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Lipase-Catalyzed Kinetic Resolution of *trans*-2,5-Disubstituted Pyrrolidine Derivatives

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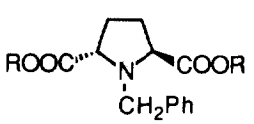
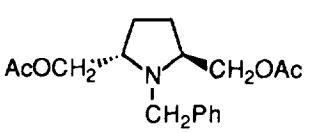
Abstract: Enantioselective preparation of C_2-symmetric (-)-(2S,5S)-N-benzyl-*trans*-2,5-bis(acetoxymethyl)pyrrolidine was carried out by the lipase-catalyzed hydrolysis of racemic diacetate. Organic co-solvent affected the enantioselectivity and 50% DMSO in the phosphate buffer was found to be the optimal solvent system. A possible active site model was also described.

Chiral *trans*-2,5-disubstituted pyrrolidine derivatives with C_2-axis of symmetry have been shown as chiral auxiliaries for various asymmetric syntheses[1] as well as chiral building blocks for the synthesis of pyrrolidine alkaloids.[2] Several stereoselective syntheses of optically active *trans*-2,5-disubstituted pyrrolidine derivatives using chiral starting materials have been reported so far.[3] Norin *et al.*[4] reported that pig liver esterase (PLE)-catalyzed hydrolysis of racemic *trans*-2,5-bis(methoxycarbonyl)pyrrolidine (**1a**) proceeded with only moderate enantioselectivity, although the hydrolysis of the corresponding meso *cis*-derivative showed high enantioselectivity. Recently lipases have been extensively utilized in organic synthesis, especially asymmetric synthesis, since lipases accept a broad structural range of substrates and are inexpensive, and easy to handle. In this context, we have previously reported on the synthesis of optically active N-benzyl-*trans*-2,5-bis(acetoxymethyl)pyrrolidine (**2**) by lipase-catalyzed kinetic resolution.[5] In this paper we describe the full details of the study, in particular the effect of organic co-solvent on the enantioselectivity in lipase-catalyzed hydrolysis.



1a : R=CH_3
1b : R=CH_2CH_3



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RESULTS AND DISCUSSION

Our initial attempts to resolve the diesters **1a** and **1b** by porcine pancreatic lipase (PPL), lipase PS (*Pseudomonas cepacia*, PCL), or *Pseudomonas fluorescens* lipase (PFL) in place of PLE were also unsuccessful. The lipase-catalyzed hydrolysis of the diesters **1a** and **1b** proceeded with a very slow reaction rate and low enantioselectivity. For example, the hydrolysis using lipase PS gave only 8% enantiomeric excess (ee) of the diester **1b** in 65% yield after 5 days. Further optimization of the reaction conditions such as pH, temperature, and organic co-solvent had no significant effect on the enantioselectivity. We therefore expected the diacetate **2** to be a more efficiently-resolved substrate for lipase-catalyzed hydrolysis, taking account of the active-site model proposed by Jones[6] for PPL-catalyzed hydrolysis of primary acetates as shown in Fig. 1.

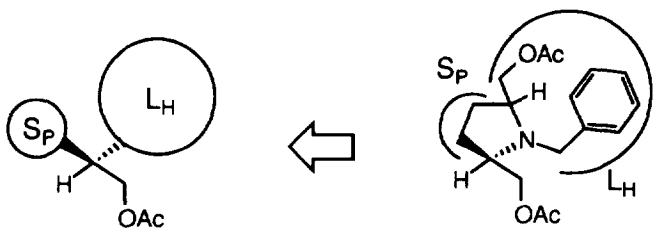

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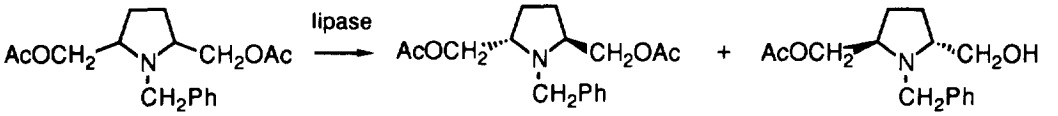
Figure 1. Jones' active site model for primary acetate and C_2-symmetric *trans*-diacetate.

The diacetate **2** was transformed from **1b** by reduction with $LiAlH_4$ and subsequent acetylation in 95% yield. The lipase-catalyzed hydrolyses of the diacetate **2** were carried out at 30 °C in 0.05 M phosphate buffer solution (pH 7.5) as shown in Scheme 1.



(±)-**2** (-)-(S,S)-**2** (+)-(R,R)-**3**

Scheme 1

The ee of the monoacetate and diacetate were determined by HPLC using a chiral column. The results are summarized in Table 1. Among the lipases screened, lipase PS was found to afford higher ee in aqueous media (entries 1, 3, and 5). It has been reported that the use of suitable quantities of organic co-solvents affected the enantioselectivity of enzymatic hydrolysis, such as 20% dimethyl sulfoxide (DMSO) for *Candida cylindracea* lipase (CCL),[7] 50% dimethylformamide (DMF) for lipase LP,[8] 25% CH_3CN for PLE,[9] or 13% *tert*-butyl alcohol for PPL.[10] Thus the diacetate **2** was hydrolyzed with lipase PS in the phosphate buffer contained 20% water-miscible organic solvent. Addition of DMSO enhanced the enantioselectivity (entry 6) and other

Table 1 Lipase-Catalyzed Hydrolysis of N-Benzyl-*trans*-2,5-bis(acetoxymethyl)pyrrolidine[a)]

Entry	lipase	Co-solvent[b)]	(-)-(S,S)-2		(+)-(R,R)-3	
			Yield(%)	% ee	Yield(%)	% ee
1	PPL	none	46	36	29	47
2	PPL	DMSO	58	45	34	55
3	PFL	none	40	42	17	86
4	PFL	DMSO	45	52	24	81
5	PS	none	38	57	21	81
6	PS	DMSO	49	59	33	81
7	PS	DMF	43	48	11	83
8	PS	CH_3CN	72	17	17	82
9	PS	acetone	58	30	23	81
10	PS	THF	63	26	20	76

a) All reactons were carried out at 30°C for 4 h.
b) 20% Organic solvent in 0.05 M phosphate buffer (pH 7.5).

solvent did not affect the enantioselectivity (entries 7-10), though CH_3CN and THF even dcreased the reaction rate (entry 8 and 10). It is noted that DMSO has smaller log P value,[11] that is, more hydrophilic than DMF and CH_3CN and these solvent effects are in contrast to that of lipase-catalyzed esterification and transesterification in organic solvent. [12]

Furthermore, we studied the effect of concentration of DMSO on the enantioselectivity in detail, because its concentration was not fully optimized in the pevious report.[5] Figure 2 shows the relationship of ee and concentration of DMSO. The highest ee was observed at 50% DMSO in phosphate buffer. Over 70% DMSO content, not only the ee but also the rate of hydrolysis decreased suddenly, which suggests that lipase PS was inactivated or denatured under the reaction condition. Thus the lipase-catalyzed hydrolysis of the diacetate **2** in 50% DMSO-phosphate buffer proceeded with high enantioselectivity to afford (-)-**2** with 96% ee in 37% yield for 8 h.

The absolute configuration of the remaining diacetate (-)-**2** was determined to be 2S,5S by comparison with the authentic diacetate (-)-(2S,5S)-**2**; $[\alpha]_D^{25}$ -71.3° (c 0.75 $CHCl_3$), which was derived from (-)-(2S,5S)-N-benzyl-*trans*-2,5-bis(methoxycarbonyl)pyrrolidine[13] by reduction with $LiAlH_4$ and acetylation. Thus the (R,R)-enantiomer of the diacetate was preferentially hydrolyzed with lipase PS, which is not contradictory to the prediction by using Jones' active site model for PPL as mentioned above. Low yield of the resulting monoacetate was due to overhydrolysis. To determine the E value[14] of the first hydrolysis in the optimum condition (50% DMSO-phosphate buffer), the reaction was terminated at low conversion and calculated to be 26 (1 h, c = 0.21, ee (**2**) = 0.24, ee (**3**) = 0.91). Also as the hydrolysis proceeded, the ee of monoacetate decreased. This result suggests that the hydrophilic hydroxyl group of monoacetate may locate in hydrophobic site (L_H) of active site model (Fig. 1) and therefore decrease the enantioselectivity of the second hydrolysis.

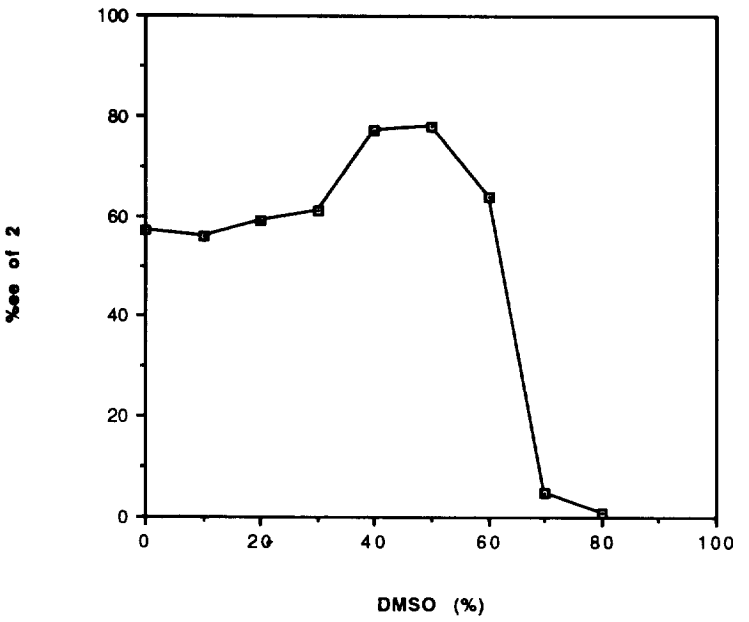


Figure 2. The effect of concentration of DMSO in lipase-catalyzed hydrolysis on % ee of (S,S)-**2**. All reactions were carried out at 30°C for 4 h.

In summary, we have disclosed that the lipase-catalyzed kinetic resolution of C_2-symmetric *trans*-2,5-disubstituted pyrrolidine using lipase PS was accomplished by the modification of structure of substrate and the optimization of organic co-solvent. We also demonstrate that Jones' active site model for PPL can be applied to the prediction of absolute configuration and the enantioselectivity in lipase PS-catalyzed hydrolysis when this type of primary acetate is used.

EXPERIMENTAL SECTION

IR spectra were determined on a Shimadzu IR-435 spectrophotometer. ^{1}H-NMR spectra were recorded at 90 MHz with JOEL JNM-EX90 spectrometer. All NMR spectra were taken in $CDCl_3$ solution with TMS as an internal standard. Optical rotations were determined on a Yanagimoto OR-50 polarimeter. HPLC analysis was carried out using a Daicel Chiralcel OJ column (0.46 X 25 cm, eluent; 20% 2-propanol-hexane) with Shimadzu LC-6A (flow rate; 1 ml/min). Tetrahydrofuran (THF) was distilled from sodium benzophenone kethyl. TLC was carried out on Merck glass plates precoated with silica gel 60F-254 (0.25 mm) and column chromatography was performed by using Merck 23-400 mesh silica gel.

PPL(Type II) was purchased from Sigma Chemical Co. PFL was obtained from Aldrich Chemical Co. Lipase PS was presented by courtesy of Amano Pharmaceutical Co. All lipases were used as received.

N-benzyl-*trans*-2,5-bis(acetoxymethyl)pyrrolidine (2). The ester **1b**[15] (1 g, 3.28 mmol) in THF (4 ml) was added to $LiAlH_4$ (187 mg, 4.92 mmol) in THF (20 ml) at 0°C and the mixture was stirred for 1h at room temperature. Addition of water (0.6 ml) and aqueous 10% NaOH solution (0.4 ml) were added to the mixture and stirred over night. The mixture was filtered through Celite and washed with dichloromethane. The filtrate was evaporated under reduced pressure to give the colorless viscous oil. Acetic anhydride (1.24 ml, 13.1 mmol) and a catalytic amount of 4-dimethylaminopyridine was added to the diol and stirred overnight at room temperature. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ solution and brine successively, and dried over $MgSO_4$. The residue was purified by flash column chromatography (hexane-ethyl acetate, 4 : 1) to give a colorless oil **2** (0.928 g, 93%); IR (neat) 2920, 1730, 1460, 1300, 1255, 698, 660 cm^{-1}; ^1H-NMR δ 1.54-2.22 (4H, m), 2.02 (6H, s), 3.08-3.40 (2H, m), 3.87, 4.03 (2H, ABq, J = 14.4 Hz), 4.02 (4H,d, J = 4.8 Hz), 7.14-7.42 (5H, m); Anal. Calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59%. Found: C, 66.63; H, 7.71; N, 4.43%.

The authentic (-)-(2S,5S)-**2**; $[\alpha]_D^{25}$ -71.3° (c 0.75 $CHCl_3$) was prepared from (-)-(2S,5S)-N-benzyl-*trans*-2,5-bis(methoxycarbonyl)pyrrolidine[13] by the similar procedure as described above.

Typical Procedure for lipase-catalyzed hydrolysis of *trans*-diacetate (2). Lipase PS (25 mg) was added to a suspension of the diacetate **2** (152.5 mg, 0.5 mmol) in 0.05 M phosphate buffer solution, pH 7.5 (2.5 ml), and DMSO (2.5 ml) and was shaken at 30 °C and 200 rpm. After 8 h, the mixture was extracted with diethyl ether and washed with water. The extract was dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the mixture by flash column chromatography with hexane-ethyl acetate (1 : 1) to afford the diacetate **2** (57.2 mg, 37%); $[\alpha]_D^{25}$ -69.9° (c 1.13, $CHCl_3$) (96% ee by HPLC, typical retention times; 10 and 22 min for the (S,S)- and (R,R)-enantiomer, respectively) and the monoacetate **3** (24.6 mg, 19%) as a colorless oil; $[\alpha]_D^{25}$ +50.6° (c 0.54, $CHCl_3$) (65% ee by HPLC, typical retention times; 7.8 and 17 min for the (S,S)- and (R,R)-enantiomer, respectively); IR (neat) 3400, 2940, 1720, 1365, 1230, 1030, 730 cm^{-1}; ^1H-NMR δ 1.55-2.25 (4H, m), 2.04 (3H, s), 2.42 (1H, br s), 3.13 (1H, m), 3.41 (1H, m), 3.42 (1H, dd, J = 1.9, 10.9 Hz), 3.63 (1H, dd, J = 3.4, 10.9 Hz), 3.87 (2H, d, J = 2.2 Hz), 4.12 (2H, d, J = 4.8 Hz), 7.16-7.50 (5H, m); Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N 6.08%. Found: C, 68.27; H, 8.29; N, 5.92%. Treatment of the monoacetate (+)-**3** with acetic anhydride afforded the diacetate (+)-**2**; $[\alpha]_D^{25}$ +46.3° (c 0.42, $CHCl_3$).

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