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The Enhancement of Enantioselectivities for Lipase-Catalyzed Reactions by Using Carbamates

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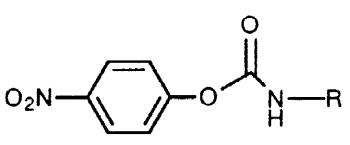
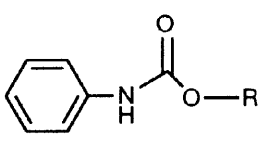
Abstract: The enantioselectivity of porcine pancreatic lipase-catalyzed resolution of 1-indanol was enhanced up to 3 fold in the presence of carbamates. The optimum incubation time for 4-nitrophenyl-*N*-hexyl carbamate and the enzyme was 18 h before this biocatalytic resolution. The optimum concentration of the inhibitor 4-nitrophenyl-*N*-hexyl carbamate in this resolution was 1 % mole equivalent of the substrate 1-indanol.

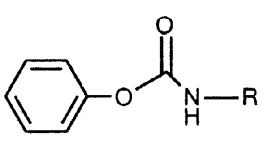
The recent development of enzyme catalysis in organic synthesis for kinetic resolutions of racemates has attracted the attention of organic chemists because of their synthetic utility.[1-8] The lipase-catalyzed acylations and transacylations have become popular methods in asymmetric synthesis.[3] We reported three methods based on azeotropic distillation,[9-11] ultrasonication,[12,13] and microwave irradiation[14] to increase the lipase-catalyzed kinetic resolution of alcohols in organic solvents. In 1930, Bamann and Laeverenz first observed that the enantioselectivity of the human liver esterase-catalyzed hydrolysis of (±)-methyl mandelate was prominently enhanced when the alkaloid strychine was added to the reaction mixture.[15] In the following year, Ammon and Fischgold also showed similar results.[16] In 1989, Guo and Sih observed that the enantioselectivity of *Candida cylindracea* lipase-catalyzed resolution of a variety of (±)-aryloxypropionic esters in the presence of either dextromethorphan or levomethorphan.[17] Recently, additives such as thiacrown ethers for enhancement of enantioselectivity have been reported.[18-21] However, a limited type of additives can be used in these biocatalytic resolutions. Therefore, we report here a method based on the addition of a more convenient achiral carbamate inhibitors (**1-8**)[22] to enhance the enantioselectivities for porcine pancreatic lipase (PPL) catalyzed resolution of 1-indanol (**9**). Indanols are used as substrates due to their high specific rotation values and their derivatives are



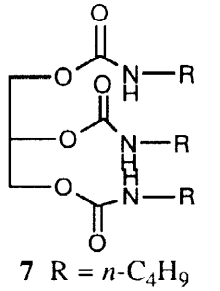
1 R = *n*-C_8H_{17}
2 R = *n*-C_6H_{13}
3 R = CH_2Ph



4 R = *n*-C_3H_7
5 R = *n*-C_4H_9



6 R = *n*-C_4H_9



7 R = *n*-C_4H_9
8 R = *n*-C_8H_{17}

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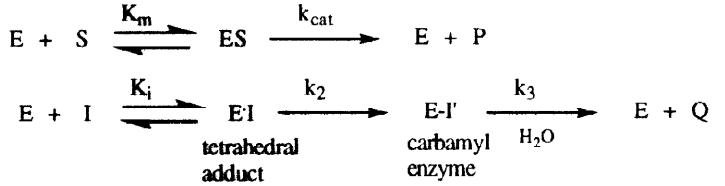
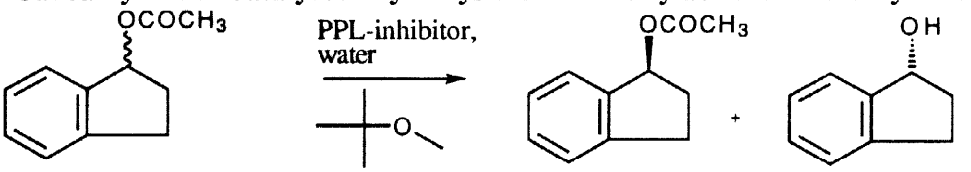
potent inhibitors for cholesterol esterase.[23] Indanols have been resolved enzymatically with moderate (E = 2.9)[24] to high (E = 45-265)[12-14] enantioselectivity for the *R* enantiomer.

In the presence of active site-directed irreversible inhibitors of a serine hydrolase such as cholesterol esterase (CEase, a homologue of PPL[25]) (Scheme I),[26-28] the modified PPL with carbamates **1-8** catalyzed the hydrolysis of *(R)*-1-indanylacetate (***R*-9**) in its racemates (*rac*-**9**) more stereoselectively than the wild type (Scheme II & Table 1).

Scheme I. Kinetic scheme for the active site-directed irreversible inhibitors of a serine hydrolase in the presence of substrate



Scheme II. Carbamyl PPL-Catalyzed Hydrolysis of 1-Indanylacetate in *t*-butyl methyl ether



rac-**9** 37°C *S*-**9** ***R*-10**

Table 1. PPL-catalyzed resolution of *rac*-**9** in the presence of carbamates **1-8**[22]

Inhibitor	K_i (μM)[a]	% ee (*R*-**10**)[b]	% ee (*S*-**9**)[b]	conv.[c]	E[d]
none	-	91	90	0.50	77
1	0.14±0.03	96	98	0.51	153
2	4.1±0.5	96	98	0.51	153
3	3.1±0.4	93	96	0.50	147
4	4.9±0.5	92	93	0.50	80
5	1.2±0.2	98	92	0.48	246
6	30±10	95	68	0.42	78
7	1.2±0.3	90	84	0.48	52
8	1.1±0.2	92	90	0.49	78

a. The inhibition constants of *pseudomona species* lipase (PSL, Sigma L9518) by carbamates **1-8** were obtained according to Hosie's procedures.[26] Carbamates **1-8** were all active site-directed irreversible inhibitors of PSL. b. % Ee was calculated by OP = %ee - (1- %ee). Optical purity of ***R*-10** was calculated by OP = $[\alpha]_{D\ exp.} / [\alpha]_{D\ lit.}$, where $[\alpha]_{D\ exp.}$ of ***R*-10** was measured from a polarimeter at 25°C, c 2.5, $CHCl_3$ and $[\alpha]_{D\ lit.}$ at 17 °C of ***R*-10** was -29 which was obtained from Aldrich Catalog 1997-1998. % Ee values of *S*-**9** was obtained from those of *S*-**10** and *S*-**10** was obtained from basic hydrolysis (0.1 N KOH, EtOH, 25 °C, 18 h, 97%) of the reactive ester *S*-**9**. c. The extent of conversion.[29] d. the enantiomeric ratio.[29]

Carbamates **1**, **2**, **3**, and **5** enhanced the enantioselectivity of this biocatalytic resolution; however, carbamates **4**, **6**, **7**, and **8** did not. The three-dimensional structure of *Candida rugosa* lipase (CRL, a homologue of PPL) was reported in 1994 and two inhibitor molecules of sulfonyl chloride were bound to the enzyme.[25] Therefore, the binding sites of lipase were relatively huge in size when compared to other serine hydrolases. Thus, the biocatalytic resolution of *rac*-**9** was enhanced by carbamates **1**, **2**, and **3** probably because these inhibitors covalently modified one of the binding sites of PPL to form the carbamyl enzyme (Scheme I). Although the carbamyl PPL decreased the size of its binding sites, the substrate was bound to the carbamyl enzyme more stereoselectively than to the wild type. When the carbamyl PPL was hydrolyzed to the wild type (k_3 step in

Scheme I), the substrate in the second binding site then entered the catalytic site immediately. Carbamate **5** was the best promoter for this resolution. When compared carbamates **5** and **6**, we found that alkyl *N*-phenyl-carbamate was the better additive than phenyl *N*-alkylcarbamate due to the fact that the former was more potent inhibitor than the latter. The fact that the enantioselectivity of the resolution was not enhanced by carbamate **6** may be explained by the weakest binding (the greatest K_i value) between the inhibitor and the enzyme. Similarly, carbamates **1-8** also promoted the enantioselectivity for the PPL-catalyzed resolution of *rac*-1-indanylbutyrate but less effectively. In the presence of carbamates **1-8**, PPL catalyzed acylation of *(R)*-1-indanol (*R*-**10**) from its racemates (*rac*-**9**) to produce *(R)*-1-indanylbutyrate (*R*-**11**) and recover *(S)*-1-indanol (*S*-**10**) with high stereoselectivity (Table 2). Both carbamates **1** and **2** enhanced about 2-fold the enantioselectivity for the lipase-catalyzed resolution of 1-indanol due to the relatively small binding sizes for the substrate in these carbamyl enzymes.

Table 2. PPL-catalyzed resolution of *rac*-**10** with vinyl butyrate in the presence of carbamates **1-7**[22,a]

Inhibitor	% ee (*R*-**11**)	% ee (*S*-**10**)	conv.	E
none	92	82	0.47	65
1	97	73	0.43	148
2	97	71	0.43	163
3	95	78	0.45	87
4	93	80	0.46	69
5	94	81	0.46	83
6	96	70	0.42	96
7	93	81	0.47	66
8	94	81	0.46	85

a. All conditions were similar to Table 1.

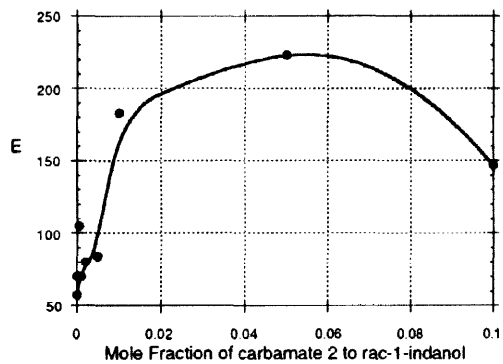


Figure 1. The plot of the enantioselectivity for PPL-catalyzed resolution of *rac*-1-indanol (scheme II) against the mole fraction of carbamate **2**.[22]

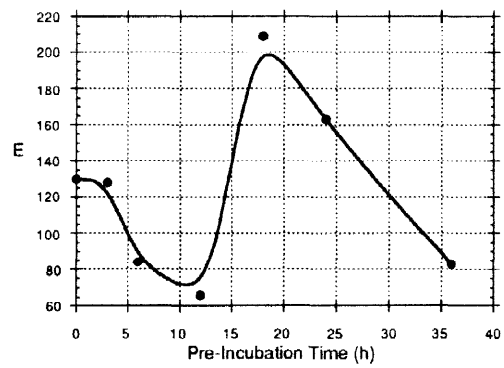


Figure 2. The plot of the enantioselectivity for PPL-catalyzed resolution of *rac*-1-indanol (scheme II) against the pre-incubation time of carbamate **2** in PPL before the reaction.[22]

We further monitored this reaction by varying both the concentration of the inhibitor and the pre-incubation time of carbamate **2** in PPL before the resolution (Figures 1 & 2). The enantioselectivity for *rac*-**10** reached a maximum when the mole fraction of carbamate **2** was 0.05 (Figure 1) because carbamate **2** was bound to PPL more tightly than *R*-**10**. The optimum concentration of the inhibitor for the resolution was 0.01 because the reaction rates for 0.05 were too slow. In the cases with carbamates **7** and **8**, we found that both compounds did not enhance the resolution at all by varying the mole fraction of carbamates to *rac*-**10** from 10^{-4} to 0.1 (data not shown). Therefore, carbamates **7** and **8** completely blocked the whole binding sites of the enzyme with their

two *N*-alkylcarbamate groups and therefore did not enhance the enantioselectivity of the resolution. The enantioselectivity of the reaction decreased at the beginning of the pre-incubation of the inhibitor in PPL but soon increased to a maximum at 18 h of pre-incubation and decreased again after that (Figure 2). The high enantioselectivity at the beginning of the pre-incubation might be due to the fact that part of the enzyme reacted with the inhibitor to form the tetrahedral adduct (Scheme I) which was more stereoselective for the substrate than the wild type. At 12 h of incubation, the enantioselectivity of the reaction was the lowest. This might be due to the fact that the departure of the leaving group in the tetrahedral adduct totally blocked the entry of the substrate. At 18 h of incubation, the highest enantioselectivity was observed. This might be because part of the enzyme was carbamylated by the inhibitor (E-I' in Scheme I) and the carbamyl enzyme was more stereoselective for the substrate than the wild type. At 36 h of incubation, the enantioselectivity of the resolution decreased again probably because the carbamyl enzyme was fully hydrolyzed to the wild type (Scheme I).

Carbamate **2** was also successfully used to enhance the PPL-catalyzed resolution of 1,2,3,4-tetrahydro-1-naphthol (E = 72 without carbamate **2**, E = 107 with carbamate **2**).

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22. General procedures: a) *Synthesis of Carbamates 1-8.* Carbamates **1-8** were prepared from the condensation of the corresponding alcohol with isocyanate in the presence of pyridine (70-90% yield). b) *PPL-catalyzed resolution of 1-indanylacetate in the presence of carbamates 1.* To a *t*-butyl methyl ether (15 mL) solution of carbamate **1** (37.6 μmol), PPL (4 g, Sigma L0382) was added and the resulting mixture was shaken at 37 °C for 24 h. To the above mixture, *rac*-**9** (3.76 mmol) and water (0.38 mol) were added and the reaction mixture was shaken at 37 °C for 24 h. The resulting mixtures were concentrated and separated by MPLC (hexane/ethyl acetate, 4/1, v/v). c) *Figure 1.* To a *t*-butyl methyl ether (20 mL) solution of varying concentration of carbamate **2**, PPL (4 g) was added. The resulting mixture was shaken at 37 °C for 24 h (pre-incubation). To this mixture, *rac*-**10** (3.76 mmol) and vinyl butyrate (4.5 mmol) were added. The reaction mixture was shaken for another 24 h at 37 °C. d) *Figure 2.* All procedures were similar to that of c) except the pre-incubation time.
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