An Optimized Sequential Kinetic Resolution of *trans*-1,2-Cyclohexanediol

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Quantitative analysis of sequential kinetic resolutions (resolutions that proceed via two or more enantioselective steps) indicates that optimal reinforcement of the enantioselectivities occurs when the rates of the two steps are equal. Under these conditions, the reinforcement is multiplicative and the overall enantioselectivity, $E_{T(max)}$, is given approximately by $[1 + (E_1E_2)]/2$. To test these ideas we optimized the resolution of *trans*-1,2-cyclohexanediol, 1. The first step of the porcine liver esterase (PLE) catalyzed hydrolysis of *trans*-1,2-diacetoxycyclohexane proceeded 47 times faster than the second step and the resolution yielded 1 with only 58% ee at 44 mol %. Addition of a hexane phase slowed the first step by selectively extracting the fast-reacting 1-diacetate (relative rate = 6) and increased the enantiomeric purity to 94% ee at 34 mol %. The resolution of 1 was further improved using lipase from *Pseudomonas cepacia* (Amano P, PCL) which showed an $E_{T(max)}$ of >2000 as compared to an $E_{T(max)}$ for PLE of 54. Resolution with PCL after equalization of the rates of the two steps gave (*R*)-1 and recovered (*S*)-1-diacetate both with >99% ee.

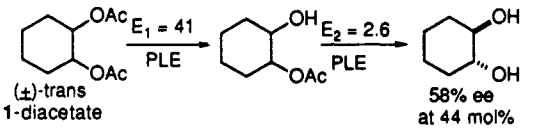
One strategy to increase the enantiomeric purity of products from enantioselective reactions is to link several enantioselective reactions that reinforce one another. An example is to use a kinetic resolution to remove mistakes from an asymmetric synthesis starting from a meso compound.[1]

A similar approach involves linking two, and potentially more, kinetic resolutions[2] to give a sequential kinetic resolution, Figure 1. This strategy applies to any molecule that can undergo two sequential reactions, especially molecules with two reactive functional groups. For example, the first step with an enantioselectivity of E_1 transforms a racemic diester (A_2, B_2) to monoester (A_1, B_1) and the second step with an enantioselectivity E_2 yields fully hydrolyzed product (A_0, B_0).

Some sequential kinetic resolution yielded products with high enantiomeric purity (>99% ee), e.g., a chiral auxiliary, 1,1′-bi-2-naphthol,[3] and several prostaglandin precursors, bicyclo[3.3.0]octane-2,6-diol[4] and a bicyclo[3.2.0]hept-2-ene derivative.[5] Other sequential kinetic resolutions have not yielded products with enhanced enantiomeric purities. A microorganism-catalyzed resolution of 1,1′-bi-2-naphthol[6] (E_1 = 12, E_2 = 55) yielded ~50 mol % product with 90% ee, not much higher than expected from a single-step resolution where E = 55.[7] Resolution of *trans*-1,2-cyclohexanediol, 1, using porcine liver esterase (PLE), Scheme I, where E_1 = 41, E_2 = 2.6, was also inefficient.[8] We obtained 1 with only 58% ee at 44 mol %, while a single-step resolution where E = 41 would give 90% ee.[7]

Roberts and co-workers suggested that the rates of the two steps in sequential kinetic resolution must be of the same order of magnitude.[5] In this paper, we quantitatively examine the effect of relative rate on the enantiomeric purity of the final product in sequential kinetic resolutions. We find that the optimum reinforcement of enantioselectivities occurs when the two rates are equal. Under these conditions, the reinforcement of the two steps is multiplicative and the overall enantioselectivity, $E_{T(max)}$, is given approximately by $[1 + (E_1E_2)]/2$.

Scheme I



We used these predictions to optimize the resolution of 1. Enantiomerically pure 1 has been used to direct the addition of organocuprates to α,β-unsaturated esters,[9] to prepare chiral phosphines[10] and chiral crown ethers,[11] and could be used to form chiral acetals and boranes. Previous resolutions of 1[8,12] have not been carried out under optimum conditions, sometimes only one enantioselective step was exploited. Our optimized resolution provides a route to both enantiomers of 1 with perfect enantiomeric purity. Further, the optimization procedure offers a general strategy for the resolution of an important class of chiral auxiliaries, namely those with C_2 symmetry,[13] and has potential for other classes of molecules.

Results

Optimum Values for the Variables in a Sequential Kinetic Resolution. Equations for sequential kinetic resolutions, reported previously,[3,14] contain three variables which completely define the amounts and enantiomeric purities of all species: the enantioselectivity of the first step, $E_1 = k_1/k_3$; the enantioselectivity of the second step, $E_2 = k_2/k_4$;[15] and the specificity of the catalyst for the first substrate (A_2, B_2) vs the second substrate (A_1, B_1), $S = (k_1$

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(15) This definition of E_2 differs from that in ref 14.

0022-3263/91/1956-7251$02.50/0

$$A_2 \xrightarrow{k_1} A_1 \xrightarrow{k_2} A_0$$
$$B_2 \xrightarrow{k_3} B_1 \xrightarrow{k_4} B_0$$

Figure 1. Two-step sequential kinetic resolution. A_2 and B_2 represent the fast- and slow-reacting enantiomers of the starting material, A_1 and B_1 represent enantiomers of the intermediate, A_0 and B_0 represent enantiomers of the final product, and k_1, k_2, k_3, and k_4 are the rate constants for the indicated irreversible reactions. For enzyme-catalyzed reactions these rate constants correspond to k_{cat}/K_M, the specificity constants for each reaction. The enantioselectivity of the first step, E_1, is k_1/k_3; the enantioselectivity of the second step, E_2, is k_2/k_4; the specificity of the catalyst for the first substrate as compared to the second substrate, S, is $(k_1 + k_3)/(k_2 + k_4)$.

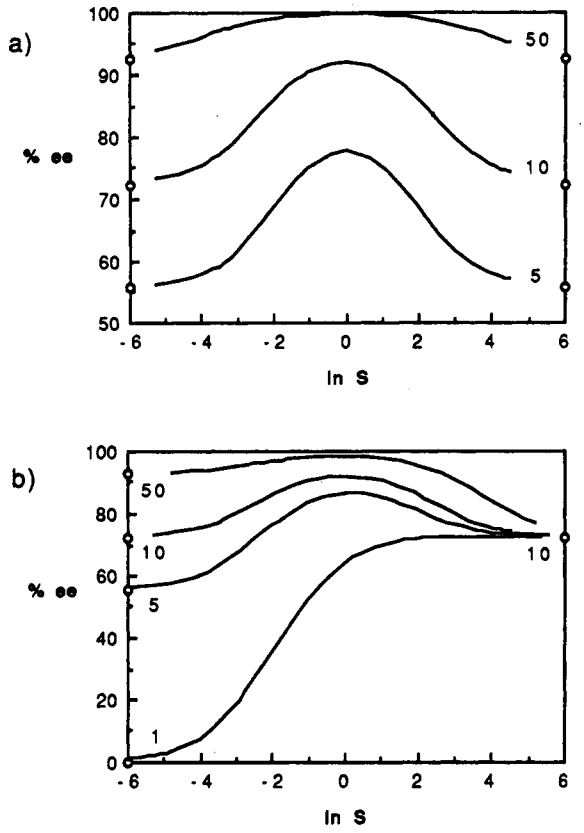


Figure 2. Optimum value of S for a sequential kinetic resolution. (a) The enantiomeric purity of the product at 40 mol % for two-step resolutions where $E_1 = E_2$ as a function of the natural logarithm of S. The highest enantiomeric purity is obtained at $\ln S = 0$, that is when the rates of the two steps are equal. When the rates are not equal the enantiomeric purity approaches that obtained from a single step resolution as indicated by the symbol o on the vertical axis. (b) The enantiomeric purity of the product at 40 mol % for two-step resolutions where $E_1 \neq E_2$ as a function of the natural logarithm of S. The maximum enantiomeric purity is obtained at $\ln S = 0$, when $S \neq 1$, higher purity product results when the slower step is the more enantioselective step.

$+ k_3)/(k_2 + k_4)$. The relative rate of the two steps is proportional to S; when $S = 1$, the rates are equal.[16] These three variables will determine the success or failure of a sequential kinetic resolution.

To determine the optimum value of S for a hypothetical synthetic-scale resolution, we calculated the enantiomeric purity of the product at 40 mol % product for different values of E_1, E_2, and S. The case where the two steps have

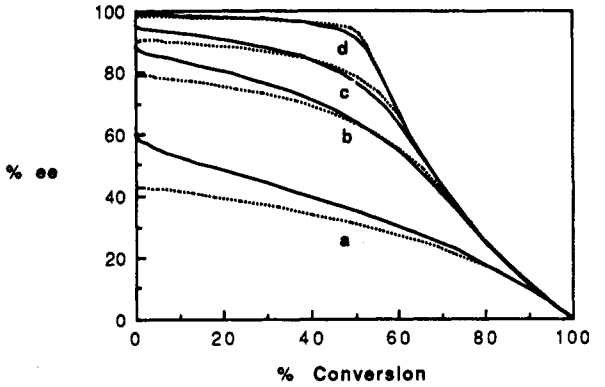


Figure 3. Comparison of the exact and approximate methods to calculate enantiomeric purity of the product from a sequential kinetic resolution. Solid lines show the exact values calculated using a spreadsheet for several possible enantioselectivities: (a) $E_1 = E_2 = 2$; (b) $E_1 = 2$, $E_2 = 8$; (c) $E_1 = 13$, $E_2 = 3$; (d) $E_1 = 5$, $E_2 = 40$. For all lines, $S = 1$. The dotted lines show approximate values calculated by treating the sequential kinetic resolution as a single-step resolution with an enantioselectivity given by eq 2. This approximate method is accurate to within 3% ee in the range 80–100% ee.

equal enantioselectivities is shown in Figure 2a. As expected, higher ee results as the enantioselectivity of the steps increases. Higher ee also results as the value of S approaches 1, i.e., $\ln S = 0$. In qualitative terms, both steps must be partly rate determining to contribute to the overall enantioselectivity. The specificity ratio need not be exactly 1 since a specificity ratio within a factor of 5 ($\ln S = \pm 1.6$) gives near optimal resolution.

Equal rates are also optimal when the two steps have different enantioselectivities, Figure 2b. When $S \neq 1$, however, enantiomeric purity is higher when the slower step is the more enantioselective step. For example, 78% ee results from a resolution where $E_1 = 50$ and $E_2 = 10$ when the first step is 150 times faster ($\ln S = 5$), but 93% ee results when the second step is 150 times faster ($\ln S = -5$). Thus, the enantioselectivity of the slower step contributes more to the overall enantioselectivity.

To determine quantitatively the advantage of a sequential kinetic resolution over a single-step resolution, we calculated an overall enantioselectivity, E_T, for a sequential kinetic resolution. We define E_T as the enantioselectivity that a hypothetical single-step resolution would need to yield the observed enantiomeric purity. The maximum value of E_T for a given enzyme, $E_{T(max)}$, will occur when $S = 1$. For given values of E_1 and E_2, a spreadsheet was used to calculate the enantiomeric purity of the product and the single-step equation was used to calculate $E_{T(max)}$. To simplify this calculation, we found an approximate equation to calculate $E_{T(max)}$:

$$E_{T(max)} \approx [1 + (E_1E_2)]/2 \qquad (1)$$

The approximate equation predicts an ee within 3% of the correct value when at >80% ee and thus is sufficiently accurate to compare different resolutions, Figure 3.[17] This equation indicates that the enhancement of enantioselectivity in a sequential kinetic resolution is multiplicative. Further, two moderately enantioselective steps yield product with higher enantiomeric purity than a highly enantioselective step combined with a poorly enantioselective step. For example, when $E_1 = E_2 = 10$, then $E_{T(max)} = 50.5$, but when $E_1 = 18$, $E_2 = 2$, then $E_{T(max)} = 18.5$.

(16) The relative rate of the two steps also depends on concentration. When $S = 1$, or when the expression in eq 2 equals 1, the relative rate is equal only when the total concentrations of each substrate are also equal. During a sequential kinetic resolution both the concentrations of the two substrates and the relative rate vary with time.

(17) At low extents of conversion, $E_{T(max)} = E_1E_2$ (Yamagata, Y. *J. Theoret. Biol.* **1966**, *11*, 495–498), but at synthetically useful extents of conversion, eq 1 is more accurate.

Table I. Enantioselectivity of Several Enzymes in the Hydrolysis of 1-Diacetate and 1-Monoacetate[a]

enzyme[b]	solvent	% c[c]	% ee[d]	E_1[e]	% c[f]	% ee[g]	E_2[e]	E_T-(max)[h]
PLE	buffer	53.7	96.1	41[i]	27.3	39.4	2.6[i]	54
PLE	satd NaCl[j]	45.2	71.7[k]	11	6.0	52.3	3.3	19
PCL	satd NaCl[j,l]	45.2	>97.9[k]	>230	23.6	85.6	17	>2000
PCL	buffer	48.2	97.8[k]	290	9.1	83.4	12	1700
CE	satd NaCl[j]	44.2	56.2	10	47.9	55.4[m]	6.9	35
CRL	buffer[l]	33.2	16.6	2.3	51.4	1.4[m]	1.0	1.6

[a] Reactions were carried out using 1 mmol of substrate in phosphate buffer (10 mL, 10 mM, pH 7) at ambient temperature unless otherwise noted; 1-monoacetate dissolved completely, 1-diacetate dissolved only partially. [b] PLE = porcine liver esterase, PCL = lipase from *Pseudomonas cepacia* (Amano lipase P; the subspecies of the microorganism producing this lipase was originally thought to be *fluorescens*, but was announced by Amano Enzyme Co. to be *cepacia* in 1989), CE = bovine cholesterol esterase, CRL = lipase from *Candida rugosa* which was previously called *Candida cylindracea*. [c] Mole percent of 1-diacetate reacted. [d] Enantiomeric purity of the unreacted 1-diacetate. The *R* mono- and diacetates were preferentially hydrolyzed by all enzymes except CRL. [e] Estimate of accuracy: −10%, +15% for $E \leq 40$; −25%, +30% for $E > 200$. [f] Mole percent of racemic 1-monoacetate that had reacted. [g] Enantiomeric purity of the product, 1. [h] Calculated using eq 1. [i] Similar values of $E_1 > 29$, $E_2 = 2.4$ were calculated from data in ref 8. [j] Buffer was saturated with sodium chloride. Both 1-monoacetate and 1-diacetate dissolved only partially. [k] Enantiomeric purity of the combined products, 1 and 1-monoacetate. [l] 5 mL of buffer were replaced by 5 mL of hexane. [m] Enantiomeric purity of unreacted 1-monoacetate.

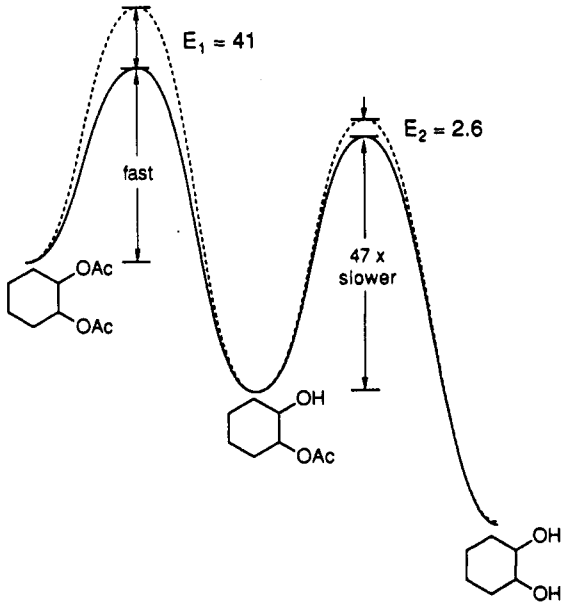


Figure 4. Reaction coordinate for the hydrolysis of 1-diacetate catalyzed by PLE. The solid line represents the reaction coordinate for the fast-reacting *R* enantiomer and the dashed line represents the reaction coordinate for the slow-reacting *S* enantiomer. Reinforcement between the two steps is poor because the second step is largely rate determining. Since the enantioselectivity of the second step is low, the overall enantioselectivity is low.

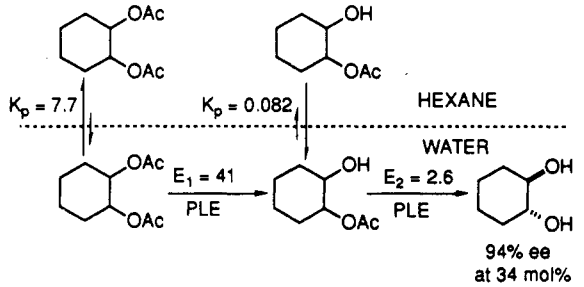
Experimental Determination of E_1, E_2, and S. The values of E_1 and E_2 were determined for the PLE-catalyzed hydrolysis of 1-diacetate using equations for single-step resolution,[7] under conditions where each step could be considered separately. Thus, the value of E_1, was determined from the enantiomeric purity of either the remaining 1-diacetate or the products of the first step: combined 1-monoacetate and 1, Table I. The value of E_2, was determined starting with racemic 1-monoacetate and measuring the enantiomeric purity of either the remaining 1-monoacetate or the product, 1. The value of S for the PLE-catalyzed hydrolysis of 1-diacetate was determined

Table II. Relative Rates of Hydrolysis of 1-Diacetate and 1-Monoacetate[a]

enzyme	aqueous phase	organic phase	relative rate[b]
PLE	buffer[c]	none	47 ± 12
PLE	buffer	excess substrate	22 ± 5
PLE	buffer	hexane[d]	6 ± 2
PLE	buffer	toluene	5 ± 2
PLE	satd NaCl	excess substrate	6 ± 1
PCL	buffer	excess substrate	86[e]
PCL	satd NaCl	hexane	3.2 ± 0.7

[a] The initial concentration of each substrate was 100 mM. See note *a* of Table I. [b] Determined directly by the competitive hydrolysis of (±)-1-diacetate and (±)-1-monoacetate or calculated using eq 2 and the partition coefficients. [c] The initial concentration of each substrate was 4 mM. [d] The organic and aqueous phases were each 5 mL. [e] Estimated from the initial rates of the two substrates measured separately.

Scheme II



to be 47 by the competing hydrolysis of (±)-1-diacetate and (±)-1-monoacetate,[18] indicating that the first step proceeds 47 times faster than the second.[19]

Using these three measured values for E_1, E_2, and S, we constructed a reaction coordinate for this sequential kinetic resolution, Figure 4,[20] which qualitatively identified the problem. The first step rapidly transforms (*R*)-1-diacetate to (*R*)-1-monoacetate, but the second step, transformation of (*R*)-1-monoacetate to (*R*)-1, is slow, causing (*R*)-1-monoacetate to accumulate at the beginning of the resolution. As we wait, substantial amounts of (*S*)-1-monoacetate are also formed, thereby wasting the enantioselectivity of the first step.

Quantitative analysis confirmed the explanation above. Figure 5a indicates that, due to the slow second step, approximately 70% of the acetate groups were hydrolyzed before 1 accumulated to ~50 mol %. Figure 5b indicates that at this extent of conversion the enantiomeric purity of 1 was only ~55% ee. The low enantiomeric purity of

(18) Equation for two competing substrates: Fersht, A. *Enzyme Structure and Function*, 2nd ed.; Freeman: New York, 1985; Chapter 3, Section G. Determination of *S* by measuring the individual kinetic constants was not sufficiently accurate. (*R*)-1-diacetate: V_{max} = 15 ± 1 U/mg, K_M = 0.8 ± 0.2 mM, V_{max}/K_M = 20 ± 6 U/mg mM. (*S*)-1-diacetate: V_{max} = 0.17 ± 0.03 U/mg, K_M = 3 ± 1 mM, V_{max}/K_M = 0.06 ± 0.03 U/mg mM. (*R*)-1-monoacetate: V_{max} = 0.94 ± 0.07 U/mg, K_M = 4.8 ± 0.6 mM, V_{max}/K_M = 0.20 ± 0.04 U/mg mM. (*S*)-1-monoacetate: V_{max} = 0.3 ± 0.1 U/mg, K_M = 10 ± 6 mM, V_{max}/K_M = 0.03 ± 0.03 U/mg mM; thus, S = 90 ± 50.

(19) Base-promoted hydrolysis of 1-diacetate at pH 10.5 in the absence of PLE proceeded at 0.56 times the rate for 1-monoacetate suggesting that the large value of *S* is not due to inherent differences in chemical reactivity of the two substrates.

(20) The free energy of hydrolysis of ethyl acetate at pH 7 is −4.72 kcal/mol (Jencks, W. P. In *Handbook of Biochemistry*; Sober, H. A., Ed.; CRC Press: Boca Raton, FL, J-183). On this basis, the level of 1-monoacetate was placed 4.7 kcal/mol below that of 1-diacetate and the level of 1 was placed 4.7 kcal/mol below that of 1-monoacetate. The free energies of activation for each step were estimated from $\Delta G^{\dagger} = -RT \ln (hk_{cat}/k_B T K_M)$. The value of k_{cat} for PLE with (*R*)-1-diacetate as the substrate was estimated to be 5×10^4 s^{-1} based on a subunit molecular weight of 60 000 Da. This estimate is consistent with a measured value of 7×10^4 s^{-1} with bisphenyl carbonate as a substrate (Farb, D.; Jencks, W. P. *Arch. Biochem. Biophys.* **1980**, *203*, 214–226).

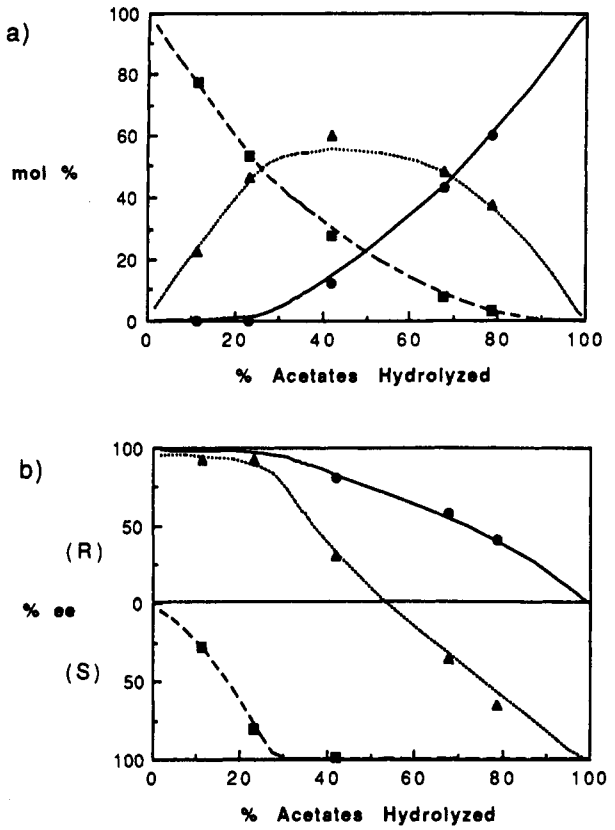


Figure 5. Quantitative analysis of the hydrolysis of 1-diacetate catalyzed by PLE. (a) The mole fraction of each species as a function of the fraction of acetates that had been hydrolyzed: diacetate (■), monoacetate (▲), and diol (●). The lines indicate the amounts predicted by $S = 47$. (b) The enantiomeric purity of each species as a function of the fraction of acetates that had been hydrolyzed. The lines represent values predicted using $E_1 = 41$, $E_2 = 2.6$, and $S = 47$.

1 in the PLE-catalyzed resolution results because first, $S = 47$, far from the optimum value of 1, which prevents efficient reinforcement of the enantioselectivities of the two steps[21] and second, because the slower step is the less enantioselective one.

Changing the Relative Rate of the Two Steps. When an organic phase is added to the reaction mixture, the two substrates partition between the organic and aqueous phases and the relative rate of the two steps[16] is proportional to

$$S[(V_{aq} + V_{org}K_{p1-m})/(V_{aq} + V_{org}K_{p1-d})] \qquad (2)$$

where V_{aq} and V_{org} are the volumes of the aqueous and organic phases, K_{p1-m} is the partition coefficient for 1-monoacetate, $[1\text{-m}]_{org}/[1\text{-m}]_{aq}$, and K_{p1-d} is a similar quantity for 1-diacetate. The relative rate was measured, as above, from the initial rate of competing racemic substrates or calculated from S and the partition coefficients using eq 2, Table II. Since the relative rate in a two-phase mixture is closer to 1, the enantiomeric purity of the product, 1, is expected to increase. Hydrolysis of 1-diacetate catalyzed by PLE in a mixture of equal volumes of buffer and hexane, Scheme II, yielded 1 with 94% ee at 34 mol % in good agreement with the predicted value of 88%.

(21) One strategy for obtaining higher enantiomeric purity material would be to carry out a single-step resolution using only the first step. An overall ee of >90% is predicted for 1-monoacetate after hydrolysis of ~25% of the acetate groups, Figure 5. This procedure would not take advantage of the enhancement of enantiomeric purity that is possible in a sequential kinetic resolution.

Another way to create a second phase is to add sodium chloride to "salt out" the excess substrate. Hydrolysis of 1-diacetate catalyzed by PLE in a two-phase mixture of buffer saturated with sodium chloride and excess substrate yielded 1 with 84% ee at 32 mol %. The enantiomeric purity of 1 under these conditions was not as high because E_1 and E_2 also changed upon addition of sodium chloride to the buffer, Table I. The predicted ee under these conditions is 79% ee in good agreement with the measured value. This increase in ee occurs despite a decrease in E_1 due to a more optimal relative rate. Both experiments demonstrate that the ee of the product increases in a sequential kinetic resolution, without an increase in the enantioselectivity of the enzyme, when the rates of the two steps are equalized.

An Optimized Resolution. Even at more favorable values of S, the enantiomeric purity of 1 obtained from a PLE-catalyzed resolution is insufficient for most synthetic purposes. To screen for an enzyme that is better suited for the resolution of 1 than PLE, we measured the values of E_1 and E_2 for several enzymes and compared the calculated values of $E_{T(max)}$, Table I. The most promising enzyme was PCL, lipase from *Pseudomonas cepacia* (Amano P), with an $E_{T(max)}$ of >1700. A resolution with PCL in buffer yielded 1 with only 95% ee at 29 mol %, $E_T = 60$. This observed value of E_T was substantially lower than $E_{T(max)}$ because the rates had not been equalized. To equalize the rates we added both a second phase of hexane and sodium chloride to saturate the aqueous phase giving a relative rate of 3.2, Table II. Under these conditions, a small increase in E_1 and E_2 was also observed, Table I, $E_{T(max)}$ is >2000. We obtained 1 with >99% ee at 47.9 mol % corresponding to an $E_T > 640$. A resolution of 1 on a 10 g scale yielded 2.4 g (84% of theory) of (*R*)-1 with >99% ee and 3.8 g (76% of theory) of recovered (*S*)-1-diacetate with >99% ee. The reaction mixture also contained 1-monoacetate (3.7 mol %) with 94.6% ee *R*, but this material was not isolated.

Discussion

The optimum sequential kinetic resolution has (1) equal rates for the two steps and (2) the highest possible value for $E_{T(max)}$. To optimize the resolution of 1, we added a hexane phase to selectively extract the faster reacting 1-diacetate and screened enzymes to find PCL that has an $E_{T(max)} > 2000$.

Most reported sequential kinetic resolutions have not been carried out under conditions where the rates are equal and this was the reason for the lack of reinforcement of enantioselectivities of the two steps. In other cases where a sequential resolution was possible, the second step was so slow that it was ignored; consequently, the multiplication of enantioselectivity was not exploited.[22] These resolutions could be improved by changing experimental conditions to equalize the rates of the two steps. Multiplicative enhancement of overall enantioselectivity is the biggest advantage of a sequential kinetic resolution.

Another advantage is that both starting material and product can be obtained with high ee at the same extent of conversion. In single-step resolutions, perfect separation

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rarely occurs and one optimizes the enantiomeric purity of either the product, by stopping the reaction at ≤45% conversion, or the enantiomeric purity of the starting material, by stopping the reaction at ≥55% conversion. For sequential kinetic resolution, this compromise may not be necessary since the impure material remains in the monoester fraction. In the PCL-catalyzed resolution of 1, both starting material and product were isolated >99% ee, while the monoester fraction, 94.6% ee, contained the mistakes.

Sequential kinetic resolutions have several potential applications. They lend themselves to molecules with C_2 symmetry which are important as chiral auxiliaries. Auxiliaries with C_2 symmetry often give higher enantioselectivity than less symmetrical auxiliaries since there are fewer competing diastereomeric transition states.[13] High enantiomeric purity is crucial for a chiral auxiliary, and the multiplicative enhancement of enantioselectivity may simplify the preparation of these molecules. Sequential kinetic resolutions should also be useful for resolution of chiral monomers used for the synthesis of chiral polymers because these molecules also contain two functional groups that can be transformed. A strategy to use sequential kinetic resolutions on molecules with only one functional group[5] may further extend the utility of these resolutions.

Experimental Section

General. 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.). Racemic 1 was purchased from Produits Chimiques Omega Inc. (Québec City, PQ). Pyridine was distilled from BaO and stored over KOH. Melting points were measured in open capillaries and are uncorrected. Enzymes and enzyme assays have been described previously.[23]

Equations for Sequential Kinetic Resolutions. A spreadsheet program was used to calculate the amounts of each species for given values of E_1, E_2, and S using the following equations

$$[A_1] = ([A_2]/(1 - k_2/k_1))(([A_2]/[A_2]_0)^{(k_2/k_1-1)} - 1)$$

$$[A_0] = (1/(1 - k_2/k_1))(k_2[A_2]/k_1 - [A_2]^{(k_2/k_1)}/[A_2]_0^{(k_2/k_1-1)}) + [A_2]_0$$

$$[A_2] = ([B_2]/[B_2]_0)^{k_1/k_3}[A_2]_0$$

$$k_3 = k_1/E_1 \qquad k_4 = (k_1 + k_3)/S_1(1 + E_2) \qquad k_2 = k_4E_2$$

where $[A_2]_0$, which represents the initial concentration of A_2, and k_1 were set to 1.

Racemic *trans*-1,2-Diacetoxycyclohexane, 1-Diacetate. Acetic anhydride (7.9 g, 78 mmol) was added over a period of 15 min to a solution of racemic 1 (3.0 g, 26 mmol) in dry pyridine (50 mL). The mixture was stirred 24 h and poured into 200 mL of ice–water. The aqueous phase was extracted with $CHCl_3$ (3 × 100 mL) and the combined organic extracts were washed with aqueous HCl (1 N, 3 × 100 mL), saturated $NaHCO_3$ (3 × 100 mL), and water (3 × 100 mL). The solution was dried over $MgSO_4$ and concentrated. Kugelrohr distillation under water aspirator vacuum afforded a colorless oil, 4.4 g (86%): bp 67 °C (10 mm) [lit.[24] 120 °C (12 mm)]; ^{1}H NMR δ 4.82 (m, 2), 2.03 (m, 8), 1.75 (m, 2), 1.38 (m, 4).

Racemic *trans*-1-Acetoxy-2-cyclohexanol, 1-Monoacetate. Acetic anhydride (26.4 g, 0.26 mol) was added over a period of 15 min to a solution of racemic 1 (10 g, 86 mmol) in ethyl acetate (300 mL) containing suspended Na_2CO_3 (27.4 g, 0.26 mol). After 14 h, the mixture was poured into 200 mL of ice–water, and the phases were separated. The aqueous phase was extracted with ethyl acetate (2 × 200 mL), and the combined organic extracts were dried over $MgSO_4$. Separation on silica gel eluting with a 33–100% gradient of ethyl acetate in hexane followed by Kugelrohr distillation under water aspirator vacuum gave a colorless oil which solidified, 8.1 g (59%): mp 39–40 °C; ^{1}H NMR δ 4.59 (m, 1), 3.58 (m, 1), 2.32 (d, J = 4.5 Hz, 1, exchanges with D_2O), 2.12 (s, 3), 2.04 (m, 2), 1.72 (m, 2), 1.31 (m, 4) [lit.[25] (80 MHz) δ 4.90 (m, 1), 3.46 (m, 1), 2.98 (m, 1, exchanges with D_2O), 2.01 (s, 3), 1.80–1.09 (m, 8)].

Quantitative Analysis of Enzyme-Catalyzed Hydrolyses. A suspension of PLE in ammonium sulfate (10–50 μL, 24–120 units with ethyl butyrate as substrate) was added to a solution of 1-diacetate (50 mg, 0.25 mmol) in phosphate buffer (10 mM, pH 7.0, 50 mL). The mixture was stirred vigorously at 25 °C, and the pH was maintained at 7.0 by automatic titration with NaOH (0.10 N) with a pHstat. The reactions were stopped after different extents of conversion by extraction with ethyl acetate (10 × 50 mL). The combined extracts were dried over $MgSO_4$ and concentrated first by rotary evaporation and second on a vacuum line. The extract was not left on the vacuum line for more than 1 min because 1-diacetate is volatile. The relative amounts of 1-diacetate, 1-monoacetate, and 1 were determined by integration of the ^{1}H NMR signals for the CH group. To determine the enantiomeric purities, the three species were separated by chromatography on silica gel eluted with a 33–100% gradient of ethyl acetate in hexane: R_f = 0.65, 0.30, and 0.06 for 1-diacetate, 1-monoacetate, and 1, respectively, on TLC eluted with 1:1 ethyl acetate/hexane. A sample of 1-monoacetate and 1 were benzoylated[26] and purified by TLC: 1-monoacetatemonobenzoate, R_f 0.33, toluene/ethyl acetate (50:1); 1-dibenzoate, R_f 0.42, hexane/ethyl acetate (5:1). The enantiomeric benzoates were separated by HPLC using a ChiralPak OT column[27] (Daicel Chemical Co., Fort Lee, NJ) eluted with methanol at 0.35–1.5 mL/min and 5 °C. The enantiomeric purity of the 1-diacetate was determined in the same manner after hydrolysis with NaOH (2 mL, 0.5 N in 1:1 water/ethanol) for 10 min followed by standard workup. Occasionally 1-monoacetate was hydrolysed to 1 before analysis since the enantiomers of 1-dibenzoate are better separated than 1-monoacetatemonobenzoate. Optical purity as high as 99.0% could be measured directly by HPLC. To determine an enantiomeric excess >99.8% of the HPLC trace of unknown was compared to an HPLC trace containing 0.2% of deliberately added racemate.

Relative Rates. A suspension of PLE in ammonium sulfate solution (50 μL, 120 units with ethyl butyrate as substrate) was added to a vigorously stirred solution of (±)-1-diacetate (50 mg, 0.25 mmol) and (±)-1-monoacetate (158 mg, 1.0 mmol) in phosphate buffer (10 mM, pH 7.0, 25 mL) at 25 °C. Both substrates dissolved completely. The pH was maintained at 7.0 by automatic titration with NaOH (0.10 N) with a pHstat. The reaction was stopped after 0.082 mmol of NaOH was consumed corresponding to hydrolysis of 5.5% of the acetate groups. The products were extracted and their relative amounts determined by ^{1}H NMR as above. The mole fractions of 1-diacetate, 1-monoacetate, and 1 were 14.3, 85.1, and 0.6, respectively; thus, the relative rate was 9.5. During the reaction, the average value for [1-m]/[1-d] was 5.0, thus, S = 47.

Partition Coefficients. Buffer saturated with hexane (5.0 mL) was added to a solution of 1-diacetate (200 mg, 1.0 mmol) in hexane saturated with buffer (5.0 mL). The mixture was stirred for 5 min, then the hexane phase was separated, dried over $MgSO_4$, and filtered. Evaporation of the solvent yielded 1-diacetate (177 mg, 0.89 mmol), thus $K_{p1-d} = [1\text{-d}]_{hexane}/[1\text{-d}]_{aq}$ = 7.7. In a similar manner, K_{p1-m} was determined to be 0.082. In toluene, K_{p1-d} = 19 and K_{p1-m} = 1.0; in diethyl ether, K_{p1-d} = 3.2 and K_{p1-m} = 2.4.

Resolution of 1. A mixture of 1-diacetate (10 g, 50 mmol), hexane (250 mL), phosphate buffer (10 mM, pH 7.0, 250 mL), and sodium chloride (150 g, not completely dissolved) was stirred vigorously with a magnetic stirrer, and the pH was readjusted to 7.0 with NaOH (0.50 N). Lipase Amano P30 (PCL, 2.0 g) was

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added and the pH kept neutral (pH paper) by addition of NaOH (0.50 N). After 7 days, 98 mL (49 mmol) of the alkaline solution had been consumed. The aqueous phase containing mostly 1 was separated and extracted continuously with diethyl ether (175 mL). The ethereal extract was concentrated to a solid (>99% ee) which was recrystallized from hot toluene (10 mL) to afford crystalline (*R*)-1, 2.4 g (42%, 84% of theory): mp 107.5–108.5 °C (lit.[28] 113–114 °C from acetone/hexane); ee > 99.8%; $[\alpha]_D^{20}$ = −35.0° (*c* 0.30, CH_3OH) [lit.[29] −36.9° (H_2O)]; ^1H NMR δ 3.68 (br s, 2), 3.31 (m, 2), 1.94 (m, 2), 1.69 (m, 2), 1.23 (m, 4). The hexane phase containing mostly 1-diacetate was dried over $MgSO_4$, filtered, and concentrated. Purification on silica gel eluted with 5:1 hexane/ethyl acetate followed by Kugelrohr distillation under water aspirator vacuum gave (*S*)-1-diacetate as a colorless oil, 3.8 g (38%, 76% of theory), >99% ee; $[\alpha]_D^{20}$ = +16.1° (*c* 0.42, CH_3OH) [lit.[29] +12.4° ($CHCl_3$)]; ^1H NMR δ 4.82 (m, 2), 2.03 (m, 8), 1.75 (m, 2), 1.38 (m, 4). The monoester (3.7 mol %, 94.6% ee *R*) was present in both the aqueous and hexane phases as shown by TLC.

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Registry No. (*R*)-1, 1072-86-2; (*S*)-1, 57794-08-8; (±)-1, 54383-22-1; (±)-1 diacetate, 79416-44-7; (±)-1 monoacetate, 62921-46-4; esterase, 9013-79-0; cholesterol esterase, 9026-00-0; lipase, 9001-62-1.

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Calixarenes. 26. Selective Esterification and Selective Ester Cleavage of Calix[4]arenes

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Methods have been developed for converting *p-tert*-butylcalix[4]arene (**1a**) in high yield to the 25-monoester **7**, the 25,26-diesters **5** and **6**, the 25,27-diester **4a**, and the 25,26,27-triesters **2** and **3** in which the aryl groups are 3,5-dinitrophenyl moieties. Concomitantly, methods have emerged whereby these esters can be selectively cleaved or rearranged. By appropriate choice of reaction conditions the 25,27-diester **4a** can be selectively cleaved with imidazole bases to the monoester **7** or rearranged to the 25,26-diester **5**; the triesters **2** and **3** can be converted to their conformationally related 25,26-diesters **5** (a chiral compound) and **6**. The effects of variations in solvent, organic base, and reaction time on the conversion of **4a** to **7**, along with semiquantitative kinetic data, suggest that two or more molecules of the imidazole are involved in the activated complex of the rate-determining step in the aminolysis. These syntheses provide easy access to the mono-, di-, and triesters, thus expanding the techniques for obtaining selectivity para-substituted calixarenes via selective de-*tert*-butylation. Thus, removal of three, two, or one *tert*-butyl group, respectively, from the monoester **7**, the diesters **4a** and **5**, and the triester **2** yields the corresponding mono-, di-, and tri-*tert*-butylated analogues **12a–d**. Acylation of **12b**, for example, can be effected at the vacated para positions to produce the diacylcalix[4]arenes **15a** and **15b**. Collectively, the system provides an example of how careful control of reaction conditions can be used to advantage in determining product formation.

As part of a program involving the synthesis of "double cavity" calixarenes[1] the 25,27-bis(3,5-dinitrobenzoyl) ester of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrahydroxycalix[4]arene (generally abbreviated as *p-tert*-butylcalix[4]arene) (**1a**) has been prepared and its complexation properties studied. Instead of forming a complex with the putative guest imidazole, however, the ester undergoes cleavage, thereby providing the starting point for the present investigation, which has led to an interesting sequence of events that makes possible the selective esterification of calix[4]arenes and the selective cleavage of esterified calix[4]arenes. As frequently happens when a research program takes an unexpected turn, the scheme of subsequent events has not been orderly. The following discussion, therefore, is a nonchronological account of how the salient features of the final picture emerged. In this discussion the procedures for preparing various esters of calix[4]arenes are first presented, followed by a commentary on the conformations of the esters, a detailed consideration of the amine-induced cleavage of the 25,27-diester, and a conclusion that summarizes the ester forming/ester cleaving processes that can be induced by the proper choice of reaction conditions.

Synthesis of 3,5-Dinitrobenzoates of *p-tert*-Butylcalix[4]arene. The procedures that are outlined below represent an end result rather than a starting point in the present investigation. They are the harvest partly of serendipity and partly of rational designs based on the various observations that are described in later sections of this paper.

25,26,27-Tris(3,5-dinitrobenzoates). *p-tert*-Butylcalix[4]arene (**1a**) was shown by Gutsche and Lin[2] to react with benzoyl chloride in the presence of pyridine to yield the tribenzoate of **1a**. 3,5-Dinitrobenzoyl chloride behaves in a qualitatively comparable fashion to yield a mixture containing some of the triester. Much more effective as the base, however, is 1-methylimidazole in acetonitrile solution which gives a 90% yield of the cone conformer of 25,26,27-tris((3,5-dinitrobenzoyl)oxy)-28-hydroxy-

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