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Sequential Kinetic Resolution of (±)-2,3-Butanediol in Organic Solvent Using Lipase From *Pseudomonas cepacia*.

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Abstract: Lipase from *Pseudomonas cepacia* (PCL, Amano PS) catalyzed the enantioselective diacetylation of (±)-2,3-butanediol in vinyl acetate. Both acetylation steps favored the (R)-enantiomer ($E_1 = 12$, $E_2 = 34$), thus the reaction is a sequential kinetic resolution. The enantioselectivities of the two steps reinforced one another because both steps proceeded at comparable rates ($S = 3$) yielding an overall enantioselectivity of approximately 200. A synthetic-scale resolution starting from 2.7 g of (±)-2,3-butanediol yielded the diacetate ester of (R)-(-)-butanediol with 96% ee (1.6 g, 30% yield) and (S)-(+)-butanediol with 99% ee (0.63 g, 23% yield). This preparation is carried out entirely in organic solvent, thereby avoiding the difficult and low yield extraction of 2,3-butanediol from aqueous solution.

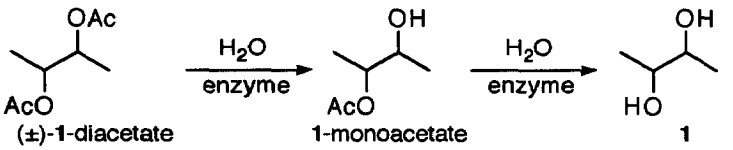
Organic chemists often use enantiomerically-pure diols, especially diols with C_2-symmetry such as (R)- or (S)-2,3-butanediol, as chiral auxiliaries. These symmetrical diols are believed to be more selective auxiliaries than unsymmetrical diols because their symmetry eliminates competing transition states that might favor other products.[1] Enantiomerically-pure *trans*-2,3-butanediol, **1**, has been used as a chiral auxiliary for cyclopropanation,[2] Diels-Alder reactions,[3] and Lewis acid-catalyzed opening[4] or β-elimination[5] of acetals.[6] Diol **1** has also been converted to other chiral auxiliaries such as diethers[7] and diamines.[8] In addition, **1** has been used to derivatize chiral ketones to measure their enantiomeric purity by ^{13}C-NMR[9] or gas chromatography.[10]

Enantiomerically-pure **1** can be prepared by microbial fermentation of sugars. While most microbes give a mixture of (R)-**1** and the *meso* isomer,[11] several strains produce enantiomerically-pure (R)-**1** and have been used on a preparative scale.[12,13] The enantiomer, (S)-**1**, was prepared from a mixture of isomers by microbial destruction of the *meso* form and (R)-**1**.[13] Unfortunately, **1** is difficult to isolate from aqueous fermentation broths. Distillation is tedious because **1** boils at a high temperature. Extraction from aqueous solution is also difficult and inefficient because **1** is hydrophilic; recovery is typically only 50%. Further, it is difficult to remove traces of water from this diol. Alternative routes to **1** include: chemical synthesis from diethyl tartrate in five steps (34% overall yield)[14] and asymmetric hydrogenation of 2,3-butanedione using BINAP as the chiral auxiliary which gave (R)-**1** in high ee, but mixed with the *meso* isomer.[15] Asymmetric hydroxylation[16] of *trans*-dialkylolefins gave 90-97% ee, but hydroxylation of *trans*-2-butene has not been reported.

In this paper we report the preparation of enantiomerically-pure (R)- and (S)-**1** by lipase-catalyzed kinetic resolution. The resolution is carried out in organic solvent to avoid difficulties in isolating **1** from aqueous solution. This resolution involves two sequential acylations and thus is a sequential kinetic resolution. This type of resolution has the advantage that the enantioselectivity of the two steps reinforce each other.

RESULTS

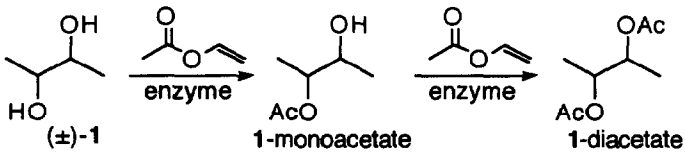
Initial screening of enzymes which catalyze hydrolysis of (±)-1-diacetate, eq 1, identified lipase from *Pseudomonas cepacia* (PCL, Amano PS) as a possible enzyme for a synthetic scale resolution.



(1)

However, this reaction was slow (activity ~3 units/g[17]) and scale-up of this reaction proved disappointing because of the low recovery (typically only 40-50%) of the water-soluble substrate and products.

To avoid water in the reaction or work-up, we turned to enzyme-catalyzed interesterification in an organic solvent, eq. 2.



(2)

Three lipases were screened as catalysts for the enantioselective acetylation of **1** with vinyl acetate: lipase from *Pseudomonas cepacia* (PCL, Amano lipase PS), porcine pancreatic lipase (PPL), and lipase from *Candida rugosa* (CRL), Table 1. Vinyl acetate was used as the acetylation reagent to ensure that the reactions were rapid and irreversible.[18] For each reaction, the relative yields of **1**, **1**-monoacetate, and **1**-diacetate and their optical purities were monitored by capillary gas chromatography, Table 1. The rate of the PCL-catalyzed acetylation reaction was 3.5 times faster than the corresponding hydrolysis reaction.[19]

Table 1. Screening of lipases for the sequential kinetic resolution of C_2-diols.[a]

		Diol		Monoacetate		Diacetate	
Lipase	Conv., %[b] (Time)	Yield, %	ee, %	Yield, %	ee, %	Yield, %	ee, %
PCL	52.9 (4 d)	33.2	nd	27.8	16.5 (S)	39.0	96.3 (R)
PCL	52.9 (4 d)	23.3	98.8 (S)	25.2	20.7 (S)	29.9	96.2 (R)
PPL	39.2 (14 d)	37.4	nd	46.8	54.9 (R)	15.8	97.5 (R)
CRL	15.1 (9 d)	72.1	nd	25.8	64.2 (R)	2.1	83.3 (R)

[a]Yields and enantiomeric purities were measured by capillary gas chromatography. nd = not determined. Absolute configurations were established by GC using authentic samples as standards. [b]Conversion is the fraction of hydroxyl groups that have been acetylated.

To compare single step kinetic resolutions, researchers compare the enantioselectivities of the reactions as measured by the enantiomeric ratio, E.[20] To compare sequential kinetic resolutions, three quantities are needed to estimate the overall enantioselectivities of the reactions: E_1, E_2, and S.[21,22] The variables E_1 and

E_2 represent the enantioselectivities of the first and second steps, respectively, while S represents the specificity of the enzyme for the first and second substrate.[23]

To measure E_1 and E_2 we used reaction conditions where each step could be considered separately and the usual single step equations could be used. The value of E_1 was determined from the combined enantiomeric purity of **1**-monoacetate and **1**-diacetate. The value of E_2 was measured using *rac*-**1**-monoacetate as substrate. The value of S was determined by the competitive acetylation of (±)-**1** and (±)-**1**-monoacetate, Table 2. In addition, we estimated the maximum overall enantioselectivity of each sequential kinetic resolution, $E_{T(max)}$. This value is approximately (E_1 x E_2)/2 and represents the enantioselectivity that a hypothetical single step resolution would need to yield the enantiomeric purity of the two step resolution.

Table 2. Kinetic parameters in the lipase-catalyzed acetylation of (±)-**1**.[a]

Substrate	Lipase	E_1	E_2	S	$E_{T(max)}$
1	PCL	12	34	3.0	200
1-diacetate[b]	PCL	nd	23	4[c]	nd
1	PPL	42	185	14	3900
1	CRL	4.0	3.2	1.8	6.4

[a] E_1, E_2 and S were measured as described in experimental section. $E_{T(max)}$ was estimated by $E_{T(max)} \sim (E_1 \times E_2)/2$. nd = not determined. [b] Hydrolysis reaction in water (NaCl-saturated water, pH 7.0, 10 mM phosphate buffer). [c] Estimate from initial rates of two separate reactions

To check that these measured values accurately describe the PCL-catalyzed acetylation of **1**, we compared the measured amounts of **1**, **1**-monoacetate, and **1**-diacetate and their enantiomeric purities to the values calculated using E_1, E_2 and S, Figure 1. The excellent agreement confirms that this quantitative analysis can be used for this sequential kinetic resolution.

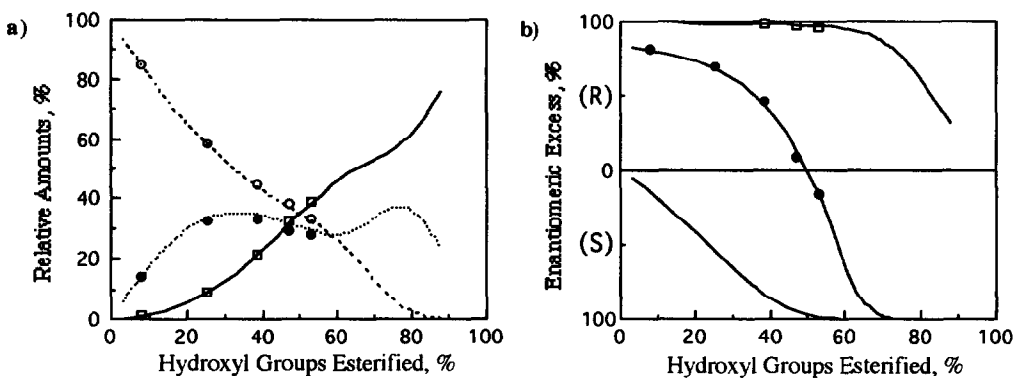


Figure 1. Quantitative analysis of the PCL-catalyzed acetylation of **1** with vinyl acetate. **a)** The measured amounts of **1** (open circle), **1**-monoacetate (filled circle), and **1**-diacetate (open square) are compared to the amounts predicted by $E_1 = 12$, $E_2 = 34$, $S = 3.0$. **b)** The measured enantiomeric purities of **1**-monoacetate (full circle), and **1**-diacetate (open square) are compared to the predicted enantiomeric purities.

The data in Tables 1 and 2 were used to choose the best resolution of **1**, that is, one which proceeds rapidly and yields both **1** and **1**-diacetate with high enantiomeric purity. The resolution catalyzed by CRL was eliminated because the overall enantioselectivity was too low, $E_{T(max)} = 6.4$.

The resolution catalyzed by PPL was also eliminated. Although the sequential kinetic resolution was highly enantioselective, $E_{T(max)} = 3900$, the esterification of the monoester was too slow (~1 U/g) for a practical reaction. A resolution that used only the first step (**1** to **1**-monoacetate) would not be sufficiently enantioselective because E_1 is 42.

The resolution catalyzed by PCL was chosen as the best resolution. The reaction is rapid (10 U/g) and overall enantioselectivity, $E_{T(max)} = 200$ is sufficient to yield high enantiomeric purity. Further, the value of S is 3, near the optimum value of 1 so that the maximum overall enantioselectivity is obtained without finding conditions to change this value as was required in other cases.[21] A small scale resolution on **1** (2.7 g) gave (R)-**1**-diacetate and (S)-**1** in 96 and 99% ee respectively in good isolated yields, Table 1.

A preliminary screening of several other vinyl esters (propanoate, butanoate, hexanoate, and octanoate) as well as anhydrides (acetic, butanoyl) showed no improvement in the overall enantioselectivity.

DISCUSSION

The major advantage of a sequential kinetic resolution is the reinforcement of the enantioselectivity of the two steps. For the PCL-catalyzed resolution of **1**, the enantioselectivity of the individual steps 12 and 34 are insufficient to efficiently resolve **1**. When combined in a sequential kinetic resolution, however, they reinforce each other giving an overall enantioselectivity of 200, which is sufficient to resolve **1**. We showed previously that to get this reinforcement, the relative rate of the two step must be equal.[21] In practice a relative rate within a factor of five is sufficient. The relative rates of most of the acylation reactions was within this range so no special strategies were required to optimize the rates.

Another advantage of a sequential kinetic resolutions is that both enantiomers can be isolated with high enantiomeric purity. A single-step resolution give either product in high ee when stopped at <50% conversion or starting material in high ee when stopped at >50% conversion. A sequential kinetic resolution can give both product and starting material in high ee at the same % conversion. The 'mistakes' end up in the middle product, in this case, **1**-monoacetate.

EXPERIMENTAL SECTION

General. Racemic **1**, vinyl acetate, and 4-dimethylaminopyridine (DMAP) were purchased from Aldrich Chemical Co. Thin-layer chromatography was done on silica gel supported on aluminum (Whatman Ltd, Maidstone, England), and column chromatography was also done on silica gel (70-230 mesh, Aldrich Chemical Co.). Enzymes and enzyme assays have been described previously.[24] The enzymic activity of crude PCL (P-30, from Amano Enzyme Company, Troy, VA) was 53 U/g with olive oil as substrate. Higher activity PCL (P-80; P-200) is now available from Amano.

Enantiomeric purity. Enantiomers of **1**-monoacetate and **1**-diacetate were separated by capillary gas chromatography using Chiraldex G-TA column (0.25 mm x 30 m, Advanced Separation Technologies, Inc., Whippany, NJ). Conditions: 91 °C, flame ionization detector: **1**-monoacetate ($\alpha = 1.16$, (S)-enantiomer elutes first); **1**-diacetate ($\alpha = 1.13$ (R)-enantiomer elutes first). To measure the enantiomeric purity of **1**, a sample of **1** was diacetylated as described below to prepare (±)-**1**-diacetate and analyzed as above.

(±)-1-monoacetate. A mixture of (±)-**1** (5.0 g, 55 mmol), ethyl acetate (100 mL), sodium carbonate (11.8 g, 111 mmol), DMAP (5.0 mg, 0.04 mmol), and acetic anhydride (6.8 g, 67 mmol) was stirred at ambient tem-

perature for 14 h. The reaction mixture was filtered and concentrated under reduced pressure. Flash chromatography on silica gel eluted with a 20-100% gradient of ethyl acetate in hexane gave **1**-monoacetate as a colorless oil, 3.2 g (44%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.71 (m, 1), 3.71 (m, 1), 2.12 (m, 1), 2.06 (s, 3), 1.17 (t, J = 7 Hz, 6).

(±)-1-diacetate. Acetic anhydride (34.0 g, 0.33 mol) was added dropwise to a solution of (±)-**1** (10.0 g, 0.11 mol) in dry pyridine (150 mL). The mixture was stirred at ambient temperature for 24 h and poured onto 200 mL of ice-water. The aqueous phase was extracted with 3 x 100 mL of chloroform and the combined organic extracts were washed successively with 3 x 100 mL each of 1 N HCl, $NaHCO_3$ satd and water. The organic phase was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The product was melted and distilled under reduced pressure to afford 14.9 g (85.5 mmol; 77%) of crystalline material; mp 42.0-42.5 °C ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.95 (m, 2), 2.10 (s, 6), 1.25 (d, 6, 7.0 Hz).

Lipase-catalyzed hydrolysis of (±)-1-diacetate. The substrate (±)-**1**-diacetate (1.0 g, 6.0 mmol) was dissolved in 10 mL of hexane. Phosphate buffer (10 mL; 10 mM; pH 7.0) and sodium chloride (5.0 g)[25] were added and the pH was adjusted to 7.0 with 0.5 N NaOH. The enzyme PCL (300 mg) was added and the mixture was vigorously stirred at 25 °C. The pH was kept neutral by addition of 0.5 N NaOH using a pHstat instrument. The hydrolysis was stopped at 51% conversion (12.2 mL of 0.5 N NaOH consumed). Water (10 mL) was added and the mixture was extracted extensively with ethyl acetate (15 x 25 mL) and dried over anhydrous sodium sulfate. Flash chromatography on silica gel eluted with a 0-100% gradient of ethyl acetate in hexane gave low yields of products: **1**, 0.24 g (23%), **1**-monoacetate 0.14 g (18%), and **1**-diacetate, 64 mg (12%).

Lipase-catalyzed acetylation of (±)-1. Solid PCL (0.50 g) was added to a solution of racemic **1** (2.7 g, 30 mmol) in vinyl acetate (50 mL) and the suspension was stirred at 25 °C. Aliquots (0.2 mL) of the mixture were diluted in diethyl ether (1.0 mL) and filtered. The relative amounts and enantiomeric purities of **1**-diacetates, **1**-monoacetates and **1** were measured by gas chromatography. After 4 d, the enzyme was filtered off and the solvent was evaporated under reduced pressure. Flash chromatography on silica gel eluting with a gradient of 100% hexanes to 100% ethyl acetate afforded **1**-diacetate (1.56 g, 29.9% yield, 96.2% ee, >97% pure by GC analysis), **1**-monoacetate (1.0 g, 25.2% yield, 20.7% ee, 87% pure by GC) and **1** (0.63 g, 23.3% yield, 98.8% ee, >97% pure by GC). Acetylations catalyzed by PPL (1.0 g, 1.8 units with olive oil as substrate) or CRL (0.5 g, 75 units with olive oil as substrate) were done similarly. Optical purities and yields for all reactions are reported in Table 1.

Measurement of E_1. The combined enantiomeric purity of **1**-monoacetate and **1**-diacetate were used to calculate E_1. For example, with PCL after 7 h, combined (R)-**1**-monoacetate and (R)-**1**-diacetate were in 83% ee and 15% yield. Therefore, E_1 was 12. With PPL after 24 h, it was 95% ee and 15% yield. Therefore, E_1 was 42. With CRL after 24 h, it was 58% ee and 10% yield. Therefore, E_1 was 4.0.

Measurement of E_2. The enantioselectivity of the second step, E_2, was measured using *rac*-**1**-monoacetate as the substrate. The enzyme (PCL: 30 mg; PPL: 100 mg; or CRL: 30 mg) was added to a solution of *rac*-**1**-monoacetate (54 mg, 0.6 mmol) in vinyl acetate (1.0 mL) and the mixture was stirred at 25 °C and monitored by GC. With PCL after 4 h, (R)-**1**-diacetate was formed in 94% ee and 11% yield. Therefore, E_2 is 34. With PPL after 26 h, it was 99% ee and 10% yield. Therefore, E_2 is 185. With CRL after 3 h, it was 52% ee and 6.0% yield. Therefore, E_2 is 3.2.

Measurement of S. For PPL: The enzyme (30 mg, 0.054 units with olive oil as substrate) was added to a solution of (±)-**1** (9.0 mg, 0.10 mmol) and (±)-1-monoacetate (132 mg, 1.0 mmol) in vinyl acetate (2.0 mL). Both

substrates dissolved completely. The mole fractions of 1, 1-monoacetate, and 1-diacetate as measured by GC were 8.4, 91.6, and 0, respectively before enzyme addition and 7.4, 91.8, and 0.8, respectively, 5.0 h after enzyme addition; thus the relative rate was 1.2. During the reaction, the average value for [1-monoacetate]/[1] was 11.7, thus S = 14. For PCL and CRL, the S values were measured in a similar way and are reported in Table 2.

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$$E_1 = \frac{(k_{cat}/K_M)_{R_1}}{(k_{cat}/K_M)_{S_1}}, \quad E_2 = \frac{(k_{cat}/K_M)_{R_2}}{(k_{cat}/K_M)_{S_2}}, \quad S = \frac{(k_{cat}/K_M)_{R_1} + (k_{cat}/K_M)_{S_1}}{(k_{cat}/K_M)_{R_2} + (k_{cat}/K_M)_{S_2}}$$

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After preparation of this manuscript related work on the resolution of 2,3-butanediol has appeared: Bisht, K. S.; Parmar, V. S.; Crout, D. H. G. *Tetrahedron: Asymmetry* **1993**, *4*, 957-958.