Table II. Summary of Structural Characteristics of Alternative Linkers from Molecular Mechanics Calculations as Potential Tethers[a]

compound		shape/length	flexibility	stability	rigidity
		cyclic compounds			
cis	$n = 3$	d	-	-	-
	$n = 4$	d	-	-	-
	$n = 5$	c	b	b	b
	$n = 6$	c	b	b	b
trans	$n = 3$	b	b	b	b
	$n = 4$	b	b	b	b
	$n = 5$	b	b	b	b
	$n = 6$	b	b	c	b
		double bond			
cis		d	-	-	-
trans		a	c	a	b

[a] a = very good, b = good, c = acceptable-poor, d = unacceptable.

of the minor groove of DNA.

The footprinting patterns and particularly the binding site size of the cis-linked structures are similar to those of distamycin indicating that in these cases only one of the arms binds at a time to the DNA in a monodentate fashion as shown in Figure 16b while the second bis-pyrrole moiety is directed away from the minor groove. In contrast to the behavior of the cis compounds, the trans isomers protect longer sequences of the DNA against MPE cleavage. This may correspond to actual bidentate binding with both arms of the ligand bound to the receptor or an "operational bidentate" binding in which one arm is bound tightly and the second arm is constrained close enough to the adjacent portion of the minor groove to prevent cleavage by the footprinting agent. However the footprinting patterns of all the trans compounds are not the same. The compounds linked by trans cyclopropane, cyclobutane, and fumaroyl moieties give evidence of bidentate binding, and those compounds linked by trans cyclopentane and trans cyclohexane rings give slightly weaker and shorter footprints. These results are in accord with the molecular mechanics analyses and indicate that conformational flexibility of the linker also plays a role in interaction of the bis trans-linked compounds with DNA.

The force field analysis indicates that while none of the tested tethers appears to be ideal (Table II) several of them have useful characteristics. The better tethers amongst those examined in this study are the trans cyclopropane, trans cyclobutane, and trans cyclopentane moieties, the fumaroic acid derivative and finally the succinate bridge. However, as explained in connection with the footprinting results, it cannot be excluded that they bind to DNA only in an operational bidentate fashion (Figure 16). Thus all of the factors discussed in this analysis which favor true bidentate binding also apply to the "dancing" mechanism. Distamycin-2 binds to DNA about 10 times weaker than distamycin-3.[43] From the experimentally determined binding constants of Dst-3 (**2**), K_a for Dst-2 can be estimated to be 4×10^4 M^{-1} with a ΔG for binding of about 6.3 kcal M^{-1}. The enthalpy of bidentate binding is expected to be about twice that for monodentate binding. Provided the entropic factors are comparable for each moiety and additive (which may not be the case) a K_a of about 10^9 is expected in the ideal case.[19,20] However because of strain in the bound ligand and less efficient binding of the longer nonideal lexitropsin, the observed K_a can be lower. Regardless of the details of the molecular recognition processes the K_a for true monodentate binding and a "dancing" process should exhibit K_a about twice as large as that for Dst-2 because of the effective doubling of the concentration of the ligand. This analysis therefore indicates a dancing mode for **6** rather than true bidentate binding. A systematic analysis of thermodynamic data from microcalorimetry as well as detailed NMR experiments should permit a more reliable differentiation of the binding modes, and such studies are ongoing.

Acknowledgment. We wish to thank Dr. M. Dobler for use of the MacMOMO program and Dr. R. S. Brown for use of his microcomputer facilities.

Registry No. **1**, 1438-30-8; **2**, 636-47-5; **3**, 130699-06-8; **4**, 130699-07-9; **5**, 130699-08-0; **6**, 130699-09-1; **7**, 130699-10-4; **8**, 130699-11-5; **9**, 130699-12-6; poly(dA-dT), 26966-61-0; lexitropsin, 121854-21-5.

Supplementary Material Available: Conformation plots of all the compounds studied in this paper (12 pages). Ordering information is given on any current masthead page.

(43) Zimmer, Ch.; Luck, G.; Birch-Hirschfeld, E.; Weiss, R.; Arcamine, E.; Guschlbauer, W. *Biochim. Biophys. Acta* **1983**, *741*, 15.

Design of α-Alkyl β-Hydroxy Esters Suitable for Providing Optical Resolution by Lipase Hydrolysis

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Received February 26, 1990

A study of the lipase-catalyzed hydrolyses of various α-substituted β-acetoxy esters revealed that a sulfur functional group in the ester, which could play an important role in the stereorecognition by lipase A6 (*Aspergillus* sp.) and an anti conformation in the ester, promotes satisfactory results in the hydrolysis.

Optically active 3-hydroxy ester derivatives are now widely recognized as highly useful chiral building blocks in the syntheses of many optically active natural and unnatural products.[1] Recently, many commercially available

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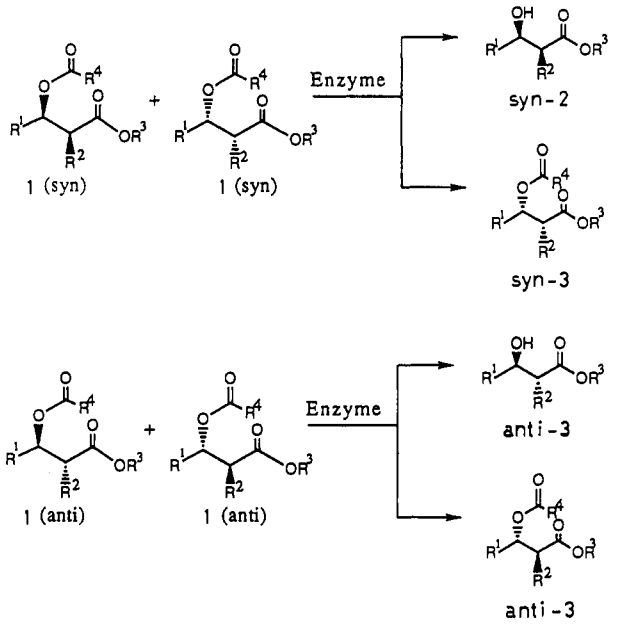
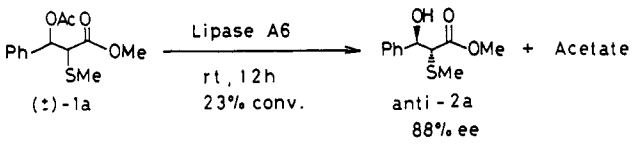
Scheme I



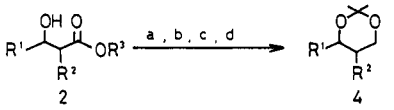
Scheme II



esterases have been successfully used as resolving agents in the preparation of opticaly active β-hydroxy esters (Scheme I). Lipases have been particularly effective in this role.[2] Lipases are uniquely stable, easy to handle, and, remarkably, can assume a variety of conformations in order to accommodate substrates of varying sizes and stereochemical complexities.[3] However, no guidelines exist for the matching of an effective lipase, from among the many, with a particular substrate needing resolution. We therefore resorted to trial and error in the selection of an appropriate enzyme. In order to do this, a knowledge of the rules of enzyme stereoselectivity was required. However, no such formula exists for the design of substrates suitable for enzyme catalyzed resolution.

Seeking an effective approach to the preparation of optically active sulfur-functionalized chiral building blocks, we examined chemo- and enantioselective hydrolyses of

Scheme III[a]



[a] (a) DHP, PPTS, room temperature; (b) $LiALH_4$, Et_2O, −78 °C; (c) *p*-TsOH, MeOH, room temperature; (d) 2,2-dimethoxypropane, *p*-TsOH, acetone, room temperature.

2-(methylthio)-3-acetoxy esters by lipases. During this investigation, we observed the interesting fact that both the enantioselectivity and speed of hydrolysis of methyl 2-(methylthio)-3-acetoxy-3-phenylpropionate (**1a**) were quite different, depending upon the diastereomer, using lipase A6 (*Aspergillus* sp.), and that only the *anti*-**1a** gave a satisfactory result. Because the rate of hydrolysis was so different with the two diastereomers, the alcohol produced after 12-h reaction was mostly the anti isomer (along with 2% of syn isomer) with 88% enantiomeric excess (Scheme II). On the other hand, it has not been reported that results of the lipase-catalyzed resolution of 2-methyl-3-acetoxy esters varied with the diastereomer using the same enzyme lipase A6.[2a] When 8 lipases were tested in our preliminary experiments using β-hydroxy esters as substrates, lipase A6 gave the most satisfactory results.[4] If we can clarify the factors which determine stereochemical preference toward lipase A6, this work might become a good guidepost in designing substrates suitable for this useful enzyme, as well as provide important information about the active site of the enzyme.

We now report the results of the kinetic resolution of various 2-substituted 3-hydroxy esters and propose a model which can account for such resolution using lipase A6 (*Aspergillus* sp.).

Results and Discussion

When methyl (2*RS*,3*RS*)-2-(methylthio)-3-phenylpentanoate (**1a**) was subjected to hydrolysis using lipase A6 as a catalyst, only the 2*R*,3*R* isomer of **2a** was hydrolyzed. Hence, it appears that the enzymes in the lipase have a diastereofavoritism, i.e. the anti isomer fits them better than does the syn isomer. Since lipase A6 consists of several proteins,[5] two different interpretations might explain the observed enanioselectivity. First, the selectivity might result from a competition for substrate hydrolysis by two different enzymes, generating alcohols of opposite configuration. Second, the selectivity could result from the differences between enantiomers of each diastereomer in binding affinity for the active site of the enzyme. In order to confirm which factors lead to the observed results of this reaction, a control experiment[6] was performed (Figure 1, parts a and b).

As it was found that the enzyme preferentially hydrolyzed the anti diastereomer, the racemic *anti*-**1a** was subjected to the resolution experiment with varying enzyme concentrations. If the enantioselectivity is a result of the presence of two or more competing enzymes of "opposite stereochemical preference", some differences in the ee of alcohols should be observed because the kinetic resolution is ruled by the difference in hydrolysis rate

(1) For reviews see: (a) Seebach, D.; Roggo, S.; Zimmermann, J. *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Ed.; VCH Verlags: Gesellschaft, 1987; p 86. (b) Mori, K. *Tetrahedron* **1989**, *45*, 3233 and references cited therein.

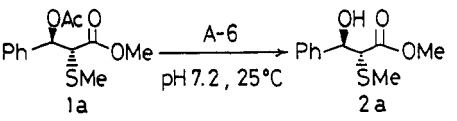
(2) (a) Akita, A.; Matsukura, H.; Oishi, *Tetrahedron Lett.* **1986**, *27*, 5241. (b) Xie, Z.-F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1987**, 839. (c) Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *Ibid.* **1988**, 966. (d) Scilimati, a.; Ngooi, T. K.; Sih, C. J.; *Tetrahedron Lett.* **1988**, *29*, 4927. (e) Feichter, C.; Faber, K.; Griengl, H. *Tetrahedron Lett.* **1989**, *30*, 551. (f) Bravdo, T.; Gutman, A. L. *J. Org. Chem.* **1989**, *54*, 5645.

(3) For examples: (a) Klivanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072. (b) Gu, Q.-M.; Reddy, D. R.; Sih, C. J. *Tetrahedron Lett.* **1985**, *26*, 5203. (c) Lauman, K.; Schneider, M. *Ibid.* **1984**, *25*, 5875. (d) Langrand, G.; Secchi, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Ibid.* **1985**, *26*, 1857. (e) Hemmerle, H.; Gais, H.-J. *Ibid.* **1987**, *28*, 3563. (f) Ramos Tombo, G. M.; Schuar, H.-O.; Busquets, X. F.; Ghisalba, O. *Ibid.* **1986**, *27*, 5707. (g) Gutman, A. L.; Zuobi, Z.; Boltansky, A. *Ibid.* **1987**, *28*, 3861. (h) Makita, A.; Nihira, T.; Yamada, Y. *Ibid.* **1987**, *28*, 805. (i) Blanco, L.; Jampel, E. G.; Roussen, G. *Ibid.* **1988**, *29*, 1915. (j) Yamamoto, K.; Nishioka, T.; Oda, J. *Ibid.* **1988**, *29*, 1717. (k) Flancalanci, F.; Cesti, P.; Cabri, W.; Bianchi, D; Martinengo, T.; Foa, M. *J. Org. Chem. Soc.* **1987**, *52*, 5079. (l) Recent review, see: Crout, D. H. G.; Christen, M. *Modern Synthetic Methods 1989*; Scheffold, R., Ed.; Springer Verlag: New York, 1989.

(4) Lipases tested are listed as follows: Lipase A, A6, P, F-AP15, Newlase F, Pancreatin F (Amano), PPL (Sigma), and MY (Meito). For the optical resolution of methyl 2-(methylthio)-3-acetoxy-3-phenylpropionate, lipase A6 gave the best results.

(5) By our simple electrophoresis experiment using the SDS disk gel method, lipase A6 was shown to consist of at least four proteins.

(6) (a) Zhou, B.; Gopalan, A. S.; VanMiddlesworth, F.; Shie, W.-Ru.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925. (b) Sih, C. J.; Chen, C.-S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570.



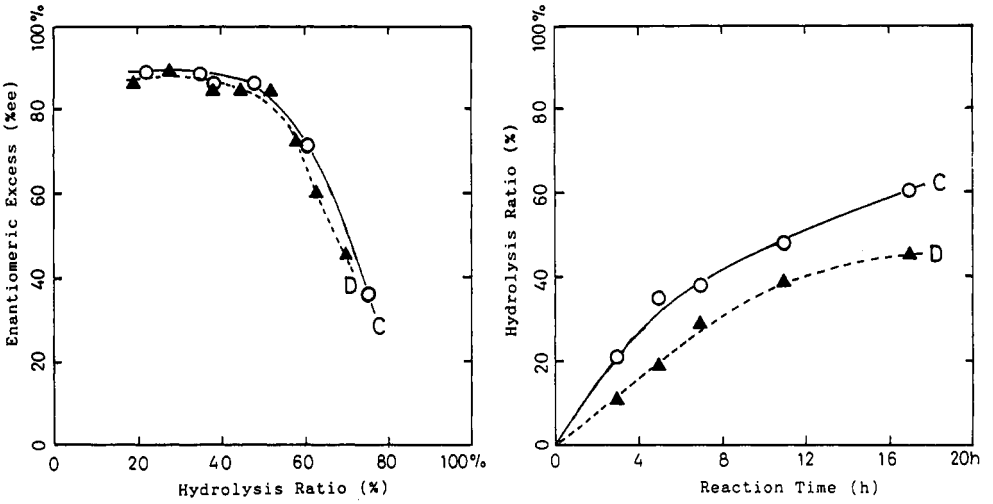


Figure 1. (a, left) Optical purity of **2a** at various points of hydrolysis ratio with different enzyme concentrations. (b, right) Time course of the hydrolysis. D: dilute condition (132 mg of enzyme/mmol). C: concentrated condition (264 mg of enzyme/mmol).

Table I. Relationship between the Coupling Constant of the Methine Proton in the 2-Position and the Chemical Shift of C-3

				anti-2 (R_f value is small)		*syn*-2 (R_f value is large)	
products	R^1	R^2	R^3	3-C (ppm)	2-H (ppm) (J = Hz)[a]	3-C (ppm)	2-H (ppm) (J = Hz)[a]
2a	Ph	SMe	Me	74.14	3.38 (8.54)	71.10	3.44 (8.41)
2b	Me	SMe	Me	67.29	3.15 (10.2)	65.25	3.00 (8.40)
2c	Et	SMe	Me	72.33	3.10 (9.72)	70.31	3.10 (8.10)
2d	Bu	SMe	Me	71.20	3.15 (9.70)	69.03	3.10 (8.10)
2e	PhCH=CH	SMe	Me	72.13	3.30 (8.00)	70.14	3.24 (7.84)
2f	Ph	SMe	Bu	74.24	3.48 (8.16)	71.24	3.41 (8.54)
2g	Ph	SMe	Oct	74.26	3.48 (8.16)	71.24	3.35 (8.51)
2j	Me	Ph	Me	69.67	3.50 (10.9)	68.56	3.40 (8.40)
2k	Et	Ph	Me	75.32	3.60 (10.4)	74.73	3.60 (7.80)
2l	Ph	Me	Me	76.37	2.82 (8.33)	73.58	2.81 (4.10)
2m	Ph	Et	Me	75.28	4.78 (8.25)[b]	74.05	4.86 (5.37)[b]

[a] J_{2H-3H}. [b] The methine proton with the 3-position.

between enantiomers.[6] The rate of hydrolysis, in turn, must be affected strongly by the enzyme concentration. As can be seen in Figure 1a, the optical purity of alcohol **2a** did not change at various points in the hydrolysis ratio with different enzyme concentrations. This experiment established that the enantioselectivity of this lipase cannot be accounted for by the first explanation. The second explanation thus seemed to be more plausible, but the question remained as to whether lipase A6 might consist of enzymes which have the same enantiopreference but different K_m and V_{max} values. It is obvious, however, that this experiment may now allow us to set forth general rules for the selectivity and to control stereoselectivity through proper modification of the substrate in an intensive study of resolution experiments using various substrates for the lipase.

Several α-substituted β-hydroxy esters (compounds **2a–m**) were prepared by the aldol reaction and successfully separated into each diastereomer using flash column chromatography on silica gel. Because the **2a** isomers with higher R_f values (thin-layer chromatography (TLC) on silica gel) showed smaller proton coupling constants for the methine proton at the 3-position than did isomers with smaller R_f values, the former were assigned as syn isomers.[7]

(7) House, H. O.; Crurine, D. S.; Teranishi, A. Y.; Olmstead, H. D. *J. Am. Chem. Soc.* **1973**, *95*, 3310.

However, ^{1}H NMR analyses of other esters gave very puzzling results, as both of the isomers with large R_f vaues by TLC of the butyl ester and octyl ester also exhibited larger J values. To establish the stereoconfiguration, acetonides **4a,d,k,m** were prepared from hydroxy esters **2a,d,k,m**, respectively (Scheme III). It was found that all of the acetonides, **4**, converted from the *syn*-**2a**, **2f**, and **2g**, (the isomers having larger R_f values by TLC), were the same compound by ^{13}C NMR analysis. The relationship between the coupling constant of the methine proton at the C-2 position and the chemical shift of the carbon at the 3-position of the esters is summarized in Table I. The stereochemistries of each of the acetonides, **4**, were determined based on the results of the coupling constant and NOE experiment in ^{1}H NMR analysis. All chemical shifts of the 3-carbon for all isomers of *syn*-**2a**, **2d**, **2k**, and **2m** occurred at higher field in the ^{13}C NMR spectra. Consequently, it was suggested that we could assign the diastereomers using the following rule, i.e. the chemical shift of the 3-carbon of the syn isomer is always at higher field than that of the anti isomer.

All of the diastereomers, **2a–n**, served as substrates for the lipase resolution as acetate (**2a–g** and **2j–n**), butyrate **2h**, and (methylthio)acetate, **2i**. To a suspension of each substrate in 0.1 M phosphate buffer (pH 7.2) was added the lipase (50 wt %), and the mixture was stirred at room temperature. Progress of the reaction was monitored by

Table II. Lipase-Catalyzed Resolution of 2-Alkyl-3-hydroxy Esters

entry	product	R^1	R^2	R^3	R^4	conv, % (time, h)	% ee of 2 (config at C-3)	E^9	$[\alpha]^{23}_D$ of 2, deg (c ca. 1 in $CHCl_3$)
1	*syn*-**2a**	Ph	SMe	Me	Me	32 (72)	10 (*S*)	1.3	−5.3
2	*syn*-**2b**	Me	SMe	Me	Me	40 (21)	22 (*R*)	1.8	−16.9
3	*syn*-**2c**	Et	SMe	Me	Me	49 (28)	34 (*R*)	2.7	−26.2
4	*syn*-**2d**	Bu	SMe	Me	Me	40 (72)	22 (*R*)	1.8	−17.7
5	*syn*-**2e**	PhCH=CH	SMe	Me	Me	38 (21)	52 (*S*)	4.3	−4.1
6	*syn*-**2f**	Ph	SMe	Bu	Me	27 (216)	4 (*R*)	1.1	0.0
7	*syn*-**2g**	Ph	SMe	Oct	Me	0 (336)	–	–	–
8	*syn*-**2h**	Ph	SMe	Me	Pr	0 (336)	–	–	–
9	*syn*-**2i**	Ph	SMe	Me	CH_2SMe	8 (528)	14 (*R*)	1.3	−4.3
10	*syn*-**2j**	Me	Ph	Me	Me	30 (143)	96 (*R*)	73.5	−97.5
11	*syn*-**2k**	Et	Ph	Me	Me	52 (249)	6 (*R*)	1.2	−14.5
12	*syn*-**2l**	Ph	Me	Me	Me	65 (48)	62 (*S*)	14.8	−21.2
13	*syn*-**2m**	Ph	Et	Me	Me	88 (120)	12 (*R*)	2.5	−2.7
14	*anti*-**2a**	Ph	SMe	Me	Me	42 (12)	88 (*R*)	30.1	+8.7
15	*anti*-**2b**	Me	SMe	Me	Me	56 (23)	34 (*R*)	3.0	+18.7
16	*anti*-**2c**	Et	SMe	Me	Me	46 (28)	52 (*S*)	4.8	−5.2
17	*anti*-**2d**	Bu	SMe	Me	Me	35 (16)	58 (*R*)	5.1	+53.2
18	*anti*-**2e**	PhCH=CH	SMe	Me	Me	43 (19)	76 (*R*)	13.0	+81.1
19	*anti*-**2f**	Ph	SMe	Bu	Me	28 (264)	76 (*R*)	10.3	+7.5
20	*anti*-**2g**	Ph	SMe	Oct	Me	2 (336)	2 (*R*)	1.0	+0.1
21	*anti*-**2h**	Ph	SMe	Me	Pr	53 (432)	38 (*R*)	3.3	+3.8
22	*anti*-**2i**	Ph	SMe	Me	CH_2SMe	38 (144)	>98 (*R*)	183.0	+11.8
23	*anti*-**2j**	Me	Ph	Me	Me	40 (116)	74 (*R*)	10.8	+57.6
24	*anti*-**2k**	Et	Ph	Me	Me	32 (172)	8 (*R*)	1.2	−4.0
25	*anti*-**2l**	Ph	Me	Me	Me	55 (38)	86 (*S*)	81.8	+51.8
26	*anti*-**2m**	Ph	Et	Me	Me	44 (192)	82 (*S*)	20.0	+67.0

silica gel TLC, and the reaction was stopped when the spots due to the ester and the alcohol became the same size. The reaction mixture was extracted with ethyl acetate and separated by silica gel TLC after the determination of conversion by ^1H NMR analysis. Optical purity of the hydrolyzed alcohol could be determined by ^1H NMR analysis in the presence of chiral $Eu(hfc)_3$ shift reagent.[8] The results of the resolution experiment using lipase A6 are summarized in Table II. As can be seen in Table II, for all of the hydrolyses cases of **1a** (entries 1 and 14), **1e** (entries 5 and 18), **1f** (entries 6 and 19), **1l** (entries 12 and 21), and **1m** (entries 13 and 26), anti isomers gave good results, except for the hydrolysis of methyl 2-phenyl-3-acetoxybutanoate (**1j**) (entries 10 and 23). When methyl 2-(methylthio)-3-phenyl-3-acetoxypropionate (**1a**) was subjected to hydrolysis, *anti*-**1a** gave the most satisfactory result (entry 14). It has been reported recently that the *tert*-butyl group was superior to the methyl group for the ester carbonyl function in the lipase resolution.[2d] In this experiment, however, the *tert*-butyl ester could not be hydrolyzed. When octyl butanoate, **1g**, was added to the reaction, the hydroxy ester (**2g**) produced only 2% after 336 h with stirring, even if *anti*-**1g** was used as a substrate (entry 20). These observations show that the structure of the ester carbonyl group at the C-1 position does not influence the substrate diastereomer preference of the lipase. In all cases previously mentioned, the anti isomers seemed to be suitable as substrates for optical resolution using lipase A6. It should also be noted that the *R* enantiomers of the 2-(methylthio)-3-acetoxy esters were more prone to hydrolysis than the syn isomers. Although the structure of the ester group at C-3 did not affect diastereomer favoritism, the enantioselectivity was affected. The contrast between the results of diastereoisomers of the 3-(methylthio)acetoxy ester, **1i**, was very marked. Although the *E* value of the resolution reaction of *syn*-**1i** was only 1.3 (entry 9), that of the anti isomer dramatically increased,

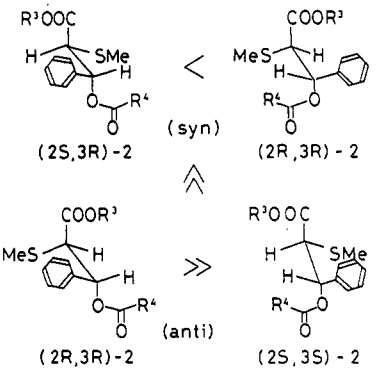


Figure 2. Comparison of enantiomers of **1a** in the ease of hydrolysis by lipase A6.

having been calculated as 183 (entry 22). For the functional group at the C-3 position, esters with substituents containing aromatic and unsaturated groups gave more satisfactory results than esters with aliphatic functional groups (entries 1, 8, 9, and 14, 21, 22). At the 2-position, it seems that a substrate involving too large a functional group does not make a good substrate (entries 11, 24) except for 2-phenyl-3-acetoxybutanoate (entries 10 and 25), because both diastereomers of methyl 2-butyl-3-acetoxy-3-phenylpropionate could not be hydrolyzed. Comparison of the results of **1a**, with a methylthio group at the 2-position, with those of esters in which methyl (**1l**) and ethyl (**1m**) groups were substituted at the same position for the methylthio group seemed to indicate that the sulfur functional group plays a critical role in the steric recognition of the enzyme. In most cases, anti isomers were more compatible with this lipase than syn isomers. In order to clarify the interaction between the size and polarity of a given substituent in the active site of the enzyme, the CPK model of syn and anti isomers of the substrates were compared. The results are summarized in Figure 2.

Whereas various lipases have been studied for the preparation of enantiomerically pure alcohol derivatives, no study has been performed on the topography of the active site and its connection with stereochemical conse-

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(9) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.

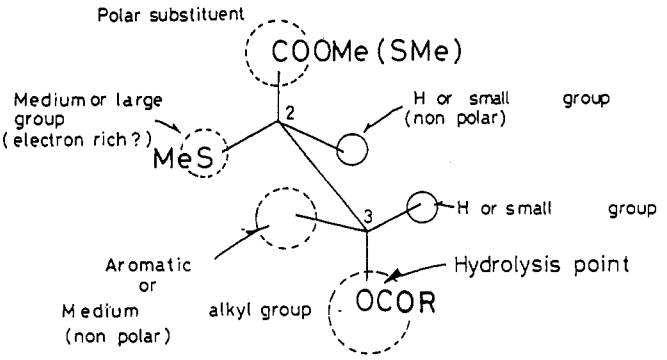

Figure 3. Structural and stereochemical requirements for fit in the cavity of the active site of lipase from *Asp. niger*.

quences.[10] These results may allow us to propose the following general formula, which summarizes the complement of an ester with the active site of the enzyme in lipase A6 (Figure 3).[11] This model predicts satisfactory results in the preparation of optically active esters when using those which bear an aromatic substituent and (methylthio)acetoxy group at the 3-position, and a methyl or methylthio substituent at the 2-position using lipase A6. This model cannot of course completely explain the stereochemistry, but simply summarizes the stereochemical requirements which we have observed to lead to high enantioselection using this inexpensive enzyme as a resolving reagent. However, this model not only represents the first attempt to describe the active site of lipase A6, but also provides important information which can be used in the design of suitable substrates for resolution using this useful enzyme.

Considering the broad substrate specificity and convenience of the lipase, the present reaction is expected to provide a versatile and efficient method for the preparation of various types of optically active β-hydroxy esters under mild conditions. It is expected that this lipase resolution might become one of the most promising methods for preparing these compounds in the optically active state.

Experimental Section

^{1}H NMR spectra were obtained at 200 and 100 MHz in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. ^{13}C NMR spectra were obtained at 50 MHz. Diethyl ether and THF were distilled from sodium benzophenone ketyl under argon. Diisopropylamine, pyridine, and methylene chloride were distilled from CaH_2. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Flash chromatography was performed on a column of silica gel (Wako gel C-300). Thin-layer chromatography (TLC) was performed using Wako gel B-5F.

2-Alkyl-3-hydroxy Esters 2. To a solution of 10 mmol of lithium diisopropylamide (LDA) in 14 mL of THF was added a solution of methyl methylthioacetate (1.20g, 10 mmol) in 3 mL of THF at −78 °C under an argon atmosphere. The solution was stirred for 2 h to produce lithium enolate solution, and then a THF (3 mL) solution of benzaldehyde (1.07 g, 10 mmol) was added dropwise using a syringe. After the addition was complete the mixture was stirred for 1 h at the same temperature. The reaction was quenched by 2 M HCl, extracted with ether, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to give a yellow oil. Purification by silica gel flash chromatography (hexane/ethyl acetate (EtOAc), 10:1 to 7:1 to 2:1) gave methyl 2-(methylthio)-3-hydroxy-3-phenylpropanoate (**2a**) in 76% yield (syn:anti = 30:70 from the isolated yield). Other alcohols, **2**, were also obtained by the same method as previously described. Chemical yield (%), boiling point (°C, mmHg), R_f value on silica gel TLC, IR spectral data (cm^{-1}), and elemental analyses are summarized below.

Methyl 2-(methylthio)-3-hydroxy-3-phenylpropionate (2a): 76%; 135 °C (1.5 mmHg), Kugelrohr; syn (0.5), anti (0.4), hexane/EtOAc, 5/1; 3450, 3030, 2950, 2920, 1720 (CO), 1430, 1150, 1020, 760, and 700 cm^{-1}; Anal. Calcd for $C_{11}H_{14}O_3S$: C, 58.39; H, 6.24. Found: C, 58.20; H, 6.30.

Methyl 2-(methylthio)-3-hydroxybutanoate (2b):[12] 73%; 105 °C (1.0 mmHg), Kugelrohr; syn (0.25), anti (0.23), hexane/EtOAc, 3/1; 3400, 2950, and 1710 (CO) cm^{-1}.

Methyl 2-(methylthio)-3-hydroxypentanoate (2c):[12] 42%; 105 °C (1.0 mmHg), Kugelrohr; syn (0.5), anti (0.4), multiple development (3 times), hexane/EtOAc, 5/1; 3500, 2900, 1710, 1440, 1160, 1120, and 980 cm^{-1}.

Methyl 2-(methylthio)-3-hydroxyheptanoate (2d): 86%; 150 °C (1.0 mmHg), Kugelrohr; syn (0.5), anti (0.4), double development, hexane/EtOAc, 8/1; 3400, 2950, and 1720 cm^{-1}. Anal. Calcd for $C_9H_{18}O_3S$: C, 52.40; H, 8.79. Found: C, 52.10; H, 8.65.

Methyl 2-(methylthio)-3-hydroxy-5-phenyl-4-pentenoate (2e): 78%; 180 °C (1.0 mmHg), Kugelrohr; syn (0.5), anti (0.45), double development, hexane/EtOAc, 2/1; 3400, 3050, 2950, 2870, 1720, 1500, 960, and 750 cm^{-1}. Anal. Calcd for $C_{13}H_{16}O_3S$: C, 61.88; H, 6.39. Found: C, 61.60; H, 6.34.

Butyl 2-(methylthio)-3-hydroxy-3-phenylpropionate (2f): 56%; 150 °C (1.0mmHg), Kugelrohr; syn (0.9), anti (0.8), multiple development (8 times), hexane/EtOAc, 5/1 (3 times) and 7/1 (5 times); 3450, 2950, 1730, 1150, 790, and 700 cm^{-1}.

Octyl 2-(methylthio)-3-hydroxy-3-phenylpropionate(2g): 89%; 200 °C (1.0 mmHg), Kugelrohr; syn (0.7), anti (0.6), double development, hexane/EtOAc, 7/1; 3400, 2860, 2800, 1710, 1480, 1440, 1260, 1140, 1000, 750, and 680 cm^{-1}.

Methyl 2-phenyl-3-hydroxybutanoate (2j):[13b] 60%; syn (0.6), anti (0.5), multiple development (3 times), hexane/EtOAc, 5/1; 3450, 3000, 1740, 1500, 1370, 1240, 1060, 960, and 730 cm^{-1}.

Methyl 2-phenyl-3-hydroxypentanoate (2k):[13a] 53%; 130 °C (1.0 mmHg), Kugelrohr; syn (0.5), anti (0.2), hexane/EtOAc, 5/1; 3450, 2850, 1720, 1600, 1440, 1340, 1030, 970, and 730 cm^{-1}.

Methyl 2-methyl-3-hydroxy-3-phenylpropionate (21):[14] 90%; syn (0.6), anti (0.5), multiple development (3 times), hexane/EtOAc, 5/1; 3450, 3000, 1720, 1460, 760, and 700 cm^{-1}.

Methyl 2-ethyl-3-hydroxy-3-phenylpropionate (2m): 88%; syn (0.7), anti (0.5), double development, hexane/EtOAc, 5/1; 3450, 3000, 1720, 1460, 1380, 1180, 1040, 760, and 700 cm^{-1}. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.28; H, 7.54. Spectral data, each diastereomers of the ester, **2**, of ^{1}H NMR (200 MHz, δ, $CDCl_3$, J = Hz) and ^{13}C NMR (50 MHz, δ, $CDCl_3$, ppm) are summarized in Table III.

Methyl 2-(Methylthio)-3-acetoxy-3-phenylpropionate (1a). To a solution of syn-**2a** (368 mg, 1.63 mmol) and acetyl chloride (150 mg, 2.0 mmol) in 2 mL of CH_2Cl_2 was added 0.6 mL of pyridine at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of crushed ice and then extracted with CH_2Cl_2. The extract was dried and concentrated in vacuo to give a yellow oil. Purification by distillation in vacuo gave *syn*-**1a** in 90% yield (393 mg, 1.46 mmol): bp 130 °C (1.0 mmHg), Kugelrohr; ^{1}H NMR (100 MHz, δ, $CDCl_3$, J = Hz) 2.1 (3 H, s), 2.2 (3 H, s), 3.6 (1 H, s), 3.8 (1 H, d, J = 11.2), 6.2 (1 H, d, J = 11.2), 7.4–7.8 (5 H, m); IR (neat, cm^{-1}) 3050, 2950, 2925, 1740 (CO), 1220, 1020, and 760. Anti isomer, **1a**, was also obtained by the same method as previously described in 90% yield: ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.9 (6 H, s), 3.6 (1 H, d, J = 12.0 Hz), 3.8 (3 H, s), 6.1 (1 H, d, J = 12.0 Hz), 7.4–7.8 (5 H, m).

Methyl 2-(Methylthio)-3-[(methylthio)acetoxy]-3-phenylpropionate (1i). *syn*-**1i**: ^{1}H NMR (200 MHz, $CDCl_3$, J = Hz) δ 2.14 (3 H, s), 2.20 (3 H, s), 3.25 (2 H, s), 3.55 (3 H, s),

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(11) This model is made with reference to that of PLE: Mohr, P.; W-Sarcevic, N.; Tam, C.; Gawronska, K.; Gawronska, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501.

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Table III. ^{1}H NMR and ^{13}C NMR Spectral Data for 3-Hydroxy Esters of 2

compd	^{1}H NMR (200 MHz, $CDCl_3$) (δ, ppm) (J = Hz)	^{13}C NMR (50 MHz, $CDCl_3$) (ppm)
syn-**2a**	2.01 (3 H, s), 3.25–3.40 (1 H, br s, OH), 3.44 (1 H, d, J = 8.41), 3.60 (3 H, s), 4.96 (1 H, s, J = 8.30), 7.2–7.4 (5 H, m)	13.61, 52.13, 52.49, 71.10 (3 C), 126.68, 128.27, 128.36, 139.71, 170.64
anti-**2a**	1.89 (3 H, s), 2.8–3.0 (1 H, br s, OH), 3.37 (1 H, d, J = 8.54), 4.86 (1 H, dd, J_1 = 8.40, J_2 = 1.62), 7.15–7.30 (5 H, m)	14.66, 52.39, 53.99, 74.14 (3 C), 126.62, 128.37, 140.37, 172.05
syn-**2b**	1.25 (3 H, d, J = 6.50), 2.20 (3 H, s), 2.8–3.0 (1 H, br s, OH), 3.00 (1 H, d, J = 8.40), 3.80 (3 H, s), 3.8–4.2 (1 H, m)	13.96, 19.90, 52.30, 55.20, 65.25 (3 C), 171.66
anti-**2b**	1.35 (3 H, d, J = 6.80), 2.20 (3 H, s), 3.15 (1 H, d, J = 10.2), 3.45–3.55 (1 H, br s, OH), 3.80 (3 H, s), 3.8–4.2 (1 H, m)	14.25, 20.43, 52.27, 53.96, 67.29 (3 C), 172.17
syn-**2c**	1.05 (3 H, t, J = 8.10), 1.4–1.7 (2 H, m), 2.20 (3 H, s), 2.85 (1 H, br s, OH), 3.10 (1 H, d, J = 8.10), 3.80 (3 H, s) 3.7–4.0 (1 H, m)	10.00, 14.03, 27.00, 52.33, 53.32, 70.31 (3 C), 171.90
anti-**2c**	1.00 (3 H, t, J = 7.45), 1.2–2.1 (2 H, m), 2.15 (3 H, s), 2.8–3.0 (1 H, br s, OH), 3.10 (1 H, d, J = 9.72), 3.80 (3 H, s), 3.7–4.0 (1 H, m)	9.73, 14.36, 26.97, 52.20, 52.25, 72.33 (3 C), 172.29
syn-**2d**	0.91 (3 H, t, J = 7.00), 1.28–1.40 (2 H, m), 1.45–1.60 (4 H, m), 2.19 (3 H, s), 2.92 (1 H, d, J = 2.00, OH), 3.15 (1 H, d, J = 7.50), 3.77 (3 H, s), 3.87–3.94 (1 H, m)	13.97, 14.07, 22.58, 27.86, 33.80, 52.30, 53.78, 69.03 (3 C), 171.86
anti-**2d**	0.961 (1 H, t, J = 7.00), 1.30–1.40 (4 H, m), 1.42–1.55 (2 H, m), 2.17 (3 H, s), 2.54 (1 H, d, J = 6.00, OH), 3.19 (1 H, d, J = 7.50), 2.79 (3 H, s), 3.88–3.95 (1 H, m)	14.00, 14.48, 22.54, 27.66, 33.85, 52.31, 52.68, 71.20 (3 C), 172.31
syn-**2e**	2.15 (3 H, s), 3.02 (1 H, d, J = 2.01, OH), 3.24 (1 H, d, J = 7.84), 3.66 (3 H, s), 4.50 (1 H, t, J = 7.33), 6.14 (1 H, dd, J_1 = 16.01, J_2 = 6.73), 6.65 (1 H, d, J = 15.63), 7.15–7.33 (5 H, m)	13.86, 52.31, 53.90, 70.14 (3 C), 126.64, 126.71, 127.95, 128.52, 127.01, 133.13, 136.23, 170.89
anti-**2e**	2.18 (3 H, s), 2.92 (1 H, d, J = 5.00, OH), 3.34 (1 H, d, J = 8.00), 3.79 (3 H, s), 4.60–4.80 (1 H, m), 6.32 (1 H, dd, J_1 = 15.68, J_2 = 7.21), 6.71 (1 H, d, J = 16.01), 7.25 (1 H, dd, J_1 = 8.60, J_2 = 6.83), 7.32 (2 H, t, J = 7.43), 7.39 (2 H, d, J = 7.81)	14.54, 52.41, 52.75, 72.13 (3 C), 126.64, 127.86, 127.95, 128.56, 132.49, 136.21, 171.85
syn-**2f**	0.83 (3 H, t, J = 7.65), 1.1–1.3 (2 H, m), 1.3–1.5 (2 H, m), 2.20 (3 H, s), 3.31 (1 H, d, J = 1.63, OH), 3.41 (1 H, d, J = 8.54), 3.99 (2 H, dt, J_1 = 6.43, J_2 = 1.79), 4.93 (1 H dd, J_1 = 6.50, J_2 = 1.41), 7.2–7.5 (5 H, m)	13.58, 18.58, 18.85, 30.40, 55.70, 64.98, 71.24 (3 C), 126.85, 128.32, 128.39, 139.72, 170.34
anti-**2f**	0.91 (3 H, t, J = 7.20), 1.2–1.4 (2 H, m), 1.5–1.7 (2 H, m), 2.04 (3 H, s), 3.15 (1 H, d, J = 5.13), 3.48 (1 H, d, J = 8.16), 4.16 (2 H, t, J = 6.60), 5.00 (1 H, dd, J_1 = 8.14, J_2 = 2.98), 7.2–7.5 (5 H, m)	13.64, 14.82, 18.98, 30.54, 54.21, 65.30, 74.24 (3 C), 126.58, 128.31, 128.41, 140,53, 171.79
syn-**2g**	0.82 (3 H, t, J = 6.50), 1.10–1.30 (10 H, m), 1.3–1.5 (2 H, m), 2.15 (3 H, s), 3.26 (1 H, d, J = 1.68, OH), 3.35 (1 H, d, J = 8.51), 3.92 (2 H, t, J = 6.56), 4.87 (1 H, dd, J_1 = 8.46, J_2 = 1.36), 7.2–7.4 (5 H, m)	13.57, 14.05, 22.59, 25.61, 28.37, 29.05, 31.71, 55.71, 65.27, 71.24 (3 C), 126.83, 128.29, 128.36, 139.76, 170.32
anti-**2g**	0.88 (3 H, t, J = 6.40), 1.21–1.28 (10 H, m), 1.50–1.70 (2 H, m), 2.03 (3 H, s), 3.13 (1 H, d, J = 5.37), 3.48 (1 H, d, J = 8.16), 4.16 (2 H, t, J = 6.70), 4.98 (1 H, dd, J_1 = 8.10, J_2 = 2.60), 7.20–7.40 (5 H, m)	14.07, 14.84, 22.61, 25.74, 28.52, 29.12, 31.74, 54.22, 65.61, 126.59, 128.30, 74.26 (3 C), 128.42, 140.56, 171.77
syn-**2j**	1.10 (3 H, d, J = 12.96), 2.90 (1 H, s, OH), 3.40 (1 H, d, J = 8.40), 3.60 (3 H, s), 4.0–4.5 (1 H, m), 7.2–7.6 (5 H, s)	20.45, 52.12, 58.57, 68.56 (3 C), 127.90, 128.76, 129.15, 135.00, 173.58
anti-**2j**	1.00 (3 H, d, J = 6.48), 3.00 (1 H, s, OH), 3.50 (1 H, d, J = 10.9), 4.10 (3 H, s), 4.2–4.5 (1 H, m), 7.3–7.5 (5 H, m)	20.31, 52.19, 60.02, 69.67 (3 C), 127.69, 128.26, 128.84, 136.20, 176.76
syn-**2k**	1.00 (3 H, t, J = 7.45), 1.2–1.6 (2 H, m), 3.00 (1 H, br s, OH), 3.60 (1 H, d, J = 7.80), 3.70 (3 H, s), 3.9–4.1 (1 H, m), 7.3–7.6 (5 H, br s)	10.14, 27.36, 52.14, 56.75, 73.56 (3 C), 127.81, 128.69, 129.23, 135.02, 173.82
anti-**2k**	0.90 (3 H, t, J = 8.10), 1.1–1.6 (2 H, m), 3.50 (1 H, br s, OH), 3.6 (1 H, d, J = 10.37), 3.70 (3 H, s), 4.0–4.3 (1 H, m), 7.4–7.7 (5 H, br s)	9.66, 26.68, 52.18, 58.15, 74.53 (3 C), 127.64, 128.34, 128.83, 136.15, 174.19
syn-**2l**	1.15 (3 H, d, J = 7.21), 2.81 (1 H, dq, J_1 = 4.10, J_2 = 7.16), 3.00 (1 H, br s, OH), 3.70 (3 H, s), 5.13 (1 H, dd, J_1 = 3.66, J_2 = 3.66), 7.25–7.40 (5 H, m)	10.68, 46.33, 51.88, 73.58 (3 C), 125.92, 127.49, 128.25, 141.35, 176.21
anti-**2l**	1.00 (3 H, d, J = 7.16), 2.82 (1 H, dq, J_1 = 8.33, J_2 = 7.27), 2.94 (1 H, d, J = 4.31, OH), 3.73 (3 H, s), 4.75 (1 H, dd, J_1 = 8.50, J_2 = 4.24), 7.25–7.36 (5 H, m)	14.47, 47.08, 51.92, 76.37 (3 C), 126.66, 128.10, 141.50, 176.25
syn-**2m**	0.80 (3 H, t, J = 7.38), 1.56–1.72 (2 H, m), 2.59 (1 H, ddd, J_1 = 10.10, J_2 = 5.76, J_3 = 5.64), 2.88 (1 H, br s, OH), 3.52 (3 H, s), 4.86 (1 H, d, J = 5.37), 7.18–7.22 (1 H, m), 7.24 (4 H, d, J = 4.34)	11.97, 20.29, 51.51, 54.58, 74.05 (3 C), 126.07, 127.62, 128.23, 141.65, 175.28
anti-**2m**	0.83 (3 H, t, J = 7.43), 1.29–1.36 (1 H, m), 1.50–1.59 (1 H, m), 2.68 (1 H, ddd, J_1 = 9.06, J_2 = 8.30, J_3 = 4.72), 2.96 (1 H, br s, OH), 3.70 (3 H, s), 4.78 (1 H, d, J = 8.25), 7.29–7.35 (5 H, m)	11.60, 22.70, 51.69, 54.78, 75.28 (3 C), 126.47, 128.01, 128.50, 142.02, 175.72

3.70 (1 H, d, J = 9.8), 6.13 (1 H, d, J = 10.0), 7.3–7.5 (5 H, m); ^{13}C NMR (50 MHz, $CDCl_3$) 13.99, 16.17, 35.76, 52.22, 52.56, 73.36 (3 C), 127.41, 128.55, 128.64, 128.91, 137.02, 168.90, 169.15 ppm. *anti*-**1i**: 1.96 (3 H, s), 2.08 (3 H, s), 3.11 (2 H, s), 3.66 (1 H, d, J = 10.8), 3.80 (3 H, s), 6.02 (1 H, d, J = 11.0), 7.4–7.5 (5 H, m); 14.16, 15.98, 35.54, 51.67, 52.51, 75.67 (3 C), 127.70, 128.48, 129.05, 136.52, 168.29, 170.19 ppm.

Lipase Resolution. To a suspension of each substrate (2.0 mmol) in 0.1 M phosphate buffer (pH 7.2, 10 mL) was added a lipase (50 wt %), and the mixture was stirred at room temperature. Progress of the reaction was monitored by silica gel TLC, and the reaction was stopped when the spots due to the ester and the alcohol became the same size. The reaction mixture was extracted with ethyl acetate and separated by silica gel TLC after determination of the conversion ratio using ^{1}H NMR analysis. When 30 mol % of Eu(hfc)$_3$ shift reagent was added to the $CDCl_3$ solution of the racemic alcohol **2**, both the ester methyl and the methylthio peaks were split into two peaks. Reaction time, conversion, yield, $[\alpha]^{25}_D$ of alcohols in $CHCl_3$, and the results of ^{1}H NMR analyses of each of the alcohols in the presence of 30 mol % of Eu(hfc)$_3$ are summarized in Table IV. The symbol (L) means lower field peak and the symbol (H) means one in a higher field.

Absolute configuration at the 3-position of the alcohols, produced by the lipase hydrolysis, was presumed by the specific rotation and ^{1}H NMR experiment in the presence of 30 mol % of the chiral shift reagent. From the comparison to the specific rotation with references, alcohols **2b**, **2c**, and **2j** were assigned as 3*R* isomers, respectively. To determine the absolute configuration of the alcohols **2a** and **2d**, both *anti*-**2a** (92% ee: $[\alpha]^{19}_D$

Table IV. Results of Hydrolysis of Esters 1 by Lipase A6 (*Aspergillus* sp.)

substrate	time, h	conv, %	yield, % 2	yield, % 1	$[\alpha]^{23}_D$, deg, of 2 in $CHCl_3$	^1H NMR of 2 (200 MHz, δ, + 30 mol % Eu(hfc)$_3$) OMe (J = Hz, L:H)	^1H NMR of 2 (200 MHz, δ, + 30 mol % Eu(hfc)$_3$) SMe (J = Hz, L:H)
syn-1a	72	32	25	44	−5.3 (*c* 1.05)		3.3 (*J* = 12, 45:55)
anti-1a	17	42	33	54	+8.7 (*c* 1.08)		3.1 (*J* = 23, 94:6)
syn-1b	21	40	33	40	−16.9 (*c* 1.16)[a]	4.8 (*J* = 8.4, 36:61)	
anti-1b	23	56	42	40	+18.7 (*c* 1.07)[b]	4.8 (*J* = 23, 33:67)	
syn-1c	25	42	17	42	−26.2 (*c* 1.00)[c]		3.1 (*J* = 12, 67:33)
anti-1c	28	46	32	46	−5.2 (*c* 1.05)[d]	4.8 (*J* = 5.4, 76:24)	
syn-1d	72	40	25	55	+17.7 (*c* 2.10)	4.6 (*J* = 21, 36:61)	
anti-1d	16	35	11	60	+53.2 (*c* 1.38)	5.9 (*J* = 6.5, 21:79)	
syn-1e	21	38	12	55	−4.1 (*c* 1.89)	4.8 (*J* = 16, 76:24)	
anti-1e	19	43	31	41	+81.1 (*c* 1.66)	4.8 (*J* = 36. 12:88)	
syn-1f	216	18	18	80	0 (*c* 1.50)		2.9 (*J* = 25, 52:48)
anti-1f	264	28	28	55	+7.5 (*c* 1.12)		2.9 (*J* = 38, 88:12)
syn-1g	336	0	0	85	-		
anti-1g	336	2	2	77	+0.1 (*c* 2.50)		2.9 (*J* = 25, 51:49)
syn-1h	528	0	0	85	-		
anti-1h	432	53	28	53	+3.8 (*c* 1.22)	4.1 (*J* = 9.3, 31:69)	
syn-1i	144	17	8	65	−4.3 (*c* 1.25)	3.9 (*J* = 67, 43:57)	
anti-1i	144	38	38	52	+11.8 (*c* 1.11)	3.9 (s, <1:>99)	
syn-1j	48	30	23	55	−97.0 (*c* 1.71)[e]	4.1 (*J* = 25, 2:98)	
anti-1j	116	40	31	55	+57.6 (*c* 1.01)	4.4 (*J* = 45, 13:87)	
syn-1k	480	52	20	41	−14.5 (*c* 1.68)	4.1 (*J* = 36, 47:53)	
anti-1k	172	32	8	58	−4.0 (*c* 1.63)	4.3 (*J* = 23, 46:54)	
syn-1l	48	65	59	30	−21.2 (*c* 1.05)	4.2 (*J* = 29, 19:81)	
anti-1l	35	55	50	40	+51.8 (*c* 1.20)	4.3 (*J* = 25, 8:93)	
syn-1m	120	88	10	57	−2.7 (*c* 1.54)	4.0 (*J* = 11, 46:56)	
anti-1m	192	50	35	44	+51.8 (*c* 1.02)	5.3 (*J* = 26, 9:91)	

[a] Lit.[12] +35° (3*S*). [b] Lit.[12] −36° (3*S*). [c] Lit.[12] +58° (3*S*). [d] Lit.[12] −8.9° (3*S*). [e] Lit.[13b] +99.8° (3*S*).

Table V. Results of NMR Analyses of Acetonide 4

compd	^1H NMR (200 MHz, δ, $CDCl_3$, J = Hz)	^{13}C NMR (50 MHz, δ, $CDCl_3$, ppm)
syn-4a	1.64 (3 H, s), 2.74 (1 H, q, *J* = 2.66), 4.07 (1 H dd, J_1 = 12.05, J_2 = 2.07), 4.44 (1 H, dd, J_1 = 12.12, J_2 = 2.77), 5.30 (1 H, d, *J* = 2.45), 7.20–7.43 (5 H, m)	15.67, 19.19, 29.23, 49.50, 65.03, 72.88, 99.53, 125.91, 127.43, 127.99, 139.68
anti-4a	1.47 (3 H, s), 1.55 (3 H, s), 1.83 (3 H, s), 2.76 (1 H, ddd, J_1 = 11.0, J_2 = 6.05, J_3 = 10.58), 3.82 (1 H, t, *J* = 11.67), 4.05 (1 H, dd, J_1 = 11.80, J_2 = 5.48), 4.63 (1 H, d, *J* = 10.55, 7.30–7.50 (5 H, m)	14.53, 18.99, 29.57, 47.50, 64.08, 72.33, 99.03, 127.64, 128.36, 128.45, 139.52
syn-4d	0.89 (3 H, t, *J* = 6.66), 1.34 (3 H, s), 1.42 (3 H, s), 1.20–1.50 (4 H, m), 1.53–1.73 (2 H, m), 2.17 (3 H, s), 2.43 (1 H, q, *J* = 2.44), 3.98 (1 H, dd, J_1 = 12.16, J_2 = 2.17), 4.03 (1 H, ddd, J_1 = 4.88, J_2 = 6.66, J_3 = 2.44), 4.21 (1 H, dt, J_1 = 12.13, J_2 = 2.82)	14.05, 15.44, 19.28, 22.60, 27.32, 29.23, 33.27, 46.84, 64.75, 71.80, 99.01
anti-4d	0.88 (3 H, t, *J* = 6.60), 1.35 (3 H, s), 1.41 (3 H, s), 1.20–1.50 (6 H, m), 2.09 (3 H, s), 2.48 (1 H, dt, J_1 = 5.37, J_2 = 10.68), 3.69 (1 H, ddd, J_1 = 10.58, J_2 = 8.03, J_2 = 2.44), 3.71 (1 H, t, *J* = 10.99), 3.93 (1 H, dd, J_1 = 11.80, J_2 = 5.43)	13.92, 14.08, 19.22, 22.57, 27.13, 29.30, 32.91, 45.32, 64.10, 72.61, 98.51
syn-4k	0.80 (3 H, t, *J* = 7.33), 1.1–1.3 (2 H, m), 1.53 (6 H, s), 2.48–2.54 (1 H, m), 3.86 (1 H, dd, J_1 = 3.37, J_2 = 6.77), 4.33 (1 H, dd, J_1 = 11.68, J_2 = 3.95), 7.2–7.3 (5 H, m)	9.70, 19.09, 26.48, 29.42, 43.37, 65.51, 72.81, 98.86, 126.47, 127.97, 129.64, 140.63
anti-4k	0.76 (3 H, t, *J* = 7.32), 1.1–1.4 (2 H, m), 1.47 (3 H, s), 1.51 (3 H, s), 2.69 (1 H, dt, J_1 = 5.43, J_2 = 10.85), 3.7–4.0 (3 H, m), 7.1–7.3 (5 H, m)	9.42, 19.54, 26.27, 29.68, 47.05, 65.61, 74.53, 98.36, 127.03, 128.22, 128.66, 139.16
syn-4m	0.68 (3 H, t, *J* = 7.30), 1.88–1.41 (2 H, m), 1.44 (3 H, s), 1.47 (3 H, s), 1.5–1.6 (1 H, m), 3.53 (1 H, dd, J_1 = 1.80, J_2 = 1.56), 4.12 (1 H, ddd, J_1 = 11.8, J_2 = 2.62, J_3 = 1.01), 5.12 (1 H, d, *J* = 2.63), 7.2–7.3 (5 H, m)	11.92, 16.22, 19.04, 29.75, 41.07, 62.35, 73.53, 98.92, 125.46, 126.73, 128.03, 141.00
anti-4m	0.63 (3 H, t, *J* = 7.39), 0.8–1.1 (2 H, m), 1.40 (3 H, s), 1.47 (3 H, s), 1.6–1.8 (1 H, m), 3.62 (1 H, dd, J_1 = 12.25, J_2 = 10.46), 3.93 (1 H, dd, J_1 = 11.72, J_2 = 5.13), 4.39 (1 H, d, *J* = 10.45), 7.2–7.3 (5 H, m)	10.71, 19.05, 20.66, 29.81, 42.10, 64.49, 77.71, 98.52, 127.60, 127.99, 128.29, 140.41

+9.4°, *c* 0.81 in $CHCl_3$) and *anti*-**2d** (19% ee: $[\alpha]^{23}_D$ +17.43°, *c* 0.51 in $CHCl_3$) were desulfurized[13] to provide methyl 3-hydroxy-3-phenylpropionate ($[\alpha]^{24}_D$ −18.0°, *c* 0.52 in EtOH, lit.[14] −18.4° (*S*)) and 3-hydroxyheptanoate ($[\alpha]^{25}_D$ −1.54°, *c* 0.39 in $CHCl_3$), respectively. To compare the specific rotation of methyl 3-hydroxyheptanoate with methyl (3*S*)-3-hydroxyhexanoate, +13.8° (*c* 3.5, $CHCl_3$),[15] it was presumed that *anti*-**2d** was assigned as the 3*R* isomer. ^1H NMR analyses of these esters in the presence of Eu(hfc)$_3$ revealed that the H peak due to the ester methyl proton and the L peak due to the methylthio proton corresponded to the *R* enantiomers, respectively. Hence, we are able to presume the absolute configuration of other esters, **2**, by the ^1H NMR analysis.[8]

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Control Experiment in Figure 1. (1) Dilute Condition. To a solution of acetate *anti*-**1** (900 mg, 3.35 mmol) in 34 mL of 0.1 M phosphate buffer (pH 7.2) and 3.4 mL of acetone was added lipase A6 (459 mg), and the mixture was stirred at 30 °C. After 3 h, a 4-mL aliquot of reaction mixture was removed and extracted with ethyl acetate. The extract was analyzed by ^1H NMR to determine the hydrolysis ratio. Enantiomeric excess of the hydrolyzed alcohol was determined by ^1H NMR analysis as previously described. Every 2 h, the hydrolysis ratio and enantiomeric excess of the alcohol were examined. **(2) Concentrated Condition.** Acetate *anti*-**1** (900 mg, 3.35 mmol) was incubated with lipase A6 (900 mg) as previously described. The results are summarized below. Reaction time (hydrolysis ratio and enantiomeric excess of the alcohol). Dilute condition: 3 h (11%, 90% ee), 5 h (19%, 86% ee), 7 h (28%, 88% ee), 11 h (39%, 84% ee), 17 h (45%, 84% ee), 23 h (52%, 84% ee), 29 h (58%, 74% ee),

36 h (70%, 45% ee), 48 h (63%, 60% ee). Concentrated condition: 3 h (21%, 88% ee), 5 h (35%, 88% ee), 7 h (38%, 86% ee), 11 h (48%, 86% ee), 17 h (61%, 74% ee), 36 h (75%, 35% ee).

Acetonide of 2-(Methylthio)-3-phenylpropane-1,3-diol (4). To a suspensiom of 40 mg (1.16 mmol) of lithium aluminum hydride in 5 mL of ether was added a solution of 245 mg (1.51 mmol) of **2d** in 5 mL of ether at −78 °C under argon, and the mixture was stirred for 5 h. After reaction was quenched, the solution was dried, and the solvent was removed under reduced pressure to give an oily product. To a solution of this oil in 5 mL of CH_2Cl_2 was added 350 mg (3.0 mmol) of 2,2-dimethoxypropane and 5 mg of *p*-toluensulfonic acid under argon and stirred for 24 h. Crushed ice was added, and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with brine, dried, and concentrated by evaporation. Distillation of the residue gave 4 in 87% yield as a colorless oil: bp 100 °C (2.5 mmHg), Kugelrohr. Other acetonides, **4**, were also prepared by the same method as previously described. Spectral data, each diastereomer of the acetonide **4** of ^1H NMR (200 MHz, δ, $CDCl_3$, J = Hz) and ^{13}C NMR (50 MHz, δ, $CDCl_3$, ppm) are summarized in Table V.

Acknowledgment. We are grateful to Professor Masanori Utaka of the Faculty of Engineering of Okayama University for helpful discussions throughout this work. We are also grateful to Mr. Noriyoshi Tateoka, Faculty of Education of Okayama University, for preparing the computer program for calculation of the E value. We also thank Amano Pharmaceutical Co., Ltd. and Meito Sangyo Co., Ltd. for providing lipases.

Cytotoxic Aromatic Alkaloids from the Ascidian *Amphicarpa meridiana* and *Leptoclinides* sp.: Meridine and 11-Hydroxyascididemin

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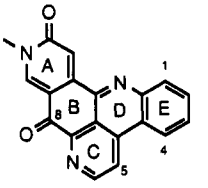
Received April 4, 1990

Three new pentacyclic aromatic alkaloids, 6–8, have been isolated from two ascidians. The structure of meridine, 6, obtained from *Amphicarpa meridiana* collected in South Australia, was determined by X-ray analysis while that of a relatively stable tautomer thereof, 7, was established by spectral analysis. The remaining alkaloid, 11-hydroxyascididemin (8), was isolated from a *Leptoclinides* sp. from Truk Lagoon. All three alkaloids are cytotoxic and one shows slight topoisomerase II activity. Limitations to the possible structures for neocalliactine acetate are discussed.

A series of structurally related and biologically active polycyclic aromatic alkaloids isolated from sponges, ascidians, and an anemone have been reported in the past few years.[1] Illustrative examples are amphimedine (**1**),[1a] shermilamines A and B (**2**, **3**),[1f,g] 2-bromoleptoclinidinone (**4**),[1b] and ascididemin (**5**),[1c] all of which have in common the tetracyclic moiety marked B–D in **1**. Cytotoxic activity has been reported for many of these metabolites. In our continuing search for cytotoxic compounds from marine organisms we have isolated three additional polycyclic aromatic alkaloids from two different ascidians. The ^{13}C NMR data confirmed for several of these alkaloids makes it possible to limit the possible structures proposed for neocalliactine acetate, a derivative of the anemone pigment calliactine. The structure of one of the new alkaloids, meridine (**6**), was confirmed by X-ray diffraction analysis.

The source of two of the new alkaloids is the ascidian *Amphicarpa meridiana*, which was collected at Stenhouse Bay, South Australia, and frozen shortly after collection.



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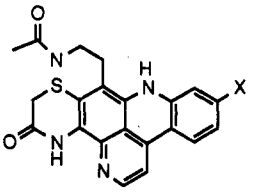

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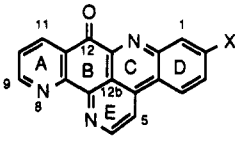
3, X = H



4, X = Br
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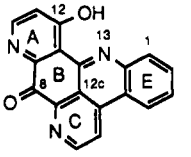
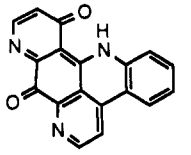


6



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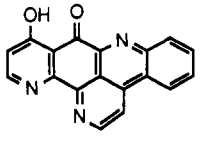

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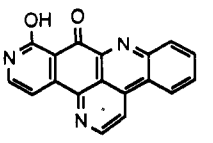

8'

The chloroform–methanol extracts yielded meridine (**6**) as a yellow, noncrystalline solid via conventional successive chromatographies on silica gel or via centrifugal countercurrent chromatography (CCCC) using a chloroform–methanol–5% aqueous HCl (5:5:3) solvent system. Meridine, $C_{18}H_9N_3O_2$ (M^+, 299.0693, +0.0305 amu), showed signals in the ^1H NMR spectrum for one exchangeable proton (15.26 ppm) and eight aromatic protons which were

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