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Lipase-catalyzed Enantioselective Acylation of Alcohols: a Predictive Active Site Model for Lipase YS to Identify which Enantiomer of an Alcohol Reacts Faster in this Acylation[1]

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Abstract: Primary alcohols having a hydroxymethyl group at an *S* stereogemic center and secondary alcohols with an *R* configuration are preferentially acylated to give the corresponding acetates by lipase YS (from *Pseudomonas fluorescens*)-catalyzed acylation using isopropenyl acetate as the acylating agent in diisopropyl ether. On the basis of enantiomer selectivities observed, a predictive 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 preparation of homochiral compounds of synthetic value using enzymes as chiral catalysts is well documented.[2] In particular, hydrolytic enzymes, which operate without expensive co-enzymes and can function in organic solvents as well as in aqueous solution, are especially attractive[3] and there have been many reports describing the enantioselective and asymmetric synthesis of homochiral alcohols and esters by lipase- and esterase-catalyzed transesterification in an organic solvent. In order for enzymes to be applied widely as chiral catalysts for the preparation of homochiral compounds, it is desirable to develop an active site model for enzyme, that is, a rule to predict accurately which is the faster-reacting enantiomer. In this regard a variety of active site models for lipases and esterases have been reported[4] and those for lipases (lipase YS and lipase AK) from *Pseudomonas fluorescens* have also been proposed.[5] We here report the lipase YS-catalyzed enantioselective and asymmetric transesterification of primary and secondary alcohols and, on the basis of the results, propose a model for active site of lipase YS.

RESULTS AND DISCUSSION

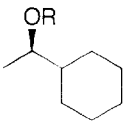
Enantioselective and asymmetric transesterifications of primary and secondary alcohols mediated by lipase YS were carried out with isopropenyl acetate as the acylating agent in diisopropyl ether at 30 °C and the progress of the reaction was monitored by GLC. The enantioselective reactions were terminated at, or close to, the 50% esterification point by removal of enzyme by filtration and the products were purified on column and/or thin layer chromatography. The enantiomeric excess (e.e.) values of acetates and alcohols isolated having a chromophore were directly determined by HPLC using a chiral column. In the cases of products without a chromophore, the determination of their e.e. values was carried out by HPLC analysis of the corresponding phenyl carbamate. The absolute configurations of the products were confirmed by comparison of their specific rotations with those of known compounds reported in literatures.

The absolute configurations of alcohols and acetates except the products (-)-**7** and (+)-**8** and e.e. values of the products together with their isolated yield are summarized in Table 1.

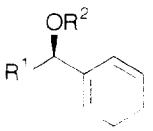
Table 1. Lipase YS-catalyzed Acylation of Racemic and Achiral Alcohols

Entry	Substrate	Products						E-value[a]
		Alcohol (yield %),		e.e.%	Acetate (yield %),		e.e.%	
1	(±)-**1**	(+)-(*S*)-**1**	(25)	87	(-)-(*R*)-**2**	(43)	88	45
2	(±)-**3**	(-)-(*S*)-**3**	(44)	96	(+)-(*R*)-**4**	(53)	75	26
3	(±)-**5**	(-)-(*S*)-**5**	(38)	65	(+)-(*R*)-**6**	(31)	83	21
4	(±)-**7**	(-)-**7**	(32)	72	(+)-**8**	(27)	98	211
5	(±)-**9**	(-)-(*S*)-**9**	(56)	66	(+)-(*R*)-**10**	(34)	98	195
6	(±)-**11**	(-)-(*S*)-**11**	(47)	68	(+)-(*R*)-**12**	(34)	99	412
7	(±)-**13**	(-)-(*S*)-**13**	(47)	66	(+)-(*R*)-**14**	(37)	92	48
8	(±)-**15**	(-)-(*S*)-**15**	(56)	66	(+)-(*R*)-**16**	(37)	82	20
9	(±)-**17**	(-)-(*S*)-**17**	(54)	51	(+)-(*R*)-**18**	(32)	97	108
10	(±)-**19**	(-)-(*S*)-**19**	(59)	62	(+)-(*R*)-**20**	(36)	99	383
11	(±)-**21**	(+)-(*S*)-**21**	(49)	92	(+)-(*R*)-**22**	(46)	93	94
12	(±)-**23**	(+)-(*S*)-**23**	(47)	94	(-)-(*R*)-**24**	(46)	83	37
13	(±)-**25**	(-)-(*S*)-**25**	(44)	35	(+)-(*R*)-**26**	(53)	42	3
14	(±)-**27**	(+)-(1*S*,2*S*)-**27**	(29)	81	(-)-(1*R*,2*R*)-**28**	(33)	92	61
15	(±)-**29**	(+)-(1*S*,2*R*)-**29**	(15)	56	(-)-(1*R*,2*S*)-**30**	(23)	75	12
16	(±)-**31**	(+)-(1*S*,2*S*)-**31**	(55)	59	(-)-(1*R*,2*R*)-**32**	(28)	57	7
					(-)-(1*R*,2*R*)-**33**	(15)	95	
17	**34**	**34**	(10)		(-)-(2*R*,2*S*)-**35**	(81)	81	12
18	(±)-**36**	(+)-(*R*)-**36**	(49)	80	(-)-(*S*)-**37**	(47)	26	2
19	(±)-**38**	(+)-(1*R*,2*R*)-**38**	(53)	25	(-)-(1*S*,2*S*)-**39**	(35)	16	2
					(-)-(1*S*,2*S*)-**40**	(8)	69	
20	**41**	**41**	(20)		(+)-(1*S*,2*R*)-**42**	(41)	37	2
21	(±)-**43**	(+)-(*R*)-**43**	(27)	11	(-)-(*S*)-**44**	(30)	21	2
22	(±)-**45**	(-)-(*R*)-**45**	(52)	64	(+)-(*S*)-**46**	(48)	75	13
23	(±)-**47**	(-)-(*R*)-**47**	(55)	86	(+)-(*S*)-**48**	(39)	79	15
24	(±)-**49**	(-)-(*R*)-**49**	(50)	74	(+)-(*S*)-**50**	(46)	86	29
25	(±)-**51**	(-)-(2*R*)-**51**	(69)	79	(+)-(2*S*)-**52**	(30)	84	13
26	(±)-**53**	(-)-(3*S*)-**53**	(72)	5	(+)-(3*R*)-**54**	(21)	21	2
27	(±)-**55**	(+)-(1*R*)-**55**	(58)	36	(-)-(1*S*)-**56**	(35)	57	5

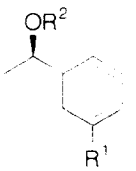
[a] E-values were calculated according to the equation described in the literature.[6]



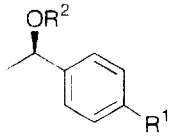
1 R=H
2 R=Ac



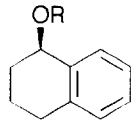
3 R^1=CH_3 R^2=H
4 R^1=CH_3 R^2=Ac
5 R^1=C_2H_5 R^2=H
6 R^1=C_2H_5 R^2=Ac



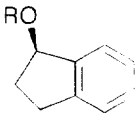
7 R^1=F R^2=H
8 R^1=F R^2=Ac
9 R^1=CF_3 R^2=H
10 R^1=CF_3 R^2=Ac



11 R^1=NO_2 R^2=H
12 R^1=NO_2 R^2=Ac
13 R^1=OCH_3 R^2=H
14 R^1=OCH_3 R^2=Ac
15 R^1=CF_3 R^2=H
16 R^1=CF_3 R^2=Ac
17 R^1=Br R^2=H
18 R^1=Br R^2=Ac
19 R^1=F R^2=H
20 R^1=F R^2=Ac



21 R=H
22 R=Ac



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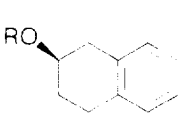
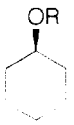
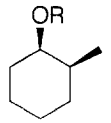
24 R=Ac



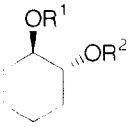
25 R=H
26 R=Ac



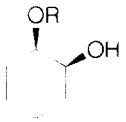
27 R=H
28 R=Ac



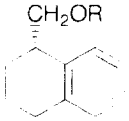
29 R=H
30 R=Ac



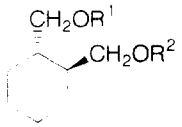
31 R^1=R^2=H
32 R^1=H, R^2=Ac
33 R^1=R^2=Ac



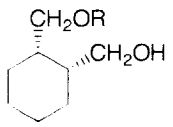
34 R=H
35 R=Ac



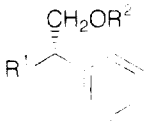
36 R=H
37 R=Ac



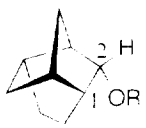
38 R^1=R^2=H
39 R^1=H, R^2=Ac
40 R^1=R^2=Ac



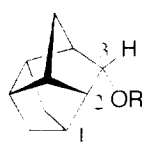
41 R=H
42 R=Ac



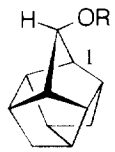
43 R^1=CH_3 R^2=H
44 R^1=CH_3 R^2=Ac
45 R^1=C_2H_5 R^2=H
46 R^1=C_2H_5 R^2=Ac
47 R^1=C_3H_7 R^2=H
48 R^1=C_3H_7 R^2=Ac
49 R^1=$CH(CH_3)_2$ R^2=H
50 R^1=$CH(CH_3)_2$ R^2=Ac



51 R=H
52 R=Ac



53 R=H
54 R=Ac



55 R=H
56 R=Ac

Figure 1 The absolute configurations of faster-reacting enantiomers of alcohols are shown

The absolute configurations of faster-reacting enantiomers of primary alcohols and secondary alcohols are given in Figure 1 and the alcohols shown in Figure 2 were inert in our studies.

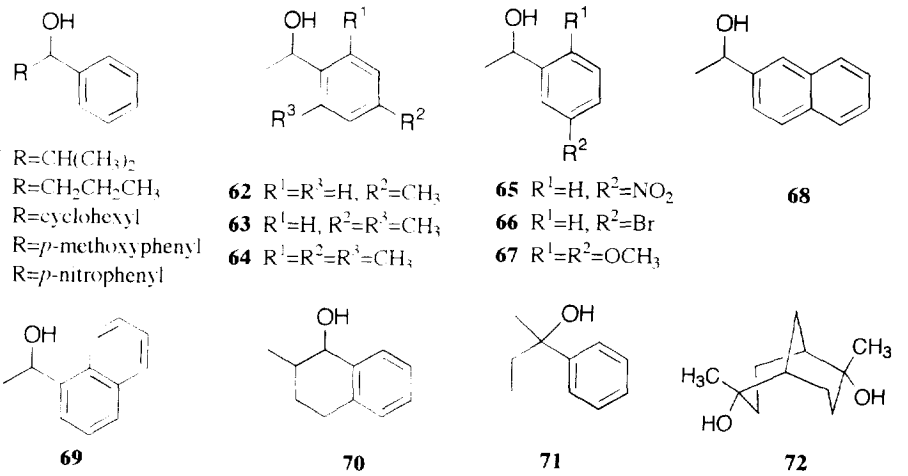


Figure 2.

From the enantioselectivities observed here together with the results previously reported,[5b,7] we propose the 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 center of the substrate. Burgess and Jenings have recently described a similar active site model for lipase AK on the basis of the specificity of transesterifications of unsaturated secondary alcohols in hexane.[5a]

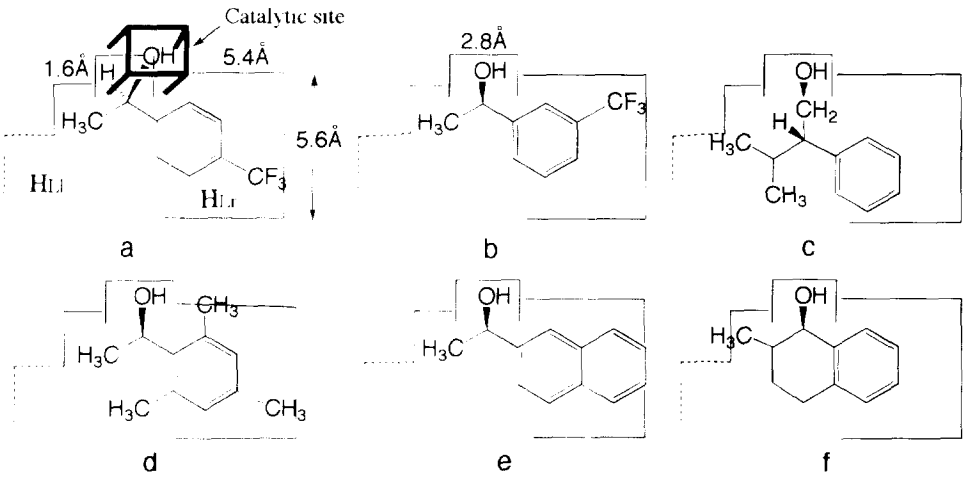


Figure 3. Top perspective view of the active site model. H_L and H_S left and right pocket of large hydrophobic binding site, respectively; (a), (b), and (c) favorable fit of the secondary alcohols (*R*)-**15** and (*R*)-**9** and the primary alcohol (*S*)-**49** into the active site; (d), (e), and (f) the secondary alcohols **64**, **68**, and **70** were not accommodated in the binding site.

First we describe the shapes and sizes of the hydrophobic binding sites on the basis of the results of transesterifications of secondary alcohols. As the larger group has a higher priority in all secondary alcohols examined here, secondary alcohols with an *R* configuration were preferentially acylated suggesting that the right hydrophobic binding site (HLr) is larger than the left hydrophobic binding site (HLl) as shown in the top perspective view of the active site model (Fig 3a). The reactions of the substrates possessing the phenyl moiety having a polar group or an electronegative atom at its *para* or *meta*-position showed high E-value. The results demonstrate that HLr pocket is more polar in character and the moiety having the polar group were favorably accommodated in this pocket resulting in high enantiodiscrimination but the substrates **65** and **66** having a larger polar group or atom at the *meta*-position of the phenyl moiety were not accepted suggesting that they are presumably a boundary compound in bulk. The inert alcohols **62**, **63**, **64**, **68**, and **69**, which were employed as probes of the HLr pocket, demonstrate that the HLr pocket can accommodate at most a phenyl moiety having a small polar group at its *para* or *meta*-position (Fig 3a, 3b, 3d, and 3e). The reactions of the substrates (*S*)-**9** and (*S*)-**15**, however, occurred and the results are interpreted in terms of the attractive interaction between the polar group of the substrate and the polar HLr pocket, which overcame disadvantage be due to the slight intrusion of the group of the substrate into the limited region, that is, the side wall of the active site leading to a productive ES complex. On the basis of the results that secondary alcohols **57** and **70** with a 'small' branched group adjacent to the stereogenic center (Fig 3f) and alcohols **58**~**61** with large substituents at the both sides of the stereogenic center were inert, we speculate that the HLl site is relatively narrow and the end of HLl widens somewhat as illustrated in Fig. 3a. The size of the HLr pocket is approximately estimated but the present results are insufficient to specify in detail the shape and size of the HLl pocket.

Although bicyclo[3.3.1]nonane-2,6-diol (E >200)[5b] and the alcohol **5** were acylated, tertiary alcohols **71** and **72** were inert and from this we infer that the hydrophobic binding site (HS) can accept a hydrogen or a methine group but cannot accommodate a methyl group.

Many data on the stereoselectivity of lipase-catalyzed 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 esterification of primary alcohols which have the same hydrophobic framework as the corresponding secondary alcohol except for substitution of a hydroxymethyl group for a hydroxyl group. It is noteworthy that all primary alcohols having a hydroxymethyl group at the *S* stereogenic center were preferentially acylated, however, the corresponding secondary *R* alcohols reacted faster. These stereoselectivity reversals are interpreted as follows.

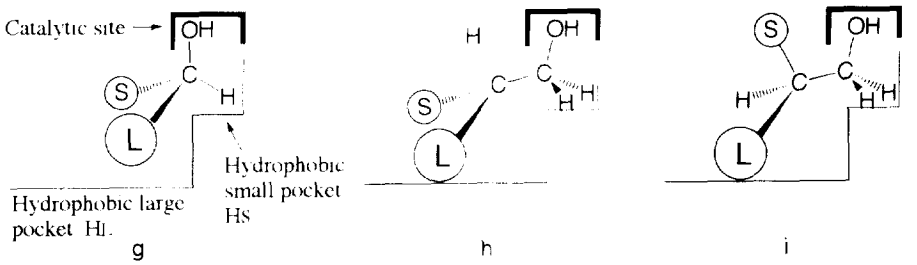


Figure 4. Side perspective view of the active site model. (g) and (h) Good fit of a secondary alcohol and a primary alcohol into the active site, respectively; (i) poor fit of a primary alcohol into the active site.

For our rule we use a working hypothesis that the hydroxyl 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 (HL). The stereoselectivity of lipase YS for secondary alcohols is interpreted straightforwardly as shown in the binding orientation (Fig 3a and 4g) in which the 'large' group on the *R* chiral center occupies the back of the HL site. For primary alcohols, the binding orientation of alcohols with the *S* stereogenic center, in which the 'large' and 'small' hydrophobic groups are located at the back of the HL and HS sites, respectively, as can be seen in Fig 3c and **4h**, gives better hydrophobic binding than that of the *R* primary alcohol in which the 'small' group of the alcohol is placed outside the large hydrophobic pocket (HL) resulting in weak hydrophobic binding (Fig 4i). Low E-values of the reactions of primary alcohols examined here are assumed to be resulted from the mobility of the binding orientation of them be due to that the stereogenic center of them is remote from the catalytic site (Fig 3c and 4h).

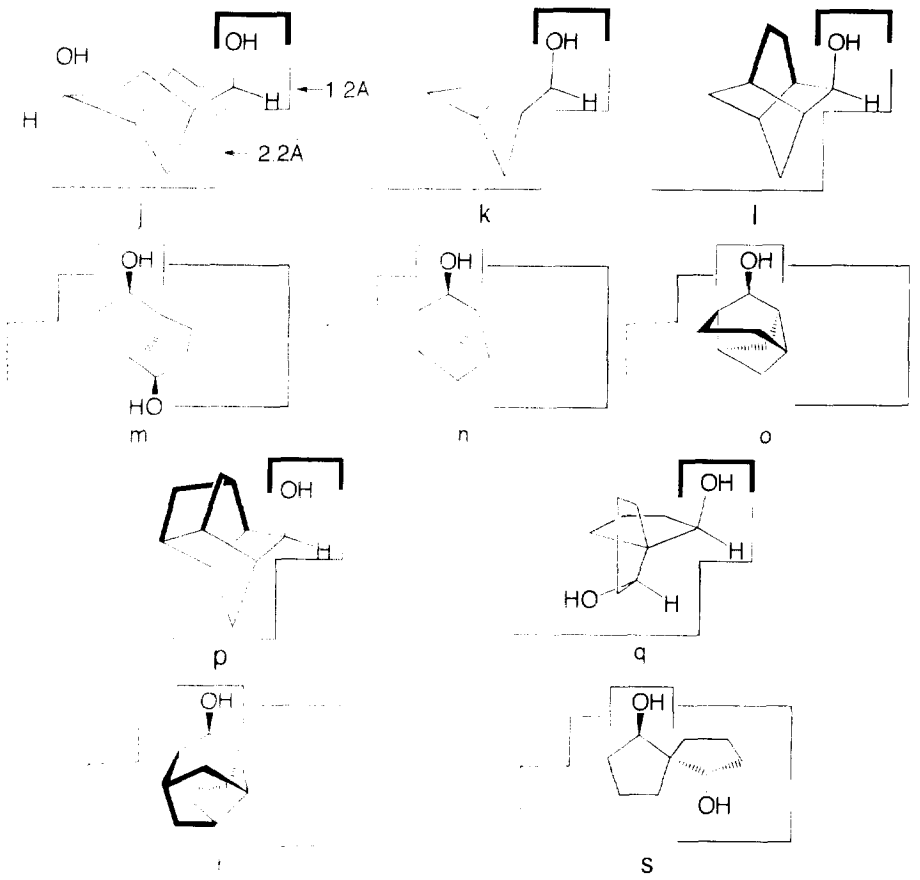


Figure 5. (j) and (m) Favorable fit of (2*R*,6*R*)-bicyclo[3.3.1]nonane-2,6-diol; (k) and (n) favorable fit of (2*R*)-bicyclo[2.2.1]heptan-2-ol; (l) and (o) favorable fit of (2*R*)-**51** into the active site; (p) and (r) favorable fit of (3*S*)-**53** into the active site; (q) and (s) binding orientation of (1*R*,5*S*,6*R*)-spiro[4.4]nonane-1,6-diol. Bonds in bold width are placed outside the large hydrophobic pocket HL.

Lipase YS is more effective for the enantioselective transesterification of alcohols with a near-planar

structure. Three dimensional cage-shaped compounds **51**, **53**, and **55**, however, were enantioselectively acylated, although very slowly, and acylations of some bicyclic compounds proceeded with high enantioselectivity.[5b] On the basis of these results, we estimate the depth of the HL site (Fig 5j) and illustrate the favorable binding modes of bicyclo[3.3.1]nonane-2,6-diol and bicyclo[2.2.1]heptan-2-ol (E 36)[5b] as shown in Fig. 5j, 5k, 5m, and 5n. While it is difficult to interpret clearly the specificity of the acylations of cage-shaped compounds **51**, **53**, and **55**, we assume that the hydrophobic portions of these substrates placed outside the HL pocket do not affect the stereospecificity of the transformation and so the stereospecificity of lipase YS for **51** and **53** is the same was that for bicyclo[2.2.1]heptan-2-ol (Fig 5l, 5o, 5p, and 5r). The previous our observation[6] that (-)-(1*R*,5*S*,6*R*)-1-acetoxyspiro[4.4]nonan-6-ol (62% e.e.) and (+)-(1*S*,5*R*,6*S*)-spiro[4.4]nonane-1,6-diol (89% e.e.) were isolated by the acylation of (±)-spiro[4.4]nonane-1,6-diol is interpreted in terms of the binding orientation (Fig 5q and 5s).

The model described here is rule of thumb to identify which enantiomer of a primary or a secondary alcohol reacts faster in the acylation mediated by lipase YS.

Experimental

General Procedure – Optical rotations were measured using a JASCO DIP-40 polarimeter at ambient temperature and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. GLC analyses were performed on a Simadzu GS 8A chromatograph using an SE-52 on Uniport HP 2 m x 2.6 mm column. HPLC analyses were carried out on Simadzu LC-6A chromatograph using a chiral column Opti-Pak AD (Waters), 250 mm x 4.6 mm. Lipase YS was supplied from the Amano Pharmaceutical Co. and used without further purification. Diisopropyl ether was stocked on Molecular sieves 4A after drying on $CaCl_2$ followed by distillation.

General Procedure for Lipase YS catalyzed Acylation (Entry 1) – A mixture of alcohol (±)-**1** (200 mg, 1.56 mmol), isopropenyl acetate (625 mg, 6.24 mmol), and lipase YS (130 mg) in dry diisopropyl ether (30 cm^3) was stirred at 30 °C and the progress of the reaction was monitored by GLC. After stirring for 69h, the enzyme was filtered off and the volatile materials were removed under reduced pressure. The residue was chromatographed on silica gel to give (+)-**2** (hexane as eluent) (113 mg, 43% yield), $[\alpha]_D^{24}$ +6.6 (*c* 2.60, $CHCl_3$) and (+)-**1** (hexane-diethyl ether 9:1 as eluent) (49 mg, 25% yield), $[\alpha]_D^{24}$ +2.6 (*c* 0.778, $CHCl_3$). A mixture of (+)-**2** (88 mg, 0.52 mmol) and $LiAlH_4$ (20 mg, 0.52 mmol) in dry diethyl ether (20 cm^3) was refluxed for 5h. After a usual work up, the products were separated by TLC on silica gel to give (+)-**1** (39 mg, 66% yield), $[\alpha]_D^{27}$ +2.7 (*c* 0.73, $CHCl_3$). The e.e. values of (+)-**1** and (-)-**1** were determined by HPLC analysis of the corresponding phenyl carbamates and the absolute configurations of (+)-**1** and (+)-**2** were confirmed by comparison of the specific rotation of **1** with that reported in the literature.[7]

General Procedure for the Preparation of the Phenyl Carbamate – A mixture of (+)-**1** (36 mg, 0.25 mmol), phenyl isocyanate (30 mg, 0.25 mmol), and five drops of pyridine was stirred for 2h at room temperature. After addition of dil. HCl, the mixture was extracted with benzene and the extract was washed with saturated aqueous solution of $NaHCO_3$, and water and then dried ($MgSO_4$). After removal of the solvent under reduced pressure, the phenyl carbamate was isolated by TLC on silica gel and HPLC analysis of the phenyl carbamate isolated was performed without further purification.

Lipase YS catalyzed Acylation of Alcohols

Entry 2 - After reaction for 19h, (-)-**3**, $[\alpha]_D^{22}$ -39.8 (*c* 1.06, $CHCl_3$)[8] and (+)-**4**, $[\alpha]_D^{24}$ +103 (*c* 4.02, $CHCl_3$) were isolated on column chromatography. Treatment of (+)-**4** with $LiAlH_4$ gave (+)-**3**, $[\alpha]_D^{22}$ +31.0 (*c* 1.20, $CHCl_3$). The e.e. values of the products were determined directly by HPLC analysis of the alcohol and the acetate isolated, unless stated otherwise.

Entry 3 - After reaction for 200h, (-)-**5**, $[\alpha]_D^{25}$ -17.8 (*c* 1.01, $CHCl_3$)[9] and (+)-**6**, $[\alpha]_D^{24}$ +75.0 (*c* 0.140, $CHCl_3$) were isolated on column chromatography. Treatment of (+)-**6** with $LiAlH_4$ gave (+)-**5**, $[\alpha]_D^{25}$ +22.8 (*c* 1.00, $CHCl_3$).

Entry 4 - After reaction for 20h, (-)-**7**, $[\alpha]_D^{26}$ -28.0 (*c* 0.979, MeOH) and (+)-**8**, $[\alpha]_D^{26}$ +80.1 (*c* 0.987, $CHCl_3$) were isolated on chromatography. Treatment of (+)-**8** with $LiAlH_4$ gave (+)-**7**, $[\alpha]_D^{24}$ +23.5 (*c* 0.750, MeOH).

Entry 5 - After reaction for 36h, (-)-**9**, $[\alpha]_D^{22}$ -18.0 (*c* 1.67, MeOH)[10] and (+)-**10**, $[\alpha]_D^{22}$ +72.2 (*c* 1.90, $CHCl_3$) were isolated on chromatography. Treatment of (+)-**10** with $LiAlH_4$ gave (+)-**9**, $[\alpha]_D^{24}$ +28.0 (*c* 0.780, MeOH).

Entry 6 - After reaction for 29h, (-)-**11**, $[\alpha]_D^{26}$ -21.2 (*c* 0.300, MeOH)[11] and (+)-**12**, $[\alpha]_D^{27}$ +92.2 (*c* 0.866, $CHCl_3$) were isolated on chromatography. Treatment of (+)-**12** with $LiAlH_4$ gave (+)-**11**, $[\alpha]_D^{26}$ +29.0 (*c* 0.305, MeOH).

Entry 7 - After reaction for 29h, (-)-**13**, $[\alpha]_D^{23}$ -27.0 (*c* 1.98, MeOH)[12] and (+)-**14**, $[\alpha]_D^{23}$ +125 (*c* 2.09, $CHCl_3$) were isolated on chromatography. Treatment of (+)-**14** with $LiAlH_4$ gave (+)-**13**, $[\alpha]_D^{24}$ +37.5 (*c* 0.673, MeOH).

Entry 8 - After reaction for 24h, (-)-**15**, $[\alpha]_D^{22}$ -20.9 (*c* 2.96, MeOH)[13] and (+)-**16**, $[\alpha]_D^{22}$ +71.3 (*c* 2.98, $CHCl_3$) were obtained. Treatment of (+)-**16** with $LiAlH_4$ gave (+)-**15**, $[\alpha]_D^{23}$ +25.1 (*c* 1.74, MeOH).

Entry 9 - After reaction for 117h, (-)-**17**, $[\alpha]_D^{27}$ -16.0 (*c* 2.20, MeOH)[14] and (+)-**18**, $[\alpha]_D^{26}$ +88.9 (*c* 1.31, $CHCl_3$) were obtained. Treatment of (+)-**18** with $LiAlH_4$ gave (+)-**17**, $[\alpha]_D^{26}$ +31.3 (*c* 0.436, MeOH).

Entry 10 - After reaction for 63h, (-)-**19**, $[\alpha]_D^{28}$ -22.2 (*c* 3.69, MeOH)[14] and (+)-**20**, $[\alpha]_D^{27}$ +96.0 (*c* 2.43, $CHCl_3$) were obtained. Treatment of (+)-**20** with $LiAlH_4$ gave (+)-**19**, $[\alpha]_D^{26}$ +35.0 (*c* 1.08, MeOH).

Entry 11 - Reaction for 7h followed by chromatography of the products gave (+)-**22**, $[\alpha]_D^{24}$ +106.1 (*c* 0.150, $CHCl_3$)[15] and (+)-**21**, $[\alpha]_D^{25}$ +30.6 (*c* 1.50, $CHCl_3$). Treatment of (+)-**22** with $LiAlH_4$ gave (-)-**21**, $[\alpha]_D^{26}$ +31.0 (*c* 1.00, $CHCl_3$)

Entry 12 - Reaction for 7h followed by chromatography of the products gave (+)-**24**, $[\alpha]_D^{24}$ +92.3 (*c* 2.39, $CHCl_3$)[16] and (-)-**23**, $[\alpha]_D^{24}$ -25.2 (*c* 0.820, $CHCl_3$). Treatment of (+)-**24** with $LiAlH_4$ gave (+)-**23**, $[\alpha]_D^{24}$ +22.2 (*c* 1.20, $CHCl_3$)

Entry 13 - Reaction for 12h followed by chromatography of the products gave (+)-**26**, $[\alpha]_D^{28}$ +21.4 (*c* 1.50, $CHCl_3$)[17] and (-)-**25**, $[\alpha]_D^{28}$ -22.2 (*c* 1.50, $CHCl_3$). Treatment of (+)-**26** with $LiAlH_4$ gave (+)-**25**, $[\alpha]_D^{27}$ +26.7 (*c* 1.50, $CHCl_3$)

Entry 14 - Reaction for 43h followed by chromatography of the products gave (-)-**28**, $[\alpha]_D^{25}$ -61.0 (*c* 1.50, $CHCl_3$)[18] and (+)-**27**, $[\alpha]_D^{26}$ +28.6 (*c* 2.60, $CHCl_3$). Treatment of (-)-**28** with $LiAlH_4$ gave (-)-**27**, $[\alpha]_D^{26}$ -32.6 (*c* 2.50, $CHCl_3$). The e.e. values of (+)-**27** and (-)-**27** were determined by HPLC analysis of the corresponding phenyl carbamates.

Entry 15 - Reaction for 70h followed by chromatography of the products gave (-)-**30**, $[\alpha]_D^{26}$ -37.4 (*c* 1.50, $CHCl_3$)[18] and (+)-**29**, $[\alpha]_D^{25}$ +10.1 (*c* 1.50, $CHCl_3$). Treatment of (-)-**30** with $LiAlH_4$ gave (-)-**29**, $[\alpha]_D^{26}$ -13.5 (*c* 2.50, $CHCl_3$). The e.e. values of (+)-**29** and (-)-**29** were determined by HPLC analysis of the

corresponding phenyl carbamates.

Entry 16 - After reaction for 29h, (-)-**33**, $[\alpha]_D^{27}$ -12.5 (*c* 1.60, $CHCl_3$),[19] (-)-**32**, $[\alpha]_D^{28}$ -30.2 (*c* 1.60, $CHCl_3$), and (+)-**31**, $[\alpha]_D^{26}$ +24.4 (*c* 0.51, H_2O) were obtained. Treatment of (-)-**33** and (-)-**32** with $LiAlH_4$ gave (-)-**31**, $[\alpha]_D^{26}$ -39.5 (c 0.52, $CHCl_3$) and (-)-**31**, $[\alpha]_D^{27}$ -24.0 (c 0.65, $CHCl_3$), respectively. The e.e. values of (+)-**31** and (-)-**31** were determined by HPLC analysis of the corresponding phenyl carbamates.

Entry 17 - After reaction for 170h, **34** and (-)-**35**, $[\alpha]_D^{22}$ -1.86 (*c* 1.50, $CHCl_3$)[20] were obtained. The e.e. value of (-)-**35** was determined by HPLC analysis of the corresponding phenyl carbamate.

Entry 18 - After reaction for 18h, chromatography of the products gave (-)-**37**, $[\alpha]_D^{24}$ -5.6 (*c* 1.36, $CHCl_3$) and (+)-**36**, $[\alpha]_D^{24}$ +3.8 (*c* 0.592, $CHCl_3$). Treatment of (-)-**37** with $LiAlH_4$ gave (-)-**36**, $[\alpha]_D^{22}$ +3.3 (*c* 0.560, $CHCl_3$). The absolute configuration of (-)-**36** was determined by oxidation with Jones' reagent in acetone to give the corresponding carboxylic acid, $[\alpha]_D^{24}$ -1.8 (*c* 0.560, $CHCl_3$).[21]

Entry 19 - After reaction for 7h, (-)-**40**, $[\alpha]_D^{24}$ -12.3 (*c* 0.794, $CHCl_3$),[22] (-)-**39**, $[\alpha]_D^{25}$ -4.6 (*c* 1.09, $CHCl_3$), and (+)-**38**, $[\alpha]_D^{24}$ +2.0 (*c* 0.712, $CHCl_3$) were obtained. Treatment of (-)-**39** and (-)-**40** with $LiAlH_4$ gave (-)-**38**, $[\alpha]_D^{26}$ -1.3 (*c* 0.420, $CHCl_3$) and (-)-**38**, $[\alpha]_D^{27}$ -5.5 (*c* 0.212, $CHCl_3$), respectively. The e.e. values of (+)-**38** and (-)-**38** were determined by HPLC analysis of the corresponding phenyl carbamates.

Entry 20 - After reaction for 28h, chromatography of the products gave **41** and (+)-**42**, $[\alpha]_D^{24}$ -10.2 (*c* 0.587, $CHCl_3$)[23] and the e.e. value of (+)-**42** was determined by HPLC analysis of the corresponding phenyl carbamate.

Entry 21 - After reaction for 7h, (-)-**44**, $[\alpha]_D^{27}$ -0.08 (*c* 1.18, $CHCl_3$)[9] and (+)-**43**, $[\alpha]_D^{26}$ +0.60 (*c* 1.17, $CHCl_3$) were obtained. Treatment of (-)-**44** with $LiAlH_4$ gave (-)-**43**, $[\alpha]_D^{25}$ -1.13 (*c* 1.56, $CHCl_3$)

Entry 22 - After reaction for 15h, (+)-**46**, $[\alpha]_D^{22}$ +12.4 (*c* 3.06, $CHCl_3$)[24] and (-)-**45**, $[\alpha]_D^{22}$ -12.4 (*c* 2.52, $CHCl_3$) were obtained. Treatment of (+)-**46** with $LiAlH_4$ gave (+)-**45**, $[\alpha]_D^{23}$ +14.5 (*c* 1.50, $CHCl_3$)

Entry 23 - After reaction for 33h, (+)-**48**, $[\alpha]_D^{24}$ +9.5 (*c* 1.17, $CHCl_3$)[25] and (-)-**47**, $[\alpha]_D^{25}$ -9.0 (*c* 2.33, $CHCl_3$) were obtained. Treatment of (+)-**48** with $LiAlH_4$ gave (+)-**47**, $[\alpha]_D^{23}$ +12.6 (*c* 2.50, $CHCl_3$).

Entry 24 - After reaction for 51h, chromatography of the products gave (+)-**50**, $[\alpha]_D^{26}$ +12.9 (*c* 3.29, $CHCl_3$) and (-)-**49**, $[\alpha]_D^{25}$ -5.0 (*c* 2.36, $CHCl_3$). Treatment of (+)-**50** with $LiAlH_4$ gave (+)-**49**, $[\alpha]_D^{25}$ +5.8 (*c* 2.50, $CHCl_3$). The absolute configuration of (+)-**49** was determined by oxidation with Jones' reagent in acetone to give the corresponding carboxylic acid, $[\alpha]_D^{22}$ +46.1 (*c* 0.500, $CHCl_3$).[26]

Entry 25 - After reaction for 20 days, chromatography of the products gave (+)-**52**, $[\alpha]_D^{26}$ +101 (*c* 0.928, $CHCl_3$) and (-)-**51**, $[\alpha]_D^{27}$ -24.6 (*c* 0.792, $CHCl_3$). Their e.e. values were determined by comparison of the specific rotation of 2-twist-brendanone. Oxidation of (-)-**51** and (+)-**51** derived from (+)-**52** with Jones' reagent in acetone gave 2-twist-brendanone, $[\alpha]_D^{24}$ -190 (c 0.450, $CHCl_3$) and $[\alpha]_D^{26}$ +203 (c 0.443, $CHCl_3$), respectively.[27]

Entry 26 - After reaction for 30 days, chromatography of the products gave (+)-**54**, $[\alpha]_D^{28}$ +28.4 (*c* 0.783, $CHCl_3$) and (-)-**53**, $[\alpha]_D^{27}$ -8.3 (*c* 1.02, $CHCl_3$). The e.e. values of (-)-**53** and (+)-**54** were confirmed by comparison of their specific rotations with those reported in the literature.[28]

Entry 27 - After reaction for 36 days, chromatography of the products gave (+)-(1*R*,3*R*,5*R*,6*S*,8*R*,10*S*)-**55**, $[\alpha]_D^{27}$ +51.5 (*c* 0.565, EtOH) and (-)-(1*S*,3*S*,5*S*,6*R*,8*S*,10*R*)-**56**, $[\alpha]_D^{26}$ -63.1 (*c* 0.570, $CHCl_3$). The e.e. values of (+)-**55** and (-)-**56** were confirmed by comparison of their specific rotations with those reported in the literature.[29]

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