version to C_{60} under vacuum.[18] This was first observed in the electron impact mass chromatogram. Heating the sample in the mass spectrometer probe under vacuum at ca. 290 °C and recording EI spectra at 0.45-min intervals gave spectra of OsO_4 and pyridine (maximum at 1.8 min) followed by the spectrum of C_{60} (maximum at 5.85 min). Prolonged heating is required to detect the C_{60} due to its low volatility; this was also observed for the spectrum of the pure cluster. A preparative version of this experiment whereby the osmate ester was heated under vacuum for 2 min (heat gun, 0.05 mmHg) gave a 47% combined yield of C_{60} and C_{70} (enriched in C_{60} relative to the starting material) as determined by HPLC with respect to a naphthalene standard. The osmate ester was free of unreacted C_{60} according to IR and TLC, so the C_{60} must have been reformed upon heating.

These experiments establish that heteroatom functionality can be added to C_{60} without disrupting the carbon framework. We are currently exploring the regiochemistry of the osmylation and converting the glycolates to other organic functional groups in order to manipulate the C_{60} framework.

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(18) $ReOCl(OCH_2CH_2O)(phen)$ similarly gives ethylene and $ReO_3Cl(phen)$ when heated under vacuum: Pearlstein, R. M.; Davison, A. *Polyhedron* **1988**, *7*, 1981.

Articles

Lipase-Catalyzed Asymmetric Hydrolysis of Esters Having Remote Chiral/Prochiral Centers

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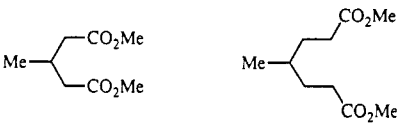
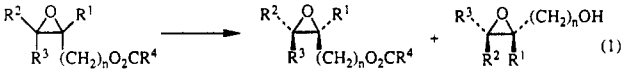
Enzymatic hydrolysis of prochiral and racemic dithioacetal esters having up to five bonds between the prochiral/chiral center and the ester carbonyl group proceeds with selectivities up to 98% enantiomeric excess when commercially available lipases are used. For lipase from *Pseudomonas* sp., chemical yields and ee's were better with the substrate having four bonds between the prochiral center and ester carbonyl than with the three-bond or five-bond analogues, demonstrating that selectivity does not necessarily diminish as the distance between the chiral center and the reaction site increases. These findings are the cornerstone of efficient chemoenzymatic syntheses of both enantiomers of a potent LTD_4 antagonist.

Use of hydrolytic enzymes to resolve racemic carboxylic esters and amides, or to stereospecifically hydrolyze prochiral or meso diesters, has become a powerful tool in organic synthesis.[1] However, in most cases, the compounds undergoing the enzymatic reaction have the prochiral or chiral center only one or two bonds away from the reacting carbonyl group, and often the ester groups are held in rigid, cyclic frameworks. Only a few examples have been reported in which the chiral/prochiral center is three or more bonds from the reacting carbonyl center. With pig liver esterase, Tamm and co-workers found that increasing the length between the prochiral center and the ester group from two bonds in **1** to three bonds in **2** resulted in a lowering of the enantiomeric excess (ee) from



1 **2**

90% to 10% in the resulting half ester product.[2] Better success was achieved by Whitesides in the porcine pancreatic lipase hydrolysis of chiral epoxy esters (eq 1).[3] In these cases, the ee's of the recovered esters were 60–90% with $n = 1$ and 70–80% with $n = 2$, while the recovered alcohols had ee's in the range of 30–80%.[3]



(1)

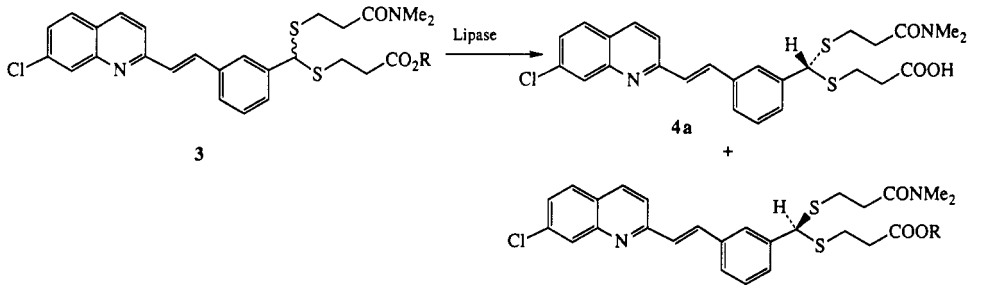
(1) Reviews: Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. Klibanov, A. M. *CHEMTECH* **1986**, 354. *Enzymes in Organic Synthesis*; Ciba Foundation Symposium 111; Porter, R., Clark, S., Eds.; Pitman: London, 1985. *Enzymes as Catalysts in Organic Synthesis*; Schneider, M., Ed.; Reidel: Dordrecht, FRG, 1986. Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695-707.

(2) Mohr, P.; Waespe-Sarcevic, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501-2511.

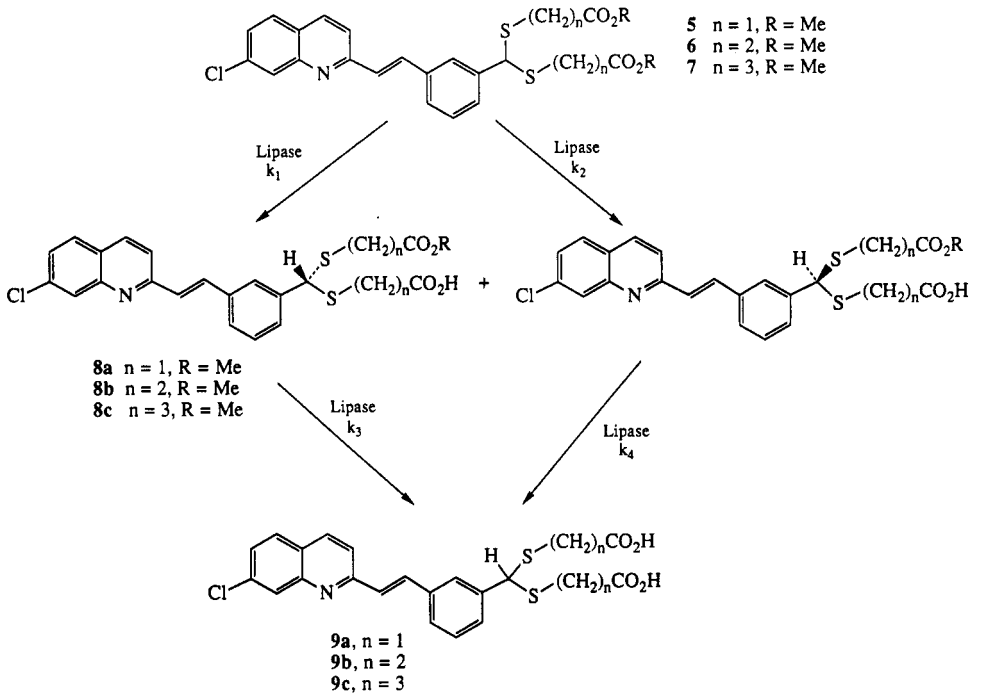
(3) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250-7251.

0022-3263/90/1955-6252$02.50/0

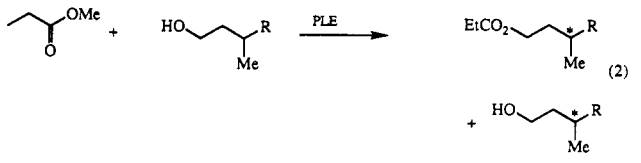
Scheme I



Scheme II



Similarly, Cambou and Klibanov[4] used pig liver esterase to esterify alcohols having three bonds between the alcohol and the chiral center with ee's generally above 90% (eq 2).



From the few examples reported of asymmetric hydrolysis of ester groups remote from the chiral/prochiral center, it is not clear whether enzymes are not suitable for these transformations or there are just few cases where they have been tried. What is clear is that asymmetric synthesis by typical chemical methods of such substrates becomes more difficult as the chiral center becomes more remote, so enzymatic methods offer great advantages if they can be made to work.

The question of using enzymes for remote ester hydrolysis arose when compounds **4a** and **4b**, both potent antagonists of LTD_4, became potential candidates for treatment of asthma, thus requiring efficient syntheses capable of making large quantities of drug. The elegant chiral syntheses of both enantiomers have been reported, which involve preparation of diastereomers early in the synthesis and separation of the diastereomers either by chromatography or by fractional crystallization, followed by conversion of each diastereomer into the appropriate enantiomer, **4a** or **4b**.[5] In principle, a more straightforward route to the enantiomers is through resolution of amide–ester **3** or selective hydrolysis of the prochiral diester **6**. However, in **3** and **6** the chiral/prochiral center is four bonds away from the reacting carbonyl group, plus the ester groups are on highly flexible chains which may not be optimal for selective fitting into a rigid enzyme active site. Despite these potential drawbacks to the enzymatic route, the rewards in terms of the synthetic simplicity, yield, and cost prompted us to explore the enzymatic route to these drug candidates. In a preliminary report we described the outcome of studies that led to highly efficient syntheses of both enantiomers with lipase-catalyzed asymmetric hydrolysis as the key step.[6]

(4) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687–2692.

(5) (a) Young, R. N.; Gauthier, J. Y.; Therien, M.; Zamboni, R. *Heterocycles* **1989**, *28*, 967. (b) Gauthier, J. Y.; Jones, T.; Champion, E.; Charette, L.; DeHaven, R.; Ford-Hutchinson, A. W.; Hoogsteen, K. K.; Lord, A.; Masson, P.; Piechuta, H.; Pong, S. S.; Springer, J. P.; Therien, M.; Zamboni, R.; Young, R. N. *J. Med. Chem.*, in press.

(6) (a) Hughes, D. L.; Bergan, J. J.; Amato, J. S.; Reider, P. J.; Grabowski, E. J. J. *J. Org. Chem.* **1989**, *54*, 1787–1788. (b) Hughes, D. L.; Bergan, J. J.; Reider, P. J.; Grabowski, E. J. J.; Amato, J. S. European Patent 89313350.4, Jan 31, 1990. (c) Presented in part by J. J. Bergan, ACS Middle Atlantic Meeting, May 1989.

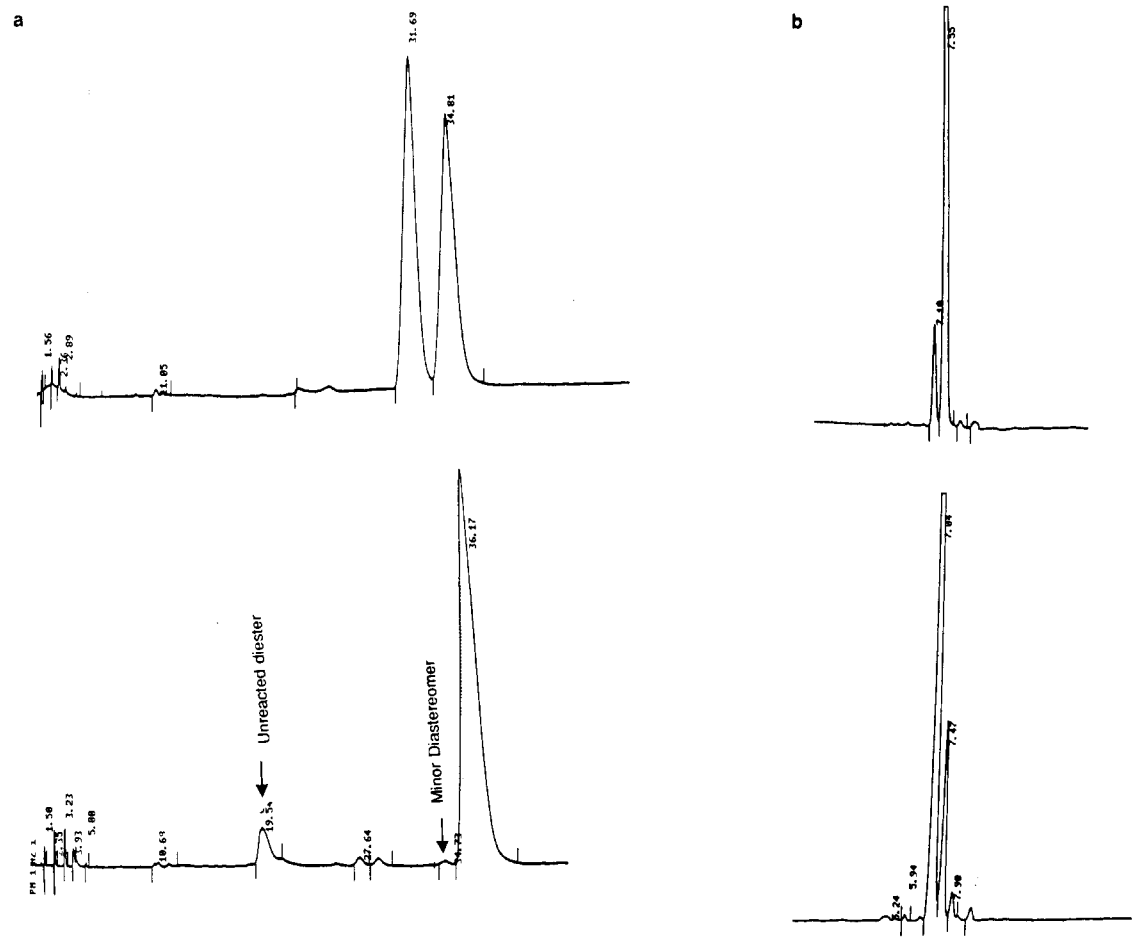


Figure 1. (a) HPLC assay of the ester–acid **8b** derivatized with (*R*)-(+)-1-(1-naphthyl)ethylamine. Top: racemic ester–acid. Bottom: chiral ester–acid (98.5% ee) from *Pseudomonas* lipase catalyzed hydrolysis. HPLC conditions: Du Pont Zorbax 25-cm C8 column; ambient temperature; detection, 350 nm; eluent, 80% acetonitrile/20% of 0.1% aqueous phosphoric acid; flow, 1.5 mL/min. (b) HPLC assay of ester–acid **8c**. Top: derivatized with (*S*)-(−)-1-(1-naphthyl)ethylamine. Bottom: derivatized with (*R*)-(+)-1-(1-naphthyl)ethylamine. HPLC conditions: Du Pont Zorbax 25-cm RX column; ambient temperature; detection, 350 nm; eluent, 70% acetonitrile/30% of 0.1% aqueous phosphoric acid; flow, 1.5 mL/min.

This paper gives full details of these studies, along with further examples of successful enzymatic hydrolyses with esters having chiral/prochiral centers up to five bonds away from the ester carbonyl group.

Results and Discussion

Since the LTD_4 antagonists **4a** and **4b** were our target molecules, most of our studies were directed toward the chemoenzymatic syntheses of these compounds. Two approaches were explored: one involving resolution of racemic ester–amide **3** (Scheme I) and the other involving selective hydrolysis of the prochiral diester **6** (Scheme II). If successful, the resolution route has the advantage of easy preparation of both enantiomers using a single enzyme, with the disadvantage of only being able to obtain a 50% yield of a single enantiomer. In contrast, the prochiral route has the advantage of potentially obtaining a 100% yield of one enantiomer.

In addition, a limited amount of work was done on analogues having three bonds (**5**) and five bonds (**7**) between the ester carbonyl group and the prochiral center. To check for substrate specificity, the simpler 1-naphthyl analogue (**10**) was also studied.

A. Enzymatic Hydrolysis of Prochiral Diester 6: Four Bonds between the Prochiral Center and the Ester Carbonyl. Table I shows the results of the enzymatic hydrolysis of various esters of **6**. Not included in Table I are several other lipases and esterases from commercial sources which gave no hydrolysis. Two enzymes, lipase from *Pseudomonas* species (Amano, Sigma)[7] and from *Chromobacterium viscosum* (Sigma), were found to selectively hydrolyze the dimethyl ester, providing ester–acid **8b** in >98% ee. The lipase from *Pseudomonas* species was also effective in hydrolyzing the (aminocarbonyl)-methyl diester **6** (R = CH_2CONH_2) with >98% ee.

The lipase from *Candida cylindracea* was sluggish toward the dimethyl ester, but was effective in hydrolyzing the activated esters. However, the selectivity was poor and overhydrolysis to the diacid was a major side reaction. Examination of Table I indicates that the CH_2CONH_2 diester gave the best results with lipase from *C. cylindracea*, giving a 90% chemical yield and an 86% ee. The ester–acid product obtained in this case could be upgraded to 95% ee by two recrystallizations from *i*-PrOH. It is not clear why the activated esters give higher enantioselectivity than the methyl ester with *C. cylindracea*. One explanation is that the hydrogen-bond-donating or -accepting ability of the activated ester moiety (a carbonyl or methoxy group) provides an additional handle for chiral binding.

(7) *Pseudomonas* sp. lipase obtained from Boehringer-Mannheim was not effective for hydrolyzing compound **6**. The *Pseudomonas* sp. lipases obtained from Amano and Sigma were different in terms of activity and selectivity. The subspecies from the Sigma lipase is not specified. The subspecies of the lipase from Amano was originally thought to be *fluorescens*, but in Sept 1989, Amano announced that the subspecies was actually *cepacia*.

Scheme III

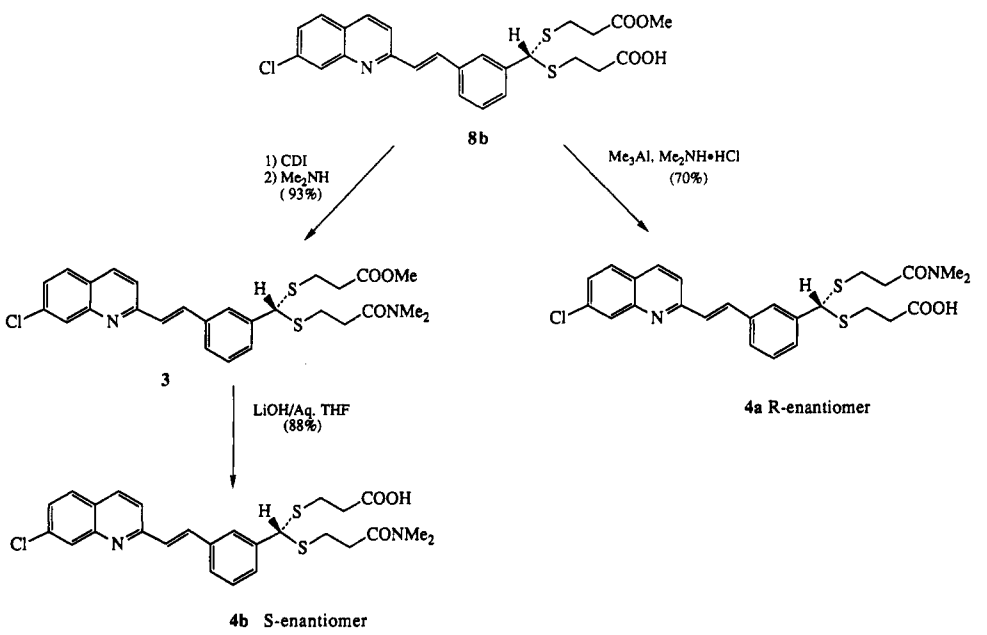


Table I. Enzymatic Hydrolysis of Prochiral Diester 6

R	enzyme	% chem yield of acid-ester 8b	% ee	% diacid 9b
Me	*Pseudomonas* (Amano)[a]	89	98.5	4
Me	*Pseudomonas* (Sigma)[b]	93	98.5	1
Me	*Pseudomonas* (BM)[c]	0	0	0
Me	*Chromobacterium viscosum*[d]	95	99	1
Me	*C. cylindracea*[e]	26	62	–
Me	pig liver esterase[f]	60	6	30
Me	pig pancreatic lipase[f]	10	0	–
CH_2CONEt_2	*C. cylindracea*[e]	30	62	10
CH_2CONEt_2	*C. cylindracea*[e]	51	86	10
CH_2CH_2OMe	*C. cylindracea*[e]	37	86	–
CH_2CONH_2	*C. cylindracea*[e]	90	86	5
CH_2CONH_2	*Pseudomonas* (Sigma)[b]	90	98	5
CH_2CONH_2	*Rhizopus javanicus*[g]	14	0	0
CH_2CONH_2	*Rhizopus arrhizus*[h]	10	28	0
CH_2CONH_2	pig pancreatic lipase[f]	13	0	0

[a] Amano P-30. [b] Sigma type XIII. [c] Boehringer-Mannheim. [d] Sigma type XII. [e] Sigma type VII. [f] Sigma. [g] Amano lipase FAP-15. [h] Sigma type XI.

In several control experiments we found that no hydrolysis of diester 6 or ester-acid 8b occurred in the absence of enzyme.

We found no chiral columns capable of resolving the underivatized enantiomers, underscoring their similarity. Therefore, the enantioselectivity was measured by preparation of the diastereomeric (*R*)- or (*S*)-1-(1-naphthyl)-ethyl amides, which were analyzed by reversed-phase HPLC on a C8 column. Figure 1 shows a chromatogram of racemic 8b prepared by chemical hydrolysis and chiral 8b prepared by using *Pseudomonas* lipase. When derivatized with (*R*)-(+)-1-(1-naphthyl)ethylamine, the *R,R* diastereomer elutes first, followed by the *R,S* diastereomer. The order of elution is reversed when the derivatization is done with the (*S*)-(−)-1-(1-naphthyl)ethylamine. This reversal in elution is shown in the chromatograms in Figure 1b for the diastereomeric amides prepared from 8c and serves as further evidence that the observed peaks in the chromatograms correspond to the two diastereomers and not to other reaction byproducts. As other control experiments, several synthetic mixtures of differing enantiomeric ratios were derivatized and analyzed to ensure that no chiral discrimination was taking place during the derivatization process. The diastereomeric mixtures could also be distinguished by ^1H NMR at 300 MHz, with the dithioacetal methine singlet resonances separated by about 0.05 ppm.

Important for these lipase-catalyzed reactions is the use of the nonionic surfactant Triton X-100. In the absence of the surfactant, the hydrolysis proceeded very slowly, presumably at the liquid–solid interface. The surfactant solubilizes the diester to a small extent and thereby increases the rate of hydrolysis. The other Triton surfactants X-405 and X-705 gave similar results in yield and enantioselectivity, but caused somewhat slower hydrolysis rates compared to X-100. No hydrolysis occurred in the presence of cationic or anionic surfactants. Addition of organic solvents such as DMF, ethanol, or 2-propanol decreased the rate of hydrolysis.

In cases where overhydrolysis to diacid occurred, the ee rose slightly as the reaction proceeded, as expected on the basis of earlier work of Sih.[8] The minor enantiomer formed in the first hydrolysis is a better substrate for the enzyme than the major enantiomer, so a kinetic resolution occurs ($k_4 > k_3$, Scheme II). As confirmation of this effect, racemic ester–acid 8b, when subjected to hydrolysis using *Pseudomonas* lipase, predominantly hydrolyzed the *R* enantiomer in a slow reaction, giving enrichment of the *S* enantiomer of the ester acid 8b.

Conversion of Chiral Ester–Acid 8b to LTD_4 Antagonists 4a and 4b. One potential drawback to the enzymatic hydrolysis of a prochiral diester is that only one enantiomer is produced. Generally, if both enantiomers are desired, then another enzyme must be found that will selectively produce the opposite enantiomer. However, in this case, the chiral nonracemic acid–ester 8b was readily converted to each enantiomer 4a and 4b by straightforward

(8) Wang, Y.-F.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *Enzymes in Organic Synthesis*; Ciba Foundation Symposium 111; Porter, R., Clark, S., Eds.; Pitman: London, 1985; pp 128–145.

Table II. Hydrolysis of Racemic Ester–Amide 3 with *C. cylindracea* Lipase

R	% yield[a]	% ee (ester–acid 4)
Me	15	20
allyl	33	36
CH_2COOEt	21	62
CH_2CONH_2	31	40
CH_2CONEt_2	30	70
CH_2COPh	5	0
CH_2CN	35	30
CH_2CH_2OMe	45	48

[a] For 100% ee, best yield would be 50%.

chemical modification of each arm of the compound (Scheme III). To make the *R* enantiomer **4a**, ester–acid **8b** was treated with 2.5 equiv of Me_3Al/Me_2NH_2Cl (Weinreb reagent)[9] in 70% yield after chromatography and crystallization from isopropyl alcohol. Alternatively, reaction with a 10% solution of Me_2NH in toluene at 100 °C for 6 h provided **4a** in 50% yield after chromatography.

The *S* enantiomer **4b** was prepared in overall 80% yield from ester–acid **8b** by activation of the acid with carbonyldiimidazole and displacement with Me_2NH to give amide–ester **3**, followed by hydrolysis with LiOH in aqueous THF (Scheme III).

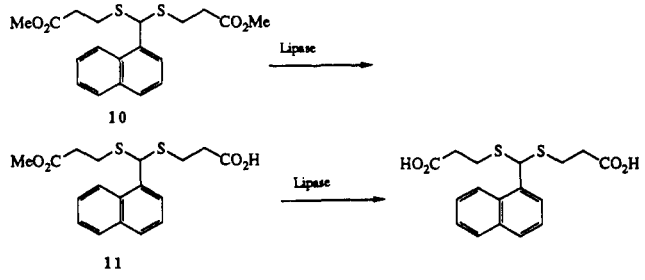
No loss in chirality was observed on taking ester–acid **8b** to final products **4a** and **4b**. The enantioselectivity was again determined by derivatization with (*R*)-(+)- or (*S*)-(−)-1-(1-naphthyl)ethylamine and analyzed by HPLC or ^1H NMR.

The absolute configurations are based on the work of Gauthier and co-workers in which an X-ray crystal structure of a synthetic diastereomeric intermediate was determined.[5]

B. Enzymatic Resolution of Racemic Ester–Amide 3. The other approach to the preparation of the enantiomers **4a** and **4b** was enzymatic resolution of racemic ester–amide **3** (Scheme I). Of many commercially available enzymes tried, none gave good results with the methyl ester. The best result was a disappointing 20% ee with *C. cylindracea* (Sigma). On the basis of the literature precedent with ibuprofen enzymatic resolutions,[10] several activated esters were prepared and subjected to hydrolysis with *C. cylindracea*. As shown in Table II, most of the activated esters gave results superior to those given by the methyl ester, with the CH_2CONEt_2 ester providing an 85/15 enantiomeric ratio. In all cases, the product was enriched in the *R* enantiomer, which has the same absolute configuration as that obtained from hydrolysis of the diester **6**. (Since the priorities change, the product is *R* from hydrolysis of **3** and *S* from hydrolysis of **6**.) This indicates that both compounds are fitting into the active site in the same way.

Because the acid–amide **4** crystallizes as a racemic compound instead of as a racemic mixture,[11] the 85/15 ratio could not be significantly upgraded by crystallization. In light of the success with the prochiral diester route, further efforts to improve the resolution route were not made.

C. Enzymatic Hydrolysis of 1-Naphthyl Diester 10. The prochiral diester **6** is a large molecule with several potential hydrophobic and hydrophilic binding sites which could be important for the high enantioselectivity observed in the enzymatic hydrolysis with *Pseudomonas* lipase. To test for substrate specificity, the simpler 1-naphthyl diester **10** was prepared and subjected to the enzymatic hydrolysis.



With *Pseudomonas* lipase (Sigma), the desired ester–acid **11** was produced in 90% yield with an ee of 94%. Although this ee is slightly less than that obtained with the quinolyl diester **6**, the result shows that the active site of *Pseudomonas* sp. lipase can accommodate both of these differing structures and lead to hydrolysis of primarily only a single ester. However, when an equal amount of enzyme is used, hydrolysis of the naphthyl diester **10** is about 10-fold slower than that of the quinolyl diester **6**, indicating perhaps a poorer binding for the smaller diester **10**.

With *C. cylindracea*, low selectivity was observed with diester **10**, and overhydrolysis to the diacid was a significant side reaction. The similar rates for the first and second hydrolyses of the ester groups by the lipase are somewhat surprising, since most ester–acids are usually not hydrolyzed by lipases.[12,13] In this case the enantioselectivity for the second hydrolysis was greater than for the first hydrolysis, resulting in a kinetic resolution of the intermediate ester–acid. For example, at the point where 30% of the diester was hydrolyzed and only 3% diacid had been formed, the ee was only 20%. At the point of maximum acid–ester yield (diester 19%, acid–ester 58%, diacid 23%), the ee had risen to 42%.

D. Hydrolysis of Diesters 5 and 7: Three and Five Bonds between Prochiral Center and Ester Carbonyl Group. In previous sections we have shown that prochiral diesters having four bonds between the prochiral center and the ester carbonyl can be hydrolyzed with high selectivity. In the two examples examined, the group attached to the dithioacetal center made little difference with the *Pseudomonas* lipase while a reduction in selectivity was observed with the *C. cylindracea* lipase. To examine the importance of the chain length in the dithioacetal, we prepared compounds **5** and **7**, in which there are three bonds (**5**) and five bonds (**7**) between the carbonyl group and the prochiral center, and subjected them to the enzymatic hydrolysis conditions which had been optimized for diester **6** (Table III).

For prochiral diester **7**, the ee's were generally lower than with diester **6**, as might be expected as the prochiral center is moved from four bonds to five bonds away from the ester carbonyl group. With lipase from *Pseudomonas* sp. (Sigma), ee's ranged from 75 to 80% with the Me ester and the CH_2CONH_2 ester, compared to an ee of >98% with prochiral diester **6**. With *C. cylindracea*, the ee for hydrolysis of the diester **7** (R = Me) was only 16%; however, hydrolysis of the second ester group proceeded with much higher selectivity, giving a kinetic resolution of the ester–acid. This kinetic resolution, which increases the ee at the expense of chemical yield, is shown in Figure 2. During the initial hydrolysis of the diester, the ee of the ester–acid produced remains virtually constant at 16%.

(9) Levin, J. I.; Turose, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
(10) Pietro, C.; Piccardi, P. European Patent 0195717, Sept 24, 1986.
(11) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates, and Resolutions*; Wiley: New York, 1981; pp 369–372.
(12) Levy, M.; Ocken, P. *Arch. Biochem. Biophys.* **1969**, *135*, 259–264.
(13) Krisch, K. *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 5, pp 43–69.

Table III. Enzymatic Hydrolysis of Prochiral Diesters 5 and 7

diester	R	enzyme	% diester	% acid-ester	% diacid	% ee ester-acid
5	Me	*C. cylindracea*[a]	0.6	92	4.2	78
5	Me	*C. cylindracea*[a]	0	69	31	86
5	Me	*Pseudomonas* sp.[b]	11	67	15	26
5	Me	*Pseudomonas* sp.[a]	46	33	10	48
7	Me	*Pseudomonas* sp.[a]	0	93	7	79
7	CH_2CONH_2	*Pseudomonas* sp.[a]	5	50	45	80
7	Me	*C. cylindracea*[a]	90	10	0	14
7	Me	*C. cylindracea*[a]	59	40	1	16
7	Me	*C. cylindracea*[a]	5	86	9	24
7	Me	*C. cylindracea*[a]	0	66	34	50
7	Me	*C. cylindracea*[a]	0	45	55	76
7	Me	*C. cylindracea*[a]	0	34	66	85
7	CH_2CONH_2	*C. cylindracea*[a]	25	50	25	90

[a] Sigma. [b] Amano P-30.

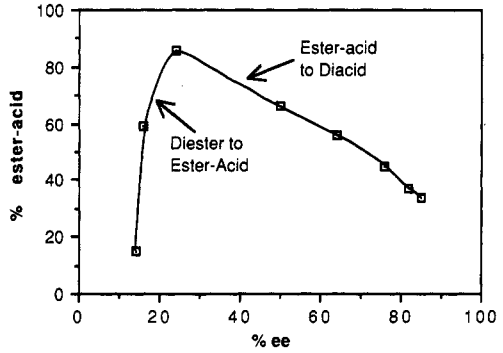


Figure 2. Plot of % ester-acid 8c vs ee for hydrolysis of diester 7 using *C. cylindracea* (Sigma).

The hydrolysis of the ester-acid to the diacid, which is several-fold slower, is more selective and results in an increase in the ee of the unreacted ester-acid. The calculated ratios for the rate constants are $k_1/k_2 = 1.3$ and $k_4/k_3 = 5.5$. This system therefore represents an unusual case wherein the ester-acid (presumably ionized at pH 7) is a good fit for the enzyme active site and gives better selectivity in the hydrolysis than the prochiral diester.

From the above discussion, it can be noted that the *Pseudomonas* sp. lipase is most effective at selectively hydrolyzing the prochiral diester while the *C. cylindracea* lipase is capable of kinetically resolving the ester-acid. This suggested that use of the enzymes together would give ester-acid 8c in highest yield and ee. The results of the dual enzyme experiment are shown in Table IV. Initially, the lipase from *Pseudomonas* sp. is added to the prochiral diester 7, resulting is a selective hydrolysis to the ester-acid 8c (88% yield) with an ee of 80%. When the diester level was below 2%, the lipase from *C. cylindracea* was added, which initiated the kinetic resolution of 8c. At the point where 28% of the ester-acid had been converted to diacid, the ee had risen to 91%, and at the point of 45% conversion to diacid, the ee had further increased to 95%.

For prochiral diester 7, R = CH_2CONH_2, hydrolysis with *C. cylindracea* lipase proceeded with much better selectivity, giving a 90% ee at the point where there was 50% ester-acid and 25% each of diacid and diester. However, in this case, the second hydrolysis proceeded at a rate that was only slightly slower than that of the first hydrolysis.

Perhaps more surprising are the poor results with diester 5, wherein there are only three bonds between the prochiral center and the ester carbonyl. The lipase from *Pseudomonas* sp., which gave excellent results with diester 6, gives ee's of only 26–48% with diester 5. With *C. cylindracea*, the hydrolysis was more selective, giving a 78% ee at a high conversion of diester to ester-acid. As with the other substrates, a kinetic resolution occurs on conversion of the acid-ester to the diacid, bringing the ee up to 86% at a point where 31% has been converted to the diacid.

Table IV. Consecutive Enzymatic Hydrolysis and Kinetic Resolution of Prochiral Diester 7 Using Lipases from *Pseudomonas* sp. and *C. cylindracea*

enzyme	time, h	% diester 7	% ester-acid 8c	% diacid 9c	% ee (8c)
Pseudomonas sp.	22	43	54	3.4	78
	48	11	82	7	80
	62	1.5	88	10	80
C. cylindracea	70	1	83	16	84
added at 62-h	85	0.5	71	28	91
point	93	0.3	66	34	93
	108	0.3	55	45	95

Summary

Lipases are capable of selectively hydrolyzing prochiral diesters with distances up to five bonds between the prochiral center and the ester carbonyl. With lipase from *Pseudomonas* sp., chemical yields and ee's were better with the substrate having four bonds between the prochiral center and the ester carbonyl than with the three-bond or five-bond analogues, demonstrating that selectivity does not necessarily diminish as the distance between the chiral center and the reaction site increase. These results, taken along with those in the literature,[14] indicate that the lipase from *Pseudomonas* sp. can hydrolyze a diverse range of ester substrates, both large and small. On the basis of this

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information, a possible model for the enzyme active site is one in which the active site contains many different binding pockets. In order to selectively hydrolyze the large prochiral diester **6**, a large, chiral hydrophobic pocket must be available for binding. However, several other smaller, chiral hydrophobic pockets must also be present near the active site that can be used to selectively bind substrates of smaller size. Alternatively, the lipases may have more than one active site which may be used depending on the size and nature of the substrate. Another possibility is that the enzyme has enough conformational flexibility to accommodate different size structures in the same active site and binding pocket.

In this work we have shown that lipases are capable of selectively hydrolyzing esters having remote chiral/prochiral centers and therefore are a very powerful tool for organic synthesis. Chiral syntheses which by conventional chemical methods would be exceedingly difficult are performed readily and inexpensively with commercially available enzymes. Further work on other remote prochiral/chiral systems is ongoing to gain further insight into the active site and the versatility of lipases. In addition, kinetic studies on the current systems are providing insight on the binding of these substrates to the enzyme, on the role of Triton X, and on product inhibition and will be reported in due course.

Experimental Section

General. ^1H and ^{13}C NMR spectra were taken on Brucker AM-250 or AM-300 instruments. Rotations were taken on a Perkin-Elmer Model 241 polarimeter using a 10-cm cell. The sources of the enzymes are given in the tables. The dithioacetals were prepared from the appropriate thiol and aldehyde as described earlier.[15]

(*S*)-3-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)-phenyl)((3-methoxy-3-oxopropyl)thio)methyl)thio)propionic Acid (8b). Two preparations are described: one using crude lipase from *Pseudomonas* sp. (Amano P-30) and one using purified lipase from *Pseudomonas* sp. from Sigma.

Crude Lipase. Amano lipase P-30 (90.5 g) was stirred for 1 h at room temperature in 600 mL of 0.1 M sodium phosphate buffer at pH 7.5 and then filtered through a bed of Solka-Floc. This pretreatment of the crude enzyme removes water-insoluble material. The filtered solution of the enzyme was added to a 1-L, three-necked flask equipped with a pH probe and a mechanical stirrer, followed by diester **6** (30.3 g, 0.059 mol) and Triton X-100 (9.3 g). The reaction mixture was warmed to 40 °C and stirred for 23 h. During this period the pH was maintained in the range 6.8–7.3 by periodic addition of 5 N NaOH. The reaction mixture remained heterogeneous throughout the reaction age, since starting material and product free acid were largely insoluble in the aqueous medium. At the end of the age, the pH was adjusted to 6.5 by addition of concentrated HCl, and the slurry was stirred for an additional 2 h. The mixture was filtered and washed with 2 × 100 mL of water. After drying at 55 °C for 8 h under 1-mm vacuum, 27.8 g of ester–acid **8b** was obtained having 95% purity and 98.5% ee (89% yield). This material was used without purification in the next step.

An analytical sample was prepared by silica chromatography using 0.7% HOAc in EtOAc as eluent, followed by crystallization from *i*-PrOH: mp 121–122 °C; ^1H NMR (CD_3CN) δ 2.54–2.61 (m, 4 H, CH_2C), 2.73–2.87 (m, 4 H, CH_2S), 3.61 (s, 3 H, CO_2CH_3), 5.12 (s, 1 H, Ar CH), 7.38–8.26 (m, 11 H, olefinic/aromatic), 9.2 (br s, 1 H, CO_2H); $[\alpha]_D^{25}$ −5.2° (*c* = 2, CH_2Cl_2).

Anal. Calcd for $C_{25}H_{24}ClNO_4S_2$: C, 59.81; H, 4.82; N, 2.79; Cl, 7.06; S, 12.77. Found: C, 59.75; H, 4.92; N, 2.73; Cl, 7.00; S, 12.69.

Pure Lipase. Compound **6** (5.27 g), Triton X-100 (5.0 g), Na_2HPO_4 (7.10 g), *Pseudomonas* sp. lipase (Sigma) (0.020 g), and 500 mL of water were combined, and the pH was adjusted to 7.5 by addition of H_3PO_4. The heterogeneous mixture was stirred for 14 days at 37 °C. The pH was then adjusted to 4.5 to crystallize the ester–acid, and the mixture was stirred for an additional 5 h at room temperature. The mixture was filtered to provide, after drying under vacuum at 55 °C, 4.85 g of the ester–acid **8b** with 97% chemical purity and 98.5% ee (92% yield).

HPLC Assays for Enantiomeric Purity. Stock solutions of 0.2 M Et_3N, ethyl chloroformate, and (*R*)-(+)-1-(1-naphthyl)ethylamine were prepared in CH_3CN. Approximately 2 mg of ester–acid **8b** was dissolved in 0.30 mL of THF, and then 0.10 mL of the stock solution of Et_3N and 0.05 mL of the stock solution of ethyl chloroformate were added and mixed for 5 min at ambient temperature. Then 0.05 mL of the solution of (*R*)-(+)-1-(1-naphthyl)ethylamine was added, and the mixture was aged for 1 min at ambient temperature. The entire sample was diluted in 100 mL of 20% aqueous CH_3CN, and 20 μL was injected for HPLC analysis. Conditions: 25-cm Du Pont Zorbax C8 column; ambient temperature; 1.5-mL flow rate; detection at 275 or 350 nm; eluent consisting of 80% CH_3CN/20% of 0.1% aqueous H_3PO_4. The *R,R* diastereomer eluted at 32 min and the *R,S* diastereomer at 35 min (Figure 1a).

The same derivatization method was used for compounds **8c**, **4a**, and **11**. For compounds **4b** and **8c**, the derivatization was done with (*S*)-(−)-1-(1-naphthyl)ethylamine in order for the minor diastereomer to elute first. For compound **8a**, the derivatization was mediated by using 2-chloro-1-methylpyridinium iodide and Et_3N in THF. HPLC conditions for **4a** and **4b** were the same as for **8b**. The conditions used for the other compounds were as follows. Compound **8a**: 25-cm Du Pont Zorbax phenyl column; ambient temperature; 1.0-mL flow rate; detection at 275 or 350 nm; eluent consisting of 80% MeOH/20% of 0.1% aqueous H_3PO_4; the diastereomers eluted at 44 and 46 min. Compound **8c**: 25-cm Du Pont Zorbax RX column; ambient temperature; 1.5-mL flow rate; detection at 275 or 350 nm; eluent consisting of 70% CH_3CN/30% of 0.1% aqueous H_3PO_4; the diastereomers eluted at 7.1 and 7.5 min. Compound **11**: 25-cm Du Pont Zorbax phenyl column; ambient temperature; 1.5-mL flow rate; detection at 275 or 350 nm; eluent consisting of 42% CH_3CN/58% of 0.1% aqueous H_3PO_4; the diastereomers eluted at 107 and 110 min.

In all cases, the separations were established by using the racemic compounds. As further confirmation, each chiral compound was derivatized with both the (*R*)- and (*S*)-(−)-1-(1-naphthyl)ethylamines. The reversal in elution order of the diastereomers (Figure 1b) upon derivatizing with the opposite amine enantiomer further confirmed the identity of the observed peaks.

(*S*)-3-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)-phenyl)((3-(dimethylamino)-3-oxopropyl)thio)methyl)-thio)propionic Acid Methyl Ester (3). To a 250-mL round-bottomed flask were added (*S*)-3-(((3-(2-(7-chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-methoxy-3-oxopropyl)thio)methyl)-thio)propionic acid (**8b**) (10.0 g, 95% pure, 0.019 mol) and THF (100 mL). After cooling to 3 °C, 1,1′-carbonyldiimidazole (10.25 g, 0.063 mol) was added and the solution was aged for 1 h at 3 °C. Then, a stream of Me_2NH was bubbled into the solution for 5 min at 3 °C. After aging for another 10 min, EtOAc (100 mL) was added. The solution was washed with saturated $NaHCO_3$ (1×) and brine (1×), dried over $MgSO_4$, and evaporated to an oil. This material was used without further purification in the next step.

An analytical sample of (*S*)-**3** was prepared by silica chromatography, initially using as eluent 1/1 hexane/EtOAc followed by increasing amounts of EtOAc: ^1H NMR ($CDCl_3$) δ 2.6 (overlapping m, CH_2C), 2.85 (overlapping m, CH_2S), 2.90 (s, $N(CH_3)_2$), 3.58 (s, OCH_3), 5.06 (s, Ar CH), 7.3–8.2 (aromatic and olefinic protons); $[\alpha]_D^{25}$ −5.1° (*c* = 2, THF).

Anal. Calcd for $C_{27}H_{29}ClN_2O_3S_2$: C, 61.29; H, 5.52; N, 5.30; Cl, 6.70; S, 12.12. Found: C, 61.02; H, 5.59; N, 5.19; Cl, 6.65; S, 12.07.

(*S*)-3-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)-phenyl)((3-(dimethylamino)-3-oxopropyl)thio)methyl)-thio)propionic Acid (4b). THF (80 mL) and the crude (*S*)-3-(((3-(2-(7-chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-(dimethylamino)-3-oxopropyl)thio)methyl)thio)propionic acid methyl ester (**3**) from the previous step were mixed and cooled to 3 °C. A solution of $LiOH \cdot H_2O$ (0.80 g, 0.019 mol) in water (20 mL) was added over a 15-min period, the reaction mixture being kept below

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5 °C. After 2.5 h, solid $LiOH·H_2O$ (0.40 g, 0.0095 mol) was added and the reaction mixture was further aged at 3 °C for 7 h. At the end of the age, water (150 mL) was added and the pH was adjusted to 4 by addition of 6 N HCl. EtOAc (100 mL) and THF (80 mL) were added, and the mixture was stirred for 3 min. The organic layer was separated, washed with brine, dried over $MgSO_4$ at 3 °C, and evaporated to an oil. The oil was dissolved in THF (30 mL) and chromatographed on 450 g of silica gel (mesh 230–400) by using 1% HOAc in EtOAc. The rich cuts were combined and concentrated, flushed twice with toluene to remove HOAc, and crystallized from *i*-PrOH (300 mL) for 36 h at ambient temperature. Isolation and vacuum drying provided 7.9 g (99% pure, 82% yield for two steps) of **4b**: mp 157–158 °C; ^1H NMR (DMSO-d_6) δ 2.5–3.4 (overlapping multiplets, 4 CH_2), 2.79, 2.89 (2 s, $N(CH_3)_2$),5.32 (s, Ar CH), 7.44 (m, 5′-H, 6′-H), 7.47 (d, J = 16.5 Hz, 3′-C=), 7.59 (dd, J = 8.7, 2.3 Hz, 6-H), 7.67 (m, 4′-H), 7.80 (br s, 2′-H), 7.87 (d, J = 16.5 Hz, 2-CH=), 7.95 (d, J = 8.6 Hz, 3-H), 8.00, 8.03 (overlapping doublets, 5-H, 8-H), 8.40 (d, J = 8.6 Hz), 12.3 (br s, CO_2H); $[\alpha]_D^{25}$ −5.0° (c = 2, THF), $[\alpha]_D^{25}$ +9.0° (c = 0.7, 1% aqueous $NaHCO_3$) (lit.[5] $[\alpha]_D^{25}$ = +9.0° (1% $NaHCO_3$)).

Anal. Calcd for $C_{26}H_{27}ClN_2O_3S_2$: C, 60.63; H, 5.28; N, 5.44; Cl, 6.88. Found: C, 60.28; H, 5.33; N, 5.32; Cl, 6.88.

(*R*)-3-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-(dimethylamino)-3-oxopropyl)thio)methyl)thio)propionic Acid (4a). To a 100-mL flask under N_2 was added anhydrous dimethylamine hydrochloride (4.11 g, 0.050 mol) and 25 mL of anhydrous toluene. The solution was cooled to −30 °C, and a 2 M solution of trimethylaluminum in toluene (25 mL, 0.050 mol) was added over a 15-min period. The solution was slowly warmed to room temperature over a 1-h period and aged for 1 h at ambient temperature.

To a 250-mL flask was added (*S*)-3-(((3-(2-(7-chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-methoxy-3-oxopropyl)thio)-methyl)thio)propionic acid (**8b**) (10.0 g, 95% pure, 0.019 mol) and CH_2Cl_2 (100 mL). The solution was cooled to 3 °C. The solution of $AlMe_3$–NMe_2 complex prepared above was added to the acid–ester **8b** solution over a 10-min period, the temperature being kept below 5 °C. The mixture was warmed to 20 °C for 5 h and then 40 °C for 15 h. The reaction was quenched by slowly adding it to an ice-cold solution containing 10% aqueous KH_2PO_4 (300 mL), THF (200 mL), and EtOAc (200 mL). This mixture was warmed to 40 °C and stirred for 1 h. The organic layer was then separated, washed with brine, dried with $MgSO_4$, filtered through Celite, and concentrated. The resulting oil was chromatographed and crystallized from *i*-PrOH to give 7.1 g of **4a** (96% purity, 72% yield). An analytical sample was prepared by recrystallization twice from 2-butanone: mp 157–158 °C; ^1H NMR spectrum identical with that of **4b**; $[\alpha]_D^{25}$ +5.2° (c = 2, THF), $[\alpha]_D^{25}$ −9.1° (c = 0.7, 1% aqueous $NaHCO_3$) (lit.[5] $[\alpha]_D^{25}$ = −9.1° (1% $NaHCO_3$)).

Anal. Calcd for $C_{26}H_{27}ClN_2O_3S_2$: C, 60.63; H, 5.28; N, 5.44; Cl, 6.88. Found: C, 60.81; H, 5.28; N, 5.43; Cl, 6.86.

2-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((2-methoxy-2-oxoethyl)thio)methyl)thio)ethanoic Acid (8a). Compound **5** (0.155 g, 0.318 mmol), Triton X-100 (0.35 g), lipase from *C. cylindracea* (Sigma crude) (0.39 g), and 12 mL of 0.1 M phosphate buffer (pH 7.5) were stirred for 3 h at ambient temperature. Then the pH was lowered to 4.5, and the aqueous solution was extracted with 25 mL of EtOAc, concentrated to an oil, and chromatographed on silica (0.5% HOAc in EtOAc) to give 121 mg (81%) of **8a** as an oil (ee = 78%): ^1H NMR (CD_3CO_2D) δ 3.3–3.7 (overlapping AB patterns, 4 H, CH_2), 3.72 (s, 3 H, CO_2CH_3), 5.46 (s, 1 H, Ar CH), 7.5–8.7 (m, 11 H, aromatic and olefinic H).

4-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((4-methoxy-4-oxobutyl)thio)methyl)thio)butanoic Acid (8c). Diester **7** (295 mg, 0.542 mmol), Triton X-100 (597 mg), lipase from *Pseudomonas* sp. (Sigma) (5.7 mg), and 30 mL of 0.1 M phosphate buffer (pH 7.5) were stirred at ambient temperature for 88 h. Assay at this point indicated 6% diacid **9c**, 3% diester **7**, and 91% ester–acid product **8c**, having an ee of 80%. The solution was filtered to remove unreacted diester, then the pH of the filtrate was adjusted to 5.5, and the product was extracted with 3 × 50% EtOAc/THF (40 mL). The combined organic layers were washed with brine and concentrated to an oil (900 mg) containing Triton X-100 and product. The oil was chromatographed on silica gel (35 g) using 50/50/0.5 EtOAc/hexanes/HOAc as eluent to provide, after concentration and flushing with toluene to remove HOAc, 224 mg (78%) of a pale yellow oil of **8c**, ee = 80%: ^1H NMR ($CDCl_3$) δ 1.9–2.1 (m, 4 H, CCH_2C), 2.45–2.6 (m, 4 H, CH_2CO_2R), 2.65–2.8 (m, 4 H, SCH_2), 3.68 (s, 3 H, CO_2CH_3), 4.97 (s, 1 H, Ar CH), 7.15–8.2 (m, 11 H, aromatic and olefinic H); $[\alpha]_D^{25}$ −1.82° (c = 1.5, THF).

3-(((1-Naphthyl)((3-methoxy-3-oxopropyl)thio)methyl)-thio)propionic Acid (11). Methyl diester **10** (373 mg, 1.08 mmol), lipase from *Pseudomonas* sp. (Sigma) (14.2 mg), Triton X-100 (1.82 g), and 40 mL of 0.1 M phosphate buffer (pH 7.4) were stirred for 7 days at 40 °C. The reaction mixture was worked up by extracting the product into EtOAc, washing with brine, drying with $MgSO_4$, and chromatographing on 30 g of silica gel using 0.2% HOAc in EtOAc as eluent to provide ester–acid **11** as a colorless oil (308 mg, 86%), ee = 94%: $[\alpha]_D^{25}$ −2.1° (c = 1.4, THF); ^1H NMR ($CDCl_3$) δ 2.55–3.05 (overlapping multiplets, 8 CH_2), 3.67 (s, 3 H, CO_2CH_3), 5.83 (s, 1 H, Ar CH), 7.4–8.3 (m, 7 H, aromatic H).

Diester 6 (R = CH_2CONH_2). Diacid **9b** (8.96 g, 0.0197 mol), anhydrous DMF (60 mL), diisopropylethylamine (8.04 g, 0.623 mol), and bromoacetamide (6.11 g, 0.0443 mol) were mixed and stirred for 18 h at ambient temperature. The mixture was poured into EtOAc (200 mL) and washed with 8% $NaHCO_3$ (200 mL), water (200 mL), and brine. The organic layer was concentrated to provide a yellow solid, which was recrystallized from 3/1 EtOAc/hexanes to provide the (aminocarbonyl)methyl diester **6** (R = CH_2CONH_2), 8.63 g (77%): mp 103–104 °C; ^1H NMR (DMSO-d_6) δ 2.7–2.9 (overlapping multiplets, 8 H, SCH_2CH_2C), 4.42 (s, 4 H, OCH_2), 5.37 (s, 1 H, Ar CH), 7.45 (br s, 4 H, NH_2), 7.5–8.5 (m, 11 H, aromatic and olefinic H).

Anal. Calcd for $C_{28}H_{28}ClN_3O_6S_2$: C, 55.86; H, 4.69; N, 6.98; Cl, 5.89. Found: C, 55.59; H, 4.44; N, 6.95; Cl, 6.12.

(*S*)-3-(((3-(2-(7-Chloroquinolin-2-yl)-(*E*)-ethenyl)phenyl)((3-((aminocarbonyl)methoxy)-3-oxopropyl)thio)-methyl)thio)propionic Acid (8b, R = CH_2CONH_2). (Aminocarbonyl)methyl diester **6** (R = CH_2CONH_2) (181 mg, 0.318 mmol), *C. cylindracea* lipase (Sigma) (337 mg), 0.1 M aqueous phosphate buffer (30 mL, pH 7.5), and Triton X-100 (2 g) were stirred for 26 h at ambient temperature. HPLC assay of the mixture indicated 1% unreacted diester, 90% ester–acid, and 9% diacid; assay of the derivatized sample indicated a 93/7 ratio of enantiomers. The solution was adjusted to pH 5 and extracted with 3 × 50 mL of 50/50 THF/EtOAc. The organic layer was concentrated to an oil and chromatographed on silica gel (20 g, 60–200 mesh) by using 1% HOAc in EtOAc. The rich cut was concentrated and crystallized from 20 mL of *i*-PrOH to provide ester–acid **8b** (R = CH_2CONH_2), 119 mg (73%) (ee = 92%): mp 159–160 °C; ^1H NMR (DMSO-d_6) δ 2.5–2.6 (m, 4 H, CH_2C), 2.65–2.85 (m, 4 H, CH_2S), 4.41 (s, 2 H, CH_2O), 5.34 (s, 1 H, Ar CH), 7.45 (s, 2 H, NH_2), 7.5–8.5 (m, 11 H, aromatic and olefinic H), 12.2 (br s, 1 H, COOH).

Anal. Calcd for $C_{26}H_{25}ClN_2O_5S_2$: C, 57.29; H, 4.62; N, 5.14; Cl, 6.50. Found: C, 57.27; H, 4.62; N, 5.10; Cl, 6.65.