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# Acyloxymethyl as an Activating Group in Lipase-Catalyzed Enantioselective Hydrolysis. A Versatile Approach to Chiral 4-Aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates.[1]

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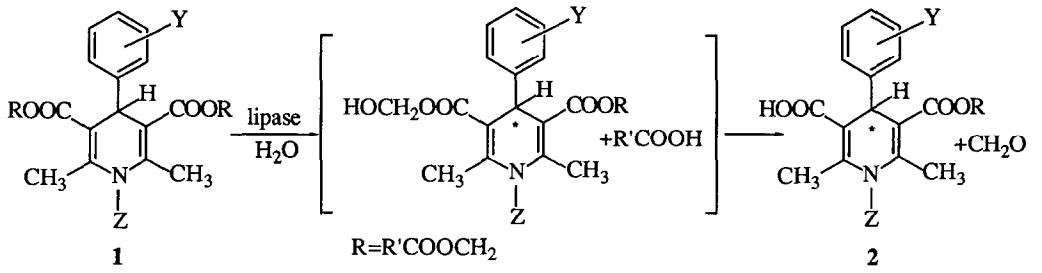
*Keywords*: 1,4-Dihydropyridine; Lipase; Enantioselective hydrolysis; Acyloxymethylester; Calcium antagonist.

***Abstract:*** The first practical syntheses of chiral 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates, which are attractive compounds as new calcium antagonists, were realized by lipase-catalyzed enantioselective hydrolysis of the acyloxymethyl esters. The monoesters obtained were revealed to have high optical purity and demonstrated to be useful chiral synthons.

Since 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates were found to be highly effective calcium antagonist about twenty years ago,[2] their derivatives have been widely investigated from the pharmacological points of view and some of them have already employed therapeutically. In the case of the analogs possessing an asymmetric carbon at the 4-position, the synthesis of optically active compounds became an important theme in synthetic chemistry because the two enantiomers were reported to show much different biological activities.[3] We focused on the enzymatic asymmetric synthesis as a practical method[4]. Our failure of preliminary enzymatic hydrolysis of the dimethyl and diphenyl esters (**1** Z=$CH_2OCH_3$, R=methyl or phenyl) suggested that lipases are not suited to hydrolysis of the esters of such weak and sterically hindered carboxylic acids as 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acids. Now, we have cleared such difficulty by designing the bis(acyloxymethyl) ester[5] and provided an efficient method for lipase-catalyzed asymmetric synthesis of chiral 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate.

Our idea is the induction of an acyloxymethyl group which has been known as a protecting group for OH or NH and hydrolyzed easily with acid or base. One of the two acyloxymethyl groups in **1** has been found to be hydrolyzed enantioselectively with lipase to give optically active monoesters **2** as shown below.

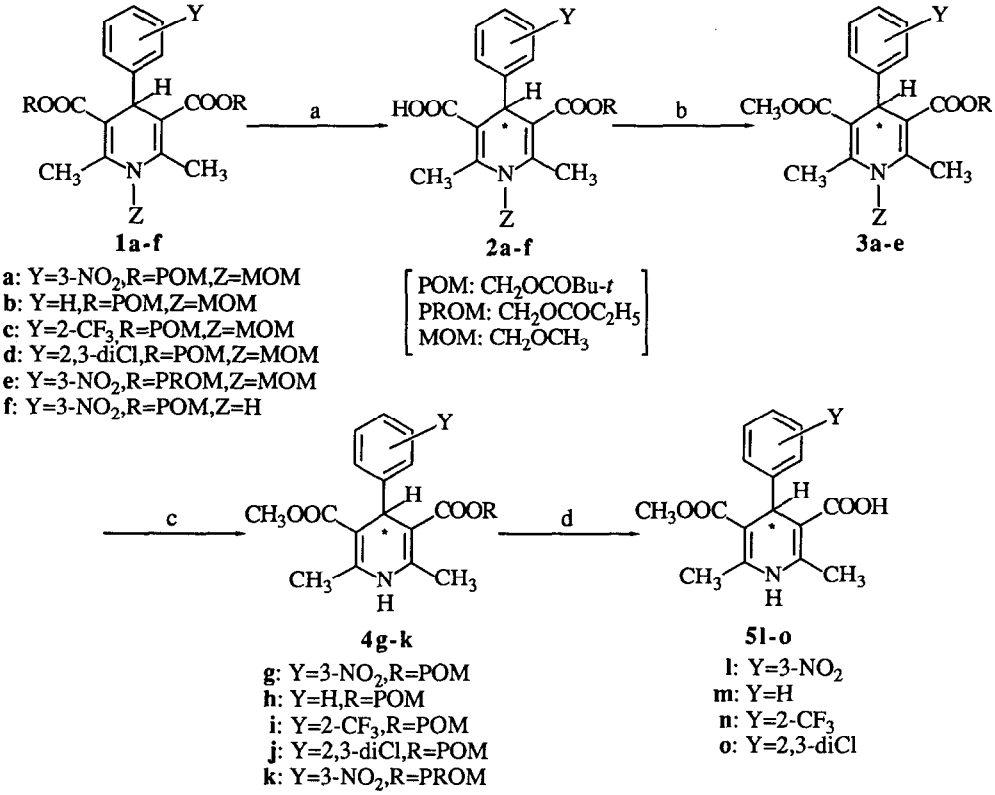



Using bis(pivaloyloxymethyl) 1,4-dihydro-2,6-dimethyl-1-(methoxymethyl)-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**1a**), the preliminary screening tests on various lipases revealed that lipase B (from *Pseudomonas fragi*) and lipase P (from *Pseudomonas fluorescens*)[6] suited well for the hydrolysis. First, the reaction was carried out in a buffer solution: A mixture of **1a** and lipase B in phosphate buffer (pH=8) containing 10% acetone was stirred for 24 hours at 25°C. Although the optical purity of the monoester (**2a**) obtained was extremely high (>99%ee),

the slow reaction rate and a serious extraction procedure seemed not to be practical. To eliminate these defects, we carried out the reaction in diisopropyl ether (IPE) saturated with water.[7] The reaction proceeded more smoothly (4 hours) and almost optically pure (+)-**2a** was obtained in a high yield (95%) (entry 1 in Table 1). The isolation procedure was so convenient that only concentration of the filtrate after removal of the enzyme by usual filtration gave the product with a very slight contaminant. The absolute configulation of (+)-**2a** was determined to be (*S*)-form by conversion to (-)-**5l** which showed the same optical rotation as that of (*R*)-(-)-**5l** reported.[3] To the best of our knowledge, there was no report that an enzyme controls exactly the stereochemistry at the reaction site fairly remote from the chiral center (7 atoms) such as this reaction.

In order to confirm the utility, phenyl (**1b**), 2-trifluoromethylphenyl (**1c**) 2,3-dichlorophenyl (**1d**) derivatives were allowed to react under the same conditions to give similar good results (entries 3, 4, and 5).

Although the reaction with lipase P proceeded very slowly, it is of interest that the lipase showed the opposite enantioselectivity resulting in formation of (*R*)-(-)-monoester [(-)-**2a**] (entry 6). In the case of the bis(propionyloxymethyl) ester (**1e**), the reaction with lipase B was carried out at 0-5°C (entry 7) because the second hydrolysis leading to the formation of dicarboxylic acid (**1** Y=$NO_2$, Z=$CH_2OCH_3$, R=H) was too fast at 25°C. On the other hand, lipase P seems to suit better for the reaction of the propionyloxymethyl ester (entry 8).




(a)lipase,$H_2O$/IPE (b)$CH_2N_2$/$CH_2Cl_2$ (c)dilHCl/acetone (d)KOH/MeOH

**Scheme 1**

These optically active monoesters serve as useful chiral synthons in the production of both enantiomers of 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates. We demonstrated that (+)-mono(pivaloyloxymethyl) esters [(+)-**2a**, (+)-**2b**, (+)-**2c**, and (+)-**2d**][8] were converted to the corresponding methyl esters [(-)-**5l**, (-)-**5m**, (-)-**5n**, and (+)-**5o**][9] through the intermediates **3** and **4** by treatment with diazomethane, hydrochloric acid and alkaline. They were able to lead to various non-symmetric diesters which have potent biological activities.[3]

Our works have opened up a new field of lipase-catalyzed enantioselective hydrolysis for syntheses of a variety of useful chiral molecules and provided a practical method for the syntheses of optically active 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylates.

**Table 1** Lipase-Catalyzed Enantioselective Hydrolysis[a]

| | | | | | Product | | | |
|---|---|---|---|---|---|---|---|---|
| Entry | Substrate | Enzyme | Solvent | Time,h | No. | C.Y.(%)[b,c] | O.Y.(%ee)[d] | $[\alpha]_D^{20}$ deg.[e] |
| 1 | **1a** | lipase B | buffer(pH8) | 24 | **2a** | 80 | >99 | +42.6 |
| 2 | **1a** | lipase B | $H_2O$/IPE | 4 | **2a** | 95 | >99 | +42.6 |
| 3 | **1b** | lipase B | $H_2O$/IPE | 5 | **2b** | 76 | >99 | +43.5 |
| 4 | **1c** | lipase B | $H_2O$/IPE | 7 | **2c** | 83 | >99 | +33.8 |
| 5 | **1d** | lipase B | $H_2O$/IPE | 6 | **2d** | 81 | 96 | +22.0 |
| 6 | **1a** | lipase P | $H_2O$/IPE | 72 | **2a** | 44 | 69 | -28.7 |
| 7 | **1e** | lipase B | $H_2O$/IPE | 8[f] | **2e** | 71 | >99 | +49.7 |
| 8 | **1e** | lipase P | $H_2O$/IPE | 10 | **2e** | 78 | 88 | -42.1 |
| 9 | **1f** | lipase B | $H_2O$/IPE | 35 | **2f** | 83 | 93 | +27.9 |

*a*. All reactions in an organic solvent were carried out by stirring a mixture of a substrate (1mmol), lipase (lipase B, 100 mg, 20,000 units or lipase P, 200 mg, 3600 units), and IPE (25 ml) saturated with water at 25°C. *b*. Isolated yield. *c*. Satisfactory elemental analyses of all products were obtained. *d*.The optical yields were determined by HPLC analyses using a column packed with Chiralcel OD (2-propanol/hexane) after conversion to the benzyl ester (**3**: R=$CH_2Ph$). *e*. acetone, *c*=1-2. *f*. at 0-5°C

## REFERENCES AND NOTES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4. Many enzymatic reactions have been applied to enantioselective syntheses. Recent reviews, see : Jones, J. B. *Tetrahedron* **1986**, *42*, 3403. Ohno, M.; Otsuka, M. *Organic Reactions*; Wiley: New York, **1989**, *37*, pp1-56.
5. Pivaloyloxymethyl esters (**1a-d**) were easily prepared by addition of chloromethyl pivalate to a mixture of the corresponding 4-aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acids and sodium hydride in dimethylformamide.
   **1a**: mp 73-74°C, $^1$H-NMR ($CDCl_3$) δ: 1.11 (18H, s, 6x$CH_3$), 2.54 (6H, s, 2x$CH_3$), 3.30 (3H, s, $OCH_3$), 4.82 (2H, s, $NCH_2O$), 5.18 (1H, s, >CH-), 5.82 (4H, ABq, J=5.5Hz, 2x$OCH_2O$), 7.32-8.00 (4H, m, $C_6H_4$).
   Propionyloxymethyl ester (**1e**) was also prepared by a similar method using chloromethyl propionate.
   **1e**: mp 86-87°C, $^1$H-NMR ($CDCl_3$) δ: 1.10 (6H, t, J=7.3Hz, 2x$CH_2C\underline{H}_3$), 2.31, 2.32 (4H, each q, J=7.3Hz, 2x$OC\underline{H}_2CH_3$), 2.55 (6H, s, 2x$CH_3$), 3.35 (3H, s, $OCH_3$), 4.82 (2H, s, $NCH_2O$), 5.14 (1H, s, >CH-), 5.80 (4H, ABq, J=5.6Hz, 2x$OCH_2O$), 7.33-8.01 (4H, m, $C_6H_4$).
   The hydrolysis with 1M hydrochloric acid of **1a** gave **1f.**
6. Lipase B was kindly supplied by Sapporo Breweries Ltd. (Nishio, T.; Chikano, T.; Kamimura, M. *Agric. Biol. Chem.* **1987**, *51*, 181). Lipase P was kindly supplied by Amano Pharmaceutical Co., Ltd.
7. Enzymatic reactions in organic media were mainly reported on lipase-catalyzed transesterification. For a recent review see: Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114.
8. **2a**: mp 84-85°C, $^1$H-NMR ($CDCl_3$) δ: 1.12 (9H, s, 3x$CH_3$), 2.55 (3H, s, $CH_3$), 2.57 (3H, s, $CH_3$), 3.33 (3H, s, $OCH_3$), 4.81 (2H, s, $NCH_2O$), 5.19 (1H, s, >CH-), 5.80 (2H, ABq, J=5.3Hz, $OCH_2O$), 7.33-8.00 (4H, m, $C_6H_4$).
9. **5l**: mp 187-188°C, $[\alpha]_D^{20}$ -19.5° (*c* 0.8, acetone), **5m**: mp 158-159°C, $[\alpha]_D^{20}$ -10.3° (*c* 0.7, acetone), **5n**: mp 109-110°C, $[\alpha]_D^{20}$ -24.4° (*c* 0.6, acetone), **5o**: mp 186-187°C, $[\alpha]_D^{20}$ +5.2°(*c* 0.7, acetone).


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