Lipase YS-catalysed Acylation of Alcohols: a Predictive Active Site Model for Lipase YS to Identify which Enantiomer of a Primary or a Secondary Alcohol Reacts Faster in this Acylation

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Primary alcohols having a hydroxymethyl group at an *S* chiral centre and secondary alcohols with an *R* configuration are preferentially acylated to give the corresponding acetates by lipase YS-catalysed acylation in diisopropyl ether; a predictive cubic-spaced active site model for lipase YS is proposed for identifying which enantiomer of a primary or a secondary alcohol reacts faster in this acylation.

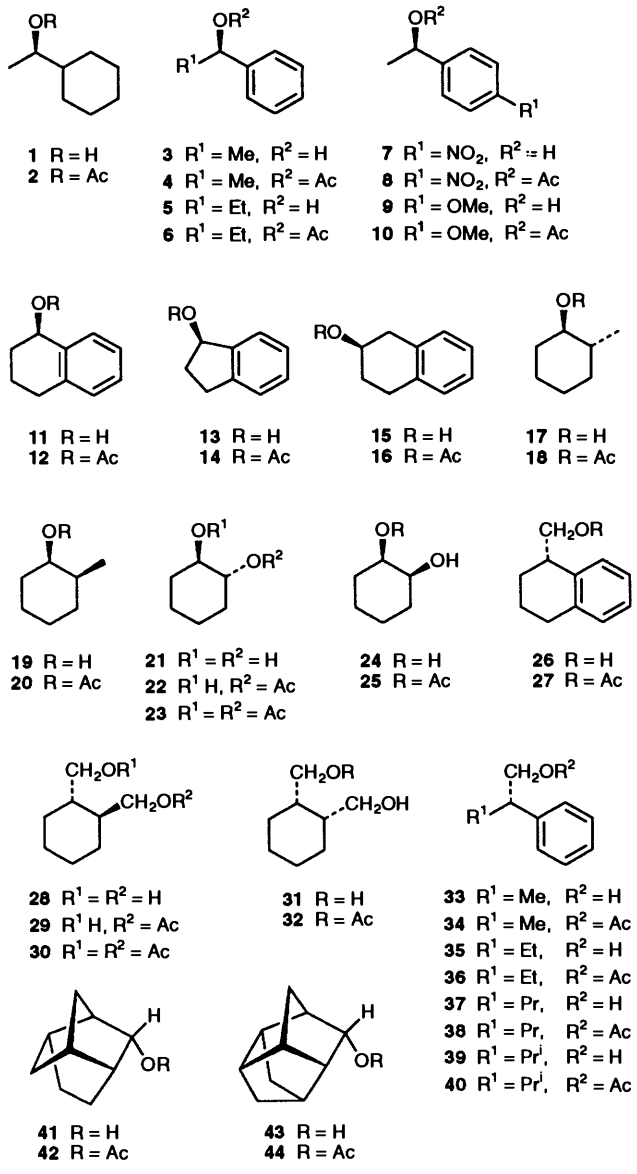
The use of enzymes as chiral catalysts for the preparation of chiral compounds of synthetic value is well documented.[1] In particular, hydrolytic enzymes which can be used in organic solvents as well as in aqueous solution are attractive[2] and there have been many reports describing the asymmetric and enantioselective synthesis of optically active alcohols and esters by lipase- and esterase-catalysed transesterification in organic media. In order for enzymes to be applied widely as chiral catalysts in organic synthesis, it is desirable to develop a rule to predict accurately which is the faster-reacting enantiomer. In this regard a variety of active site models for hydrolytic enzymes have been reported[3] and those for lipases (lipase YS and lipase AK) from *Pseudomonas fluorescens* have also been proposed.[4] Here we report the stereospecificity of the lipase YS-catalysed transesterification of primary and secondary alcohols and, on the basis of the results, propose a cubic-spaced model for the active site of lipase YS.

Transesterifications of alcohols mediated by Lipase YS† were carried out with isopropenyl acetate as the acylating agent in diisopropyl ether at 30 °C and terminated at, or close to, the 50% esterification point by filtering off the enzyme. The absolute configurations of products were confirmed by comparison of their rotations with those of known compounds and their enantiomeric excess (e.e.) values were determined by HPLC using a chiral column. The results are shown in Table 1.

From the present results together with the data previously reported,[4b] we propose a cubic-spaced active site model for lipase YS which predicts the faster-reacting enantiomer of the alcohol on the basis of the sizes of the substituents at the chiral centre of the substrate. Burgess and Jenings[4a] have recently described a similar model for lipase AK on the basis of the specificity of transesterifications of unsaturated secondary alcohols.

First we describe the shapes and sizes of the hydrophobic binding sites. Secondary alcohols **45** and **49** with a 'small' branched group adjacent to the chiral centre were inert but the corresponding primary alcohol **39** was acylated at a moderate reaction rate, and from these results we speculate that the HL_1 site is relatively narrow and that the end of this site widens somewhat as illustrated in the top perspective view of the active site model [Fig. 1(*c*)]. Tertiary alcohols **50** and **51** were inert and from this we infer that the hydrophobic site HS can accept a hydrogen or a methine group but cannot accommodate a methyl group. The present results are insufficient to specify in detail the size of the HL_r site.

Extensive data on the stereospecificity of the lipase-catalysed reactions of secondary alcohols have been reported, but there have been few results of those of primary alcohols. Therefore, we next examined the specificity of the esterification of primary alcohols which have the same hydrophobic framework as the corresponding secondary alcohol except for substitution of CH_2OH for OH. It is noteworthy that all primary alcohols

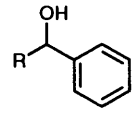


† Lipase YS was supplied by the Amano Pharmaceutical Co. and used without further purification.

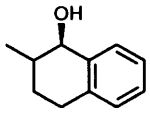
Table 1 Lipase YS-catalysed acylation of alcohols

Entry	Substrate	Reaction time (h)	Products	Isolated yield (%)	E.e. (%)	*E* value[a]
1	(±)-**1**	69	(−)-(*S*)-**1**	25	87	45
			(+)-(*R*)-**2**	43	88	
2	(±)-**3**	19	(−)-(*S*)-**3**	44	96	26
			(+)-(*R*)-**4**	53	75	
3	(±)-**5**	200	(−)-(*S*)-**5**	38	65	21
			(+)-(*R*)-**6**	31	83	
4	(±)-**7**	29	(−)-(*S*)-**7**	47	68	>200
			(+)-(*R*)-**8**	34	99	
5	(±)-**9**	29	(−)-(*S*)-**9**	47	66	48
			(+)-(*R*)-**10**	37	92	
6	(±)-**11**	7	(−)-(*S*)-**11**	49	92	94
			(−)-(*R*)-**12**	46	93	
7	(±)-**13**	7	(−)-(*S*)-**13**	47	94	37
			(+)-(*R*)-**14**	46	83	
8	(±)-**15**	12	(−)-(*S*)-**15**	44	35	3
			(+)-(*R*)-**16**	53	42	
9	(±)-**17**	43	(+)-(1*S*,2*S*)-**17**	29	81	61
			(−)-(1*R*,2*R*)-**18**	33	92	
10	(±)-**19**	70	(+)-(1*S*,2*R*)-**19**	15	56	12
			(−)-(1*R*,2*S*)-**20**	23	75	
11	(±)-**21**	29	(+)-(1*S*,2*S*)-**21**	55	59	7
			(−)-(1*R*,2*R*)-**22**	28	57	
			(−)-(1*R*,2*R*)-**23**	15	95	
12	**24**	170	(−)-(1*R*,2*S*)-**25**	81	84	12
13	(±)-**26**	18	(+)-(*R*)-**26**	49	30	2
			(−)-(*S*)-**27**	47	26	
14	(±)-**28**	7	(+)-(1*R*,2*R*)-**28**	53	25	2
			(−)-(1*S*,2*S*)-**29**	35	16	
			(−)-(1*S*,2*S*)-**30**	8	69	
15	**31**	28	(+)-(1*S*,2*R*)-**32**	41	37	2
16	(±)-**33**	7	(+)-(*R*)-**33**	27	11	2
			(−)-(*S*)-**34**	30	21	
17	(±)-**35**	15	(−)-(*R*)-**35**	52	64	13
			(+)-(*S*)-**36**	48	75	
18	(±)-**37**	33	(−)-(*R*)-**37**	55	56	15
			(+)-(*S*)-**38**	39	79	
19	(±)-**39**	51	(−)-(*R*)-**39**	50	74	29
			(+)-(*S*)-**40**	46	86	
20	(±)-**41**	20 days	(−)-(2*R*)-**41**	69	9	13
			(+)-(2*S*)-**42**	30	84	
21	(±)-**43**	30 days	(−)-(2*S*)-**43**	72	5	2
			(+)-(2*R*)-**44**	21	21	

[a] *E* Values were calculated according to the equation described in the literature.[5]



45 R = Pr^i
46 R = c-C_6H_{11}
47 R = *p*-$MeOC_6H_4$
48 R = *p*-$NO_2C_6H_4$



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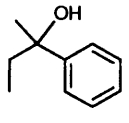

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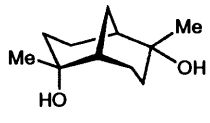

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having a hydroxymethyl group at the *S* chiral centre were preferentially acylated, however, the corresponding secondary *R* alcohols reacted faster. These stereospecificity reversals are interpreted as follows. For our rule we use a working hypothesis that the hydroxy group being acylated is always positioned at the catalytic site and the hydrophobic moieties of the substrate are located at the back of the large hydrophobic pocket. The stereospecificity of lipase YS for secondary alcohols is interpreted straightforwardly as shown in the binding orientation [Fig. 1(*a*), (*c*) and (*f*)] in which the 'large' group on the *R* chiral centre occupies the wide HL_r site. For primary alcohols, the binding orientation of alcohols with an *S* chiral centre, in which the 'large' and 'small' hydrophobic groups are located at the back of the HL_r and HL_l sites, respectively, as can be seen in Fig. 1(*b*) and (*g*), gives better hydrophobic binding resulting in preferential *S* acetate formation. In contrast the binding orientation of its enantiomer gives weak hydrophobic binding, because the 'small' group of the *R* alcohol is placed outside the HL site [Fig. 1(*h*)].

Lipase YS is more effective for the enantioselective transformation of alcohols with a near-planar structure. Three dimensional cage-shaped compounds **41** and **43**, however, were enantioselectively acylated, although very slowly, and acylations of some bicyclic compounds[4b] proceeded with high enantioselectivity. On the basis of these results, we estimate the depth of the HL site [Fig. 2(*a*)] and illustrate the favourable binding modes of bicyclic substrates as shown in Fig. 2(*a*) and (*b*). While it is difficult to interpret clearly the specificity of the acylations of **41** and **43**, we assume that the hydrophobic portions of these substrates placed outside the HL

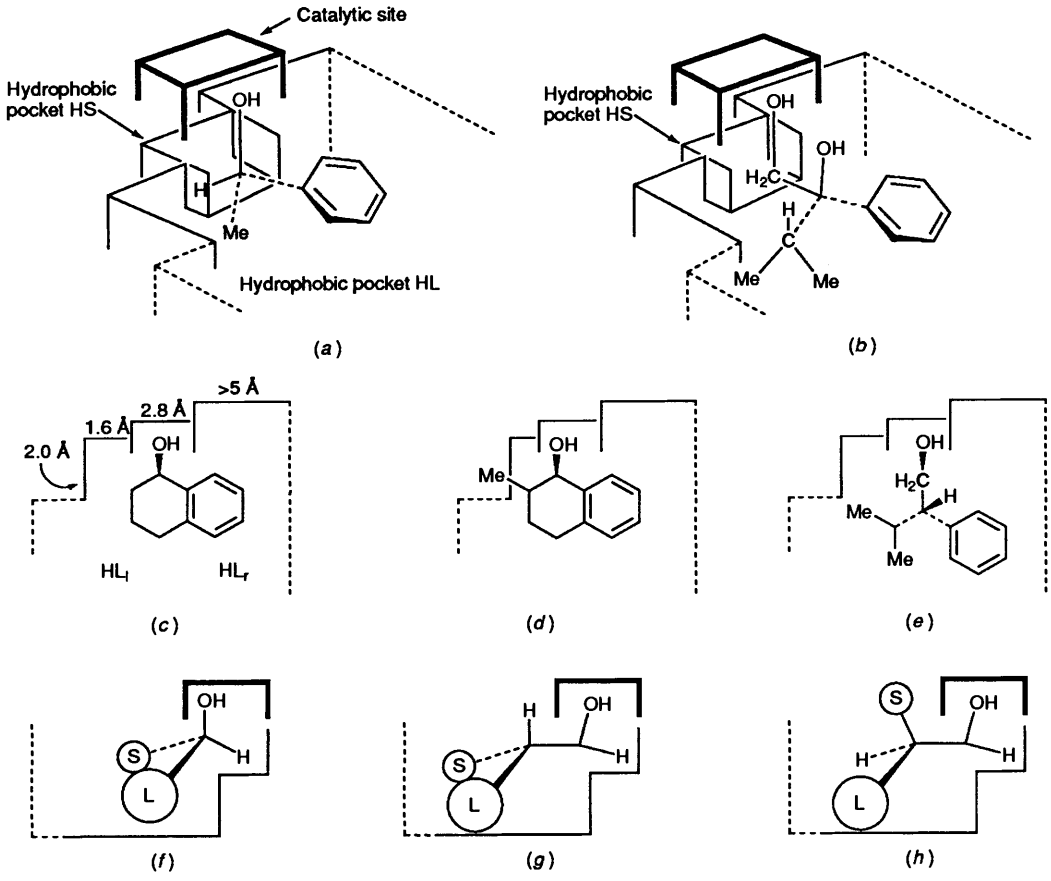

Fig. 1 (*a*) and (*b*) Three-dimensional representations of the active site model illustrated for the secondary alcohol (*R*)-**3** and the primary alcohol (*S*)-**39**, both of which are the faster-reacting enantiomer; (*c*), (*d*) and (*e*) top perspective view of the active site model. HL_l and HL_r = left and right site of large hydrophobic binding site, respectively, HS = small hydrophobic binding site; (*c*) and (*e*) favourable fit of the secondary alcohol (*R*)-**11** and the primary alcohol (*S*)-**39** into the active site; (*d*) the secondary alcohol **49** cannot be accommodated in the binding site; (*f*), (*g*) and (*h*) side perspective view of the active site model; (*f*) good fit of a secondary alcohol into the active site; (*g*) and (*h*) good and poor fit of a primary alcohol into the active site, respectively.

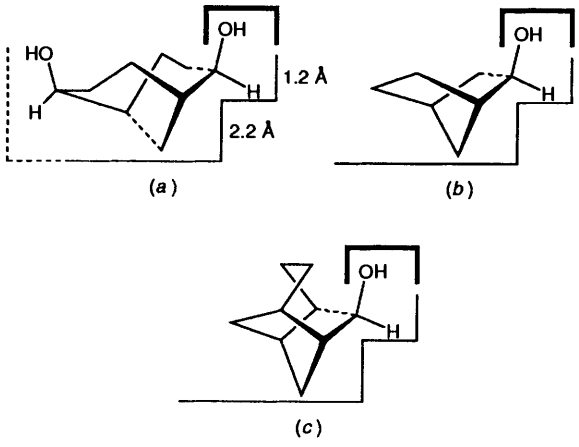

Fig. 2 Favourable fit of (*a*) (2*R*,6*R*)-bicyclo[3.3.1]nonane-2,6-diol (*E* > 200), (*b*) (2*R*)-bicyclo[2.2.1]heptan-2-ol (*E* 36) and (*c*) (2*R*)-**41** into the active site

site do not affect the specificity of the transformation and so the specificity of lipase YS for them is the same was that for bicyclo[2.2.1]heptan-2-ol [Fig. 2(*b*) and (*c*)].

Experimental

General Procedure for Lipase YS-catalysed Acylations.—A mixture of alcohol (±)-**7** (200 mg, 1.35 mmol), isopropenyl acetate (540 mg, 5.40 mmol), lipase YS (130 mg) and dry diisopropyl ether (30 cm^3) was stirred at 30 °C for 7 h and then the enzyme was filtered off. The solvent and isopropenyl acetate were evaporated under reduced pressure and silica gel chromatography of the residue gave ester (+)-**8** (hexane) (118 mg, 46%), $[\alpha]_D^{24}$ + 106 (*c* 1.5 $CHCl_3$) and alcohol (+)-**7** (hexane–diethyl ether, 9:1) (98 mg, 49%), $[\alpha]_D^{24}$ + 30.6 (*c* 1.5 $CHCl_3$).* E.e. values of (+)-**7** and (+)-**8** were directly determined by HPLC. For products without an aromatic moiety, HPLC analyses were carried out after conversion into the corresponding phenyl carbamate.

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* $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

References

1 H. G. Davies, R. H. Green, D. R. Kelly and S. M. Roberts, *Biotransformations in Preparative Organic Chemistry*, Academic Press, London, 1990; A. M. Klibanov, *Acc. Chem. Res.*, 1990, **23**, 114; S. Servi, *Synthesis*, 1990, **1**; L.-M. Zhu and M. C. Tedford, *Tetrahedron*, 1990, **46**, 6587; E. J. Toone, E. S. Simon, M. B. Bednarski and G. M. Whitesides, *Tetrahedron*, 1989, **45**, 5365; C.-H. Wong, *Science*, 1989, **244**, 1145; D. H. G. Crout and M. Chrisen, in *Modern Synthetic Methods*, ed. R. Scheffold, Springer-Verlag, Berlin,

1989, vol. 5, pp. 1–114; A. Akiyama, M. Bednarski, M. J. Kim, E. S. Simon, H. Waldmann and G. M. Whitesides, *Chem. Br.*, 1987, **23**, 645; M. P. Schneider, *Enzymes as Catalysts in Organic Synthesis*, Reidel, Dordrecht, 1986; J. B. Jones, *Tetrahedron*, 1986, **42**, 3351; A. M. Klibanov, *CHEMTECH*, 1986, 354.

2 A. M. Klibanov, *Acc. Chem. Res.*, 1990, **23**, 114.

3 J. Zemlicka and L. E. Craine, *J. Org. Chem.*, 1988, **53**, 937; J. Ehrler and D. Seebach, *Liebigs Ann. Chem.*, 1990, 379; E. J. Toone, M. J. Werth and J. B. Jones, *J. Am. Chem. Soc.*, 1990, **112**, 4946; C. R. Johnson, A. Golebiowski, T. K. McGill and D. H. Seensma, *Tetrahedron Lett.*, 1991, **32**, 2597; R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport and L. A. Cuccia, *J. Org. Chem.*, 1991, **56**, 2656; D. O'Hagan and N. A. Zaidi, *J. Chem. Soc., Perkin Trans. 1*, 1992, 947; Z. Wimmer, *Tetrahedron*, 1992, **48**, 8431; K. Naemura, *J. Synth. Org. Chem. Jpn.*, 1993, **52**, 49 and references cited therein.

4 (*a*) K. Burgess and L. D. Jennings, *J. Am. Chem. Soc.*, 1991, **113**, 6129; (*b*) K. Naemura, H. Ida and R. Fukuda, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 573; (*c*) K. Naemura, R. Fukuda, N. Takahashi, M. Konishi and Y. Tobe, *Tetrahedron: Asymmetry*, 1993, **4**, 911.

5 C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.

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