Enhancing the Enantioselectivity of Lipase in Transesterification by Substrate Matching: An Enzyme Memory Based Approach

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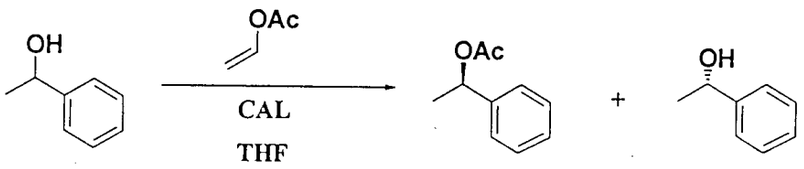
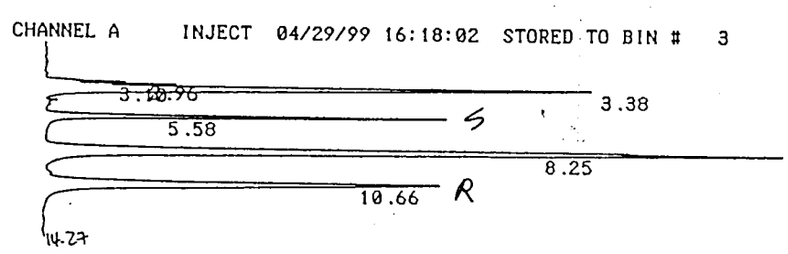
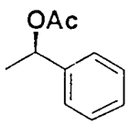
Supplementary Materials

General procedure for determining the enantioselectivity of lipase. The enzymatic reactions were carried out at room temperature with enol acetate (2-8 equiv.), alcohol (30-50 mg), and enzyme (100mg) in dry solvent (1-1.5 mL). The reactions were carried to approximately 40-50% completion. An aliquot was withdrawn from the reaction mixture, concentrated, and then subjected to the hplc analysis using a chiral column (Chiralcel OD or Welko-O-1) to determine the enantiomeric excesses of the acetylated products and remaining substrates. The hplc analysis was done under the following analytical conditions: **3a**, Chiralcel OD, hexane/isopropanol = 98/2, flow rate 1.0 mL/min, UV 217 nm; **3b** Chiralcel OD, hexane/isopropanol = 98/2, flow rate 0.5 mL/min, UV 217 nm; **3c**, Chiralcel OD, hexane/isopropanol = 99/1, flow rate 0.5 mL/min, UV 217 nm; **4a**, Welko-O-1, hexane/isopropanol = 98/2, flow rate 0.5 mL/min, UV 217 nm; **4b,c**, determined after hydrolyzed to **3b,c**, respectively. See the attached chromatograms for some representative hplc data. The E values for the enantioselectivity were calculated using the equation, $E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$ or $\ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$, where $c = ee_s/(ee_s + ee_p)$,[8] and described together with the values for ee_s, ee_p, and c in Tables1-2.







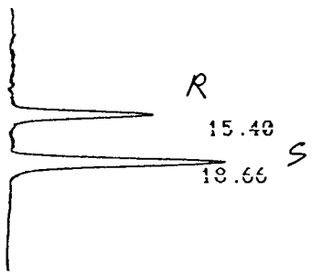
Column :Whelk-O-1
Eluent: n-Hexane/IPA=98/2
Flow rate:1.0mL/min
UV : 217nm

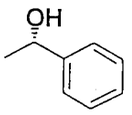
04/29/99 16:18:02 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 3 INDEX 3 BIN 3

PEAK#	AREA%	RT	AREA BC	
1	1.351	2.96	20763 02	
2	1.726	3.1	26534 02	
3	23.78	3.38	365548 03	→ solvent
4	11.189	5.58	171998 01	→ (S)-OAc
5	39.485	8.25	606954 01	→ alcohol
6	22.469	10.66	345386 01	→ (R)-OAc
TOTAL	100.00		1537183	

ee_p = 33.51(%)





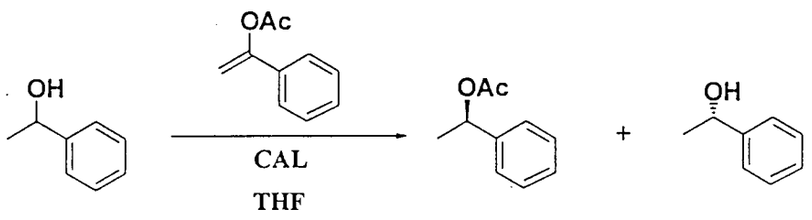
Column :Daicel OD
Eluent: n-Hexane/IPA=98/2
Flow rate:1.0mL/min
UV : 217nm

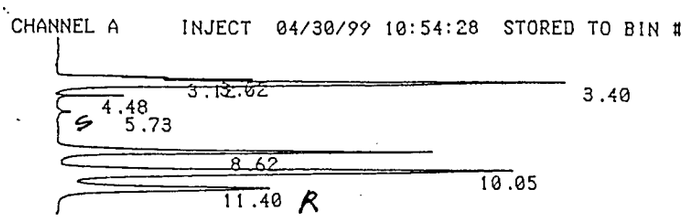
04/28/99 20:09:45

FILE 1. METHOD 0. RUN 17 INDEX 11

PEAK#	AREA%	RT	AREA BC	
1	2.698	3.79	11793 02	
2	10.482	4.62	45163 02	
3	10.118	5.79	43574 02	→ Acetate
4	10.059	6.11	43973 03	
5	4.357	7.52	19046 02	
6	1.069	7.84	4673 03	
7	14.84	15.4	64872 01	→ (R)-alcohol
8	28.378	18.66	124051 01	→ (S)-alcohol
TOTAL	100.		437145	

ee_s = 31.32(%)





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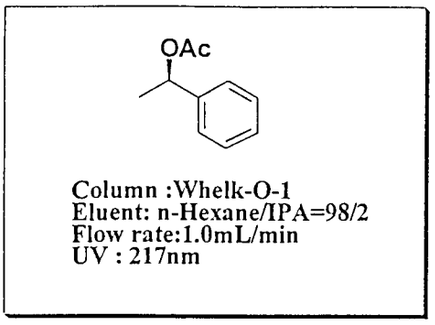
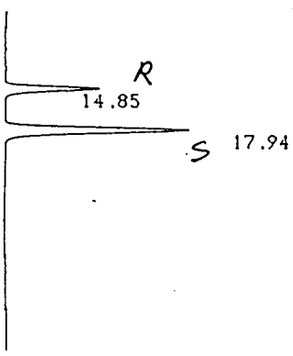


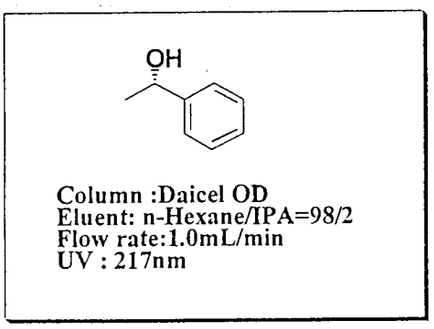
04/30/99 10:54:28 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 4 INDEX 4 "N 4

PEAK#	AREA%	RT	AREA	BC	
1	1.36	3.02	16283	02	
2	4.016	3.12	48089	02	
3	28.082	3.4	336287	03	
4	0.81	4.48	9697	01	
5	0.244	5.73	2924	01	→ (S)-OAc
6	17.171	8.62	205628	01	: alcohol
7	32.54	10.05	389672	02	: enol-ester/ketone
8	15.776	11.4	188922	03	→ (R)-OAc
TOTAL	100.		1197502		

ee_p = 96.95(%)





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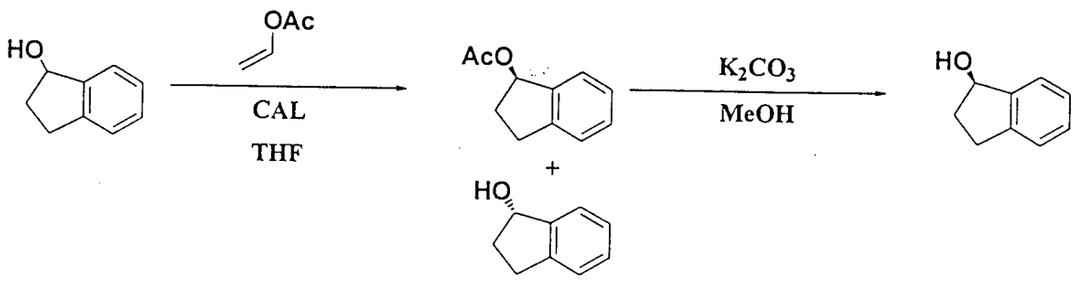
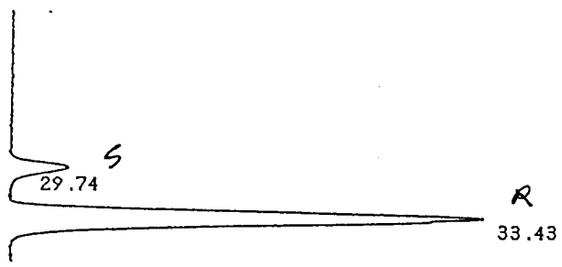
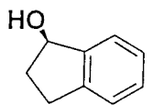
FILE 1. METHOD 0. RUN 17 INDEX 17 BIN 14

PEAK#	AREA%	RT	AREA	BC	
1	0.364	4.66	4109	01	
2	7.407	5.82	83664	01	→ acetate
3	69.874	7.33	789225	01	→ enol-ester/ketone
4	6.603	14.85	74579	01	→ (R)-alcohol
5	15.752	17.94	177918	01	→ (S)-alcohol
TOTAL	100.		1129495		

ee_s = 40.93(%)







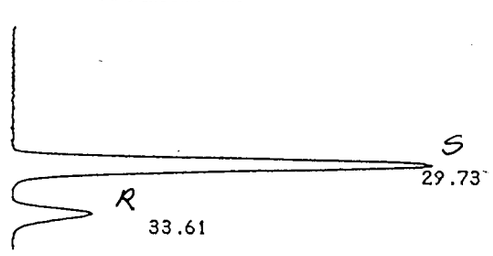
Column :Daicel OD
Eluent: n-Hexane/IPA=98/2
Flow rate:0.5mL/min
UV : 217nm

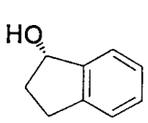
08/02/99 16:57:44 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 10 INDEX 10 BIN 7

PEAK#	AREA%	RT	AREA	BC	
1	0.93	11.7	23521	01	
2	8.835	29.74	223524	01	→ (S)-alcohol
3	90.235	33.43	2282909	01	→ (R)-alcohol
TOTAL	100.		2529954		

ee_p = 82.16(%)





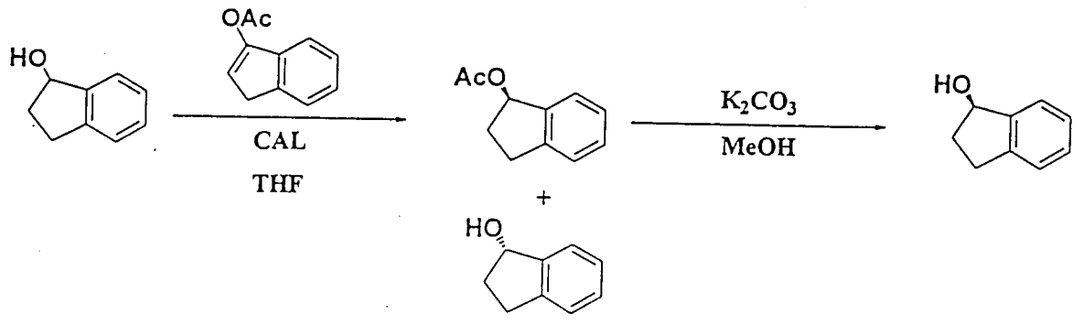
Column :Daicel OD
Eluent: n-Hexane/IPA=98/2
Flow rate:0.5mL/min
UV : 217nm

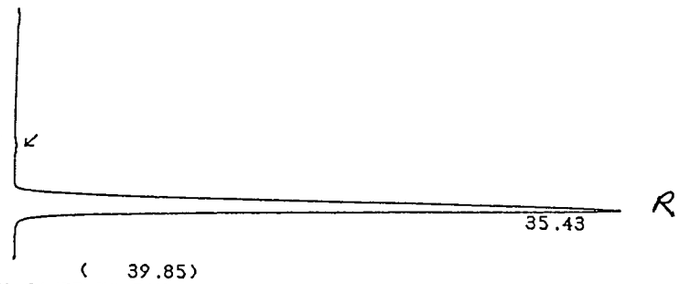
08/02/99 16:15:56 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 8 INDEX 8 BIN 6

PEAK#	AREA%	RT	AREA	BC	
1	0.387	11.7	8383	01	
2	84.297	29.73	1826385	01	→ (S)-alcohol
3	15.316	33.61	331837	01	→ (R)-alcohol
TOTAL	100.		2166605		

ee_s = 69.35(%)





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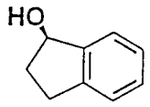


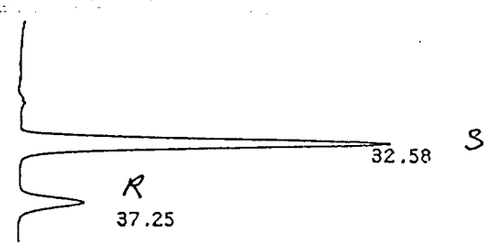
Column :Daicel OD
Eluent: n-Hexane/IPA=98/2
Flow rate:0.5mL/min
UV : 217nm

06/23/99 13:15:53 CH= "A" PS= 1.

ILE 1. METHOD 0. RUN 1 INDEX 1 BIN 1

EAK#	AREA%	RT	AREA BC	
1	2.122	5.05	25795 01	
2	1.978	7.65	24051 02	
3	1.185	10.01	14404 03	
4	0.166	13.32	2024 01	
5	1.723	15.93	20950 01	
6	92.825	35.43	1128483 01	→ (R)-alcohol
OTAL	100		1215707	

ee_p = > 99.5(%)



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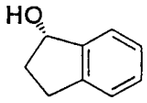

Column :Daicel OD
Eluent: n-Hexane/IPA=98/2
Flow rate:0.5mL/min
UV : 217nm

06/22/99 09:40:40 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 1 INDEX 1 BIN 1

PEAK#	AREA%	RT	AREA BC	
1	0.023	7.72	763 01	
2	0.091	9.43	2984 01	
3	15.744	11.93	516806 02	→ acetate
4	0.482	12.75	15832 03	
5	0.681	13.7	22367 02	
6	0.117	14.4	3833 03	
7	0.117	15.97	3846 01	
8	60.42	21.22	1983346 01	→ enol-ester/ketone
9	0.188	29.27	6170 01	
10	18.603	32.58	610669 01	→ (S)-alcohol
11	3.533	37.25	115969 01	→ (R)-alcohol
TOTAL	100.		3282585	

ee_s = 68.08(%)

Table 1. The enantioselectivity in the lipase-catalyzed reactions of **3a** in dry organic solvents.

entry	lipase	solvent	donor	ee_s	ee_p	c	E
1	CAL	toluene	**VA**	0.443	0.491	0.474	4
			1a	0.971	0.874	0.499	42
2		benzene	**VA**	0.451	0.727	0.383	9.8
			1a	0.334	0.893	0.272	25
3		*t*-butyl methyl ether	**VA**	0.252	0.217	0.537	2
			1a	0.475	0.840	0.361	18
4		methylene chloride	**VA**	0.121	0.666	0.154	5.6
			1a	0.132	0.898	0.128	21
5		tetrahydrofuran	**VA**	0.313	0.335	0.483	2.7
			1a	0.409	0.970	0.297	98
6	PCL	toluene	**VA**	0.968	0.987	0.495	639
			1a	>0.995	>0.995	0.500	>2390
7		*t*-butyl methyl ether	**VA**	0.994	0.948	0.512	216
			1a	0.877	0.987	0.470	445
8		methylene chloride	**VA**	0.822	0.971	0.458	175
			1a	0.724	>0.995	0.421	>870
9		acetonitrile	**VA**	0.798	0.970	0.451	161
			1a	0.775	0.955	0.438	943
10		tetrahydrofuran	**VA**	0.766	0.933	0.451	67
			1a	0.876	>0.995	0.468	>1160

Table 2. The enantioselectivity in the lipase-catalyzed reactions of **1** with **3** in THF.

entry	lipase	donor	acceptor	ee_s	ee_p	c	E
1	CAL	**VA**	**3b**	0.693	0.822	0.457	21
2		**1b**	**3b**	0.681	>0.995	0.406	>818
3	PCL	**VA**	**3b**	0.613	0.916	0.401	43
4		**1b**	**3b**	>0.995	>0.995	0.500	>2390
5		**VA**	**3c**	0.994	0.791	0.557	48
6		**1c**	**3c**	0.909	>0.995	0.477	>2130
7		**1b**	**3a**	0.498	0.991	0.335	364
8		**1c**	**3a**	0.952	0.983	0.492	390
9		**1a**	**3b**	0.695	0.984	0.414	255
10		**1a**	**3c**	0.970	0.982	0.497	459