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PROTEASE-CATALYZED ENANTIOSELECTIVE SYNTHESIS OF OPTICALLY ACTIVE 1,4-DIHYDROPYRIDINES

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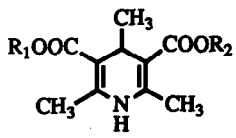
Abstract: The first enantioselective protease-catalyzed hydrolyses of 1,4-dihydropyridine-3,5-dicarboxyric diesters were developed. The monoesters obtained had high optical purity and were useful chiral building blocks which could easily lead to optically active Ca-blockers.

4-Aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates were shown to be highly effective calcium antagonists about twenty years ago. Many derivatives have been investigated under clinical development worldwide and some of them have already employed for the treatment of cardiovascular diseases.[1] 4-Alkyl derivatives in a series of 1,4-dihydropyridines, PCA 4248 (**1a**) and PCA 4233 (**1b**), have been reported as the new types of PAF antagonists.[2] In the case of the analogs possessing an asymmetric carbon at the 4-position, the two enantiomers were reported to differ in their pharmacological effects. In most cases, the (*S*)-enantiomer is more active than the (*R*)-enantiomer.[3] Almost 1,4-dihydropyridines were purchased as racemates and the first optically active 1,4-dihydropyridine appeared on the market in 1992. Optically active 1,4-dihydropyridines have been prepared by chemical resolution of racemates, chromatographic separation and enzymatic hydrolyses.

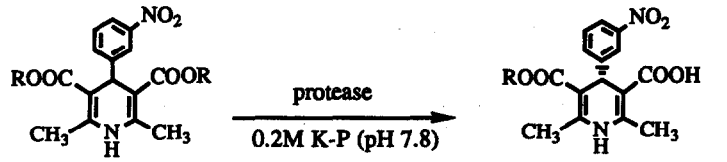
Previously, Sih *et al* and our group independently reported lipase-catalyzed enantiomeric hydrolyses of 1,4-dihydropyridines induced acyloxymethyl groups at 3,5-positions.[4] This idea provided a useful method for the compounds with ester groups which lipases can't hydrolyze on account of their steric hyndrance or inactivity, since it was reported that dimethyl or diethyl 4-aryl-2,6-dimethyl-3,5-pyridine dicarboxylates were hardly hydrolyzed in an alkali or acid solution,[5] and also inert to enzymatic hydrolysis.[6] We have investigated and found that the prochiral 1,4-dihydropyridines with new activated ester groups which can be easily removed with base or acid and susceptible to enzymatic hydrolysis. First of all, we examined bis(cyanoethyl) 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate (**2a**) and bis(methylsulfoneethyl) dicarboxylate (**2b**)as substrates for the screening of enzymes because both cyanoethyl group and methylsulfoneethyl group can be easily removed in an alkali solution. The enzymatic reaction was carried out with various kinds of lipases, esterases, proteases and others in 0.2M phosphate buffer (pH 7.8). No lipases and esterases hydrolyzed these compounds but the enzymatic hydrolyses were observed in experiments using some proteases and others, for instance, seaprose S(*Aspergillus melleus*), protease A(*Aspergillus oryzae*), proleather(*Bacillus subtilis*), deamizyme(*Aspergillus sp.*) and acylase(*Aspergillus sp.*).[7]



1a-d

1a : R_1=CH_3, R_2=CH_2CH_2SPh
1b : R_1=C_2H_5, R_2=CH_2CH_2SPh
1c : R_1=R_2=CH_2CH_2CN
1d : R_1=R_2=$CH_2CH_2SO_2CH_3$

The best result was obtained by using seaprose S.[8] The reaction rate was not so fast, but this enzyme afforded the hydrolyzed products in good yields and enantiomerically pure form. These results were summarized in Table 1. The absolute configurations of the monocarboxylic acids (**3a-b**) were assigned to be (*R*)-form by the optical rotation of compound **5** derived from **3a-b** and the optical purities were more than 99%. Compounds **3c-e** thus obtained seemed to have the same absolute configuration as (*S*)-**3a-b**.[9]



2a-e

2a: R=CH_2CH_2CN
2b: R=$CH_2CH_2SO_2CH_3$
2c: R=CH_2CONH_2
2d: R=$CH_2COOC_2H_5$
2e: R=CH_2OCH_3

3a-e

3a: R=CH_2CH_2CN
3b: R=$CH_2CH_2SO_2CH_3$
3c: R=CH_2CONH_2
3d: R=$CH_2COOC_2H_5$
3e: R=CH_2OCH_3

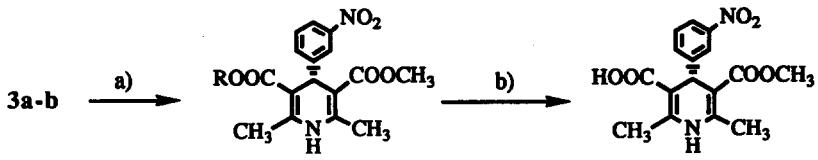
Scheme 1

Table 1 Seaprose S - Catalyzed Enantioselective Hydrolyses [a]

			Product (3a-e)			
Entry	Substrate	Time (hr)	No.	Yield (%)[b]	ee (%)[c]	$[\alpha]_D^{20}$(deg.)[d]
1	**2a**	72	**3a**	87	>99	-44.0
2	**2b**	72	**3b**	50	>99	-60.9
3	**2c**	20	**3c**	83	>99	-61.9
4	**2d**	72	**3d**	60	>99	-55.8
5	**2e**	72	**3e**	54	98	-35.0

a.) All reactions were carried out by stirring a mixture of a substrate (200mg) in 1ml of dimethylsulfoxide, 1.0g of seaprose S and 50ml of 0.2M phosphate buffer (pH7.8) at room temperature. b.) Isolated yield. c.) (Entries 1 and 2) The optical yields were determined by HPLC on a Chiralcel OJ (Daicel, Japan) column (EtOH/hexane) after conversion to the benzyl esters of **5** (Scheme 2). (Entry 3) The optical yield was determined as above method after transesterification to the methyl ester ((*R*)-**5**) (Scheme 3). (Entries 4 and 5) The optical yields were determined by HPLC on a Chiralcel AS (Daicel, Japan) column (EtOH/hexane) after conversion to the methyl esters. d.) (Entries 1-2 and 4-5) c=0.4 - 0.8, acetone. (Entry 3) c=0.473, EtOH.

This is a first report on the enzymatic hydrolysis of carboxylates at 3,5-positions of 1,4-dihydropyridines. The methyl ester (**5**) can be also an important intermediate for commercial products, such as manidipine, nitrendipine and valnidipine. It is reported that (*R*)-**5** is more effective than (*S*)-**5** for optically active 1,4-dihydropyridines.[3] Unfortunatelly, the methyl ester (**5**) derived from **3a-b** was (*S*)-form (Scheme 2).



4a-b **(*S*)-5**

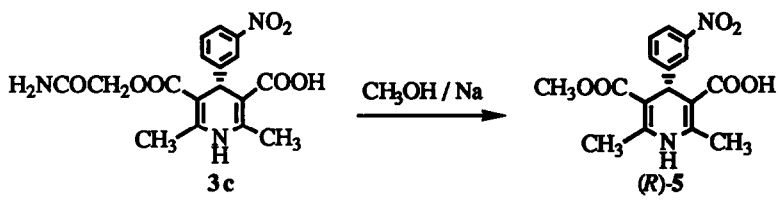
4a: R=CH_2CH_2CN $[\alpha]_D$ -32.6° (c=0.825, acetone) $[\alpha]_D$ +21.9° (c=0.845, acetone)

4b: R=$CH_2CH_2SO_2CH_3$ $[\alpha]_D$ -64.0° (c=0.83, acetone) (Lit.[3] $[\alpha]_D$ +19.1° (c=0.556, acetone))

a) CH_2N_2 / CH_2Cl_2 b) 1N-NaOH / acetone

Scheme 2

After much experimentation on direct conversion of (*R*)-**3** to (*R*)-**5**, we found that carbamoylmethyl group was a good group for this conversion.[10] The enzymatic reaction of bis(carbamoylmethyl) 1,4-dihydro-2,6-dimethyl 4-(3-nitrophenyl)-3,5-pyridine dicarboxylate (**2c**) was carried out with seaprose S to afford the (*R*)-monocarboxylic acid ((*R*)-**3c**) in a good yield and this half ester ((*R*)-**3c**) was transesterificated in the presence of sodium methoxide to give the methyl ester ((*R*)-**5**) in a moderate yield without racemization. The desired compound ((*R*)-**5**), which has the appropriate stereochemistry for the synthesis of optically active 1,4-dihydropyridines, was obtained in 54% yield based on **2c**.



3c **(*R*)-5**

$[\alpha]_D$ -19.5° (c=1.0, acetone)

(Lit.[3] $[\alpha]_D$ -19.6° (c=0.542, acetone))

Scheme 3

We also carried out the enzymatic hydrolyses of 4-methyl-1,4-dihydropyridines (**1c-d**) with seaprose S, however, the reactions were unsuccessful. These results suggested that the aryl group at 4 position was necessary for seaprose S-catalyzed hydrolysis.

In summary, it has been found that protease-catalyzed hydrolyses of bis(cyanoethyl) and bis(carbamoylmethyl) 1,4-dihydropyridine dicarboxylates were carried out in good yields. 1,4-Dihydropyridines are kinds of amino acids and were considered as good substrates for the proteases.

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7. These enzymes are products of Amano Pharmaceutical Co., Ltd.
8. No reaction was observed in hydrolysis of bis(pivaloyloxymethyl) 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate[4] using seaprose S.
9. The absolute configurations of **3d** and **3e** were assigned to be (*R*)-form by comparing the optical rotations of the methyl esters of **3d** and **3e** with those of the corresponding esters from (*S*)-**5**.
   The methyl ester of **3d**; $[\alpha]_D$= -34.8° (c=1.0, acetone), (from (*S*)-**5** $[\alpha]_D$= -32.1° (c=1.0, acetone)).
   The methyl ester of **3e**; $[\alpha]_D$= -15.5° (c=0.8, acetone), (from (*S*)-**5** $[\alpha]_D$= -16.1° (c=0.8, acetone)).
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