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Kinetic Resolutions Concentrate the Minor Enantiomer and Aid Measurement of High Enantiomeric Purity.

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Abstract: Although many methods can measure enantiomeric purity, only a few can measure high enantiomeric purity, >98% ee, because in most methods the signal for the major enantiomer overwhelms the signal for the minor enantiomer. We use a kinetic resolution to concentrate the minor enantiomer into the product and thereby extend the ability of all existing techniques to measure high enantiomeric purity. The original enantiomeric purity is calculated using the enantioselectivity of the kinetic resolution and the extent of conversion. We verified this method with samples of (1S)-menthol using an acetylation with vinyl acetate catalyzed by lipase from *Candida rugosa*. The enantiomeric purities determined by capillary gas chromatography directly and after kinetic resolution agreed for samples with 90-99.9% ee. Error analysis suggests that the usual accuracies for conversion and enantiomeric ratio are sufficient for accurate determination of enantiomeric purity with this method. In another example, we used a kinetic resolution followed by a simple optical rotation measurement to accurately quantify 98.5% ee for a commercial sample of (S)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid (naproxen). Thus, this kinetic resolution method allows simple techniques such as optical rotation to measure high enantiomeric purity.

The growing appreciation for the differing biological effects of enantiomers has increased interest in the synthesis and analysis of enantiomerically-pure materials, especially those with high enantiomeric purity, >98% ee. For example, the (R)-enantiomer of a Japanese beetle pheromone attracts male beetles, while the (S)-enantiomer interferes, even as a 1% impurity.[1] Thus, pheromone researchers must accurately measure the enantiomeric purity of their samples. New regulatory guidelines require researchers in the pharmaceutical industry to measure the biological effects of each enantiomer.[2] Traces of the minor enantiomer can mask the effects of the major enantiomer, especially when measuring the absence of a biological effect. For example, the enantiomers of α-(*p*-chlorophenoxy)propionic acid show opposite effects on the chloride conductance, and a 1.5% impurity of the more potent (R)-enantiomer can distort the effect of the (S)-enantiomer.[3] Manufacturing labs must also measure high enantiomeric purity to control quality. Synthetic chemists must measure high enantiomeric purity during the development of new enantioselective reactions. Even natural product chemists measure the enantiomeric purity of isolated samples due to the growing realization that many natural products are not enantiomerically-pure.[4]

Most methods to determine enantiomeric purity are not suitable for samples with high enantiomeric purity, >98% ee.[5,6] Optical rotation methods would require high chemical purity and better than 0.1% precision to measure this level of enantiomeric purity. Methods that require derivatization of the unknown with a chiral reagent are limited by the enantiomeric purity of the chiral reagent, possible racemization during the derivatization reaction, as well as possible kinetic resolution during the derivatization reaction.

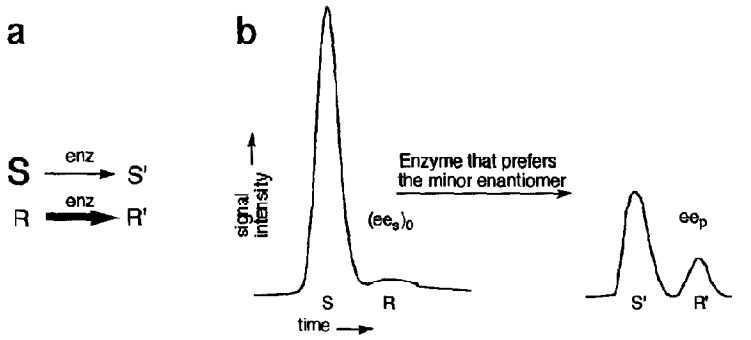


Figure 1. Kinetic resolution of an (S)-enantiomer-enriched sample with an enzyme that favors the (R)-enantiomer. **a)** Schematic of the kinetic resolution. The (R)-enantiomer reacts faster; thus, the reaction enriches the product in the (R)-enantiomer relative to the starting material. **b)** Hypothetical chromatograms to measure the enantiomeric purity of the initial starting material, $(ee_S)_O$, and the product after kinetic resolution, ee_P. Accurate integration of $(ee_S)_O$ may be difficult because the major peak overlaps the minor peak. The enantiomeric purity of the product, ee_P, is lower and therefore easier to measure.

The most convenient methods to determine high enantiomeric purity are chromatographic methods (HPLC or GC) using chiral stationary phases. When the resolution factor[7] is ≥1.5, these methods can measure enantiomeric purities of 98% ee, that is, 1% of the minor enantiomer.[8] Capillary GC often gives even higher resolution factors allowing routine measurement of 99.9% ee.[4b,9] However, with many chromatographic methods, the resolution factor is <1.5, causing the minor peak to overlap the major peak, and making accurate integration difficult. This problem is most severe in chromatography when a minor peak appears on the tail of the major peak.

Another convenient method to measure enantiomeric purity is NMR with chiral shift reagents.[10] In favorable cases, this method can measure enantiomeric purities >99% ee,[11] but the resolution and detection limits vary with each compound.

Other methods to determine high enantiomeric purity are less convenient. Radiotracer methods require isotopically labeled standards.[6] Enzymic methods require perfectly enantioselective enzymes, which are available only for a few compounds, mostly amino acids. Using this method, workers measured enantiomeric purities as high as 99.98% ee[12] for L-alanine and L-serine and as high as 99.8% ee for several other amino acids. Calorimetric methods detect a low-melting eutectic mixture caused by the presence of impurities and require a crystalline sample.[6] Although researchers measured >99.9% ee with this method,[13] it does not distinguish between chemical and enantiomeric purity.

In this paper we describe a method, based on an enzymic kinetic resolution, to concentrate the minor enantiomer into the product, Figure 1. This sieving removes much of the major enantiomer and extends the detection limit of existing methods to determine enantiomeric purity. The enantiomeric purity of the original material can be calculated from the enantioselectivity of the sieving enzyme, the degree of conversion, and the enantiomeric purity of the product. This method does not require perfectly enantioselective enzymes.

RESULTS

Theory

When an (R)-enantioselective enzyme acts on a sample containing both (R)- and (S)-enantiomers, it enriches the product with (R)-enantiomer. For an irreversible reaction that follows Michaelis-Menton kinetics, eq 1 gives

the relative rate of reaction of the two enantiomers (v_R/v_S), where $(k_{cat}/K_M)_R$ and $(k_{cat}/K_M)_S$ represent the specificity constants and [R] and [S] represent the concentrations of the fast- and slow-reacting enantiomers,

$$\frac{v_R}{v_S} = \frac{(k_{cat}/K_M)_R[R]}{(k_{cat}/K_M)_S[S]} = E\frac{[R]}{[S]} \tag{1}$$

respectively.[14] The ratio of the specificity constants is the enantiomeric ratio, E. This equation is the starting point for all quantitative analyses of kinetic resolutions.

At a low extent of conversion, the ratio of enantiomers in the product, [R']/[S'], is given approximately by eq 2, where $[R]_o/[S]_o$ represents the initial ratio of the two enantiomers in the starting material. This expression shows that the resolution enriches the product in (R)-enantiomer by approximately a factor of E.

$$\frac{[R']}{[S']} \sim E\frac{[R]_o}{[S]_o} \tag{2}$$

This equation shows that a kinetic resolution enriches the product fraction with the favoured enantiomer by a factor of E. This enrichment can concentrate a small amount of the minor enantiomer into the product fraction. This concentration procedure can extend the detection limit of the minor enantiomer by a factor of 50 or more. For example, if the detection limit of the minor enantiomer is 1 part in 50 or 96.1% ee,[5] then the maximum value of E that can be accurately measured is ~50. A kinetic resolution with an enzyme that has an enantiomeric ratio of 50 enriches the product fraction in the minor enantiomer by a factor of 50; thus the new detection limit is 1 part in $(50)^2$ or 99.92% ee. Similarly, if the original detection limit is 1 part in 200 or 99.0% ee, then concentration of the minor enantiomer with a kinetic resolution can extend the detection limit to 1 part in $(200)^2$ or 99.995% ee.

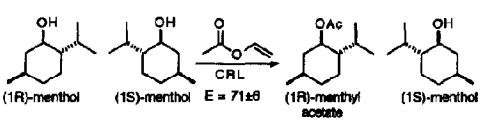
Chen, *et al.*[15] derived an exact form of eq 2 by integration of eq 1 and substitution of experimentally measurable variables: $(ee_s)_o$, the initial enantiomeric purity of the starting material; ee_p, the enantiomeric purity of the product fraction; and c, the extent of conversion, eq 3. We use this equation for all the quantitative analyses

$$\left[1 - c\left(\frac{1+ee_p}{1+(ee_s)_0}\right)\right] = \left[1 - c\left(\frac{1-ee_p}{1-(ee_s)_0}\right)\right]^{\frac{1}{E}} \tag{3}$$

in this paper. We calculated the unknown, $(ee_s)_o$, by an iterative solution of eq 3 using measured values of ee_p and c and a value of E determined in a separate experiment. Note that this equation describes kinetic resolutions that favour the minor enantiomer in the starting material. To describe kinetic resolutions that favour the major enantiomer,replacethe exponent 1/E by E.

(1S)-Menthol

To test the accuracy of the sieving method, we compared the enantiomeric purity of samples of (1S)-menthol (unnatural menthol) determined directly to the enantiomeric purity measured by sieving with a kinetic resolution. The sieving reaction was an enantioselective acetylation of menthol with vinyl acetate catalyzed by lipase from *Candida rugosa* (CRL), eq 4. This reaction favored (1R)-menthol, thereby concentrating (1R)-menthol in the



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product, menthyl acetate. The enantioselectivity (enantiomeric ratio[15]) for this reaction was 71±6, similar to other esterifications[16] or transesterifications[17,18] of menthol in organic solvents. Hydrolysis of menthyl acetate in aqueous solution was less enantioselective.[17] We chose vinyl acetate as the acetylation reagent to ensure that the reaction was irreversible, as required by the assumptions made to derive eq 3.

Capillary gas chromatography using a Chiraldex G-TA column (modified γ-cyclodextrin stationary phase) resolved the enantiomers of both menthol and menthyl acetate, Figure 2a. The resolution factor for menthol was 2.0, allowing direct measurement of 97.5±0.5% ee for the commercial sample of (1S)-menthol, Figure 2b. We

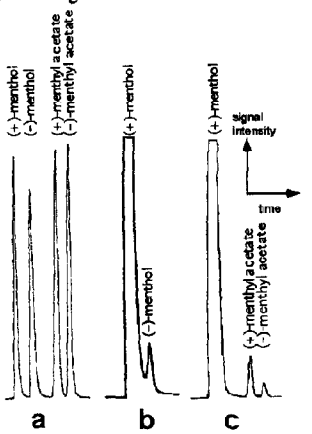


Figure 2. Determination of enantiomeric purity of a commercial sample of (1S)-menthol. **a)** Gas chromatogram showing the separation of enantiomers of menthol and menthyl acetate. Enantiomers were separated by capillary gas chromatography using a Chiraldex G-TA column (menthol: α = 1.050, resolution = 2.0; menthyl acetate: α = 1.034, resolution = 1.5). **b)** Direct determination of enantiomeric purity of a commercial sample of (+)-menthol. Integration of the peaks showed that this sample had 97.5±0.5% ee. **c)** Determination of enaniomeric purity of the same sample of (1S)-menthol using the sieving method. The sample in panel b was sieved by a CRL-catalyzed acetylation to 4.75% conversion. The measured enantiomeric purity of product menthyl acetate, ee_p, was 52.8% ee. The calculated enantiomeric purity of the commercial menthol sample, $(ee_s)_o$, was 97.6%, in good agreement with the direct measurement.

estimate that enantiomeric purities up to 99.5% ee (0.25% of the minor enantiomer) can be measured directly. The detection limit was ~0.01 mol%, but accurate integration at this level was difficult.

We concentrated the minor (1R)-enantiomer in this sample by the CRL-catalyzed kinetic resolution. At 4.75% conversion, we measured 52.8% ee for the product, (1S)-menthyl acetate, Figure 2c. A back-calculation using eq 3 showed that the original material had 97.6% ee. The average of seven such measurements was 97.7% ee with a range (2σ) of ±0.1%ee, in excellent agreement with the direct measurement.

We further tested this method with samples of (1S)-menthol having enantiomeric purities between 90 and 99.99% ee. We prepared known samples by adding a measured amount of racemate to 100% ee (1S)-menthol, which we prepared from the commercial (1S)-menthol by resolution with the same CRL-catalyzed acetylation. For each sample, we measured ee_p and c several times over the course of the sieving reaction and averaged the results, Table I. The values of ee_p were lowest at small extents of conversion (2-15%), so we made most measurements in this range. The known values, directly measured values, and values measured using the sieving reaction all agreed to within the error limits. Even at 99.99% ee, the sieving reaction gave values close to those expected. Note that potential errors in the preparation of this sample made it impossible to establish the true enantiomeric purity of this sample.

Estimate of errors

The effects of errors in E, ee_p and c on the calculated enantiomeric purity, $(ee_s)_o$, were calculated using eq 3 for typical experimental values, Figure 3. At a fixed conversion of 5%, changes in E had little effect on $(ee_s)_o$, Fig 3a. For example, at ee_p = 95%, an E of 71 gave $(ee_s)_o$ = 99.743% ee, while an E of 50 gave $(ee_s)_o$ = 99.728% ee. Similarly, changes in ee_p had little effect on $(ee_s)_o$. The slope of the plot of ee_p vs $(ee_s)_o$, Fig 3a, was approximately 0.05 indicating that an error of ±1% ee in ee_p leads to only a ±0.05% error in $(ee_s)_o$.

A different type of plot, Figure 3b, which includes experimental data for menthol as well as calculated curves,

Table I. Comparison of Enantiomeric Purities of (+)-Menthol Determined by GC both Directly and After Sieving the Enantiomers.[a]

$(ee_s)_o$ (%) weight	$(ee_s)_o$ (%)[b] direct integration	conversion (%)	ee_p (%)	$(ee_s)_o$ (%)[c] calculated
90±0.4	90.4±0.2	14.75	22.22	89±2
94.0±0.4	94.1±0.2	7.33	30.91	94.7±0.7
98.0±0.4	97.9±0.5	13.29	84.93	98.2±0.4[d]
e	97.5±0.5	4.63	53.93	97.7±0.1
99.0±0.3	98.7±0.3	5.31	78.57	98.8±0.3
99.99±0.2	99.7±0.2	4.00	97.43	99.92±0.1

[a] Measurement by capillary gas chromatography using a Chiraldex G-TA column. [b] Estimated errors in integration. [c] Average and twice the standard deviation for three to seven determinations. The %c and ee_p values listed are typical values. [d] Only two measurements were made; the error limit is an estimate. [e] Commercial sample, labeled 99% pure.

shows the effect of changes in c and ee_p on $(ee_s)_o$. A line calculated assuming $(ee_s)_o$ of 98.8 best fit the experimental data points. Lines calculated assuming that $(ee_s)_o$ was 99.1% ee or 98.5% ee clearly did not fit the experimental data. The lines show that a change of approximately ±1% in the conversion and a change of approximately ±5% in ee_p would still keep the calculated value of $(ee_s)_o$ within ±0.3% ee. Thus, the calculated enantiomeric purity is relatively insensitive to errors in the measured values of E, ee_p, and c. This sieving technique can accurately measure enantiomeric purity.

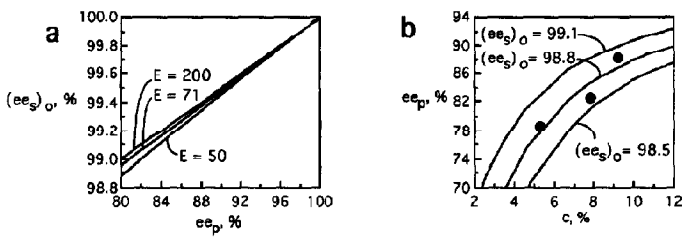
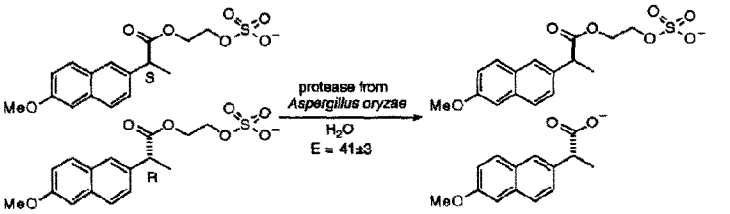


Figure 3. Effect of errors in the measured values of E, ee_p, and c on the calculated enantiomeric purity, $(ee_s)_o$. **a)** Effect of errors in ee_p and E on $(ee_s)_o$ while c is fixed at 5.0%, a typical value. The lines calculated using E = 50, 71, or 200 all lie near one another showing that changes in E have little effect on $(ee_s)_o$. The slope of the lines is approximately 0.05 indicating that a change in ee_p of 1% ee results in a change in $(ee_s)_o$ of only 0.05% ee. **b)** Effect of errors in ee_p and c on $(ee_s)_o$ while E is fixed at 71, a typical value. The calculated lines predict the positions of experimental points for three different values of $(ee_s)_o$. Experimental points (•) are best fit by $(ee_s)_o$ = 98.8. This value is not sensitive to experimental errors in ee_p and c because changes of ±5% in ee_p or ±1% in c would be needed to change $(ee_s)_o$ by ±0.3% ee.

(S)-(+)-Naproxen

Naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid) is a nonsteroidal antiinflammatory and an analgesic. The (R)-enantiomer is toxic to the liver; for this reason, the commercial product is the enantiomerically pure (S)-(+)-enantiomer. We applied the concentration method to the determination of enantiomeric purity of naproxen and demonstrated that optical rotation in combination with an enzyme catalyzed concentration of the minor enantiomer can accurately measure the enantiomeric purity of commercial samples of naproxen (98.5% ee).

To concentrate the minor enantiomer in commercial samples of (S)-naproxen, we required a kinetic resolution that favored the (R)-enantiomer. A literature search revealed that a protease from *Aspergillus oryzae*



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(AOP) favored the (R)-enantiomer in the enantioselective hydrolysis of water-soluble esters of naproxen,[19] eq 5.

We prepared racemic ethyl sulfate ester of naproxen and measured the enantioselectivity of the AOP-catalyzed hydrolysis. We measured the enantiomeric purity of the product, naproxen, by HPLC using a Chiralcel OD column eluted with hexane/isopropanol/formic acid (90: 9.5: 0.5). Our measured enantioselectivity, 41±3, was higher than the reported enantioselectivity of 15.5,[19] which was measured under slightly different conditions.

To measure the enantiomeric purity of a commercial sample of naproxen, we prepared the ethyl sulfate ester and concentrated the (R)-enantiomer with AOP-catalyzed hydrolysis to 4.0% conversion. The naproxen was separated from remaining starting material by extraction. An optical rotation of this sample showed $[\alpha]_D = +47.5$, indicating an enantiomeric purity of 72%; enantiomerically-pure naproxen showed $[\alpha]_D = +66°$.[20] Back calculation using eq 3 indicated that the original sample contained 98.5% ee. An alternate method (HPLC of the methyl ester) indicated that this sample was 98.5% ee, in excellent agreement with the enzyme-catalyzed concentration method.

DISCUSSION

We described a method that extends the detection limit of all existing methods to measure enantiomeric purity. This method uses a kinetic resolution to concentrate the minor enantiomer, making it easier to measure. The original enantiomeric purity can be accurately calculated from the extent of conversion and the enantioselectivity of the kinetic resolution. Although we were initially concerned that the propagation of errors would render this approach useless, we found that errors in E, conversion, and % ee of the product did not create serious errors in the calculated values of enantiomeric purity. Normal care in the determination of these values is sufficient. The simplest way to estimate errors is to substitute different values in eq 3 and observe the effect on the final value.

In order for the concentration method to be accurate, the assumptions made in the derivation of eq 3 must be valid. These assumptions are indeed valid for most kinetic resolutions, but not for reversible reactions[21] and those that follow more complex kinetics.[22] Even for these reactions, the deviations at low conversions appear small and it may still be possible to use the same method, but we did not examine this possibility.

Kinetic resolutions[23] have been used to calculate the specific rotation of enantiomers[24] and to determine very low enantiomeric purity (0.2 - 1% ee).[25] As far as we know, ours is the first application of kinetic resolution to aid measurement of high enantiomeric purity.

One disadvantage of our method is that it requires extra steps: kinetic resolution and isolation of the product.

Recently, Zhang and Wainer[26] coupled an enzyme reactor containing an immobilized lipase to an analytical HPLC column. If this enzyme reactor was used for the kinetic resolution, then both the concentration and analysis steps could be carried out in a single, automated procedure.

Although calculation of the original enantiomeric purity requires an iterative solution to eq 3, we also used an approximate method for quick and dirty calculations. We assumed that the sieving reaction had converted *all* of the minor enantiomer to product. From the area of the minor enantiomer in the product and the area of all other species, we estimated $(ee_S)_O$. This method provides a good estimate of $(ee_S)_O$ when the enantioselectivity is high, but it does not replace eq 3, which gives the exact value.

The sieving method described in this paper requires an enzyme that favors the minor enantiomer. Another sieving method, one that uses an enzyme that favors the major enantiomer, is also possible. In this case, the enantiomeric purity of the remaining starting material decreases as the reaction proceeds. We estimate that this method would give a similar enhancement in the dynamic range of ee measurements.

EXPERIMENTAL SECTION

General. Chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, unless otherwise noted. Lipase from *Candida rugosa* (L-1754, 0.16 units/mg solid using olive oil, also known as *C. cylindracea*[27]) and protease from *Aspergillus oryzae* (Type XXIII, P 4032, activity 0.008 units/mg solid for hydrolysis of (±)-naproxen ethyl sulfate ester) were purchased from Sigma Chemical Co., St. Louis, MO. One unit of enzymic activity corresponds to one μmol of ester hydrolyzed per min. The Solver function of Excel software (v 4.0, Microsoft, Redmond, WA) was used to solve eq 3 by iteration.

Gas chromatographic analysis of menthol and menthyl acetate. Enantiomers of menthol and menthyl acetate were separated by capillary gas chromatography on a 30 m Chiraldex G-TA (Advanced Separation Technologies, Whippany, NJ) which contained 2,6-di-*O*-pentyl-3-*O*-trifluoroacetyl derivative of γ-cyclodextrin as the chiral stationary phase. Conditions: 1 μL injection, split ratio 100: 1, 80 °C, flame ionization detector. Peak areas for the data in Table 2 were measured by cutting and weighing the peaks. To maximize accuracy, the peak for the major enantiomer was cut from a chromatogram printed with high attenuation, while the peak for the minor enantiomer was cut from the same chromatogram printed with low attenuation. We used the cut and weigh technique because electronic integration did not accurately detect the beginning and end of the peaks. To calculate the percent conversion, the area of the menthyl acetate peaks were divided by 1.122 to account for the different response of the FID detector for menthol and menthyl acetate.

Enantioselectivity of CRL-catalyzed acetylation of (1S)-menthol. CRL powder (0.10 g) was suspended in a solution of (±)-menthol (0.153 g, 1.0 mmol) in vinyl acetate (3 mL) and stirred at room temperature. Periodically, an aliquot of the reaction mixture (0.1 mL) was removed, filtered through a plug of cotton to remove enzyme and concentrated with stream of nitrogen. The residue was dissolved in anhydrous ethyl ether (2 mL) and analyzed by capillary GC. A typical analysis showed 95.8% ee at 26.1% conversion, E = 65. The average enantiomeric ratio was 71 with an estimated error limit of ±6.

Preparation of 100% ee (1S)-menthol. CRL powder (0.65 g) was suspended in a solution of (1S)-menthol (Aldrich, 0.50 g, 3.2 mmol) in vinyl acetate (10 mL) and stirred at room temperature for three days, approximately 10% conversion by GC. The reaction mixture was filtered through a cotton plug in a Pasteur pipette to remove enzyme. The filtrate was concentrated to an oil by rotary evaporation and purified by flash chromatography on silica gel. The column was eluted with two column volumes of hexane, then 10: 1 hexane: ethyl acetate. The fractions containing menthol were pooled and concentrated by rotary evaporation to oily crystals. The crystals were dried on a vacuum pump to give a solid mass of white crystals, 0.1 g, 20% yield. No (1R)-menthol could be detected by capillary GC, estimated limit of detection was 0.01 mol%. Based on an initial enantiomeric purity of 97.5% ee, an enantioselectivity of 71 for this reaction and 10% conversion, the enantiomeric purity calculated using eq 3 was

99.997% ee.

Sieving of menthol using CRL-catalyzed acetylation. CRL powder (400 mg) was suspended in a solution of (1S)-menthol (0.625 g, 4.00 mmol) in vinyl acetate and stirred at room temperature for three days. Periodically, aliquots of this reaction mixture (0.1 mL) were removed, filtered through a plug of cotton to remove enzyme and concentrated with stream of nitrogen. The residue was dissolved in anhydrous ethyl ether (2 mL) and analyzed by capillary GC.

Ethylene sulfate (1,3,2-dioxathiolane, 2,2-dioxide) was prepared by a modification of the method of Gao and Sharpless.[28] A 1-L three-necked round-bottom flask equipped with a dropping funnel, a sodium hydroxide trap and a stopper was charged with 1,2-ethanediol (12.4 g, 0.20 mol) and carbon tetrachloride (200 mL). Thionyl chloride (20 mL, 0.27 mol) was added from the dropping funnel over a period of 1 hour. The solution was stirred another hour and the solvent was evaporated under reduced pressure. The yellowish liquid was distilled under reduced pressure to afford the sulfite ester as a colorless oil (15 g, 70%). ^{1}H-NMR ($CDCl_3$, 200 MHz): d 4.30 (m, 2 H), 4.62 (m, 2 H). A solution of the sulfite ester (5.4 g, 50 mmol) in acetonitrile (50 mL) was cooled in an ice bath and $RuCl_3 \cdot 3H_2O$ (100 mg, 0.38 mmol), sodium periodate (16 g, 75 mmol) and cold water (75 mL) were added. The mixture was stirred for 5 min. Ethyl acetate (400 mL) and satd sodium bicarbonate (40 mL) were added and the two phases were separated. The aqueous phase was extracted with ethyl acetate (2 x 100 mL) and the combined organic extracts were washed with water (30 mL), dried over anhydrous sodium sulfate and filtered. Evaporation of the solvent under reduced pressure afforded ethylene sulfate as a white solid, 5.6 g, 70% yield. This crude product was used for synthetic reactions. A sample was recrystallized from ethyl acetate/petroleum ether; mp 96-97 °C. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 4.72 (s).

(S)-Naproxen ethyl sufate ester was prepared by a modification of the method of Dodds, *et al.*[19] Potassium *t*-butoxide (0.31 g, 2.8 mmol) followed by ethylene sulfate (0.35 g, 2.8 mmol, in 5 mL of warm 2-propanol) were added to a solution of (S)-naproxen (0.58 g, 2.5 mmol) in 20 mL of warm (~45 °C) 2-propanol. A solid formed immediately after ethylene sulfate addition. The mixture was stirred for 10 min and ether (50 mL) was added. The product was filtered and washed with ether (3 x 5 mL) affording a white solid (0.75 g, 80%); mp 176.5-178.5 °C. ^{1}H-NMR (D_2O, 200 MHz): δ 1.29 (d, 3 H, 7.2 Hz), 3.61 (s, 3 H), 3.70 (q, 1 H, 7.2 Hz), 4.11 (m, 4 H), 6.90 (m, 2 H), 7.13 (d, 1 H, 7.0 Hz), 7.45 (m, 3 H).

Racemic naproxen. A solution of (S)-naproxen (4.0 g, 17 mmol) in KOH (3 N, 100 mL) was refluxed for 18 h. The solution was cooled and then neutralized by dropwise addition of conc HCl (25 mL) to the well-stirred solution. The resulting white precipitate was filtered, washed with water (20 mL) and dried under reduced pressure. Recrystallisation from acetone/hexane afforded racemic naproxen (3.5 g, 88%); mp 159-160 °C. ^{1}H-NMR (D_2O, 200 MHz) was identical to that of (S)-naproxen: δ 1.68 (d, 3 H, 7.2 Hz), 4.0 (m, 4 H), 7.22 (m, 2 H), 7.50 (d, 1 H, 7.0 Hz), 7.78 (m, 3 H). HPLC analysis showed equal amounts of both enantiomers.

Enantiomeric purity of naproxen by HPLC. Enantiomers of naproxen were separated by HPLC using a Chiralcel OD chiral column eluted with hexane/isopropanol/formic acid (90: 9.5: 0.5) at a flow rate of 0.75 mL/min: $\alpha = 1.07$, $R = 1.4$, (R)-enantiomer eluted first. Enantiomers of naproxen methyl ester were separated on the same column eluted with hexane/isopropanol (90: 10) at a flow rate of 0.50 mL/min: $\alpha = 1.10$, $R = 2.0$, (R)-enantiomer eluted first. Peaks were detected by UV absorbance at 254 nm.

Enantiomeric ratio of protease from Aspergillus oryzae. The potassium salt of racemic naproxen ethyl sulfate (mp 166-168 °C) was prepared as above for the optically pure (S) enantiomer. A sample of this ester (75 mg, 0.20 mmol) was dissolved in phosphate buffer (10 mL, 10 mM, pH 7.0) and AOP (75 mg) was added. The pH was kept constant during enzyme hydrolysis through addition of sodium hydroxide 0.10 N using a pH stat. The reaction was stopped after 1.5 h at 26% conversion (0.53 mL of NaOH consumed). The pH was lowered to 3.0 with HCl 1 N and the

solution was extracted with chloroform (2 x 3 mL). The organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated. The optical purity was found by HPLC to be 93.5±1.0% ee; therefore, E = 41±3.

Enantiomeric purity of (S)-naproxen by optical rotation. The potassium salt of (S)-naproxen ethyl sulfate (0.75 g, 2.0 mmol) was dissolved in phosphate buffer (100 mL, 10 mM, pH 7.0) and AOP (0.30 g) was added. The pH was kept constant during enzyme hydrolysis through addition of sodium hydroxide 0.10 N using a pH stat. The reaction was stopped after 2.3 h at 4.0% conversion (0.80 mL of NaOH consumed). The pH was lowered to 3.0 with HCl (1 N) and the solution was extracted with chloroform (2 x 10 mL). The organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated. $[\alpha]_D^{20} = +47.5$ (*c* 2.2, $CHCl_3$) [lit.[20] 66 ($CHCl_3$)]. Back calculation using eq 3 and a E of **41** gave 98.5% ee. Direct measurement of the enantiomeric purity of naproxen methyl ester (prepared by acid-catalyzed esterification in methanol) using HPLC gave 98.5% ee.

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