# Enzymatic Resolution Coupled with Substrate Racemization Using a Thioester Substrate

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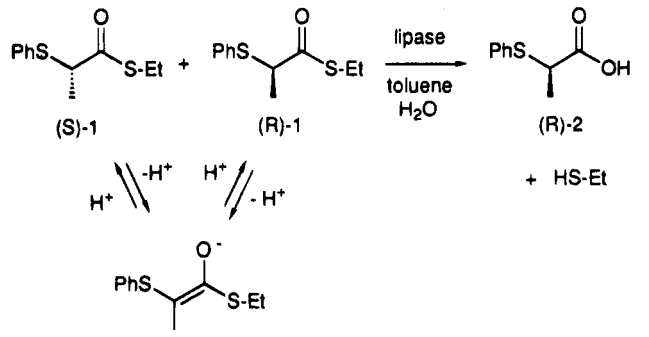
The resolution of racemic compounds using hydrolytic enzymes is probably the most widespread application of enzymes as synthetic catalysts.[1−4] Normal enzymatic resolution processes, like other resolution methods, afford a maximum 50% yield of optically pure product based on racemic starting material. In a few examples, enzymatic kinetic resolution has been coupled with substrate racemization.[3,5−9] Performing the resolution reaction under conditions in which the substrate continuously racemizes allows quantitative conversion of racemic starting material into one enantiomer of the product.[10] Racemization-coupled enzymatic resolution has been limited to substrates either for which a second enzyme (a racemase) is available to catalyze the racemization or in which a proton at the chiral center of the substrate is sufficiently acidic to permit racemization by deprotonation/reprotonation under neutral or mildly basic conditions.

Enzymatic resolution processes have generally used esters and sometimes amides as substrates. The α-hydrogens of thioesters are more acidic than those of oxoesters and amides, though the rates of base-catalyzed hydrolysis of thioesters and oxoesters are very similar.[11−14] We envisioned that thioesters of certain carboxylic acids having a chiral center at the α-carbon could be sufficiently acidic for deprotonation and resulting racemization under the conditions of enzymatic resolution. While thioesters have been used as substrates in enzymatic resolution, their α-proton acidity has apparently never been exploited for resolution coupled with substrate racemization.[15−17] We report here the use of a thioester substrate to achieve enzymatic resolution under substrate-racemizing conditions.

**Scheme 1**




To demonstrate that a thioester could be used for racemization-coupled enzymatic resolution, we chose the ethyl thioester of α-(phenylthio)propionate **1** (Scheme 1). The corresponding (*R*)-acid **2** has been subjected to diastereoselective oxidation to the sulfoxide in the synthesis of a chiral vinyl sulfoxide.[18] The α-phenylthio substituent should enhance the acidity of the α-hydrogen to facilitate racemization of the thioester.[19] Accomplishing the desired resolution required finding both an enzyme that would hydrolyze the substrate with high enantioselectivity and developing conditions under which the substrate would undergo continuous racemization during the course of the enzymatic resolution. Due to the insolubility in water of **1** and most other substrates of interest, it was decided to use a lipase as catalyst in a biphasic system, while inducing racemization of the substrate in the organic phase.

Initially, conditions for racemization were studied by observing exchange of the α-proton of the thioester with deuterium from $CD_3OD$ by $^1$H-NMR. In a solution of 0.25 mmol of **1**, 0.25 mmol of $CD_3OD$, and 0.125 of mmol trioctylamine in 0.5 mL of toluene-$d_8$, a half-life for deuterium exchange of 80 min was observed. A halflife of 60 min was observed when the $CD_3OD$ was replaced with a 2 mL solution of 0.1 M PIPES buffer (pD 7.0) in a continuously stirred biphasic system. No measurable difference in rate was observed when a pD 8.0 buffer was used. In all cases, no exchange was observed in the absence of the trioctylamine. Control experiments with an oxoester and acid showed no measureable proton exchange over 24 h under similar conditions in the presence of trioctylamine. This indicated that the thioester, but not the oxoester, was suitable for the *in situ* racemization method and that the product acid was configurationally stable under thioester-racemizing conditions. Furthermore, no thioester hydrolysis was observed over a course of 24 h in the deuterium exchange experiments, indicating that nonenzymatic hydrolysis would not be a problem.

The resolution of **1** was initially studied under nonracemizing conditions by adding a solution of 0.50 mmol of racemic **1** in 1 mL of toluene to a solution of lipase in 30 mL of 0.01 M PIPES buffer at pH 7.0. The pH was controlled and hydrolysis monitored by measured addition of 0.2 M aqueous NaOH using a pH stat apparatus. Reactions were stopped at <50% conversion. The product acid and unreacted thioester substrate were isolated by acidification of the aqueous layer and extraction with ethyl acetate. $^1$H-NMR integration was used to accurately determine the percent conversion. The acid and thioester were separated by chromatography on silica gel ($CH_2Cl_2$/EtOAc, 1:1). Optical purity was determined by $^1$H-NMR analysis of the complex of the acid with (1*R*,2*R*)-1,2-diphenylethylenediamine in benzene-$d_6$.[20] For assignment of absolute stereochemistry, an authentic sample of (*R*)-**2** was prepared from (*S*)-2-bro-

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**Table 1.** Resolution of **1** with *Pseudomonas cepacia* Lipase under Nonracemizing and Racemizing Conditions

| conditions | reactn time (h) | convrsn (%) | ee (*R*, %) |
|---|---|---|---|
| nonracemizing | 48 | 18.7 | 96.1[a] |
| racemizing | 65 | >99 | 96.3[b] |

[a] *E* value, 62.4; calculated as described in ref 4. [b] Expected ee, 96.8% for a racemization-coupled resolution with an *E* value of 62.4; calculated from ee = $(E - 1)/(E + 1)$.

mopropionic acid and submitted to the same analysis.[21] Upon survey of several lipases, excellent enantioselectivity was obtained with the lipase from *Pseudomonas cepacia* (Amano lipase PS-30). Using the reaction conditions described above with 11.4 mg of lipase PS-30, the reaction proceeded to 18.7% completion in 48 h (Table 1). The resolution was repeated under racemizing conditions by addition of 0.25 mmol of trioctylamine to the enzyme reaction described above, using 176 mg of Amano lipase PS-30. The reaction was complete after 65 h, and the product was isolated in quantitative yield and 96.3% enantiomeric excess (ee).

In a normal resolution, the optical purity of product decreases as the reaction proceeds, due to increasing enrichment of substrate in the less reactive enantiomer.[4] For resolution coupled with substrate racemization, the optical purity should depend only on the enantiomeric ratio, *E* (ee = $(E - 1)/(E + 1)$), and should be independent of extent of conversion. The ee of product formed under racemizing conditions should equal the initial ee under nonracemizing conditions. This requires that racemization is rapid relative to hydrolysis, so that the substrate never becomes enriched in the less reactive enantiomer. Based on the *E* value measured under nonracemizing conditions, a maximum ee of 96.8% was predicted for the product of resolution under racemizing conditions. This matches the measured value of 96.3%, within experimental error. In contrast, an ee of only 93% would be expected for a first-order resolution with an *E* value of 62.4 carried to 45% conversion. The resolution coupled with substrate racemization thus gives product in both higher yield and higher optical purity than a normal resolution.

The resolution reaction under racemizing conditions was 50% complete in 22 h. This is 17 times the half-life of racemization predicted on the basis of the deuterium exchange experiments described above. The high optical purity of the product confirms that racemization was rapid relative to hydrolysis. The presence of trioctylamine does not appear to affect the enantioselectivity of the lipase, at least not in a negative way. This could not be assumed prior to these experiments, as diminished enantioselectivity has been reported upon addition of trioctylamine in protease-catalyzed Ketorolac resolution.[7] Chiral amines have also been shown to act as enantioselective inhibitors of *Candida rugosa* lipase-catalyzed hydrolysis of α-aryl and α-(aryloxy)-propionate esters.[22] While the experiments described here revealed no effect of trioctylamine on enantioselectivity, some inhibition was observed, so that a larger quantity of enzyme was required.

(21) The ee was determined from relative integration of the α-proton signals of each isomer with methyl proton decoupling: (*R*)-isomer, $\delta$ = 3.78; (*S*)-isomer, $\delta$ = 3.62.

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This work demonstrates that thioesters are attractive substrates for enzymatic resolution coupled with substrate racemization with apparent broad potential, though some limitations. A thioester of an active having only saturated alkyl substituents on the α-carbon would not be sufficiently acidic to permit racemization under the conditions used in the example described here. However, most chiral acids of interest have a nonalkyl substituent at the α-carbon, and a variety of nonalkyl functional groups may provide substantial carbanion stabilization including *S*-alkyl, *S*-acyl, *O*-aryl, aryl, and halide.[23] In the present example, the rate of racemization is 17-fold greater than the hydrolysis rate. A somewhat lower rate of racemization relative to hydrolysis may be acceptable without substantially compromising optical purity, and the rate of hydrolysis may be slowed by using less enzyme. Thus, thioester substrates having $pK_a$ values somewhat higher than that of the α-phenylthio thioester should also be useful substrates for enzymatic resolution under substrate-racemizing conditions. One feature of normal kinetic resolutions is that either the reactive or the unreactive substrate/product can be isolated, and thus access to either enantiomer of product is possible. Resolution under substrate-racemizing conditions, while giving higher yield and optical purity, provides access to only the more reactive enantiomer. This may be overcome if a different enzyme with selectivity for the opposite enantiomer can be found.

Work is underway to further expand the scope of the enzymatic resolution of thioesters. Several α-thiocarboxylic acid derivatives have proven useful as chiral synthons and as components of enzyme inhibitors.[24–27] Oxoesters of many other valuable carboxylic acids having a chiral center and a potential carbanion-stabilizing substituent at the α-carbon have been subjected to enzymatic resolution.[28–32] Use of thioesters in racemization-coupled processes may provide these products in increased yield and optical purity. The enzymatic resolution of thioesters coupled with substrate racemization is expected to become a useful, powerful, and practical addition to the methods available for enzymatic resolution.

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