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# Significant Effect of Acyl Groups on Enantioselectivity in Lipase-Catalyzed Transesterifications

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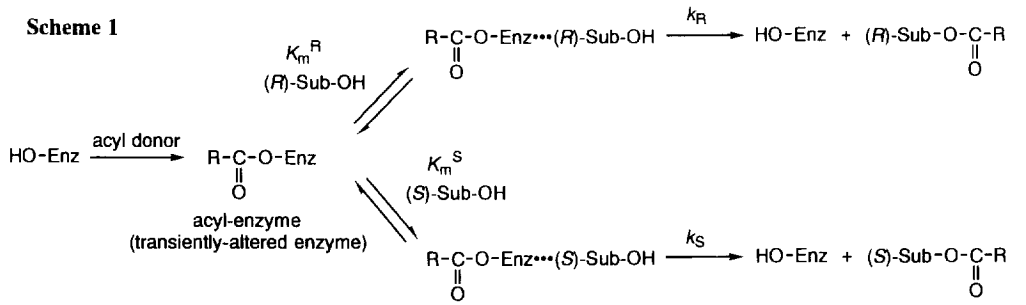
***Abstract:*** The effect of the acyl group of acylating agents on the enantioselectivity in the lipase-catalyzed transesterifications of racemic 2-[(*N*,*N*-dimethylcarbamoyl)methyl]-3-cyclopenten-1-ol in diisopropyl ether was found to be significant. The enantioselectivity was enhanced markedly by changing the acylating agent from vinyl acetate to vinyl butyrate, and dropped substantially with longer acyl donors. Other acyl donors were also examined.


The kinetic resolution of racemic compounds catalyzed by hydrolytic enzymes has received considerable attention in recent years.[1] Klibanov *et al.* have demonstrated that organic solvents have a marked effect on the stereoselectivity of enzymatic reactions.[2] The transesterification by immobilized enzymes in a wide range of organic solvents allows one to optimize the reaction conditions.[2,3] However, satisfactory improvement in the enantioselectivity is not necessarily attained by changing the solvent. Hence, other rational strategies to alter the thermodynamic stability of the enzyme–substrate complex and/or the transition state need to be pursued.

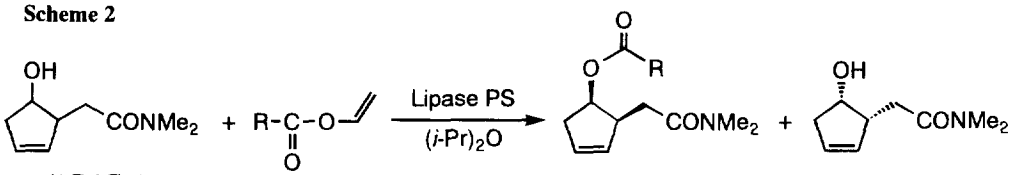
**Scheme 1**




By changing the acyl group of acylating agents, a hydrolytic enzyme is transformed into a variety of acyl-enzyme intermediates in an organic solvent.[4] Sih *et al.* have proposed that various acyl-enzyme intermediates thus produced *in situ*, whose active site structures differ from one another, can in principle exert various chiral discrimination abilities in the course of the enantioselective acylation of racemic alcohols.[1b] The simplified scheme is shown above, where R represents a variety of substituents (*vide infra*). Much about the potential of this method remains to be investigated,[1b,5] though this is an easier method to modify an enzyme (transiently *in situ*) compared with conventional chemical modification methods[6] or site-directed mutagenesis.[7]

Here we report the effect of the acyl group of acylating agents on the enantioselectivity in the lipase-catalyzed transesterifications of 2-[(*N*,*N*-dimethylcarbamoyl)methyl]-3-cyclopenten-1-ol (**1**)[8] shown in Scheme 2. The alcohol **1** and the ester **2** can be readily lactonized to 2-oxabicyclo[3.3.0]oct-6-en-3-one, a useful chiral synthon for prostaglandins.[8] Among various types of acyl donors, vinyl esters are considered to be the most suitable for investigating the effect of the acyl group of acylating agents because of their high reactivity and irreversibility.[9]

**Scheme 2**




(1*R*,2*S*)-**1**
(1*S*,2*R*)-**1**

(1*R*,2*S*)-**2**

(1*S*,2*R*)-**1**

We examined several vinyl esters with different alkyl chain lengths in the kinetic resolution of **1** using lipase PS (a lipase from *Pseudomonas cepacia*) in (*i*-Pr)$_2$O. The enantioselectivities (*E* values[10]) obtained are shown in Table 1. It is interesting to note that a slight elongation of the alkyl chain of the vinyl esters caused dramatic changes in the enantioselectivity. The enantioselectivity was successfully improved by changing the acylating agent from vinyl acetate (*E* = 30) to vinyl butyrate (*E* = 156), and dropped substantially with longer acyl donors. We employed vinyl butyrate in various organic solvents, and obtained higher *E* values (e.g., (1*S*,2*R*)-**1** 40% (97%ee), (1*R*,2*S*)-**2** 51% (96%ee), *E* = 207, in $Et_2O$).[11] These results encouraged us to investigate further other types of vinyl esters listed in Table 1. With vinyl chloroacetate, the reaction rate was found to be fast and the enantioselectivity was excellent, which can be ascribed, respectively, to the

**Table 1.** Lipase-Catalyzed Transesterification of **1** by Use of Various Acylating Agents[a]

| Acylating agent R | Time, h | Conversion[b] % | (1*S*,2*R*)-**1** % Yield[c] (% ee[d]) | (1*R*,2*S*)-**2** % Yield[c] (% ee[d]) | *E* |
|---|---|---|---|---|---|
| $CH_3$ | 3 | 50 | 46 (83) | 47 (84) | 30 |
| *n*-$C_2H_5$ | 3 | 42 | 48 (69) | 41 (95) | 81 |
| *n*-$C_3H_7$ | 3 | 45 | 51 (78) | 42 (97) | 156 |
| *n*-$C_5H_{11}$ | 3 | 51 | 47 (72) | 43 (70) | 12 |
| *n*-$C_7H_{15}$ | 2 | 45 | 40 (70) | 51 (86) | 28 |
| *n*-$C_9H_{19}$ | 1.5 | 49 | 42 (84) | 44 (89) | 45 |
| $ClCH_2$ | 0.5 | 51 | 43 (95) | 48 (92) | 89 |
| $CF_3$ | 0.75 | 40 | 45 (8) | 35 (12) | 1.4 |
| *tert*-$C_4H_9$ | 48 | 24 | 10[e] (27) | 19 (84) | 15 |
| $C_6H_5$ | 48 | 14 | 53[e] (15) | 19 (89) | 20 |
| $CH_3CH{=}CH$ | 48 | 7 | 16[e] (7) | 12 (92) | 26 |

[a] Conditions; lipase PS (400 mg), **1** (1.2 mmol), acylating agent (2.4 mmol), dry (*i*-Pr)$_2$O (10 mL), 30 ℃. [b] Calculated from 100xee(**1**)/(ee(**1**)+ee(**2**)) according to ref. 10. [c] Isolated yield. [d] Determined by capillary GC with Chrompack CP-Cyclodextrin-β-2,3,6-M-19, after **1** and **2** were lactonized. [e] Large amounts of lactone were obtained due to the prolonged reaction time. The lactone was not formed by the lipase-catalysis, but by the spontaneous lactonization.

electronegativity and to the size of the chlorine atom. Surprisingly, vinyl trifluoroacetate gave a very poor result. Some interaction characteristic of the trifluoromethyl group might operate to cancel the steric effect.[12] The very bulky acylating agent, vinyl pivalate, did acylate the lipase, although the reaction rate was very slow. The enantioselectivity was moderate in this particular case, but the steric effect of the pivaloyl group may be effective for other substrates not examined. The reactions using vinyl benzoate and vinyl crotonate were very slow probably because of the low reactivity of the ester due to the conjugation, and the prolonged reaction time led to the lactonization of **1**.[8]

The enantioselectivity in the lipase-catalyzed transesterification results from the diastereomeric interaction either in the acyl-lipase–substrate complex or in the following transition state (Scheme 1), and it seems evident from the above results that not only the chiral binding site itself but also the acyl group in the acyl-lipase intermediate play an important role in the enantioselective acylation of **1**, although the detailed structure of the active site in the lipase PS is at present unclear.[7,13] Accordingly, it is important to elucidate the mode of the interaction operating around the acyl group in the acyl-lipase in organic solvents. The nature of the intermolecular force of enzymes in nonaqueous media is the focus of current interest.[2] To gain an insight into this aspect, a preliminary kinetic study was performed. The pseudo-first-order rate constants for the acylation of the fast- and slow-reacting enantiomers, (1*R*,2*S*)-**1** and (1*S*,2*R*)-**1**, were determined as listed in Table 2. The acylation rate of (1*R*,2*S*)-**1** using vinyl butyrate was slower than that using vinyl acetate, which may suggest the direct steric repulsion between the acyl moiety in the acyl-lipase and (1*R*,2*S*)-**1**. However, interestingly, the acylation rates of (1*R*,2*S*)-**1** became faster when acyl donors longer than

**Table 2.** Pseudo-first-order Rate Constants for Lipase-Catalyzed Transesterification of (1*R*,2*S*)-**1**[b] and (1*S*,2*R*)-**1**[b] by Use of Various Acylating Agents[a]

| Acylating agent R | $k_{obs}^{(1R,2S)}$ x $10^2$, $min^{-1}$ [c] | $k_{obs}^{(1S,2R)}$ x $10^2$, $min^{-1}$ [c] | $k_{obs}^{(1R,2S)}/k_{obs}^{(1S,2R)}$ |
|---|---|---|---|
| $CH_3$ | 1.2 | 0.02 | 60 |
| *n*-$C_2H_5$ | 0.78 | 0.05 | 16 |
| *n*-$C_3H_7$ | 0.68 | 0.05 | 14 |
| *n*-$C_5H_{11}$ | 0.78 | 0.13 | 6 |
| *n*-$C_7H_{15}$ | 5.3 | 0.17 | 31 |
| *n*-$C_9H_{19}$ | 12 | 0.17 | 71 |

[a] Conditions; lipase PS (80 mg), **1** (0.24 mmol), acylating agent (5.0 mmol), dry (*i*-Pr)$_2$O (10 mL), 30 °C. [b] The optically pure enantiomers (1*R*,2*S*)-**1** and (1*S*,2*R*)-**1** were prepared by the lipase-catalyzed transesterification using vinyl butyrate in (*i*-Pr)$_2$O. [c] Aliquots were withdrawn periodically and assayed by HPLC. Pseudo-first-order rate constants were calculated from the equation $-\ln(1-c) = k_{obs}t$.

vinyl butyrate were used,[14] indicating that the acyl moiety in the acyl-lipase did not cause steric repulsion predominantly but contributed either to attractive interactions or to activation of the lipase. Induced-fit mechanism[15] might be a plausible candidate for the latter, and further investigations are necessary for a better understanding. The trend of the enantioselectivities determined by the kinetic measurements (Table 2) is different from that obtained in the preparative resolutions (Table 1), which may be ascribed to the difference in the reaction conditions (concentration and ratio of the reactants[16], presence or absence of the antipodal enantiomer).

In summary, the active site of the lipase can be manipulated by using various acylating agents, enabling an easy search for the *transiently-altered* lipase (acyl-lipase) suitable for asymmetric organic syntheses. We are investigating further the potential of this strategy.

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## REFERENCES AND NOTES:

1. For leading reviews and books, see: (a) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (b) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Eng.* **1989**, *28*, 695. (c) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114. (d) *Preparative Biotransformations: Whole Cell and Isolated Enzymes in Organic Synthesis*; Roberts, S. M.; Wiggins, K.; Casy, G. Eds.; John Wiley & Sons: Chichester, 1993. (e) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. (f) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, 1995.
2. (a) Tawaki, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1992**, *114*, 1882. (b) Terradas, F.; Teston-Henry, M.; Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1993**, *115*, 390.
3. (a) Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 4941. (b) Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron: Asymmetry* **1992**, *3*, 267.
4. Nakatani, T.; Hiratake, J.; Yoshikawa, K.; Nishioka, T.; Oda, J. *Biosci. Biotech. Biochem.* **1992**, *56*, 1118.
5. For the effect of chain length of the acyl donors on the enantioselectivity in the lipase-catalyzed acylation, see: (a) Stokes, T. M.; Oehlschlager, A. C. *Tetrahedron Lett.* **1987**, *28*, 2091. (b) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200. (c) Okahata, Y.; Fujimoto, Y.; Ijiro, K. *Tetrahedron Lett.* **1988**, *29*, 5133. (d) Inagaki, M.; Hiratake, J.; Nishioka, T.; Oda, J. *Agric. Biol. Chem.* **1989**, *53*, 1879. (e) Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4942. (f) Yamazaki, Y.; Hosono, K. *Tetrahedron Lett.* **1990**, *31*, 3895. (g) Miyazawa, T.; Kurita, S.; Ueji, S.; Yamada, T.; Kuwata, S. *J. Chem. Soc., Perkin Trans. 1*, **1992**, *18*, 2253.
6. Takahashi, K.; Ajima, A.; Yoshimoto, T.; Okada, M.; Matsushima, A.; Tamaura, Y.; Inada, Y. *J. Org. Chem.* **1985**, *50*, 3414.
7. Hirose, Y.; Kariya, K.; Nakanishi, Y.; Kurono, Y; Achiwa, K. *Tetrahedron Lett.* **1995**, *36*, 1063.
8. Sakai, T.; Iida, Y.; Kikuyama, S.; Tsuboi, S.; Utaka, M. *Chem. Lett.* **1991**, 1651.
9. Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.
10. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
11. Solvent effect was investigated using isopropenyl acetate and vinyl butyrate. The plots of the *E* values against the log*P* values of solvents gave bell-shaped curves having a peak maximum around log*P* ~ 1 in both cases. See also ref. 3a.
12. Bumgardner, C. L.; Whangbo, M.-H. In *Fluorine-Containing Molecules: Structure, Reactivity, Synthesis, and Applications*; Liebman, J. F.; Greenberg, A.; Dolbier, W. R., Jr. Eds.; VCH: New York, 1988; pp. 83-97.
13. Recent X-ray chrystallographic studies have revealed that several lipases have a catalytic triad in the active site. (a) Brady, L.; Brzozowski, A. M.; Derewenda, Z. S.; Dodson, E.; Dodson, G.; Tolley, S.; Turkenburg, J. P.; Christiansen, L.; Huge-Jensen, B.; Norskov, L.; Thim, L.; Menge, U. *Nature*, **1990**, *343*, 767. (b) Winkler, F. K.; D'Arcy, A.; Hunziker, W. *Nature*, **1990**, *343*, 771.
14. It has recently been reported that in nonaqueous media such as isooctane the rates of the lipase-catalyzed esterification of the longer carboxylic acids were faster. Okahata, Y.; Fujimoto, Y.; Ijiro, K. *J. Org. Chem.* **1995**, *60*, 2244.
15. Koshland, D. E., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98.
16. To check the effect of the concentration, we performed a preparative resolution of rac-**1** using vinyl acetate under the same reaction conditions as those in the kinetic measurements, with the result that the *E* value was enhanced up to 147.

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