ENZYME-CATALYZED SYNTHESIS OF BIOLOGICALLY ACTIVE (*S*)-NILVADIPINE

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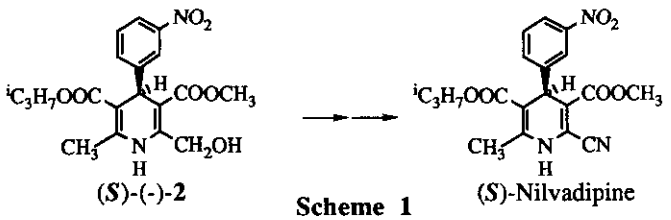
Abstract- Optically active 2-hydroxymethyl-1,4-dihydropyridine was obtained by lipase-catalyzed transesterification of isopropyl methyl 1,4-dihydro-2-hydroxymethyl-6-methyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate. This chiral dihydropyridine was readily converted into biologically active (*S*)-nilvadipine.

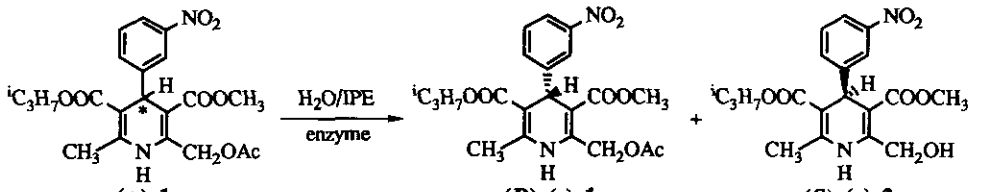
Hydrolytic enzymes, lipases and esterases have been widely used for organic synthesis of chiral compounds. Especially, lipases are one of the most suitable biocatalysts for organic synthesis, because they can use without inactivation, and keep acceptable catalytic activities even in organic solvents.[1]



Scheme 1

4-Aryl-1,4-dihydropyridinedicarboxylic diesters are known as calcium antagonists, and this series of derivatives have been widely used as an antihypertensive drug.[2] When the two ester groups are different, C_4 of the dihydropyridine ring becomes chiral, and their enantiomers have been reported to show much different biological activities.[3,4] In previous papers, we reported asymmetric synthesis of their derivatives from prochiral substrates (bisacyloxymethyl 4-aryl- and 4-alkyl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates) using lipase catalysts,[5,6] and the enzyme-catalyzed synthesis of optically active 2-substituted 1,4-dihydropyridines,[7] where absolute configuration was assumed on the lipase-catalyzed hydrolysis mechanism of the related 4-aryl-1,4-dihydropyridinedicarboxylic diacyloxymethyl esters. In this paper, we report the synthesis of (*S*)-(+)-isopropyl 1,4-dihydro-2-cyano-3-methoxycarbonyl-4-(3-nitrophenyl)-5-pyridinecarboxylate (nilvadipine)[8] using lipase-catalyzed kinetic resolution from the racemic materials and the stereochemical pathway of these lipase-catalyzed transesterification with vinyl acetate as shown in Scheme 2.

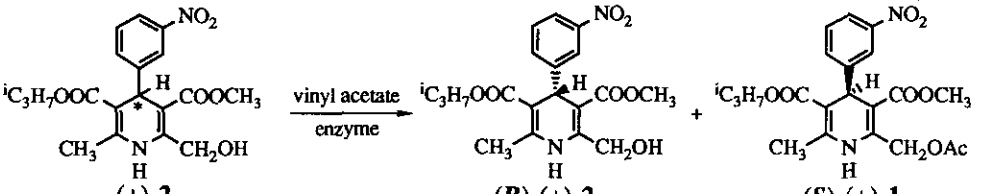
Table 1. Enzyme-catalyzed Optical Resolution of Racemic Isopropyl 2-Acetoxymethyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3-methoxycarbonyl-5-pyridinecarboxylate[a]



				(*R*)-(-)-1		(*S*)-(-)-2	
Entry	Enzyme	Solvent	Time(h)	C.Y(%)[b,c]	O.Y.(% ee)[d]	C.Y(%)[b,c]	O.Y.(% ee)[d]
1	lipase PS	IPE	96	41	74[e]	36	81[f]
2	CHE	IPE	24	32	39	50	25

a All reactions were carried out by stirring a mixture of substrate(1 mmol), enzyme(200 mg), and IPE(10 ml) saturated with H_2O containing 10% acetone. *b* Isolated yields. *c* Satisfactory elemental analyses of all products were obtained. *d* Optical yields were determined by hplc analysis using a column packed with Chiralcel AS (IPA/hexane) after conversion to **1**. *e* $[\alpha]_D$ -9.8°(*c* 1.8, acetone). *f* $[\alpha]_D$ -2.2°(*c* 1.4, acetone).

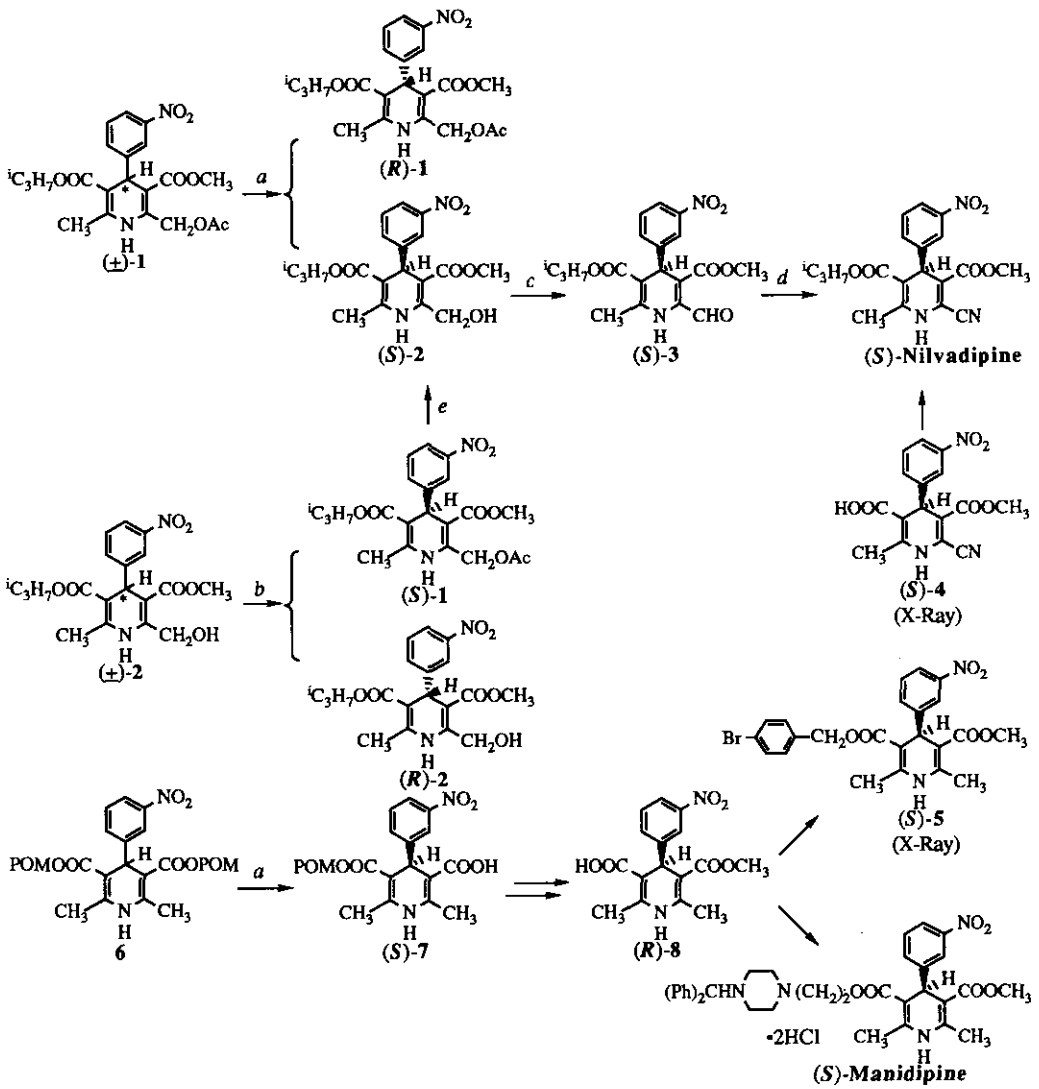
Table 2. Enzyme-catalyzed Optical Resolution of Racemic Isopropyl 2-Hydroxymethyl-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3-methoxycarbonyl-5-pyridinecarboxylate[a]



				(*R*)-(+)-2		(*S*)-(+)-1	
Entry	Enzyme	Solvent	Time(h)	C.Y(%)[b,c]	O.Y.(% ee)[d]	C.Y(%)[b,c]	O.Y.(% ee)[d]
1	lipase AH	Acetone	33	49	68	46	90[f]
2	CHE	Acetone	19	49	29	41	35
3	lipase PS	Acetone	41	46	78	40	89
4	lipase PS	DMF	136	52	40	26	46
5	lipase PS	THF	71	46	78	44	89
6	lipase PS	Acetone[g]	136	40	76	39	89
7	lipase PS	Acetone[g]	64	40	68	38	82
8	lipase PS	Acetone[h]	44	42	97[e]	55	72

a All reactions were carried out by stirring a mixture of substrate(1 mmol), enzyme(200 mg),vinyl acetate (25 ml), and solvent(10 ml). *b* Isolated yields. *c* Satisfactory elemental analyses of all products were obtained. *d* Optical yields were determined by hplc analysis using a column packed with Chiralcel AS(IPA/hexane) after conversion to **1**. *e* $[\alpha]_D$ +3.0°(*c* 1.6, acetone). *f* $[\alpha]_D$ +12.6°(*c* 1.8, acetone). *g* at 0°C. *h* at 40°C.

Enzyme-catalyzed Synthesis of Biologically Active (*S*)-Nilvadipine



Reagents; *a* lipase/H_2O/IPE *b* lipase/vinyl acetate *c* 1) $(COCl)_2$/DMSO/CH_2Cl_2,-78°C 2) $(C_2H_5)_3N$ *d* 1) NH_2OH•HCl/AcOH 2) Ac_2O *e* NH_3/CH_3OH

Scheme 2

The preliminary screening tests of various enzymes revealed that lipase PS[9] (from *Pseudomonas cepacia*) and CHE[9] (cholesterol esterase) were effective for hydrolysis of **1**,[10] and transesterification of **2**.[11] The enzymatic hydrolysis of **1** was carried out by stirring a mixture of **1** and a crude enzyme in diisopropyl ether (IPE) saturated with water containing 10% acetone, and the transesterification of **2** was carried out in vinyl acetate containing 40% solvent shown in Table 2. Table 1 shows the results of hydrolysis of **1**. The reaction with lipase PS proceeded to give (***R***)-**1**[12] in 74% ee and (*S*)-**2**[12] in 81% ee, respectively. But the reaction rate was very slow. The successive tranesterification of **2** was run with vinyl acetate. This transesterification proceeded more faster

than the hydrolysis to give (*R*)-**2**[13] and (*S*)-**1**[13] in high optical yields (Table 2). The absolute configuration of **1** and **2** was assigned after conversion to (*S*)-nilvadipine[14] whose absolute configuration was already determined by X-ray crystallographic analysis of (*S*)-**4**.[15] In previous paper, we assigned the absolute configuration of (*S*)-**7** by conversion to (*R*)-**8** whose absolute configuration was already determined by X-ray crystallographic analysis of (*S*)-**5**.[4] These results clearly indicate that enzyme-catalyzed hydrolyses of **1** and **6** were undergone at the same side of the dihydropyridine ring.

REFERENCES AND NOTES

1. Many enzymatic reactions have been applied to enantioselective synthesis. Recent reviews, see: J. B. Jones, *Tetrahedron* 1986, **42**, 3403. M. Ohno, and M. Otsuka, *Organic Reactions*, Wiley: New York, 1989, **37**, pp. 1-56. Z. Xie, *Tetrahedron: Asymmetry*, 1991, **2**, 733.
2. Recent review, see: S. Goldmann, and J. Stoltefuss, *Angew. Chem., Int. Ed. Eng.*, 1991, **30**, 1559.
3. T. Shibanura, M. Iwatani, K. Takenaka, and M. Murakami, *Chem. Pharm. Bull.*, 1980, **28**, 2809.
4. M. Kajino, T. Wada, Y. Nagai, A. Nagaoka, and K. Meguro, *Chem. Pharm. Bull.*, 1989, **37**, 2225.
5. H. Ebiike, Y. Terao, and K. Achiwa, *Tetrahedron Lett.*, 1991, **32**, 5808.
6. H. Ebiike, K. Maruyama, and K. Achiwa, *Chem. Pharm. Bull.*, 1992, **40**, 1083.
7. H. Ebiike, K. Maruyama, and K. Achiwa, *Tetrahedron: Asymmetry*, 1992, **3**, 1157.
8. Y. Satoh, K. Okumura, and Y. Shiokawa, *Chem. Pharm. Bull.*, 1992, **40**, 1799.
9. Lipase AH,lipase PS, and CHE are products of Amano Pharmaceutical Co., Ltd.
10. **1**: mp 95-97°C, ^{1}H-nmr ($CDCl_3$) δ: 1.11 (3H, d, J=6.4 Hz, $\underline{CH_3}CH<$), 1.27 (H, d, J=6.4 Hz, $\underline{CH_3}CH<$), 2.21 (3H, s, CH_3), 2.38 (3H, s, CH_3CO), 3.67 (3H, s, OCH_3), 4.92-5.01 (1H, m, >CHO-), 5.10 (1H, s, >CH-), 5.34 (2H, ABq, J=14.9 Hz, CH_AH_BO), 6.71 (1H, s, NH), 7.36-8.12 (4H, m, C_6H_4).
11. **2**: mp 161-166°C, ^{1}H-nmr ($CDCl_3$) δ: 1.10 (3H, d, J=6.4 Hz, $\underline{CH_3}CH<$), 1.26 (3H, d, J=5.9 Hz, $\underline{CH_3}CH<$), 2.39 (3H, s, CH_3), 3.63 (3H, s, OCH_3), 4.91-5.00 (1H, m, >CHO-), 5.08 (1H, s, NH), 7.24-8.12 (4H, m, C_6H_4).
13. (*R*)-**1**: yellow oil, $[\alpha]_D$ -9.8° (*c* 1.8, acetone). (*S*)-**2**: yellow oil, $[\alpha]_D$ -2.2° (*c* 1.4, acetone).
14. (*S*)-Nilvadipine: $[\alpha]_D$ +218.0° (*c* 0.9, methanol), mp 120-121°C, ^{1}H-nmr ($CDCl_3$) δ: 1.09 (3H, d, J=5.9 Hz, $\underline{CH_3}CH<$), 1.26 (3H, d, J=6.3 Hz, $\underline{CH_3}CH<$), 2.42 (3H, s, CH_3), 3.78 (3H, s, OCH_3), 4.91-5.01 (1H, m, >CHO-), 5.18 (1H, s, >CH-), 6.55 (1H, s, NH), 7.42-8.12 (4H, m, C_6H_4). Ir (nujol) : 3300cm^{-1} (NH), 2234cm^{-1} (CN). [lit.,[15] mp 120-122°C, $[\alpha]_D$ +222.4° (*c* 1.0, methanol).]
15. Y. Sato, *Farumashia*, 1989, **25**, 343.

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