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Enzymatic Hydrolyses of Acetoxy- and Phenethylbenzoates by *Candida cylindracea* Lipase

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Abstract: The lipase from *Candida cylindracea* (CCL) deacetyles rapidly and selectively all three regioisomer methyl acetoxybenzoates. In the enzymatic hydrolyses of analogous aryl acetoxybenzoates, the difference of reactivity between the acetoxy and benzoyloxy functionalities is reduced and a methoxy group in meta position of the aryl group reverses the reactivity order making the compounds aspirin or aspirin-like prodrugs. The degree of enantioselectivity of the enzymatic hydrolysis of phenethylbenzoates is related to the position of the stereogenic center.

In the realm of enzymes, lipases are those most used in organic synthesis because they are commercially available, inexpensive (cofactors are not required), easy to use and chemically versatile.[1] These hydrolytic enzymes perform selective hydrolysis[2] of esters, amides, anhydrides, and esterifications,[3] they exhibit stability in both water and organic solvents,[4] and accept a broad range of substrates often coupled with a high chemo-, regio- and stereoselectivity.[5]

The regioselective ability of lipases to discriminate between chemically identical hydroxy or acyl groups of aliphatic and cycloaliphatic substances by acylation and deacylation, respectively, has been frequently investigated,[6] less is known about their chemoselective capability.[7]

In order to make a contribution to this less studied problem and to the problem of prodrugs, we have investigated the hydrolysis of substrates containing chemically different ester groups in the same molecule, *i.e.* aliphatic and aromatic.

The selected compounds were some alkyl *ortho*-, *meta*- and *para*-acetoxybenzoate (**1a, 1b**) and aryl *ortho*-, *meta*- and *para*-acetoxybenzoate (**1c-h**). Some of these compounds are interesting from a biological and pharmacological view-point because they are potential aspirin and aspirin-like prodrugs.[8]

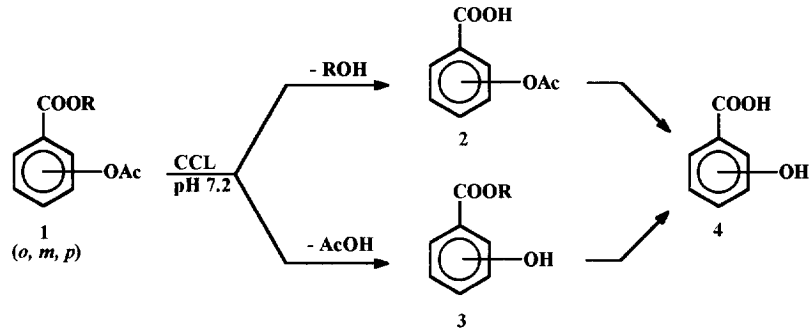
A promising approach to minimize the side effects of aspirin (gastric irritation and bleeding) is to block the carboxylic group by esterification.[9] This derivatized aspirin is not always a bioreversible derivative or "aspirin prodrug" which undergoes enzymatic cleavage to regenerate the parent drug because the rate of enzymatic hydrolysis of acetyl ester functionality is greatly increased when the carboxy group is esterified.[9] Therefore a fundamental requisite for a true "aspirin prodrug" is that the rate of hydrolysis of ester carboxylate functionality should be much greater than the rate of deacetylation of the acetyl group. Otherwise, the compound behaves like a "salicylic acid prodrug" and not as an "aspirin prodrug".

Results and Discussion

The hydrolysis of all acetoxybenzoates was carried out at room temperature (20-22°C) in a phosphate buffered heterogeneous aqueous medium in the presence of catalytic amounts of lipase from *Candida cylindracea* (CCL). The pH was kept at 7.2 with the aid of an autoburette. In control experiments without enzyme, no hydrolysis was observed either under the same conditions or with prolonged time and increased reaction temperature. The reaction products were isolated and characterized by using the usual procedures and techniques (see experimental).

As shown in scheme 1, the hydrolysis of **1** can give, in principle, three different products: **2**, **3** and **4**. The hydroxy acid **4** can originate from the hydrolysis of **2** and/or from the hydrolysis of **3**.

SCHEME 1



	R		R
a	**Me**	**e**	**C_6H_4-*o*OMe**
b	**$(CH_2)_{10}$OMe**	**f**	**C_6H_4-*m*OMe**
c	**Ph**	**g**	**C_6H_4-*p*OMe**
d	**C_6H_4-*p*NO_2**	**h**	**C_6H_4-*m*Et**

The CCL catalyzed hydrolysis of acetoxybenzoic acid **2** is slow and competitive reactions of acids **2** with methyl hydroxybenzoates **3-a** and with phenyl hydroxybenzoates **3-c**, show that the later substrates react completely to give **4** before that the hydrolyses of compounds **2** occur.

The results of enzyme-catalyzed hydrolysis of *ortho*-acetoxybenzoates *o*-**1**, *meta*-acetoxybenzoates *m*-**1**, and *para*-acetoxybenzoates *p*-**1**, are summarized in Tables **1**, **2** and **3**, respectively.

Methyl *ortho*-acetoxybenzoate *o*-**1a** deacetylates rapidly and totally (Table 1, entry 1) to give only the corresponding hydroxyester *o*-**3a**. The same result was found in the chemical hydrolysis[10] and in the hydrolysis in *vitro* in the presence of human plasma.[8]

A long alkyl chain (Table 1, entry 2) does not affect greatly the chemoselectivity of the reaction while it strongly reduces the rate of hydrolysis.

With entries 3, 4 it can be seen (Table 1) that by replacing the Me group with the Ph group (*i*) the reaction rate of hydrolysis was lowered, (*ii*) the hydroxybenzoate (*o*-**3c**) was absent and (*iii*) a good amount of *o*-acetoxybenzoic acid (*o*-**2**) was present. One can also speculate that the hydrolysis of benzoyloxy functionality of *o*-**1c** is faster than that of the accompaining acetoxy one differently to that previously observed for *o*-**1a** and that *o*-**2** is hydrolyzed more slowly than *o*-**3c**, as expected.

The compound *o*-**1c** is therefore a good candidate for an aspirin prodrug only when the conversion of the reaction is about 30% .

Table 1. Enzymatic hydrolysis of *ortho*-acetoxybenzoates[a] *o*-**1**

Entry	R		Conv [b](%)	time (h)	**2**[c] (%)	**3**[c] (%)	**4**[c] (%)
1	Me	(*o*-**1a**)	100	3	-	100	-
2	$(CH_2)_{11}$-OMe	(*o*-**1b**)	55	90	-	92	8
3	C_6H_5	(*o*-**1c**)	85	60	50	-	50
4	C_6H_5	(*o*-**1c**)	30	8	67	-	33
5	C_6H_4-NO_2	(*o*-**1d**)	38	132	25	-	75
6	C_6H_4-*o*OMe	(*o*-**1e**)	56	48	2	45	53
7	C_6H_4-*m*OMe	(*o*-**1f**)	79	23	56	-	44
8	C_6H_4-*p*OMe	(*o*-**1g**)	42	108	21	21	58
9	C_6H_4-*m*Et	(*o*-**1h**)	83	34	20	39	41

a. In phosphate buffer at pH 7.2 and at room temperature.
b. Conversion determined by GC after consumption of proper amount of NaOH 0.2N by automatic titration with the aid of pH-Stat.
c. Determined by GC.

Substituents in the phenyl ring such as NO_2, OMe and Et (Table 1, entries 5-9) affect the reactivity and selectivity of enzymatic hydrolysis in such a way that cannot be easily explained on the basis of their electronic effects. In terms of aspirin prodrug the best result is given by *meta*-metoxyphenyl *ortho*-acetoxybenzoate (*o*-**1f**), in which about 50% of the substrate is converted into aspirin.

The *meta*-acetoxy derivatives *m*-**1a, -c, -d, -g,** hydrolyze (Table 2) faster than the corresponding *ortho*-ones. The chemoselectivity of the reaction is the same except for *p*-methoxyphenyl *meta*-acetoxybenzoate (*m*-**1g**) which gives the acid *m*-**2** quickly and regioselectively and therefore it is a very good aspirin-like prodrug compound.

Table 2. Enzymatic hydrolysis of *meta*-acetoxybenzoates[a] *m*-**1**

R		Conv. (%)[b]	time(h)	**2** (%)[c]	**3**(%)[c]	**4**(%)[c]
Me	(*m*-**1a**)	100	2	-	100	-
Ph	(*m*-**1c**)	64	3	65	-	35
C_6H_4*p*NO_2	(*m*-**1d**)	38	7	52	-	48
C_6H_4*p*OMe	(*m*-**1g**)	100	4	100	-	-

For footnotes a, b, c, see Table 1

We hypothesize that the presence of OMe group and the relative geometry of two ester functionalities increase the chelating properties of the substrate *m*-**1g** which binds to the protein molecule in such a way that the group of the serine residue at the active site of lipase attacks only the carbonyl of the benzoyloxy function giving selectively the *meta*-acetoxybenzoic acid *m*-**2**.

Table 3 shows the results of CCL-catalyzed hydrolysis of *para*-acetoxybenzoates *p*-**1**. The reactivities of these compounds resemble those of corresponding *meta* isomers. Once again the presence of metoxy group greatly influences the selectivity of the enzymatic reaction. The acetoxy *p*-**1g** gives the acid *p*-**2** with a yield higher than 50% and can be considered an aspirin-like prodrug.

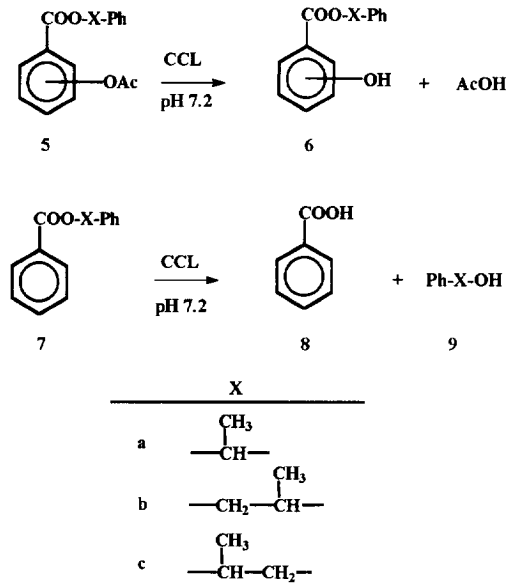
Table 3. Enzymatic hydrolysis of *para*-acetoxybenzoates[a] *p*-**1**

R		Conv. %[b]	time h	2%[c]	3%[c]	4%[c]
Me	(*p*-**1a**)	100	4	-	100	-
Ph	(*p*-**1c**)	65	3	59	25	16
C_6H_4-pNO_2	(*p*-**1d**)	56	7	11	-	89
C_6H_4-pOMe	(*p*-**1g**)	90	3.5	63	15	22

For footnotes a, b, c, see Table 1

We also investigated the effect of the presence of a stereogenic center on the stereoselectivity of enzymatic hydrolysis of racemic *sec*-phenethyl, β-methylphenethyl, and α-methylphenethyl *ortho*-, *meta*- and *para*-acetoxybenzoates **5a**, **5c**, **5b** and of corresponding unsubstituted phenethylbenzoates 7**a**, 7**b**, 7**c** (Scheme 2 and Table 4).

SCHEME 2



The hydrolysis of phenethyl acetoxybenzoates **5** was fast and the deacetylation reaction was greatly favored with respect to the dealkoxylation one.

Table 4. Enzymatic hydrolysis of Phenethyl acetoxybenzoates[a] (**5a, b, c**) and Phenethyl benzoates (**8a, b, c**)

Substrate	Conv. %[b]	time (h)	Product	Yield %[c]	ee
(±)*o*-**5a**	50	2	*o*-**6a**	85	-
(±)*m*-**5a**	55	4	*m*-**6a**	88	-
(±)*p*-**5a**	50	6	*p*-**6a**	75	-
(±)*o*-**5b**	50	4	*o*-**6b**	85	-
(±)*m*-**5b**	55	3	*m*-**6b**	88	-
(±)*p*-**5b**	45	4	*p*-**6b**	75	-
(±)**7a**	45	36	(+)**7a**	68	77
			(+)**9a**	71	81
(±)**7b**	50	20	(+)**7b**	70	22
			(+)**9b**	75	26
(±)**7c**	60	36	**7c**	78	-
			9c	82	-

For footnotes a,b, see Table 1
c. Determinated by isolated compounds

The corresponding phenols **6** were isolated with good yields but the CCL lipase was unable to discriminate between the two starting enantiomers. The CCL lipase hydrolyzes phenethylbenzoates **7** slowly and its ability to recognize the two diastereotopic faces of carbonyl group of benzoyloxy functionality depends on the position of the stereogenic center. When the stereocenter is bonded to the oxygen of the ester and to the phenyl group like *sec*-phenethylbenzoate (**7a**), the hydrolysis allows to obtain either the unreacted (+)**7a** and the alcohol (+)**9a** with a good degree of enantioselectivity (ee 77-81%), but the insertion of a methylene group causes a strong reduction or a total loss of enantioselectivity.

In conclusion, the acetoxy functionality of alkyl acetoxybenzoates is hydrolyzed by CCL faster than the benzoyloxy one. The difference in reactivity between the two ester functionalities is less in aryl than in alkyl acetoxybenzoates and a methoxy group in meta position of the aryl group reverses the reactivity order making the compounds aspirin or aspirin-like prodrugs.

The degree of enantioselectivity of the enzymatic hydrolysis of phenethylbenzoates is closely related to the position of the stereogenic center.

Experimental

Compounds *p*-**1a**, *o*-**2**, *p*-**2**, *o*-**3a**, *m*-**3a**, *p*-**3a**, **4**, were purchased from Aldrich. Compounds *o*-**1a**, *o*-**1c**, *o*-**1g**, *m*-**1a** and *m*-**2** were prepared according to the literature.[10,11,12] Lipase from *Candida cylindracea* (CCL, Type VII) was purchased from Sigma. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution (TMS as internal

standard) on a Brucker FT 80 SY spectrometer. GLC analyses were performed on a Hewlett-Packard 5890 chromatograph with HP-5 fused silica capillary column (30m, 0.25 mm internal diameter, 0.25μm film thickness) an "on column injector system", an FID detector and hydrogen as the carrier gas. Infrared spectra were recorded on a Perkin-Elmer Model 983 spectrophotometer.

n-OMe-undecyl o-acetoxybenzoate (*o*-**1b**). A mixture of *o*-**3b** (34 mmol), acetic anhydride (15 ml) and 2-3 drops of concentrated H_2SO_4 was stirred at 50°C for 30 min. The solution was then diluted with water (70 ml), cooled at 0°C and the solid precipitate filtered and recrystallized from ethanol-water: yield 60%, ^{1}H-NMR δ : 1.1-1.9 (m, 20H, CH_2), 2.38 (s, 3H, COMe), 3.26-3.50 (m, 5H, CH_2OMe), 4.29 (t, 2H, $COOCH_2$), 7.00-8.09 (m, 4H, ArH). Anal.calcd for $C_{21}H_{32}O_5$: C,69.19 ; H,8.85 . Found : C,69.01 ; H,8.95 .

Aryl acetoxybenzoates and phenethylbenzoates

The proper acetoxybenzoic acid (55 mmol) and thionyl chloride (30 ml) was mixed at room temperature and stirred at 60°C for 6 h. The excess of thionyl chloride was evaporated at reduced pressure and after cooling the residue at 0°C, dry pyridine (10 ml) and the proper phenol or alcohol (50 mmol) were added. The mixture was left at room temperature for 2 h, acidified and the solid filtered off. The organic phase was washed with diluted NaOH, dried (Na_2SO_4) and evaporated at reduced pressure. The crude product was chromatographed on the column eluting with a n-hexane-ethylacetate 9:1.

Phenyl m-acetoxybenzoate (*m*-**1c**) : yield 75%, mp 60°C. ^{1}H-NMR δ : 2.3 (s, 3H, COMe), 7.1-8.2 (m, 9H, ArH). IR (thin film) 1747, 1776 cm^{-1}. Anal. calcd for $C_{15}H_{12}O_4$: C, 70.29; H, 4.72. Found : C,70.21; H,4.86.

Phenyl p-acetoxybenzoate (*p*-**1c**) : yield 70%, mp 80°C. ^{1}H-NMR δ : 2.3 (s, 3H, COMe), 7.1-8.3 (m, 9H, ArH). IR (thin film) 1743, 1774 cm^{-1}. Anal. calcd for $C_{15}H_{12}O_4$: C,70.29; H,4.72. Found : C,70.18; H,4.90.

p-NO_2-Phenyl o-acetoxybenzoate (*o*-**1d**) : yield 70%, mp 93-95 °C. ^{1}H-NMR δ : 2.34 (s, 3H, COMe), 7.32-8.25 (m, 8H, ArH). Anal. calcd for $C_{15}H_{11}NO_6$: C,59.79; H,3.68. Found : C,59.91; H,3.75.

p-NO_2-Phenyl m-acetoxybenzoate (*m*-**1d**) : yield 72%, mp 81-84 °C. ^{1}H-NMR δ : 2.32 (s, 3H, COMe), 7.25-8.42 (m, 8H, ArH). Anal. calcd. for $C_{15}H_{11}NO_6$: C,59.79; H,3.68. Found : C,59.93; H,3.77.

p-NO_2-Phenyl p-acetoxybenzoate (*p*-**1d**) : yield 66%, mp 145-146°C. ^{1}H-NMR δ : 2.34 (s, 3H, COMe), 7.18-8.40 (m, 8H, ArH). Anal. calcd. for $C_{15}H_{11}NO_6$: C,59.79; H,3.68. Found : C,59.61; H,3.16.

o-OMe-Phenyl o-acetoxybenzoate (*o*-**1e**) : yield 70%, mp 65-66°C. ^{1}H-NMR δ : 2.33 (s, 3H, COMe), 3.82 (s, 3H, OMe), 6.80-8.20 (m, 8H, ArH). Anal. calcd. for $C_{16}H_{14}O_5$: C,67.11; H,4.93. Found : C,67.23; H,4.81.

m-OMe-Phenyl o-acetoxybenzoate (*o*-**1f**) : yield 68%, mp 64-65°C. ^{1}H-NMR δ : 2.32 (s, 3H, COMe), 3.82 (s, 3H, OMe), 6.7-8.3 (m, 8H, ArH). Anal. calcd. for $C_{16}H_{14}O_5$: C,67.11; H,4.93. Found : C,67.23; H,4.81

p-OMe-Phenyl m-acetoxybenzoate (*m*-**1g**) : yield 70%, mp 78-79 °C. ^{1}H-NMR δ : 2.35 (s, 3H, COMe), 3.84 (s, 3H, OMe), 6.82-7.22 (m, 4H, ArOMe), 7.33-7.69 (m, 4H, ArOAc). Anal. calcd. for $C_{16}H_{14}O_5$: C,67.11; H,4.93. Found : C,67.34; H,4.75.

p-OMe-Phenyl p-acetoxybenzoate (*p*-**1g**) : yield 73%, mp 109-110°C. ^{1}H-NMR δ: 2.34 (s, 3H, COMe), 3.85 (s, 3H, OMe), 6.83-7.00 (m, 4H, ArOMe), 7.20-8.29 (m, 4H, ArOAc). Anal. calcd. for $C_{16}H_{14}O_5$: C,67.11; H,4.93. Found : C,67.25; H,4.70.

m-Et-Phenyl o-acetoxybenzoate (*o*-**1h**) : yield 66%, mp 42-44°C. ^{1}HNMR δ : 1.3 (t, 3H, CH_3), 2.32 (s, 3H, COMe), 2.72 (q, 2H, CH_2), 6.90-8.20 (m, 8H, ArH). Anal. calcd. for $C_{17}H_{16}O_4$: C,71.80; H,5.68. Found : C,71.92; H,5.53.

sec-Phenethyl m-acetoxybenzoate (*m*-**5a**) : yield 75%, ^{1}H-NMR δ : 1.7 (d, 3H, CH_3), 2.32 (s, 3H, COMe), 6.15 (q, 1H, CH), 7.16-8.05 (m, 9H, ArH). Anal. calcd. for $C_{17}H_{16}O_4$: C,71.80; H,5.68. Found : C,71.98; H,5.51.

sec-Phenethyl p-acetoxybenzoate (*p*-**5a**) : yield 74%, mp 49-51°C. ^{1}H-NMR δ : 1.68 (d, 3H, CH_3), 2.31 (s, 3H, COMe), 6.13 (q, 1H, CH), 7.15-8.2 (m, 9H, ArH). Anal. calcd. for $C_{17}H_{16}O_4$: C,71.80; H,5.68. Found : C,72.00; H,5.72.

β-Methylphenethyl m-acetoxybenzoate (*m*-**5b**) : yield 73%, liquid. ^{1}H-NMR δ : 1.38 (d, 3H, CH_3), 2.31 (s, 3H, COMe), 3.05-3.5 (m, 1H, CH), 4.28-4.57 (m, 2H, CH_2), 7.2-7.95 (m, 9H, ArH). Anal. calcd. for $C_{18}H_{18}O_4$: C,72.47; H,6.08. Found : C,72.59; H,5.95.

β-Methylphenethyl p-acetoxybenzoate (*p*-**5b**) : yield 68%, mp 48-50°C. ^{1}H-NMR δ : 1.39 (d, 3H, CH_3), 2.31 (s, 3H, COMe), 3.18-3.29 (m, 1H, CH), 4.25-4.56 (m, 2H, CH_2), 7.21-7.99 (m, 9H, ArH). Anal. calcd. for $C_{18}H_{18}O_4$: C,72.47; H,6.08. Found : C,72.35; H,5.98.

α-Methylphenethyl m-acetoxybenzoate (*m*-**5c**) : yield 84%, liquid. ^{1}H-NMR δ : 1.33 (d, 3H, CH_3), 2.31 (s, 3H, COMe), 2.7 -3.3 (m, 1H, CH_2), 5.15-5.6 (m, 2H, CH), 7.1- 8 (m, 9H, ArH). Anal. calcd. for $C_{18}H_{18}O_4$: C,72.47; H,6.08. Found : C,72.55; H,6.13.

α-Methylphenethyl p-acetoxybenzoate (*p*-**5c**) : yield 70%, liquid. ^{1}H-NMR δ : 1.3 (d, 3H, CH_3), 2.32 (s, 3H, COMe), 2.8-3.2 (m, 1H, CH_2), 5.2-5.5 (m, 2H, CH), 7- 8.1 (m, 9H, ArH). Anal. calcd. for $C_{18}H_{18}O_4$: C,72.47; H,6.08. Found : C,72.60; H,6.18.

sec-Phenethyl benzoate (**7a**) : yield 80%,. ^{1}H-NMR δ : 2.17 (d, 3H, CH_3), 6.15 (q, 1H, CH), 7.2-8.2 (m, 10H, ArH). IR (thin film) 1718 cm^{-1}. Anal. calcd. for $C_{15}H_{14}O_2$: C 79.61; H,6.24. Found : C,79.81; H,6.37.

α-Methylphenethyl benzoate (**7c**) : yield 87%, ^{1}H-NMR δ : 1.35 (d, 3H, CH_3), 2.71-3.25 (m, 2H, CH_2), 5.13-5.5 (m, 1H, CH), 7.2-8.1 (m, 10H, ArH). IR (thin film) 1718 cm^{-1}. Anal. calcd. for $C_{16}H_{16}O_2$: C,79.96; H,6.72. Found : C,79.83; H,6.51

β-Methylphenethyl benzoate (**7b**) : yield 83%, ^{1}H-NMR δ : 1.4 (d, 3H, CH_3), 3-3.4 (m, 1H, CH), 4.4 (d, 2H, CH_2), 7.2-8.1 (m, 10H, ArH). IR (thin film) 1718 cm^{-1}.Anal. calcd. for $C_{16}H_{16}O_2$: C,79.96; H,6.72. Found : C,79.83; H,6.85.

Enzymatic hydrolysis

In a standard experiment commercial *Candida cylindracea* lipase (120 mg) was suspended in phosphate buffer (NaH_2PO_4-Na_2HPO_4 12 ml, 0.1 M, pH 7.2) and stirred for 15 min. at room temperature. Substrate (1.0 mmol) was added and the mixture maintained at pH 7.2 by automatic titration with NaOH 0.2 N using a Mettler DK pH-Stat.The reaction was stopped when was consumpted 1.0 mmol of NaOH, adding a saturated solution of NaCl (20 ml) and the mixture was extracted three times with diethyl ether. The products were purified and separed by usual chemical work-up and then identified by GLC comparison with authentic samples. Reaction times and yields are reported in the Tables. The ee were determined by known specific optical rotation and by ^{1}H-NMR chiral shifts experiments using Eu(hfc)$_3$.[13]

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References

1. Jones, J.B. *Tetrahedron* **1986**, 42, 3351.
   Boland, W.; Frobl, C.; Lorenz, M. *Synthesis* **1991**, 1049.
   Chen, C.S.; Sih , C. J.Angew. *Chem. Int.* Ed. Engl., **1989**, 28, 695.
   Klibanov, A.M. *Acc. Chem. Res.* **1990**, 23, 114.
2. Holmberg, E.; Dahlen, E.; Norin, T.; Hult, K. *Biocatalysis* **1991**, 4, 305.
   Bevinakatti, H.S.; Banerji, A.A.; Newadkar, R.V.; Mukesh, D. *Biocatalysis* **1991**, 5, 99.
   Gu. Q.M.; Sih, J.C. *Biocatalysis* **1992**, 6, 115.
   Ader, U.; Schaneider, M. *Tetrahedron Asymmetry* **1992**, 3, 201.
   Furutani, A. J. *Chem. Research.* **1992**, 174.
3. Chen, C.S.; Wu,S.H.;Girdaukas, G.; Sih, C.J. *J.Am. Chem. Soc.* **1987**, 109, 2812.
   Bjorkling, F.; Godtfredsen, S.E.; Kirk, O. *J.Chem. Soc.Chem.Commun.* **1989**, 934.
   Reslow, M.; Adlercreutz, P.; Mattianson, B. *Biocatalysis* **1992**, 6, 307.
4. Klibanov, A.M. *Trends Biochem. Sci.* **1989**, 14, 141.
   Dordick, J.S. *Enzyme Microb. Technol.* **1989**, 11,194.
   Aldercreutz, P.; Mattiasson, B. *Biocatalysis* **1987**, 1,99.
5. Sih, C.J.; Wu, S.H. *Top. Stereochem.* **1989**, 19, 63.
   Wu, S.H.; Guo, Z.W.; Sih, C.J. *J.Am.Chem.Soc.***1990**, 112, 1990.
6. Kitazume, T.; Sato, T.; Kobayashi, T.; Lin, J.T. *J. Org. Chem.* **1986**, 51, 1003.
7. Pedrocchi-Fantoni, G.; Servi, S. *J.Chem. Soc. Perkin Trans. I* **1992**,1029.
8. Nielsen, N.M.; Bundgaard, H. *J.Med. Chem.* **1989**, 32, 727.
   Jones, G. In *Design of Prodrugs;* Bundgaard, H.,Ed.; Elsevier: Amsterdam, **1985**; 199-241.
9. Hausen, A.B.; Senning, A. *Acta Chemica Scandinavica* **1983,** 37, 351.
10. Fersht, A.R.; Kirby, A.J. *J.Am. Chem.Soc.* **1968**, 90, 5826.
11. Chattaway, F.D. *J. Chem. Soc.* **1981**, 2495.
12. Diaz-Mondéjar, M.R., Miranda, M.A. *Tetrahedron* **1982**, 38, 1523
13. Meyers, A.I; Yamamoto, Y. *Tetrahedron* **1984**, 40,2309.

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