. (%ee)[d]	$[\alpha]_D^{20}$ deg[e]
1	**1a**	Me	^{t}Bu	B (100mg)	8	**2a**	76	91	+25.8
2	**1b**	Bn	^{t}Bu	B (200mg)	151	**2b**	67	0	+2.6
3	**1c**	Bn	Et	B (50mg)	4	**2c**	56	48	-27.2
4	**1c**	Bn	Et	P (100mg)	48	**2c**	71	91	-42.9
5	**1d**	Cy	^{t}Bu	B (200mg)	96	**2d**	62	56	+11.5
6	**1e**	Cy	Et	B (50mg)	89	**2e**	16	20	-13.4
7	**1e**	Cy	Et	P (100mg)	118	**2e**	29	26	-16.3

a) All reactions were carried out by stirring a mixture of substrate, lipase, and IPE saturated with water at 25°C. *b*) Isolated yields. *c*) Satisfactory elemental analyses of all products were obtained. *d*) Optical yields were determined by HPLC analyses using a column packed with Chiralcel OJ (IPA/hexane) after conversion to methyl POM ester (entries 1,5), or benzyl methyl ester (entries 2-4,6,7). *e*) Acetone, *c* 0.5-1.

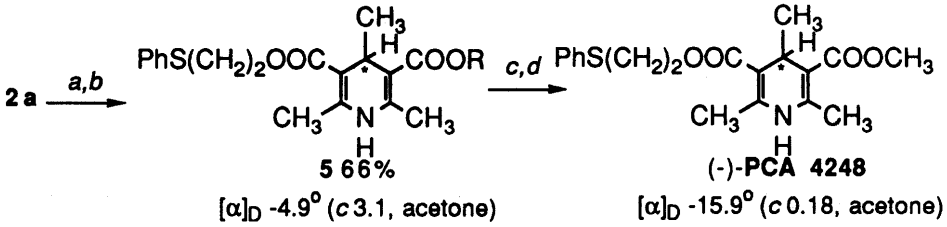
The optical purities of (+)- and (-)-PCA4248 were determined by HPLC analysis,[12] and both of them were revealed to be almost optically pure.

Thus, we have achieved the first asymmetric synthesis of both (+)- and (-)-PCA 4248, and demonstrated that the lipase-catalyzed enantioselective hydrolysis of acyloxymethyl esters was applicable to a wide range of syntheses of optically active medicines.[13]



Reagents; *a*, lipase B/H_2O/IPE *b*, CH_2N_2/acetone *c*, recrystallization *d*, KOH/MeOH *e*, $SOCl_2$, 2-(phenylthio)ethanol/DMF, CH_2Cl_2

Chart 2



Reagents; *a*, $SOCl_2$, 2-(phenylthio)ethanol/DMF, CH_2Cl_2 *b*, recrystallization *c*, KOH/MeOH *d*, CH_2N_2/acetone

Chart 3

REFERENCES AND NOTES

1) F. Bossert and W. Yater, *Naturwissenschaften*, **58**, 578 (1971). For reviews see: F. Bossert, F. Mayer, and E. Wehinger, *Angew. Chem. Int. Ed. Eng.*, **20**, 762 (1981). S. Goldmann, and J. Stoltefuss, *ibid.*, **30**, 1559 (1991).

2) R. Towart, and E. Wehinger, *Arzneim-Forsch.*, **32(I)**, 338 (1982). T. Tamazawa, H. Arima, T. Kojima, Y. Isomura, M. Okuda, S. Fujita, T. Furuya, T. Takenaka, O. Inagaki, and M. Terai, *J. Med. Chem.*, **29**, 2304 (1986).

3) T. Shimamura, M. Iwatani, K. Okuda, T. Takenaka, and M. Murakami, *Chem. Pharm. Bull.*, **28**, 2809 (1980). K. Muto, T. Kuroda, H. Kawato, A. Karasawa, K. Kubo, and M. Nakamizo, *Arzneim-Forsch.*, **38(II)**, 1662 (1988). M. Kajino, T. Wada, Y. Nagai, A. Nagaoka, and K. Meguro, *Chem. Pharm. Bull.*, **37**, 2225 (1989). A. Ashimori, T. Uchida, Y. Ohtaki, M. Tanaka, K. Ohe, C. Fukuya, M. Watanabe, M. Kagitani, and K. Yokoyama, *Chem. Pharm. Bull.*, **39**, 108 (1991).

4) H. Ebiike, Y. Terao, and K. Achiwa, *Tetrahedron Lett.*, **32**, 5805 (1991).

5) K. Suzuki, Y. Yoshida, and H. Inada, *Japan Patent (Kokai)* 31780 (1989). *ibid.*, 31781 (1989). H. Suzuki, *Iyaku Journal*, **27**, 519 (1991).

6) S. Fernandez, M. D. P. Ortega, J. G. Priego, M. F. D. Casajuana, C. Sunkel, and M. S. Crespo, *J. Pharmacol. Exp. Ther.*, **255**, 34 (1990). M. P. Ortega, M. D. C. Garcia, M. A. Gijon, M. F. Casa-Juana, J. G. Priego, M. S. Crespo, and C. Sunkel, *ibid.*, **255**, 28 (1990). C. Sunkel, M. F. Casa-Juana, F. J. Cillero, J. G. Priego, and M. P. Ortega, *J. Med. Chem.*, **31**, 1886 (1988).

7) Pivaloyloxymethyl ester (**1a**) was prepared by addtion of chloromethyl pivalate to a mixture of 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylic acid and sodium hydride in dimethylformamide.
1a: mp 82-83°C, ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, d, *J*=6.4Hz, CH_3), 1.21 (18H, s, 6xCH_3), 2.27 (6H, s, 2xCH_3), 3.81 (1H, q, *J*=6.4Hz, >CH-), 5.85 (4H, ABq, *J*=5.4Hz, 2xOCH_2O), 6.17 (1H, s, NH).

8) Initial attempt to enzymatically hydrolyze dimethyl 1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate was unsuccessful.

9) **2a**: mp 113-114°C, $[\alpha]_D^{20}$ +25.4° (*c* 1.0, acetone), ^{1}H-NMR (d_6-acetone) δ: 0.94 (3H, d, *J*=6.4Hz, CH_3), 1.19 (9H, s, 3xCH_3), 2.27 (6H, s, 2xCH_3), 3.85 (1H, q, *J*=6.4Hz, >CH-), 5.83 (2H, ABq, *J*=5.5Hz, OCH_2O), 7.91 (1H, s, NH).

10) Optical yield was determined by HPLC analysis using a column packed with Chiralcel OJ (2-propanol/hexane) after conversion to **3**.

11) **(+)-PCA 4248**: $[\alpha]_D^{20}$ +15.6° (*c* 0.2, acetone), ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, d, *J*=6.4Hz, CH_3), 2.26, 2.27 (6H, s, 2xCH_3), 3.20 (2H, t, *J*=6.8Hz, CH_2SPh), 3.72 (3H, s, CH_3), 3.81 (1H, q, *J*=6.4Hz, >CH-), 4.26 (1H, dt, *J*=11.2, 6.8Hz, OCH_AH_BO), 4.33 (1H, dt, *J*=11.2, 6.8Hz, OCH_AH_BO), 5.62 (1H, s, NH), 7.19-7.42 (5H, m, C_6H_5).
(-)-PCA 4248: $[\alpha]_D^{20}$ -15.8° (*c* 0.18, acetone).

12) Optical yield was determined by HPLC analysis using a column packed with Chiralcel OJ (2-propanol/hexane).

13) M. Murata, and K. Achiwa, *Tetrahedron Lett.*, **32**, 6763 (1991).

(Received March 2, 1992)