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Optical Resolution of a *d*-Biotin Chiral Intermediate by Use of Lipoprotein Lipase

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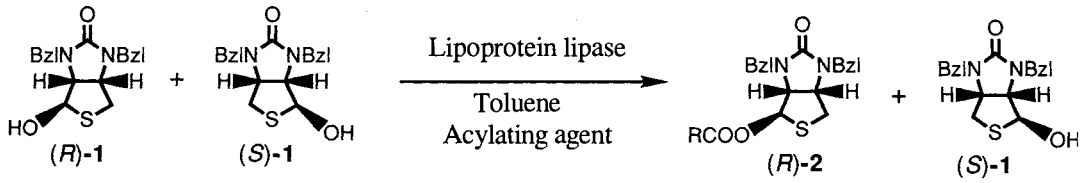
An efficient optical resolution of (±)-(3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*RS*)-**1**] was accomplished by acylation with lipoprotein lipase from *Pseudomonas aeruginosa* TE3285 in toluene. The lipase acylated (*R*)-**1** enantioselectively, and unreacted (*S*)-**1**, which is a chiral *d*-biotin intermediate, was isolated in excellent chemical and optical yields (>99% e.e.). Effects of acylating agents, water content and molecular sieves were also investigated.

Enzyme-catalyzed syntheses in non-aqueous media have been recognized to be an efficient method to obtain optically pure compounds.[1] Especially, the stereoselective acylation or transesterification by lipase has been well studied due to its remarkable stereoselectivity and broad applicability.

Since the first total synthesis of *d*-biotin, which is an essential nutrient and co-factor, was accomplished in 1949,[2] it has been a focal point of interest due mainly to its unique structure.[3] We have assumed that the effective synthesis of chiral intermediates is significant. Accordingly, a kinetic resolution of a *d*-biotin chiral intermediate by lipase-catalyzed acylation was investigated.

We applied enzymes such as lipases and esterases to the acylation of (±)-(3aα,4α,6aα)-1,3-dibenzyl-3a,4,6,6a-tetrahydro-4-hydroxy-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one (*RS*)-**1**,[4] which is a key *d*-biotin intermediate, as depicted in Scheme 1.



Scheme 1. Lipoprotein lipase-catalyzed acylation.

Among enzymes tested, immobilized lipoprotein lipase from *Pseudomonas aeruginosa* TE3285 (TOYOBO immobilized lipase, **LIP**), which was adsorbed on Hyflo Super-Cel by TOYOBO Co. Ltd.,[5] was found to afford (-)-[3a*R*-(3aα,4α,6aα)]-4-acetoxy-1,3-dibenzyl-3a,4,6,6a-tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one [(*R*)-**2**] in excellent enantioselectivity. Hence, the desired stereoisomer, (*S*)-**1**, was isolated from the reaction mixture in excellent chemical and optical yields(99.8%e.e.) as follows. **LIP** (2500 mg), vinyl acetate (1.0 ml) and molecular sieves 4A 1/16 (5.0 g) were added to the solution of (*RS*)-**1** (1000 mg) in toluene (500 ml). The reaction mixture was incubated for 16 hours at 37 °C with stirring. After filtration of lipase and

molecular sieves, the filtrate was analyzed by HPLC using the chiral column (Chiralcel OD, hexane (60) : 2-propanol (40), 0.4 ml/min, 220 nm). It contained (*S*)-**1** (460 mg, 99.0%e.e.) and (*R*)-**2** (600 mg). Then, the filtrate was evaporated to dryness, and the residue was recrystallized from hexane-ethyl acetate (2/1) to give (*S*)-**1** crystals (344 mg, 99.8%e.e.). $[\alpha]^{27}_{D}$ + 71.7 (*c* 0.87, chloroform).

It has been argued that lipase-catalyzed acylations are often slow due to their reverse reactions. In addition, it has been also demonstrated that the acyl transfer from an acylating agent to a lipase is a rate determining step.[5] Accordingly, various acylating agents, such as enol esters,[6] acid anhydrides,[7] oxime esters[8] and trifluoroethyl esters,[9] were subjected to the acylation of (*RS*)-**1** (Table 1). Among acylating agents tested, vinyl acetate and

Table 1. Effect of acylating agents.

Acylating agent	Conv. [b), c)] %	E.e. of **1** [c)] %
$CH_3COOCH=CH_2$	56	96
$C_3H_7COOCH=CH_2$	62	87
$C_5H_{11}COOCH=CH_2$	58	97
$ClCH_2COOCH=CH_2$	18	14
$PhCOOCH=CH_2$	6	1
$(CH_3CO)_2O$	55	89
$(C_2H_5CO)_2O$	58	99
$(C_3H_7CO)_2O$	62	98
$[CH_2COOCOCH_2]$	9	8
$CH_3COON=C(CH_3)COCH_3$	34	41
$C_3H_7COOCH_2CF_3$	8	25

a) The reaction mixture containing (*RS*)-**1** (8.1 mg, 0.024 mmol), **LIP** (48 mg, lipoprotein lipase content 7.4%, H_2O content 0.3%), and acylating agent (0.43 mmol) in toluene (4.0 ml) was incubated for 5 h at 37 °C with stirring. b) Determined by HPLC (Novapak C_{18}, 0.05M KH_2PO_4 (60) : CH_3CN (40), 1.0 ml/min, 220 nm). c) Determined by HPLC(Chiralcel OD, hexane (60) : 2-propanol (40), 0.4 ml/min, 220 nm).

vinyl hexanoate exhibited excellent reactivity and stereoselectivity. The introduction of a phenyl group or an electron withdrawing chlorine atom in acylating agents caused a detrimental effect on reactivity and stereoselectivity. On the other hand, acid anhydrides, such as propionic anhydride and butyric anhydride, showed excellent stereoselectivities and reactivities.

The effect of the water content on the reactivity of enzymes in non-aqueous media has been a focus of recent research.[10] In this regard, the acylation of (*RS*)-**1** by **LIP** with various water contents was carried out. The optimal reactivity was observed with **LIP** containing 0.3% of water (Fig.1). The enhancement of the reactivity by a small amount of water would be attributed to the optimized conformational flexibility of the enzyme, as described by Kitaguchi et al.[10b]

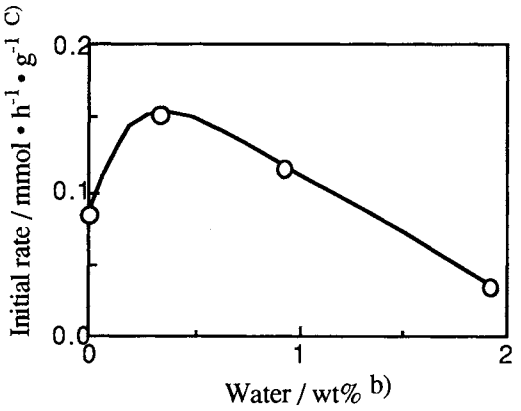


Fig.1. Effect of water content in the absence of molecular sieves. a)

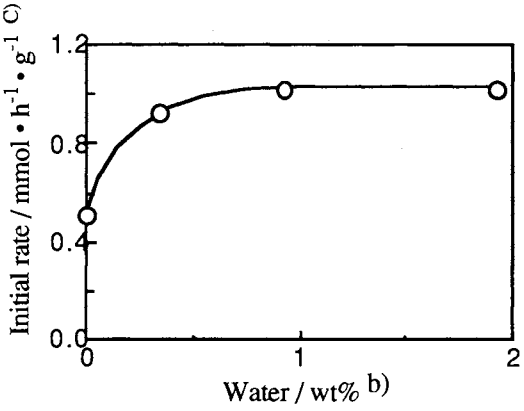
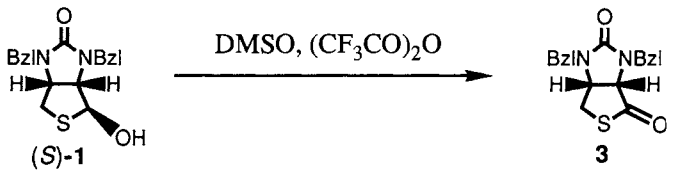


Fig.2. Effect of water content in the presence of molecular sieves. d)

a) The reaction mixture containing (*RS*)-**1** (8.3 mg), **LIP** (48 mg, lipoprotein lipase content 7.4%), vinyl acetate (0.040 ml), and toluene (4.0 ml) was incubated at 37 °C with stirring. b) Determined by thermal analysis. c) Determined by HPLC (Chiralcel OD, hexane (60) : 2-propanol (40), 0.4 ml/min, 220 nm). d) **LIP** (12 mg) and molecular sieves 4A (65 mg) were added to the reaction mixture.

Addition of molecular sieves 4A to the reaction mixture improved the reactivity by 6 to 30-fold. Even in the presence of molecular sieves, a small amount of water was actually beneficial for the reaction. In this case, the initial rate leveled off at more than 0.35% of water(Fig.2). It therefore seems that molecular sieves serve to trap excess water from the reaction mixture.

The (*S*)-**1** thus obtained was oxidized to the known (+)-(3aα,6aα)-1,3-dibenzyl-6,6a-dihydro-1*H*-thieno[3,4-*d*]imidazole-2(3*H*),4(3a*H*)-dione (**3**), which is commercially utilized as a *d*-biotin intermediate,[2] by Swern oxidation (Scheme 2).[4] Moreover, undesired stereoisomer, (*R*)-**2**, can be converted to a precursor of



Scheme 2. Oxidation of the alcohol (*S*)-**1**.

the (*RS*)-**1** by deacylation followed by dehydroxylation, and re-utilized.[11] Thus, the lipoprotein lipase-catalyzed optical resolution provides an efficient synthetic procedure for *d*-biotin.

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References

1) For a recent review: A. M. Klibanov, *Acc.Chem.Res.*, **23**, 114 (1990); E. Santaniello, P. Ferraboschi, P. Grisenti, and A. Manzocchi, *Chem.Rev.*, **92**,1071 (1992).

2) M. W. Goldberg and L. H. Sternbach, *U.S.Patent* 2 489 232, 2 489 234, 2 489 235 (1949).

3) For a recent report: a) E. J. Corey and M. M. Mehrotra, *Tetrahedron Lett.*, **29**, 57 (1988); b) M. Senuma, T. Fujii, M. Seto, K. Okamura, T. Date, and A. Kinumaki, *Chem.Pharm. Bull.*, **38**, 882 (1990).
4) H. A. Bates and S. B. Rosenblum, *J.Org.Chem.*, **51**, 3447 (1986).
5) T. Nakatani, J. Hiratake, K. Yoshikawa, T. Nishioka, and J. Oda, *Biosci. Biotech. Biochem.*, **56**, 1118 (1992).
6) Y.-F. Wang, J. J. Lalonde, M. Momongan, D. E. Bergbreiter, and C.-H. Wong, *J.Am.Chem.Soc.*, **110,** 7200 (1988).
7) D. Bianchi, P. Cesti, and E. Battistel, *J.Org.Chem.*, **53**, 5531 (1988).
8) A. Ghogare and G. S. Kumar, *J.Chem.Soc., Chem.Comm.*, **1989**, 1533.
9) B. Morgan, A. C. Oehlschlager, and T. M. Stokes, *J.Org.Chem.*, **57**, 3231 (1992).
10) a) P. A. Fitzpatrick and A. M. Klibanov, *J.Am.Chem.Soc.*, **113**, 3166 (1991); b) H. Kitaguchi, I. Itoh, and M. Ono, *Chem.Lett.*, **1990**, 1203; c) T. M. Stokes and A. C. Oehlschlager, *Tetrahedron Lett.*, **28**, 2091 (1987).
11) R. Bihovsky and V. Bodepudi, *Tetrahedron*, **46**, 7667 (1990).

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