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Optically-active Dihydropyridines via Lipase-catalyzed Enantioselective Hydrolysis

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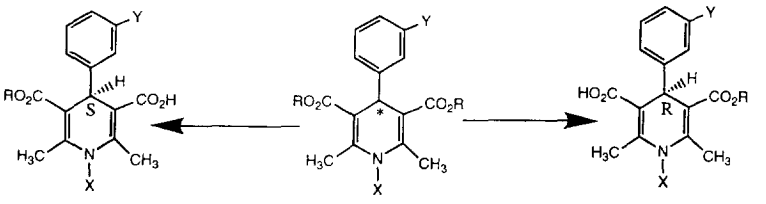
Abstract: Several prochiral esters of 1,4-dihydropyridines were enantioselectively hydrolyzed by *Pseudomonas* lipases (AK, P-30, and K-10) and *Candida cylindracea* lipase (OF-360). The stereochemical preferences of the lipases P-30 and K-10 were found to be always 4-Pro R and that of OF-360 to be 4-Pro S. In contrast, the prochiral preference of the lipase AK varied depending on the substitution on the dihydropyridine ring. The N-methoxymethyl derivatives afforded the 4S isomers (95% ee) whereas the N-unsubstituted compounds yielded the 4R isomers (50-70% ee).

Calcium antagonists of the type 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylates are important peripheral vasodilators and are commonly used for the treatment of cerebro-circulatory disorders and hypertension.[1] In many cases, the enantiomers have been shown to have different pharmacological properties. [2,3] Optically-active 1,4-dihydropyridines have been prepared by conventional chemical resolution of racemates or via chiral column chromatographic separation of the antipodes.[3]

In 1991, our laboratory reported the first biocatalytic synthesis of optically-active 1,4-dihydropyridines by taking advantage of the enantioselective properties of the microbial lipases.[4] Although initial attempts to enzymatically cleave the esters (methyl, ethyl, and allyl) at the C-3 and C-5 positions of the dihydropyridine ring were unsuccessful, highly enantiotopically-selective lipase-catalyzed hydrolyses were achieved using the sterically unhindered prochiral acetoxymethyl ester, **1**, to give either monoester **1a** or **1b**. The stereopreferences of the *Pseudomonas* lipases (AK, P-30, and K-10) were found to be opposite to that of *Candida cylindracea* (OF-360).

In continuing our studies on the lipase-catalyzed enantiotopically-selective hydrolysis of dihydropyridine esters, we noted that the prochiral preference of the *Pseudomonas* lipase AK may be altered by different substituents on the nitrogen of the dihydropyridine nucleus. The experimental details of this interesting observation are reported herein.

We had previously shown that the *Pseudomonas* lipases AK, P-30, and K-10 all have the same pro-R stereopreference towards **1**, whereas *Candida* OF-360 lipase preferred the pro-S chirality.[4] To further examine the stereochemical behavior of these lipases toward other substituted 4-aryl-1,4-dihydropyridines-3,5-dicarboxylates, compounds **2-7** were synthesized by known methods.[4-6] The experimental conditions were



b **a**

1 X = CH_2Ph; Y = NO_2
2 X = CH_2OCH_3; Y = H
3 X = Y = H

R = $-CH_2OCOCH_3$

4 X = CH_2OCH_3; Y = NO_2
5 X = H; Y = NO_2
6 X = CH_2Ph; Y = H
7 X = CH_2Ph; Y = NO_2

similar to those previously described using tert-butyl methyl ether as solvent and methanol was used as the nucleophile for the *Pseudomonas* lipases and water for the *Candida* lipase.[7] The results in Table 1 clearly showed that while the stereochemical preferences of the lipases P-30 and K-10 (pro-R) and OF-360 (pro-S) for substrates **2** and **3** were retained, a dramatic reversal of stereochemical preference was observed with lipase AK in the methanolysis of the N-protected **2** (65% yield, 96% ee, pro-S) and the N-unprotected **3** (67% yield, 72% ee, pro-R).

To determine the generality of this substitution effect, the 4-(3-nitrophenyl) derivatives, **4** and **5**, were prepared. Again, Lipase AK converted the N-methoxymethyl derivative, **4**, into the S-enantiomer, **4b**[8], in 95% ee and the unsubstituted compound, **5**, into the R-enantiomer, **5a**, in 54% ee. The stereochemical behaviors of lipases P-30 and K-10 (pro-R) towards **4** and **5** were similar but were markedly different for lipase OF-360 (pro-S). The methoxymethyl derivative, **4**, was transformed by OF-360 into the S-enantiomer in 90% ee but with the corresponding unsubstituted compound, **5**, the product was virtually racemic.

We then examined the effect of other N-substituents and the N-benzylated derivatives, **6** and **7**, were prepared and exposed to the lipases. Low yields and poor enantioselectivity were obtained for both substrates with lipase AK. However, hydrolysis of **7** afforded **7a** with optical yields of over 85% with lipases P-30 and K-10 whereas OF-360 gave **7b** in 83% ee. For compound **6**, it was surprising to find that all the *Pseudomonas* lipases gave R-monoesters of poor optical purity.

We have shown that the stereopreference of lipase AK for the methanolysis of 4-aryl-1,4-dihydropyridine diesters is markedly influenced by substituents on the ring nitrogen atom. The methoxymethyl group caused a dramatic change in the stereopreference of enzyme-catalyzed solvolysis as compared to the unsubstituted compounds. This reversal of chiral preference was not observed for lipases P-30 and K-10 which always retained the pro-R stereoselectivity. It is worthy of note that a dramatic change in the stereochemical preference of hydrolysis of dihydropyridine diesters catalyzed by lipase AH (*Pseudomonas sp.*) was achieved by simply altering the reaction media. In water saturated diisopropyl ether, the S-monoesters were obtained, whereas in moist cyclohexane the R-monoesters were preferentially formed.[8,9]

Table 1. Reaction of 4-aryl-1,4-dihydropyridine diesters with lipases.

Substrate	Lipase	Nucleophile	Temp (°C)	Time (h)	% Monoester (ee)	Configuration[8]
2	AK	MeOH	22	15	65 (96)	S
2	AK	MeOH	50	5	63 (87)	S
2	P-30	MeOH	50	8	71 (94)	R
2	K-10	MeOH	50	23	75 (95)	R
2	OF-360	H_2O	50	12	50 (61)	S
3	AK	MeOH	50	65	67 (72)	R
3	P-30	MeOH	50	17	82 (96)	R
3	K-10	MeOH	50	63	91 (99)	R
3	OF-360	H_2O	50	24	45 (53)	S
4	AK	MeOH	22	12	63 (95)	S
4	AK	MeOH	50	4	26 (95)	S
4	P-30	MeOH	50	7	60 (85)	R
4	K-10	MeOH	50	14	68 (95)	R
4	OF-360	H_2O	50	14	40 (90)	S
5	AK	MeOH	50	41	69 (54)	R
5	P-30	MeOH	50	12	77 (97)	R
5	K-10	MeOH	50	40	88 (98)	R
5	OF-360	H_2O	50	15	50 (8)	S
6	AK	MeOH	22	48	49 (15)	R
6	P-30	MeOH	50	24	69 (35)	R
6	K-10	MeOH	50	87	62 (24)	R
6	OF-360	H_2O	50	17	42 (59)	S
7	AK	MeOH	22	43	20 (8)	S
7	P-30	MeOH	50	12	44 (86)	R
7	K-10	MeOH	50	48	72 (88)	R
7	OF-360	H_2O	50	24	53 (83)	S

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7. The *Pseudomonas* lipases (P-30, AK, and K-10) were products of Amano International Enzyme Co. and *Candida cylindracea* lipase (OF-360) was purchased from the Meito Sangyo Co. The reaction mixture contained: 50 mg of the respective 1,4-dihydropyridine diester and 50 mg of lipase, suspended in 7.5 mg of *t*-Bu-OMe and a suitable nucleophile (5 eq. of MeOH or 20 eq. of H_2O). The mixture was stirred vigorously at the indicated temperature, and the progress of the reaction was monitored on TLC using CH_2Cl_2-EtOAc (1:1) as the solvent system. To facilitate the analysis of the products, the chiral monoesters were purified by flash-chromatography on silica gel (40 μM) and were methylated with diazomethane. The enantiomeric purity of the resulting mixed ester was determined by ^1H NMR in the presence of Eu(hfc)$_3$.
8. The absolute configuration of **4b** ($[\alpha]_D^{20}$ -45.7 (c, 1.0, $CHCl_2$)) obtained from the lipase AK reaction was methylated with diazomethane in ethyl ether. The mixed ester was stirred with K_2CO_3 in methanol for 2h at 23 °C and acidified with 3N HCl solution. The resulting monomethyl ester was identified as the known (4R)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridine carboxylic acid, $[\alpha]_D^{20}$ -18.3 (c, 0.75, acetone), lit.[2e] $[\alpha]_D^{23}$ -18.8 (c, 0.825, acetone). Hence, **4b** has the S configuration. Similarly **5a**, $[\alpha]_D^{20}$ -33.2 (c, 0.98 acetone), derived from the lipase K-10 hydrolysis of **5** was transformed into (4S)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridine carboxylic acid, $[\alpha]_D^{20}$ +23.4 (c, 0.76, acetone). Reported[2e] $[\alpha]_D^{23}$ +19.8 (c, 0.615, acetone) using a similar sequence of reactions. Consequently, **5a** has the R configuration.
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