VOLUME 120, NUMBER 23
JUNE 17, 1998

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Dynamic Enzymatic Resolution of Thioesters

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Received February 9, 1998

Abstract: A detailed investigation of several issues related to the enzymatic resolution of thioesters under conditions of continuous racemization of substrate was conducted. The kinetic acidity of the α-protons of a series of α-substituted propionate thioesters was studied. It was found that the rate of α-proton exchange could be enhanced as much as 20-fold by variation of the thiol moiety, increasing the range of compounds to which enzymatic dynamic resolution may be applied. The relative rates of hydrolysis of ethyl butyrate and ethyl thiobutyrate by several enzymes commonly used in enzymatic resolution were determined. All of the enzymes studied exhibited similar rates of thioester and oxoester hydrolysis except for the esterase from pig liver, which showed very low activity in thioester hydrolysis. Dynamic resolution of the propargyl and trifluoroethyl thioesters of α-phenylpropionate was conducted using subtilisin Carlsberg as a catalyst. These examples demonstrated that enzymatic dynamic resolution can be applied even when the rate of α-proton exchange and the enantioselectivity of the enzyme are fairly low. A dynamic enzymatic transesterification procedure was demonstrated in the resolution of the trifluoroethyl thioester of α-(2,4-dichlorophenoxy)propionate, and product was obtained in 93% ee. This work helps expand and define the scope of enzymatic dynamic resolution of thioesters.

The resolution of racemic compounds continues to be a valuable method for obtaining chiral compounds in high optical purity,[1−7] with both enzymatic[3,7] and nonenzymatic[1,4−6] catalysts and reagents being widely utilized. A disadvantage of standard kinetic resolution procedures is that a maximum 50% yield of the desired product is obtained based on racemic starting material. To overcome this limitation, recovered starting material may in some cases be racemized and resubmitted to the resolution procedure. As a potentially more efficient procedure, resolution processes have been coupled with continuous in situ racemization of the starting material.[5−8] This permits quantitative conversion of racemic starting material into one isomer of the product in a single deracemization process. This process has been termed dynamic resolution.[5,6] Despite the potential practicality of such a process, it has thus far been limited to fairly specific examples.

We previously reported the use of a thioester of a carboxylic acid having a chiral center at the α-carbon in an enzymatic resolution procedure.[9] In contrast to an oxoester, the α-protons of the thioester were sufficiently acidic to permit continuous racemization of the substrate by base-catalyzed deprotonation at the α-carbon.[10] This was demonstrated with an α-phenylthio

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S0002-7863(98)00445-4 CCC: $15.00
Published on Web 05/28/1998

Scheme 1

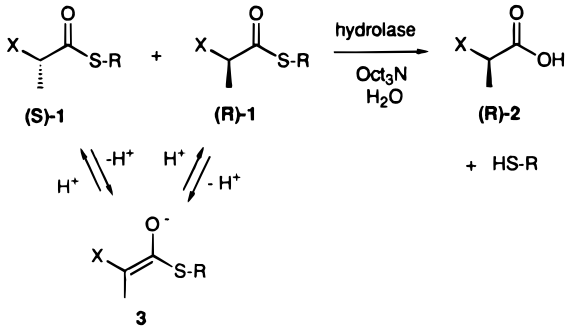

Table 1. Rates of α-Proton Exchange for α-Substituted Ethyl Thiopropionates (**1**, R = CH_2CH_3)

compd	k_{exch} (h^{-1})	$t_{1/2}$ (h)
1a (X = PhS)	0.53	1.3
1b (X = $PhCH_2S$)	0.17	4.0
1c (X = Cl)	0.16	4.3
1d (X = Br)	0.10	6.7
1e (X = N_3)	0.059	12
1f (X = 2,4-dichlorophenoxy)	0.011	63
1g (X = 3-benzoylphenyl)	0.0075	92
1h (X = Ph)	0.0064	108

propionate thioester **1a** (R = Et, X = PhS), the phenylthio group also contributing to the acidity of the α-proton (Scheme 1). It was expected that this procedure could be applicable to a variety of carboxylic acids having a chiral center and a proton at the α-carbon. However, several factors regarding the general applicability of this procedure were unknown, including the acidity of the α-proton of thioesters of other carboxylic acids and the general utility of hydrolytic enzymes as catalysts for enantioselective thioester hydrolysis. We report here studies directed at further understanding and developing the utility of thioesters as substrates in enzymatic dynamic resolution procedures. These studies include further analysis of the α-proton acidity and enzymatic hydrolysis of thioesters and additional examples of enzymatic dynamic resolution using thioester substrates.

Results

A series of α-substituted propionate ethyl thioesters (**1a**−**h**) were prepared and their kinetic acidity determined by measuring the rate of deuterium substitution of the α-proton upon reaction with CD_3OD catalyzed by triethylamine in toluene-d_8 (Scheme 2). The extent of deuteration was monitored by ^1H NMR, and results are shown in Table 1. The α-thio derivatives (**1a,b**), as well as the halo (**1c,d**) and azido (**1e**) compounds showed half-lives for α-proton exchange of a few hours while the α-dichlorophenoxy (**1f**) and α-aryl (**1g,h**) compounds showed half-lives of 2.5 days or more.

α-Phenyl propionate was chosen for further study, and thioesters of this acid with different thiols (**1i**−**m**) were prepared and their rates of α-proton exchange were studied. Results are shown in Table 2, with time courses for α-proton exchange shown in Figure 1. The allyl thioester (**1i**) showed a slightly enhanced rate of proton exchange relative to the ethyl thioester (**1h**), while the benzyl (**1j**) and phenyl (**1k**) thioesters and especially the propargyl (**1l**) and trifluoroethyl (**1m**) thioesters showed substantially enhanced rates.

A comparison of the rates of enzymatic hydrolysis of thioesters vs oxoesters was made by measuring the rates of hydrolysis of ethylbutyrate **4a** and ethylthiobutyrate **4b** catalyzed by several hydrolytic enzymes (Scheme 3). Results are shown in Table 3. The five lipases studied, the horse liver acetone powder, and the protease subtilisin Carlsberg all showed fairly similar rates of hydrolysis of each substrate, the Candida lipases and subtilisin exhibiting slightly lower rates of thioester hydrolysis with the others showing slightly greater rates of thioester hydrolysis relative to oxoester hydrolysis. In contrast, the pig liver esterase showed very low relative activity in thioester hydrolysis.

The ethyl (**1h**) and allyl (**1i**) thioesters of α-phenylpropionate and the allyl thioester of ketoprofen were tested as substrates for the lipase from *Candida rugosa*, which has been used efficiently in the enantioselective hydrolysis of oxoesters of

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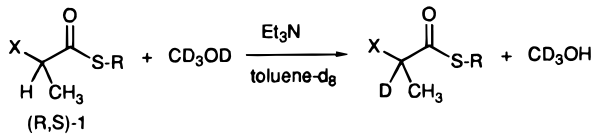
Table 2. Rates of α-Proton Exchange for α-Phenylpropionate Thioesters (**1**, X = Ph)

compd	electroneg[a]	thiol pK_a[b]	k_{exch} (h^{-1})
1h (R = CH_2CH_3)	2.3	10.6[c]	0.006
1i (R = $CH_2CH{=}CH_2$)	3.0	10.0[c]	0.007
1j (R = CH_2Ph)	3.0	9.43[c]	0.011
1k (R = Ph)		6.52[c]	0.015
1l (R = $CH_2C{\equiv}CH$)	3.3		0.032
1m (R = CH_2CF_3)	3.35	6.8[d]	0.13

[a] The group electronegativity value for the X-group of R = CH_2X (ref 14). [b] The aqueous pK_a value of the thiol (RSH) component of the thioester. [c] Reference 15. [d] Estimated value (ref 16).

Scheme 2



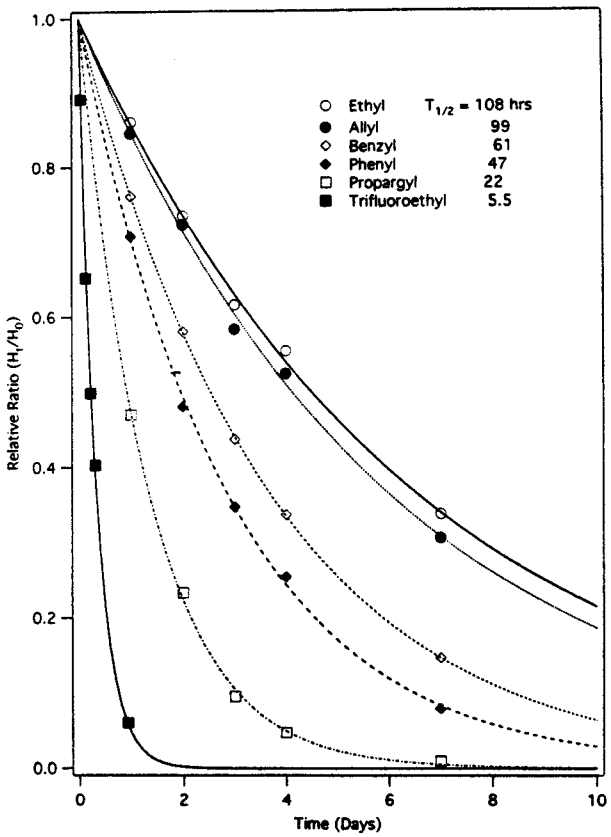


Figure 1. Time course for α-proton exchange for thioesters of α-phenylpropionic acid as monitored by ^1H NMR. H_t = α-proton signal intensity at each time point. H_0 = initial α-proton signal intensity.

Table 3. Rates of Enzymatic Hydrolysis of Ethyl Butyrate and Ethyl Thiobutyrate (μmol/(min·g of enzyme))

enzyme	k_{oxo}	k_{thio}	k_{thio}/k_{oxo}
C. rugosa lipase	295	134	0.45
Candida antarctica lipase	3088	926	0.30
porcine pancreatic lipase	308	327	1.1
Aspergillus niger lipase	4	11	2.8
Pseudomonas cepacia lipase	366	730	2.0
horse liver acetone powder	139	236	1.7
pig liver esterase	2934	88	0.03
subtilisin Carlsberg	103	65	0.63

Scheme 3

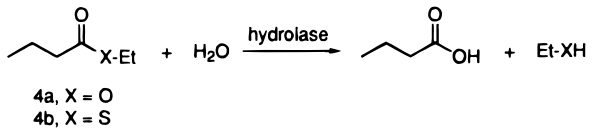


Table 4. Resolution of **1l,m** with Subtilisin Carlsberg under Nonracemizing and Racemizing Conditions

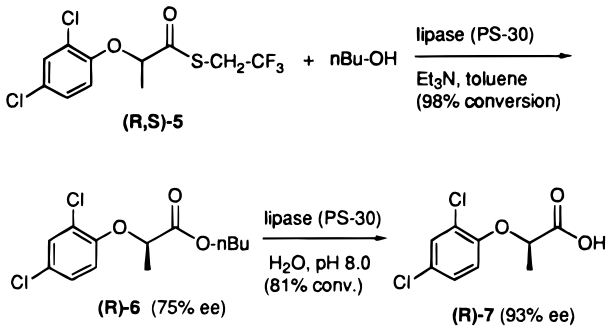
	conditions	conversion (%)	ee (*R*) (%)	*E*/max ee
1l	nonracemizing	43	74	12[a]
1l	racemizing	95	80	84%[b]
1m	nonracemizing	35	73	11[a]
1m	racemizing	97	83	83%[b]

[a] *E* value, calculated as described in ref 3. [b] Optimal ee for a second-order resolution with the *E* value determined under nonracemizing conditions, calculated from ee = $(E - 1)/(E + 1)$.

α-arylpropionates.[11−13] The Candida lipase was utilized in nonimmobilized form, immobilized on silica gel, and in cross-linked crystalline form. Very low activity was observed in all cases relative to reported activities with oxoesters, but by using large amounts of enzyme, products were obtained after about 20% hydrolysis. Enantiomeric purity determination showed low enantioselectivity in all of these reactions, with typical *E* values of 4−5. Several other hydrolytic enzymes were screened for enantioselective hydrolysis of thioesters of α-phenylpropionate, and subtilisin Carlsberg was identified as a useful catalyst for this reaction. Resolution of the propargyl thioester of α-phenylpropionate **1l** was conducted using this enzyme. Resolution was initially conducted without racemization in the absence of an amine base and gave the (*R*)-acid in 74% ee after 43% hydrolysis (Table 4). The reaction was repeated under racemizing conditions by inclusion of trioctylamine and carried to 95% conversion. The product was obtained in 80% ee. Resolution of the trifluoroethyl thioester under nonracemizing conditions gave an *E* value of 11 and under racemizing conditions gave product in 83% ee after 97% hydrolysis (Table 4).

Resolution of thioesters of 2,4-dichlorophenoxypropionate was also studied. Hydrolysis of the ethyl thioester **1f** under nonracemizing conditions gave the (*R*)-acid with good enantioselectivity. α-Proton exchange studies of the trifluoroethyl thioester **5** gave a half-life of 2.1 h for racemization. However, hydrolysis of **5** under racemizing conditions gave low and variable enantiomeric purities. It was subsequently found that substantial nonenzymatic hydrolysis occurred under the reaction conditions. A transesterification reaction was then performed using *n*-butyl alcohol as the acyl acceptor in the presence of triethylamine (Scheme 4). The (*R*)-butyl ester **6** was obtained in 75% ee at 98% conversion. Hydrolysis of this enantiomerically enriched butyl ester using the same enzyme under nonracemizing conditions gave the (*R*)-acid **7** in 93% ee when stopped at 81% conversion.

Scheme 4



Discussion

Kinetic α-Proton Acidity of Thioesters. The application of the previously reported coupling of enzymatic resolution of a thioester with substrate racemization requires that racemization of the substrate be at least as fast and preferably faster than the enzymatic hydrolysis reaction. If racemization is too slow, the substrate will become enriched in the slower reacting isomer over the course of the reaction. This will result in decreased enantiomeric purity of the product, especially if the enantioselectivity of the enzyme is only modest.[7] The rate of the enzymatic hydrolysis reaction may be controlled by the amount of enzyme that is added. A reaction time of 2 days or more may be attractive to minimize the amount of enzyme that is required. However, much longer reaction times may be disadvantageous as nonenzymatic hydrolysis may become significant, resulting in reduced enantiomeric purity of product.

The previously published example of thioester resolution employed a thioester having an acidity enhancing phenylthio group on the α-carbon.[9] The rate of racemization of the phenylthiopropionate ethyl thioester was deduced from the rate of α-proton exchange with deuterated solvent to be 0.53 h^{-1} ($t_{1/2}$ = 1.3 h). This rate was demonstrated, in an enzymatic resolution reaction proceeding over the course of 65 h, to be sufficient to avoid a drop in ee below the theoretical value, determined from the *E* value in a reaction under nonracemizing conditions. A major goal of subsequent work has been to investigate the potential applicability of this procedure to thioesters of acids having inherently lower α-proton acidity. Studies of α-proton exchange with deuterated solvent were undertaken with the ethyl thioesters of several carboxylic acids to evaluate their potential for racemization during the course of enzymatic resolution. These studies were conducted using triethylamine in toluene-d_8 with CD_3OD added as a source of exchangeable deuterium (Scheme 2). Previous studies have shown the rate of α-proton exchange under these conditions to be similar to that under biphasic conditions which mimic the enzymatic resolution, in which racemization occurs in the organic phase.[9] The ethyl thioesters of the α-thio, α-halo, and α-azido propionates all appear sufficiently acidic for resolution coupled with racemization over 2−3 days. In contrast, the dichlorophenoxy, 3-benzoylphenyl (ketoprofen), and phenyl compounds all appear insufficiently acidic for practical dynamic resolution.

The phenylpropionate thioester was chosen for further study, as it serves as a model for the α-arylpropionate class of

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nonsteroidal antiinflammatory drugs. While the identity of the acid moiety of a thioester substrate for dynamic enzymatic resolution is dictated by the desired carboxylic acid product, the thiol moiety can be varied. Studies were undertaken to determine if modification of the *S*-alkyl moiety could enhance the α-proton acidity. The allyl thioester showed slightly enhanced α-proton acidity relative to the ethyl thioester. The benzyl and phenyl thioesters showed further enhanced rates while the propargyl and trifluoroethyl thioesters exhibited rates of α-proton exchange enhanced approximately 5-fold and 20-fold relative to the ethyl thioester. The propargyl and trifluoroethyl thioesters appeared sufficiently acidic to allow racemization-coupled resolution over the course of a few days.

The relative acidity of the thioesters in Table 2 can be correlated with the group electronegativity values[14] of the functional group attached to the thiomethylene group. While the ethyl thioester **1h** has a methyl group attached to this methylene, the allyl **1i** and benzyl **1j** thioesters have the more electronegative vinyl and phenyl groups, respectively. This greater electronegativity and the resulting enhanced acidity of **1i**,**j** relative to the ethyl thioester **1h** is attributed to the greater S-character of the orbitals used in the bond to the thiomethylene carbon. The propargyl thioester **1l**, having the more electronegative alkynyl group, and the trifluoroethyl thioester **1m**, having the very electronegative trifluoromethyl group, exhibit even greater acidity. The phenyl thioester **1k** has the phenyl group attached directly to the thioester sulfur atom with no intervening methylene group. However, the kinetic acidity of this thioester was only slightly enhanced over that of the benzyl thioester. Except for the phenyl thioester, the relative rates of α-proton exchange also follow the trends expected on the basis of the acidity of the thiol from which the thioester is derived[15,16] and the inductive parameters for the substituent attached to sulfur,[17] though a good linear fit with these parameters was not observed.

Rates of nonenzymatic hydrolysis of thioesters appear to correlate well with the acidity of the thiol component.[18] The phenyl thioesters are thus expected to be especially prone to nonenzymatic hydrolysis. In contrast, the allyl, benzyl, and propargyl thioesters are expected to have only moderately enhanced rates of nonenzymatic hydrolysis relative to the ethyl and other saturated alkyl thioesters on the basis of the modest differences in acidity of the thiols. The trifluoroethyl thioesters are expected to have rates of nonenzymatic hydrolysis similar to those of the phenyl thioesters, even though the trifluoroethyl thioesters have much greater α-proton acidity. Phenyl thioesters are thus probably less attractive substrates for dynamic resolution than the others.

Thiophenol and allyl and benzyl thiols are commercially available and are very inexpensive. Trifluoroethanethiol is somewhat expensive while propargyl thiol is to our knowledge not commercially available but is readily prepared from the inexpensive propargyl chloride. The results of α-proton exchange studies suggest that thioesters of these thiols may be valuable substrates for dynamic enzymatic resolution of acids for which the simple ethyl thioesters are not sufficiently acidic.

Thioesters as Substrates for Hydrolytic Enzymes. To achieve enzymatic resolution of a thioester, an enzyme must be identified which catalyses hydrolysis of the thioester substrate and which exhibits sufficient enantioselectivity for the desired stereoisomer. Only a few examples of enzymatic resolution using thioesters as substrates have been reported,[19−22] and the ability of common hydrolytic enzymes to hydrolyze thioester substrates has not been well documented. Thioesters are thermodynamically less stable than oxoesters, the free energy of hydrolysis of thioesters being more than 2 kcal/mol greater than that of oxoesters.[23,24] However, the rates of base-catalyzed hydrolysis of thioesters and oxoesters are virtually identical, though thioesters are much more reactive toward amine nucleophiles.[24,25] A survey of several enzymes commonly used in enzymatic resolution showed that ethyl thiobutyrate was generally accepted as a substrate with activity comparable to that with the oxoester. The only exception found was pig liver esterase, which was a very poor catalyst for hydrolysis of the thioester. It is possible that the lower solubility of the thioester in aqueous solution contributes to the low activity. These studies suggested that the enzymes used in resolution of oxoesters should also be useful in the dynamic resolution of thioesters.

The lipase from *C. rugosa* in purified soluble form, in immobilized form, and in cross-linked crystalline form has shown very general applicability in the enantioselective hydrolysis of oxoesters of α-arylpropionates.[11−13] Hydrolysis of the methyl and chloroethyl esters have given *E* values ranging from about 40 to greater than 100. Studies of the hydrolysis of thioesters of α-phenyl propionate and ketoprofen reported here show that this enantioselectivity does not extend to the thioesters. These results are somewhat surprising, as it was expected that recognition of the oxoesters and thioesters would be similar. These results indicate that established enzyme−substrate combinations in oxoester resolution cannot always be directly extended to thioester resolution, even for enzymes which have been shown to efficiently hydrolyze simple thioesters. A more complete understanding of these observations awaits further study.

Demonstration of Dynamic Enzymatic Resolution of Thioesters. Previous work has demonstrated that enzymatic dynamic resolution can be accomplished efficiently with a thioester substrate having a fairly rapid rate of racemization, as indicated by α-proton exchange experiments. The final objective of this work was to demonstrate that dynamic resolution could also be applied to acids for which the thioesters have inherently lower rates of racemization. α-Phenyl propionate and α-(2,4-dichlorophenoxy)propionate were selected for further demonstration of dynamic enzymatic resolution. α-Phenyl propionate is representative of the α-arylpropionates, which are important as nonsteroidal antiinflammatory drugs.

A survey of several enzymes identified subtilisin Carlsberg as having moderate enantioselectivity in hydrolysis of the propargyl thioester of α-phenyl propionate, though the enantiomer hydrolyzed does not correspond to the active isomer of the nonsteroidal antiinflammatory drugs.[11−13] The modest

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enantioselectivity made this a further challenging test for dynamic resolution, as enrichment of substrate in the slower reacting enantiomer over the course of the reaction resulting from a slow rate of substrate racemization relative to hydrolysis could in turn result in substantially diminished enantiomeric purity of the product. Hydrolysis of the propargyl thioester **1l** under racemizing conditions taken to 95% completion gave product in 80% ee. This is only slightly lower than the theoretical value of 84% expected on the basis of the *E* value for a reaction in which substrate remains racemic throughout the reaction. The trifluoroethyl thioester, which has a greater rate of racemization, gave product in 83% ee upon hydrolysis to 97% completion under racemizing conditions. This is equivalent to the theoretical value and demonstrates the effect of racemization rate on enantiomeric purity of product.

In addition to the low rate of racemization, the dichlorophenoxypropionate thioesters posed further challenges due to their susceptibility to nonenzymatic hydrolysis. This enhanced rate of nonenzymatic hydrolysis relative to other thioesters studied is attributed to the inductive effect of the dichlorophenoxy group, which is much greater than the inductive effect of the phenyl group. In proton exchange studies, the resonance effect of the phenyl group on stabilization of the enolate makes up for the smaller inductive effect so that the rate of α-proton exchange is similar to that of the dichlorophenoxypropionate thioester. The nonenzymatic hydrolysis precluded efficient enzymatic dynamic resolution by hydrolysis. Instead, a transesterification reaction was performed using *n*-butyl alcohol as the acyl acceptor. Nonenzymatic transesterification was apparently less prevalent than nonenzymatic hydrolysis, as the product was obtained in moderate enantiomeric purity. A second advantage of the transesterification procedure is that it provides a product which can be used in a second enzymatic enantiomeric purity enhancing step by hydrolysis using the same enzyme. This was clearly demonstrated, as hydrolysis to 81% completion under nonracemizing conditions increased the product ee from 75 to 93%.

Conclusion

Studies reported here demonstrate that the choice of thiol moiety can have a large effect on the α-proton acidity of thioesters. The ethyl thioesters of some of the α-substituted propionates studied in this work appear sufficiently acidic for dynamic resolution while the acidity of thioesters of other acids can be sufficiently enhanced by modification of the thiol moiety. Thioesters of these thiols should be practical substrates for enzymatic dynamic resolution.

This work has also shown that most hydrolytic enzymes will readily accept thioesters as substrates. However, studies with the *C. rugosa* lipase have shown that high activity and enantioselectivity in oxoester hydrolysis does not guarantee high activity and enantioselectivity in hydrolysis of a thioester of the same acid. Enzymatic dynamic resolution may often require the rediscovery of a suitable catalyst, even if one or more enzymes have been previously identified for oxoester resolution. Enzymatic dynamic resolution has been further demonstrated in hydrolysis of a pair of α-phenylpropionate thioesters using the protease subtilisin Carlsberg. Enzymatic dynamic resolution has also been demonstrated in a transesterification reaction using an alcohol rather than water as the acyl acceptor. This minimizes nonenzymatic reactions, for substrates which are prone to nonenzymatic hydrolysis under the basic racemizing conditions, and facilitates further enhancement of enantiomeric purity by enzymatic hydrolysis of the oxoester product of the transesterification reaction. These examples have demonstrated that product can be obtained in good ee, even with substrates for which racemization is slow and enzyme enantioselectivity is modest.

This study demonstrates that substantial work is involved in developing optimal enzymatic dynamic resolution procedures. However, the potential to vary the choice of enzyme, the substrate structure, and the reaction conditions should make this a widely applicable and powerful technology.

Experimental Section

General Experimental. CH_2Cl_2 was distilled from calcium hydride prior to use. ^{1}H NMR were taken at 200 MHz and ^{13}C NMR at 50 MHz using TMS as an internal standard. Lipase PS-30 (*Pseudomonas cepacia*) was obtained from Amano. *C. rugosa* lipase as cross-linked crystals was obtained from Altus. Other enzymes were from Sigma. *C. rugosa* lipase immobilized on silica gel was prepared as previously described.[13]

Synthesis of Thioesters of 2-Substituted Propionic Acids (1a−m). To a solution of a 2-substituted propionic acid (10 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (1.4 mL, 10 mmol), the mixture was cooled to 4 °C, and oxalyl chloride (0.87 mL, 10 mmol) was added dropwise over 15 min. After additional stirring for 2 h at 4 °C, a solution of triethylamine (1.5 mL, 11 mmol) and the appropriate thiol (11 mmol) in CH_2Cl_2 (5 mL) was added. After stirring overnight at room temperature the solution was filtered, the filtrate was washed with 5% aqueous $NaHCO_3$ (2 × 20 mL) and saturated aqueous NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel using ethyl actate/hexanes (1:9) as eluent. Products were obtained in 81−94% yield.

Propargyl Mercaptan (Prop-2-yne-1-thiol). To a solution of propargyl chloride (2.07 mL, 30 mmol) in DMF (30 mL) was added a solution of sodium thiophosphate[26] (23.76 g, 60 mmol) in water (150 mL). The mixture was stirred for 5 h at room temperature, and the pH was lowered to 4.0 with 1 N HCl. After the mixture was stirred overnight at room temperature, the product, which separated off as a yellow oil, was collected and further purified by distillation under reduced pressure (1.4 g, 65%): bp 35−37 °C (100 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.28 (dd, 2H, J = 2.7, 7.5 Hz), 2.32 (t, 1H, J = 2.7 Hz), 2.08 (t, 1H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 81.9, 70.7, 11.9.

(*R*,*S*)-Butyl 2-(2,4-Dichlorophenoxy)propanoate (5). To a solution of a 2-(2,4-dichlorophenoxy)propionic acid (2.35 g, 10 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (1.4 mL, 10 mmol). The mixture was cooled to 4 °C, and oxalyl chloride (0.87 mL, 10 mmol) was added dropwise over 15 min. After additional stirring for 2 h at 4 °C, a solution of triethylamine (1.5 mL, 11 mmol) and 1-butanol (1.0 mL, 11 mmol) in CH_2Cl_2 (5 mL) was added. After the mixture was stirred overnight at room temperature, the solution was filtered, the filtrate was washed with 5% aqueous $NaHCO_3$ (2 × 20 mL) and saturated aqueous NaCl (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel using ethyl actate/hexanes (1:9) as eluent to give (*R*,*S*)-**5** (2.4 g, 82%): ^{1}H NMR ($CDCl_3$) δ 7.40 (d, 1H, J = 2.6 Hz), 7.17 (dd, 1H, J = 2.6, 8.8 Hz), 6.80 (d, 1H, J = 8.8 Hz), 4.75 (q, 1H, J = 6.8 Hz), 4.18 (m, 2H), 1.70 (d, 3H, 6.8 Hz), 1.62 (m, 2H), 1.33 (m, 2H), 0.92 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 171.4, 152.2, 130.3, 127.4, 126.9, 124.7, 115.9, 74.3, 65.3, 30.5, 18.9, 18.4, 13.6.

Deuterium Exchange Experiments. To a solution of the thioester (0.25 mmol) and CD_3OD (1.25 mmol) in toluene-d_8 (0.5 mL) was added trioctylamine (0.125 mmol). The decrease in intensity of the α-proton signal was monitored over time by ^{1}H NMR integration.

Measurement of Enzyme Activity for Oxoethyl and Thioethyl Butyrate. To a biphasic mixture of aqueous PIPES buffer (20 mL, 0.01 M, pH 7.0) and 2 mL of toluene (or 2 mL of acetonitrile for subtilisin Carlsberg) were added substrate (1 mmol) and enzyme. The pH was maintained at 7.0 by addition of 0.02 M KOH solution using a pH stat. Enzyme activity was determined by monitoring the volume of base added vs time.

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Enzymatic Hydrolysis without Racemization. To a biphasic mixture of aqueous PIPES buffer (20 mL, 0.01 M, pH 7.0) and 2 mL of organic solvent (toluene for lipases, acetonitrile for subtilisin) were added substrate (1 mmol) and enzyme (100 mg). The pH was maintained at 7.0 by addition of 0.02 M KOH solution using a pH stat. When 0.3−0.4 equiv of base had been added, the pH was adjusted to 1.0 by the addition of 1 N aqueous HCl. The solution was saturated with NaCl and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The extent of conversion was determined by ^{1}H NMR analysis. The product was redissolved in ethyl acetate (50 mL) and extracted with aqueous $NaHCO_3$ (5%, 3 × 15 mL). The combined aqueous layers were acidified with 4 N aqueous HCl and extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over $MgSO_4$ to give the acid. The optical purity of the acid 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.[27]

Enzymatic Hydrolysis with Racemization. To a biphasic mixture of aqueous PIPES buffer (20 mL, 0.01 M, pH 7.0) and 2 mL of organic solvent (toluene for lipases, acetonitrile for subtilisin) were added substrate (1 mmol), trioctylamine (0.5 mmol), and enzyme (50 mg). The pH was maintained at 7.0 by addition of 0.02 M KOH solution using pH stat. When the conversion was over 95%, the reaction was stopped and product isolated as described above for nonracemizing conditions. Isolated product yields ranged from 84% to 92%. 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.[27]

Transesterification of Thiotrifluoroethyl 2-(2,4-Dichlorophenoxy)propionate with Racemization. To a solution of (*R*,*S*)-**5** (1.0 mmol) in toluene (10 mL) was added butanol (5 mmol), triethylamine (2 mmol), and lipase PS-30 (50 mg). The suspension was stirred at room temperature. Samples (0.5 mL) were removed periodically (five times over the course of the reaction), aqueous HCl (1 mL, 1 M) was added, and the mixture was extracted with ethyl acetate (2 × 1 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The extent of conversion was determined by ^{1}H NMR analysis. When the conversion was over 95%, the reaction was stopped by addition of ethylenediamine (1.0 mmol) and the solution was extracted with aqueous HCl (2 × 3 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to give (*R*)-**6** (0.19 g, 0.66 mmol, 87% yield considering volume removed for analysis). Optical purity was determined by ^{1}H NMR analysis of the complex of the oxoester with $Eu(hfc)_3$ in $CDCl_3$.

Enzymatic Hydrolysis of Transesterification Product without Racemization. To a biphasic mixture of aqueous PIPES buffer (20 mL, 0.01 M, pH 7.0) and toluene (2 mL) were added (*R*)-**6** (0.15 g, 0.52 mmol) and lipase PS-30 (100 mg). The pH was maintained at 7.0 by addition of 0.02 M aqueous KOH using a pH stat. When 0.8 equiv of base had been added the reaction was stopped by acidification to pH 1 by addition of 1 N aqueous HCl. The solution was saturated with NaCl and extracted with ethyl acetate (3 × 20 mL). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated. The degree of conversion was determined by ^{1}H NMR analysis. Hydrolyzed acid was separated from unreacted thioester by extraction as described above to give (*R*)-**7** (0.088 g, 0.38 mmol, 73% yield). 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.[27]

Acknowledgment. This work was supported by National Institutes of Health Grant GM45831 (D.G.D.). D.G.D. is a fellow of the Alfred P. Sloan Foundation (1996−1998). We thank Derek S. Tan for contribution to initial experiments.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra and spectral data for compounds **1c**−**m**, **4b**, (*R*,*S*)-**5**, (*R*)-**6**, and propargyl mercaptan (28 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980445B

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