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CONVENIENT PRACTICAL RESOLUTION OF RACEMIC ALKYL-ARYL ALCOHOLS VIA ENZYMATIC ACYLATION WITH SUCCINIC ANHYDRIDE IN ORGANIC SOLVENTS

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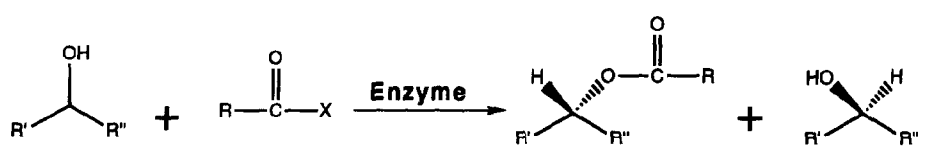
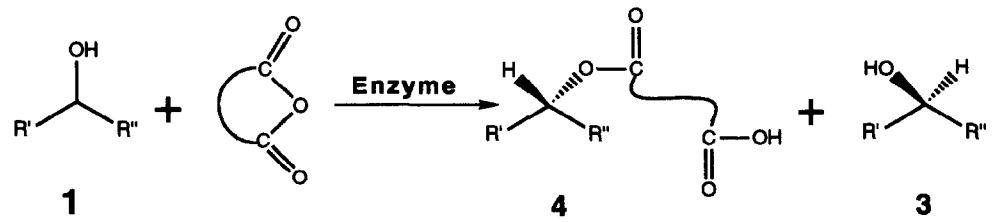
Abstract: Enantiomerically pure alkyl-aryl secondary alcohols were conveniently obtained on a kilogram scale from their racemic mixtures by enzymatic acylation with succinic anhydride in organic solvents. A major advantage of this acylation method is the ease of separating the ester from the unreacted alcohol. This is achieved by extracting the organic solution with aqueous $NaHCO_3$ after the enzymatic reaction is completed.

Chiral secondary alcohols are very common as intermediates and are valuable chiral auxiliaries in organic synthesis, and are of great synthetic utility for the pharmaceutical and the fine-chemical industries.[1] Unlike the chiral acids or amines, which can be resolved via diastereomeric crystallisation of appropriate salts, alcohols do not form salts. Hence, chiral alcohols cannot be obtained easily by this direct method. It is therefore not surprising that much effort has been devoted to the enzymatic resolution of racemic alcohols. Enzymatic hydrolysis of the corresponding racemic esters has been used extensively for this purpose.[2] The discovery of enzymatic catalysis in organic solvents[3] and Klibanov's first reports on lipase catalysed stereoselective esterifications and transesterifications,[4] prompted a great deal of work on the kinetic resolution of racemic secondary alcohols via enzymatic acylation in organic solvents. The acylating agents included carboxylic acids,[4] various activated and enol esters,[5] or acyclic aliphatic acid anhydrides.[6] The enzymatic reactions amounted to conversion of one enantiomer of the alcohol (1) into an ester (2) with the other one remaining as the unreactive alcohol (3) (Scheme 1). Separation of the unreacted alcohol (3) from the ester (2) (followed by hydrolysis of the latter), enabled to resolve racemic mixtures into their enantiomers.



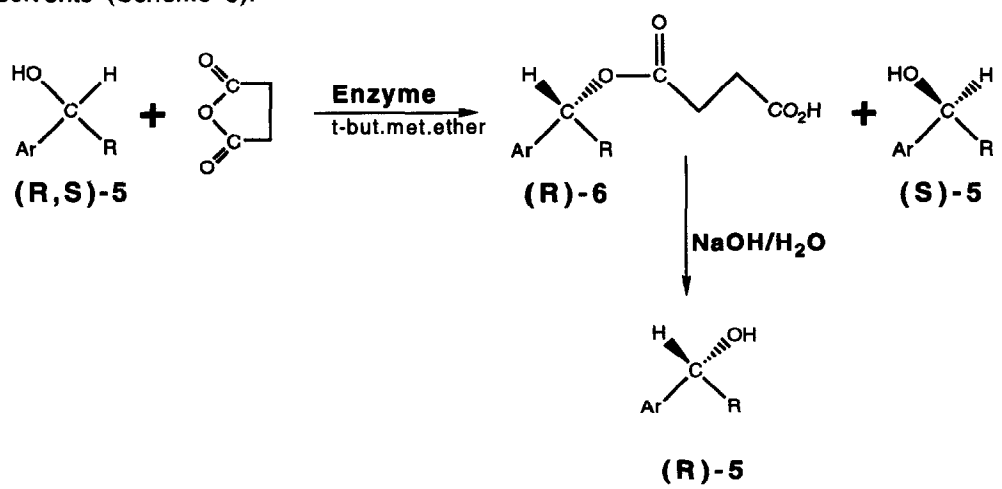
Scheme 1

A major problem with this approach is the difficulty of separating the ester from the unreacted alcohol. In many cases both have similar boiling points, making it impossible to separate them by distillation, and tedious chromatographic separation becomes necessary. This makes the procedure impractical for large scale preparation. This problem may be solved, in principal, by choosing a cyclic anhydride as an acylating agent. Thus, enzymatic acylation according to Scheme 2, if stereospecific, would convert one enantiomer of the alcohol into the half acid (4) which can be separated from the unreacted alcohol (3) by extraction with an aqueous base. The feasibility of this approach has recently been investigated for the preparation of enantiomerically enriched glycerol derivatives[7] and substituted cyclobutylidenethanols.[8]



Scheme 2

As an extension of our studies on enzymatic reactions in organic solvents,[9] in the present work we undertook to explore this approach with a view to developing a facile, large-scale method for the preparation of several optically pure alkyl-aryl secondary alcohols by enzymatic acylation with succinic anhydride in organic solvents (Scheme 3).



Scheme 3

The substrate racemic alcohols (5a-5e) are either commercially available or were obtained as described in the literature. Screening of several commercially available lipase preparations[10] with succinic anhydride in several organic solvents revealed that the lipases Amano P, Amano PS and Amano Sam 2 catalysed the acylation reaction in tert-butyl methyl ether (Table 1).

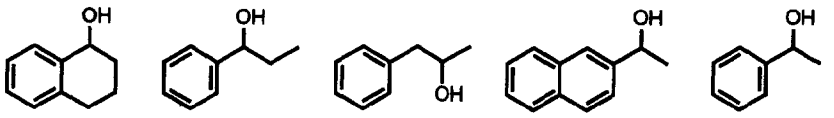
Table I. Acylation of alkyl-aryl alcohols with succinic anhydride catalysed by lipases in organic solvents[a]

Substrate	Lipase	Time (hr)	% convers.[b]	ee of (S)-5[c]	ee of (R)-5[c]
	Sam 2	0.75	47.2	88.3	94.3
5a	Sam 2	170	59.5	98.6	71.1
	PS	1.85	32	44.6	84.6
	Sam 2	118		59	94
5b	PS	70.7	49.6	72.5	93.8
	PS	118		97	87.5
	Sam 2	32.8	56	99.6	97.4
5c	PS	29.1	49	63	99
	PS	34.3	58	89	78
	Sam 2	26.9	51.6	99	95
5d	Sam 2	599	60	99.8	71
	PS	599	58	99.6	63
	Sam 2	1.0	44.1	72	98
5e	Sam 2	259	56.2	100	80.6
	PS	4.1	43	67.8	97.3

a. The experimental protocol was as follows: 6.7 mmole of the racemic alcohol (5) and 8 mmole of succinic anhydride were dissolved in 8 ml tert-butyl methyl ether dried over molecular sieves in a 50 ml screw-cap vial. 116 mg of lipase Amano Sam 2, or 265 mg of lipase Amano PS were added and the suspension was shaken at 40 °C and 200 rpm. The reaction was stopped by filtering off the enzyme and separating between the unreacted alcohol and half acid by extraction with a saturated solution of sodium bicarbonate. (R)-5 was obtained by basic hydrolysis of the half ester (6), as described in the experimental section. No reaction took place in the absence of enzyme under the conditions used.

b. % conversion was monitored to within ±5% accuracy by comparing the relative intensities of the ^{1}H NMR signals corresponding to the hydrogen adjacent to the hydroxyl in (5) (at about 4.7 ppm) with that of the same hydrogen in the resultant half ester (6) (at about 6 ppm).

c. Absolute configurations of the alcohols were determined by comparing their optical rotations with those described in the literature.[11] Optical purities were determined by HPLC on a chiral column as described in experimental section.



5a 5b 5c 5d 5e

As can be seen in Table 1, the two lipases, Amano Sam 2 and Amano PS, exhibited a similar high enantiomeric specificity, the (R) enantiomer of (5) undergoing acylation in preference to the (S) enantiomer in all cases. The high degree of stereospecificity is illustrated by the very considerable retardation of the reaction towards 50% conversion. For example, for (5e), 44% conversion was achieved after 1 hour, but 56% conversion required an additional 258 hours. Following the general theory of enzyme-catalysed kinetic resolution, when it is desired to increase the optical purity of the unreactive (S) alcohol the reactions were stopped at a relatively late stage of conversion, whereas in order to increase the optical purity of the (R) enantiomer, the reactions were stopped at a relatively early stage of conversion. After the removal of the enzyme, the unreacted alcohol was very easily separated from the half ester by extracting the organic solution with an aqueous solution of $NaHCO_3$ and then the half acid (6) was hydrolysed with aqueous NaOH to give the (R) enantiomer of the alcohol.

Unlike several other enzymatic reactions in organic solvents,[9] reactions with SAM 2 required only small quantities of enzyme and in preparative experiments we worked with a substrate:enzyme ratio of 100:3. Since the price of this enzyme is less than $1/gram, $30 worth of enzyme enabled us to carry out a reaction on 1 KG of sec-phenethyl alcohol (5e) to obtain 280 grams and 420 grams of the optically pure R and S enantiomers respectively (see experimental below). This high substrate to enzyme ratio coupled with the extreme ease of separation between the reacted and unreacted enantiomers, make the economics of the process plausible for industrial preparation of various optically pure phenyl-alkyl and naphthyl-alkyl secondary alcohols.

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EXPERIMENTAL

General.

^1H NMR spectra were recorded on a bruker AM 200-MHz spectrometer in $CDCl_3$. All chemical shifts were reported in ppm with tetramethylsilane as internal standard. Enantiomeric excess (ee) was determined by Merck Hitachi HPLC on a chiral column (chiralcel OJ, Daicel). Optical rotations were determined on a JASCO digital polarimeter DIP-370. Distillations were performed on a glass tube oven Buchi GKR50. The shaker used for enzymatic experiments was a G24 environmental shaker incubator from New Bronswick Scientific Co.

The lipases Amano PS and Amano Sam 2 were purchased from Amano Pharmaceutical Co. Unless otherwise stated, all solvents and other chemicals were obtained from commercial suppliers and were used without further purification. Substrates (5b) and (5e) were commercially available. (5a), (5c) and (5d) were prepared by sodium borohydride reduction of the corresponding commercially available ketones by the standard procedure. All substrates were distilled prior to enzymatic reactions and their ^1H NMR spectra were consistent with structure.

Kinetic measurements and determination of enantiomeric excess.

6.7 mmole of the racemic alcohol (5), 8 mmole of succinic anhydride and 116 mg of lipase Amano Sam 2 in 8 ml tert-butyl methyl ether (TBME), dried over molecular sieves, were placed in a 50 ml screw-cap vial and the suspension was shaken at 40 °C and 200 rpm. The reaction was stopped by filtering off the enzyme, which was extracted twice with 10 ml diethyl ether. An aliquot of the combined organic solutions was evaporated and its NMR spcectrum used to evaluate the degree of conversion. The organic solution was extracted with 2 X 50 ml saturated solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated to give the S-enriched unreacted alcohol, S-(5). The water phase was stirred for 3 hours with 6 g NaOH to hydrolyse the half ester (6), and then extracted with 3 X 30 ml ether. This ether solution was dried over Na_2SO_4 and evaporated to give the R-enriched alcohol, R-(5). The enriched alcohols were purified by vacuum bulb-to-bulb distillation and their optical purities were determined by HPLC analysis on a chiral column (Chiralcel OJ, Daicel) with a mixture of hexane and 2-propanol 95:5 as the mobile phase at the flow rate of 0.9 ml/min using detection at 258 nm. The R_t values were: for (5a) - 12 min for the R- and 14 min for the S-enantiomer; for (5b) - 11.5 min for the R- and 12.5 min for the S-enantiomer; for (5d) - 10 min for the R- and 12 min for the S-enantiomer; for (5e) - 13 min for the R- and 15 min for the S-enantiomer. The ee of (5c) could not be determined directly and it was converted into the corresponding acetate with excess of acetyl chloride, which had R_f 7.7 min for the R- and 7.3 min for the S-enantiomer.

Preparative resolution of sec-phenethyl alcohol (5e).

1 liter (1.01 KG) of sec-phenethyl alcohol (5e) in 5 liters of t-butyl methyl ether (TBME) were placed in a polyethylene wide mouth bottle equipped with a powerful magnetic stirrer. To the solution were added 0.994 KG of succinic anhydride and 30 g of lipase SAM 2. A suspension was formed, since succinic anhydride is poorly soluble in TBME and the enzyme is insoluble in this or other organic solvents. The suspension was stirred at room temperature and progress of the reaction was followed by NMR: (5e) has a quartet at 4.77 ppm corresponding to the benzylic hydrogen, while the quartet of this hydrogen in the product (6e) appears at 5.90 ppm. Accurate integration of the appropriate signals enabled us to determine the ratio between starting material and product and thus determine the degree of conversion. After 38 hours the enzymatic reaction reached 30.4% conversion and was stopped by filtering off the solids, which were washed with TBME, dried at room temperature in the air and kept aside (to reuse the enzyme). The filtrates were extracted with a 1M solution of sodium carbonate (3 x 1 l) and with 1 l of water. The aqueous phase containing (6e) was washed with TBME (2 x 600 ml) and then hydrolysed by adding 300 g of sodium hydroxide and stirring for 6 hours at room temperature. Then, the basic aqueous solution was extracted with 3 x 500 ml dichloromethane, the organic phase was washed with water (500 ml), dried over Na_2SO_4, evaporated, and the residue distilled (98°C/20 mm Hg) to give 280 g of R-(+)-sec-phenethyl alcohol, $[\alpha]_D^{20}$=+40.8 (neat). The ee of this alcohol was determined to be 98% by HPLC on a chiral column. The combined organic phases (which contained the unreacted S-enriched alcohol) were poured into the 10 l polyethylene wide mouth bottle, the enzyme and the succinic anhydride recovered from the previous experiment, together with 15 g of fresh enzyme and 50 g of succinic anhydride were added and the suspension was stirred at room temperature. Periodically aliquotes were withdrawn and the increase in optical purity of the

unreacted S enantiomer was monitored by HPLC on the chiral column. The reaction was continued for 3 days until complete disappearance of R-alcohol. The insoluble enzyme and succinic anhydride were then filtered off, the organic solution washed with 1M sodium carbonate (3 x 1 l), and the organic solvent was removed under reduced pressure. The residue was distilled (98°C/20 mm Hg) to give 420 g of S-(-)-sec-phenethyl alcohol, $[\alpha]_D^{20}$=-42.7 (neat). The ee of this alcohol was determined to be 99.6%.

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