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Enzymatic Resolution of *trans*-2-Arylcyclohexan-1-ols Using Crude Chicken Liver Esterase (CCLE) as Biocatalyst†

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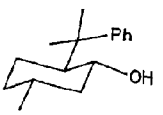
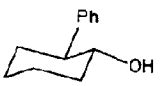
ABSTRACT : Homochiral trans-2-arylcyclohexan-1-ols were synthesized via crude chicken liver esterase (CCLE) mediated enantioselective hydrolysis of the corresponding racemic acetates.

The exponential growth of asymmetric synthesis[1] in the last two decades has resulted mainly from the rational designing of a large variety of chiral auxiliaries and their utilization in a large number of reaction types. Among the various chiral auxiliaries, the cyclohexyl -based chiral auxiliaries[2] such as Corey's 8-phenylmenthol[3] and Whitesell's *trans*-2-phenylcyclohexan-1-ol[4] occupy a special position because of their versatility and the high levels of stereocontrol they offer. The *trans*-2-phenylcyclohexan-1-ol, in particular, has been extensively used in a wide variety of asymmetric reactions such as the ene reaction, the Diels-Alder reaction, the zinc-Reformatsky reaction, the Pauson-Khand reaction, Darzen's glycidic ester condensation, *etc.*, with remarkable success.[2,5] This useful chiral auxiliary, *trans*-2-phenylcyclohexan-1-ol can be obtained homochiral in both (+)- and (-)-forms *via* enzymatic resolution[5-7] or directly *via* asymmetric hydroboration of 1-phenylcyclohexene.[8]





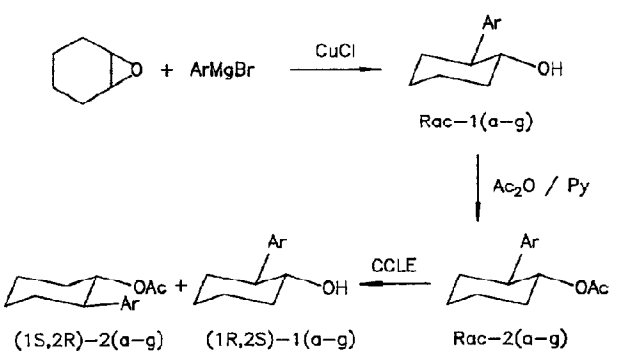
However *trans*-2-phenylcyclohexan-1-ol has failed to offer good asymmetric induction in the case of the Baylis-Hillman reaction.[9] It occurred to us that sterically more demanding analogs of *trans*-2-phenylcyclohexan-1-ol could be better chiral auxiliaries for our purpose. The

biocatalytic approach[10] to the synthesis of homochiral molecules has become an accepted methodology. We have recently reported enzymatic resolutions of various racemic secondary alcohols using crude esterases.[11-15] We herein report the synthesis of homochiral *trans*-2-arylcyclohexan-1-ols *via* crude chicken liver esterase (CCLE) mediated enantioselective hydrolysis of corresponding racemic acetates.

Results and discussion

Whitesell *et al.*[6] have synthesized homochiral *trans*-2-phenylcyclohexan-1-ol in both (+)- and (-)-forms *via* pig liver acetone powder catalyzed enantioselective hydrolysis of (±)-*trans*-1-acetoxy-2-phenylcyclohexane (**2a**). All our attempts to use PLAP for hydrolysis of other trans-1-acetoxy-2-arylcyclohexanes *viz* **2b** and **2c** have resulted in failure. The failure of PLAP to catalyze the hydrolysis of sterically more demanding substrates despite its broad substrate specificity, as we see, has something to do with the size of the active-site. This failure has driven us to a search for an alternative and cheap esterase. We have been using liver acetone powders from pig,[12] goat,[13] bovine,[14] and chicken[15] as substitutes for the respective pure esterases with remarkable success. Our experience with chicken liver esterase shows that its tolerence towards structural variations of substrates is considerably high.[16] Prompted by this observation the acetate (**2a**) of racemic *trans*-2-phenylcyclohexan-1-ol was first subjected to CCLE mediated hydrolysis to afford (1R,2S)-2-phenylcyclohexan-1-ol [(-)-**1a**] in homochiral form (Scheme 1).

SCHEME 1 :



Ar = a) phenyl, b) 1-naphthyl, c) 4-methylphenyl, d) 4-methoxyphenyl, e) 4-bromophenyl, f) 2,4,6-trimethylphenyl, g) 2-methylphenyl

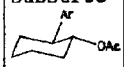
In order to show the generality of the transformation, a variety of racemic *trans*-1-acetoxy-2-arylcyclohexanes **2**(b-g) were prepared (Scheme 1) and subjected to crude chicken liver esterase mediated hydrolysis to produce the required homochiral (-)-*trans*-2-arylcyclohexan-1-ols **1**(b-g) and enantiomerically enriched unhydrolyzed acetates (Scheme 1 & Table 1) The recovered acetates upon hydrolysis with KOH/MeOH furnished the corresponding (+)-alcohols.

The enantiomeric purities of both (-)-**1a** and (-)-**1g** were determined by optical rotation measurements. The enantiomeric purities of (-)-**1**(b-e) were determined by ^{1}H NMR analysis of corresponding Mosher's esters[17] using the Mosher's esters of corresponding racemic alcohols as reference. The enantiomeric purity of (-)-**1f** was determined by ^{1}H NMR analysis of corresponding Mosher's esters from (-)-**1f** and (±)-**1f** in presence of chiral shift reagent Eu(hfc)$_3$.

The absolute stereochemistry of (-)-*trans*-2-phenylcyclohexan-1-ol[6] was established by Whitesell and Lawrence and that of (-)-*trans*-2-(2-methylphenyl)cyclohexan-1-ol[18] by Galpin and Huitric as 1R,2S. We have assigned the absolute configurations of (-)-**1b**, **1c**, **1d** and **1e** following the method of Yasuhara and Yamaguchi.[19] The partially enriched (+)-*trans*-2-phenylcyclohexan-1-ol was converted into corresponding (R)-MTPA derivatives. The ^{1}H NMR spectrum of this material showed two signals for diastereomeric OMe groups of MTPA portions with the downfield signal being more intense. As the starting enantiomerically enriched alcohol contains more (1S,2R)-enantiomer the high intensity downfield OMe signal was attributed to (1S,2R)-enantiomer. Then the partially enriched (+)-*trans*-2-arylcyclohexanols **1**(b-e) were converted into corresponding (R)-MTPA derivatives. In the ^{1}H NMR spectra of all these (R)-MTPA derivatives the OMe signals have shown the same tendency. Moreover the ^{1}H NMR spectra of (R)-MTPA derivatives of (-)-*trans*-2-arylcyclohexanols do not contain the down field signal. Since the conformation of all the (R)-MTPA derivatives of (+)-*trans*-2-arylcyclohexanols in consideration should be more or less same, as the structural variations are trivial in nature, the absolute sterochemistry of all the (-)-alcohols **1**(b-e) were assigned, by analogy with (-)-(1R,2S)-2-phenylcyclohexan-1-ol, as 1R,2S and that of (+)-*trans*-2-arylcyclohexan-1-ols as 1S,2R. We have tentatively assigned the configuration of (-)-**1f** also as (1R,2S) and that of (+)-**1f** as (1S,2R).

In summary we have shown that the inexpensive CCLE is a very suitable enzyme system for the synthesis of homochiral *trans*-2-arylcyclohexan-1-ols. Applications of these molecules as chiral auxiliaries are in progress. Other applications of CCLE will be published elsewhere.

Table 1 : Enantioselective Hydrolysis of (±)-*trans*-1-Acetoxy-2-aryl-cyclohexanes **2**(a-g) Mediated by Crude Chicken Liver Esterase (CCLE).[a]

Substrte  (±)-2	Time in days	Conversion Ratio[b] OH:OAc	(1R,2S)-(-)-1			(1S,2R)-2	
			Yield[c] %	$[\alpha]_D^{22}$	Ee %	Yield[c] %	Ee %
2a	10	35:65	30	-58.6 (c 1.19, MeOH)	>99[d]	56	50[e]
2b	12	26:74	21	-72.9 (c 1.47, MeOH)	>99[f]	67	39[e]
2c	10	40:60	33	-59.5 (c 1.37, MeOH)	>99[f]	53	65[e]
2d	10	37:63	32	-55.4 (c 1.46, MeOH)	>99[f]	56	55[e]
2e	12	28:72	22	-26.2 (c 1.67, $CHCl_3$)	>99[f]	61	42[e]
2f	12	25:75	21	-32.4 (c 1.26, MeOH)	>99[g]	65	30[h]
2g	10	28:72	22	-63.9 (c 1.45, $CHCl_3$)	90[i]	59	34[j]

a) All reactions were carried out in 5 mM scale with 1 g of CCLE.

b) Conversion ratios were determined by HPLC analysis.

c) Yields of pure isolated products after column purification.

d) Based on specific rotation reported[6] for optically pure (1R,2S)-1a, $[\alpha]_D^{27}$ - 58.4 (c 10.0, MeOH).

e) Determined by ^1H NMR analysis of Mosher's ester of corresponding (+)-alcohol obtained after hydrolysis with KOH/MeOH.

f) Determined by ^1H NMR (200 MHz) analysis of Mosher's ester (the OMe signal of other diastereomer is absent).

g) Determined by ^1H NMR analysis of Mosher's ester in the presence of chiral shift reagent, $Eu(hfc)_3$.

h) Determined by ^1H NMR analysis of Mosher's ester of corresponding (+)-alcohol obtained after hydrolysis with KOH/MeOH in the presence of chiral shift reagent, $Eu(hfc)_3$.

i) Based on specific rotation reported[18] for optically pure (1R,2S)-1g, $[\alpha]_D^{26}$ - 71.1 (c 10.0, $CHCl_3$).

j) Based on specific rotation reported[18] for optically pure (1S,2R)-1g, $[\alpha]_D^{26}$ + 70.6 (c 10.0, $CHCl_3$).

Experimental

Melting points and boiling points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1310 or 297 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded either on JEOL-FX-100 (100 MHz) or Bruker

200 (200 MHz) spectrometer using chlorofom-d as solvent and TMS as internal reference. HPLC analysis was carried out on a SHIMADZU LC-10AD equipped with SPD-10A UV-VIS detector using special grade solvents. Column chromatography was carried out using Acme's silica gel (100-200 mesh). Mass spectra were recorded on Finnigon MAT instrument. Optical rotations were measured either on Autopol II automatic polarimeter or JASCO DIP 370 Digital polarimeter at the wave length of the sodium D-line (589 nm).

Crude Chicken Liver Esterase (CCLE) :

Freshly purchased chicken liver (500 g) was homogenised in chilled acetone (2 L) using kitchen juicer. The brown mass obtained after filtration was homogenised again with chilled acetone. The residue obtained was air dried at room temperature and powdered using juicer. The fibrous material was removed by seiving to afford 80-90 g of CCLE as fine powder. This powder (chicken liver acetone powder) can be stored in refrigerator for 2-3 months without any significant loss of activity.

(±)-*trans*-2-Arylcyclohexan-1-ols 1(a-g) :

These compounds were prepared following the procedure, similar to the one reported by Whitesell *et al.*[6] for *trans*-2-phenylcyclohexan-1-ol.

General procedure:

To a stirred solution of arylmagnesium bromide (100 mM) in dry THF (100 mL) [prepared from bromoarene (100 mM) and magnesium turnings (2.43 g, 100 mM)] at -20°C, cuprous chloride (0.49 g, 5 mM) was added. After 10 min., a solution of cyclohexene oxide (10.11 mL, 100 mM) in dry THF (10 mL) was added dropwise at the same temperature. After the addition is complete, the reaction mixture was allowed to warm to 0°C. After stirring 2h at 0°C, the reaction was quenched with saturated $(NH_4)_2SO_4$ solution. Organic layer was separated and washed with saturated $(NH_4)_2SO_4$ solution until the aqueous layers were no longer blue. The combined aqueous layer was extracted with ether (3 x 50 mL). The extracts were combined and dried over anhydrous Na_2SO_4 and concentrated. The solid obtained was crystallized from pentane or hexane to furnish the racemic alcohol as a white crystalline solid (distilled in case of liquid).

(±)-*trans*-2-Phenylcyclohexan-1-ol (1a) : Yield : 79%; m.p. 56-57 (lit.[6] m.p. 56-57°C); IR (KBr) : 3300 cm^{-1}; ^1H NMR (200 MHz): δ 1.27-2.17 (m, 9H, 1H D_2O washable), 2.37-2.48 (m, 1H), 3.60-3.71 (m, 1H), 7.20-7.38 (m, 5H); ^{13}C NMR : δ 25.11, 26.06, 33.41, 34.43, 53.18, 74.29, 126.83, 128.12, 128.77, 143.71; Mass (M^+) : 176.

(±)-*trans*-2-(1-Naphthyl)cyclohexan-1-ol (1b) : Yield : 65%; m.p. 129-130°C (lit.[20] m.p. 129-130°C); IR (KBr) : 3220 cm^{-1}; ^1H NMR (200 MHz): δ 1.49-2.30 (m, 9H, 1H D_2O washable), 3.40 (m, 1H), 4.00 (m, 1H), 7.40-8.25 (m, 7H); ^{13}C NMR (50 MHz) : δ 25.24, 26.49, 33.99, 34.96, 47.03, 74.28, 122.94, 123.35, 125.64, 125.71, 126.02, 127.07, 128.99, 132.81, 134.29, 139.70; Mass (M^+) : 226.

(±)-*trans*-2-(4-Methylphenyl)cyclohexan-1-ol (1c) : Yield : 74%; m.p. 70-72°C (lit[21] m.p 72-73°C); IR (KBr): 3300 cm^{-1}; ^1H NMR (200 MHz): δ 1.25-2.14 (m, 9H, 1H D_2O washable), 2.32 (s, 3H), 2.32-2.44 (m, 1H), 3.58-3.63 (m, 1H), 7.14 (s, 4H); ^{13}C NMR : δ 21.00, 25.06, 26.06, 33.47, 34.47, 52.76, 74.35, 127.89, 129.47, 136.30, 140.48; Mass (M^+) : 190.

(±)-*trans*-2-(4-Methoxyphenyl)cyclohexan-1-ol (1d): Yield : 71%; m.p. 70-72°C (lit.[22] m.p. 71-72°C); IR (KBr) : 3400 cm^{-1}; ^1H NMR (200 MHz): δ 1.25-2.14 (m, 9H, 1H D_2O washable) 2.30-2.41 (m, 1H) 3.54-3.62 (m, 1H) 3.78 (s, 3H), 6.83-6.90 (m, 2H), 7.13-7.20 (m, 2H); ^{13}C NMR : δ 24.94, 26.00, 33.41, 34.41, 52.18, 55.06, 74.35, 114.06, 128.77, 135.42, 158.47; Mass (M^+) : 206.

(±)-*trans*-2-(4-Bromophenyl)cyclohexan-1-ol (1e) : Yield : 60%; m.p. 107°C; IR (KBr) : 3300 cm^{-1}; ^1H NMR (200 MHz): δ 1.25-2.14 (m, 9H, 1H D_2O washable), 2.33-2.46 (m, 1H), 3.57-3.65 (m, 1H), 7.09-7.16 (m, 2H), 7.41-7.48 (m, 2H); ^{13}C NMR : δ 26.23, 27.11, 34.53, 35.94, 53.26, 75.41, 121.72, 131.00, 133.06, 144.01; Mass(M^+) : 254 and 256.

(±)-*trans*-2-(2,4,6-Trimethylphenyl)cyclohexan-1-ol (1f) : Yield : 78%; m.p. 76°C (lit.[22] m.p. 76.5°C); IR (KBr) : 3400 cm^{-1}; ^1H NMR (200 MHz): δ 1.22-2.18 (m, 9H, 1H D_2O washable), 2.23 (s, 3H), 2.33 (s, 3H), 2.45 (s, 3H), 2.92-3.05 (m, 1H), 4.13-4.23 (m, 1H), 6.82 (d, 2H, J = 4 Hz); ^{13}C NMR : δ 20.64, 21.76, 21.94, 25.33, 26.26, 29.82, 35.53, 49.23, 71.35, 129.77, 131.53, 135.36, 135.77, 136.18, 138.59; Mass(M^+) : 218.

(±)-*trans*-2-(2-Methylphenyl)cyclohexan-1-ol (1g): Yield : 70%; b.p. 96°C at 2 mm (lit.[18] b.p. 81-82°C at 0.20 mm); IR (neat) : 3400 cm^{-1}; ^1H NMR (200 MHz): δ 1.20-2.20 (m, 9H, 1H D_2O washable), 2.38 (s, 3H), 2.68-2.86 (m, 1H), 3.70-3.90 (m, 1H), 7.00-7.40 (m, 4H); ^{13}C NMR : δ 19.64, 24.94, 26.06, 32.94, 34.35, 47.53, 74.06, 125.47, 126.00, 126.30, 130.41, 137.00, 141.65; Mass(M^+) : 190.

(±)-*trans*-1-Acetoxy-2-phenylcyclohexane (2a) :

To a mixture of (±)-*trans*-2-phenylcyclohexan-1-ol (1a) (8.81 g, 50 mM), pyridine (8.5 mL, 105 mM) and DMAP (0.24 g, 2 mM) in dry dichloromethane (50 mL), acetic anhydride (9.4 mL, 100 mM) was added slowly with stirring at room temperature. After stirring for 2h, the reaction mixture was taken up in ether (75 mL) and washed successively

with ice-cold 2N HCl (3 x 30 mL) and saturated K_2CO_3 solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The liquid obtained was distilled under reduced pressure to afford pure racemic acetate as a colourless liquid; Yield : 10 g (92%); b.p. 90-92°C at 0.5 mm; IR (neat) : 1740 cm^{-1}; ^1H NMR (100 MHz): δ 0.96-2.24 (m, 11H), 2.32-2.80 (m, 1H), 4.64-5.12 (m, 1H), 7.16 (s, 5H).

(±)-*trans*-1-Acetoxy-2-(1-naphthyl)cyclohexane (2b) : Yield : 96%; IR (neat) : 1740 cm^{-1}; ^1H NMR (100 MHz): δ 1.20-2.32 (m, 11H), 3.32-3.72 (m, 1H), 4.96-5.40 (m, 1H), 7.08-8.24 (m, 7H).

(±)-*trans*-1-Acetoxy-2-(4-methylphenyl)cyclohexane (2c) : Yield : 83% m.p. 46-48°C; IR (melt) : 1740 cm^{-1}; ^1H NMR (100 MHz): δ 1.04-2.32 (m, 14H), 2.40-2.76 (m, 1H), 4.68-5.08 (m, 1H), 7.04 (s, 4H).

(±)-*trans*-1-Acetoxy-2-(4-methoxyphenyl)cyclohexane (2d) : Yiled : 91%; m.p. 56-57°C; IR (KBr) : 1720 cm^{-1}; ^1H NMR (100 MHz) : δ 1.04-2.28 (m, 11H), 2.36-2.76 (m, 1H), 3.76 (s, 3H), 4.68-5.08 (m, 1H), 6.80 (d, 2H, J = 8 Hz), 7.08 (d, 2H, J = 8 Hz).

(±)-*trans*-1-Acetoxy-2-(4-bromophenyl)cyclohexane (2e) : Yield : 95%; IR (neat) : 1740 cm^{-1}; ^1H NMR (100 MHz): δ 0.96-2.20 (m, 11H), 2.32-2.76 (m, 1H), 4.64-5.08 (m, 1H), 6.80-7.60 (m, 4H).

(±)-*trans*-1-Acetoxy-2-(2,4,6-trimethylphenyl)cyclohexane (2f) : Yield : 92%; m.p. 63-64°C; IR (neat) : 1740 cm^{-1}; ^1H NMR (100 MHz): δ 0.96-2.48 (m, 20H), 2.84-3.32 (m, 1H), 5.16-5.56 (m, 1H), 6.72 (s, 2H).

(±)-*trans*-1-Acetoxy-2-(2-methylphenyl)cyclohexane (2g) : Yield : 92%, b.p. 98-99°C at 1 mm; IR (neat) : 1740 cm^{-1}; ^1H NMR (100 MHz) : δ 1.02-2.40 (m, 14H), 2.64-3.08 (m, 1H), 4.70-5.16 (m, 1H), 6.84-7.28 (m, 4H).

CCLE-catalyzed hydrolyses of (±)-*trans*-1-acetoxy-2-arylcyclohexanes (2):
General procedure :

To 0.5 M, pH 8.0, KH_2PO_4/K_2HPO_4 buffer (40 mL), racemic acetate (5 mM) in ether (10 mL) was added with stirring at room temperature. After 10 min., CCLE (1 g) was added and the stirring was continued. The progress of the hydrolysis was monitored by HPLC. When an appropriate degree of hydrolysis was accomplished, the reaction was quenched with 2N HCl (10 mL). To this sodium chloride (5g) and dichloromethane (50 mL) were added and the resulting suspension was vigorously stirred for 0.5 h Then the enzyme was removed by filtration with suction and the layers were separated. The aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4. Removal of solvent followed by column chromatography (silica gel, 10% ethyl acetate in hexane) afforded (-)-alcohol and unhydrolyzed acetate.

Mosher's esters -- General procedure :

Mosher's esters of all the alcohols were prepared according to the following procedure.

To a suspension of oil free sodium hydride (10 mg) in pyridine (0.5mL) were added alcohol (0.05 mM) and DMAP (5mg) and stirred for 15 min. To this 0.1 M solution of (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPACl) in dichloromethane (1 mL, 0.1 mM) was added and stirred for 24 hr at room temperature. Then the reaction mixture was poured into cold 4N HCl (5 mL) and extracted with ether (3 x 5 mL). The ether layer was washed with saturated K_2CO_3 solution and dried over anhydrous Na_2SO_4. Removal of solvent followed by column purification (silica gel, 10% ethyl acetate in hexane) of the residue afforded pure Mosher's ester.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-phenylcyclohexane (2a) :

Hydrolysis of (±)-**2a** (1.09 g, 5 mM) with CCLE (1 g) in 10 days afforded (-)-alcohol and unhydrolyzed acetate in 35:65 ratio.
(-)-Alcohol: Yield : 0.26 g (30%); m.p. 64-65°C (lit.[6] m.p. 64-65°C); $[\alpha]_D^{22}$ - 58.6 (c 1.19, MeOH), >99% ee, ((lit.[6] $[\alpha]_D^{27}$ - 58.4 (c 10.0, MeOH) 100% ee).

The above recovered acetate (0.6 g, 56% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.47 g, 97% yield), $[\alpha]_D^{22}$ + 29.2 (c 2.46, MeOH) (lit.[6] $[\alpha]_D^{27}$ + 58.3 (c 10.0 MeOH), 100% ee).
Mosher's ester of (+)-1a : ^{1}H NMR (200 MHz): δ 1.20-2.36 (m, 8H), 2.52-2.88 (m, 1H), 3.10 & 3.21 (two singlets, 3H), 5.08-5.48 (m, 1H), 6.88-7.40 (m, 10H).

The two singlets at δ 3.10 & 3.21 with integration in the ratio of 3.2:9.6 (50% ee) establish that the downfield signal arises due to the dextrorotatory (1S,2R)-enantiomer.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(1-naphthyl)cyclohexane (2b):

Hydrolysis of (±)-**2b** (1.34 g, 5 mM) with CCLE (1 g) in 12 days afforded (-)-alcohol and unhydrolyzed acetate in 26:74 ratio.
(-)-Alcohol: yield : 0.24 g (21%); m.p. 101-102°C; $[\alpha]_D^{22}$ - 72.9 (c 1.47, MeOH), >99% ee.

The above recovered acetate (0.9 g, 67% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.72 g, 95% yield), $[\alpha]_D^{22}$ + 27.6 (c 1.51, MeOH).
Determination of enantiomeric purity:
Mosher's ester of (±)-1b : ^{1}H NMR (200 MHz): δ 1.40-2.40 (m, 8H), 2.84 &

3.10 (two singlets, 3H), 3.60-3.80 (m, 1H), 5.48-5.70 (m, 1H), 6.88-8.15 (m, 12H).

Two distinct singlets of almost equal integration appeared at δ 2.84 and 3.10 due to OMe protons indicating that the compound is a 50:50 mixture of two diastereomers.

Mosher's ester of (-)-1b : The ^{1}H NMR spectrum of this compound showed only one singlet at δ 2.84 (OMe protons) establishing the enantiomeric purity of (-)-1b to be >99%.

Mosher's ester of (+)-1b: The ^{1}H NMR spectrum of this compound contained both the singlets (δ 2.84 & 3.10) in the ratio of 2.7:6.2, establishing the enantiomeric purity of (+)-1b to be 39%.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(4-methylphenyl)cyclohexane (2c):

Hydrolysis of (±)-2c (1.16 g, 5 mM) with CCLE (1 g) in 10 days afforded (-)-alcohol and unhydrolyzed acetate in 40:60 ratio.

(-)-Alcohol : yield : 0.31 g (33%); m.p. 54-55°C; $[\alpha]_D^{22}$ - 59.5 (c 1.37, MeOH), >99% ee.

The above recovered acetate (0.61 g, 53% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.49 g, 98% yield), $[\alpha]_D^{22}$ + 40.1 (c 1.89, MeOH).

Determination of enantiomeric purity:

Mosher's ester of (±)-1c : ^{1}H NMR (200 MHz): δ 1.20-2.40 (m, 11H), 2.60-2.80 (m, 1H), 3.15 and 3.25 (two singlets, 3H), 5.16-5.36 (m, 1H), 6.94-7.32 (m, 9H).

The two singlets at δ 3.15 and 3.25 arising from OMe protons are of equal intensities indicating that they arise from two diastereomers.

Mosher's ester of (-)-1c: The ^{1}H NMR spectrum of this compound contained only one singlet at δ 3.15 establishing the enantiomeric purity of (-)-1c to be >99%.

Mosher's ester of (+)-1c: The ^{1}H NMR spectrum of this compound contained both the singlets (δ 3.15 & 3.25) in the ratio of 2.3:10.7, establishing the enantiomeric purity of (+)-1c to be 65%.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(4-methoxyphenyl)cyclohexane (2d):

Hydrolysis of (±)-2d (1.24 g, 5 mM) with CCLE (1 g) in 10 days afforded (-)-alcohol and unhydrolyzed acetate in 37:63 ratio.

(-)-Alcohol: yield : 0.33 g (32%); m.p. 83-84°C; $[\alpha]_D^{22}$ - 55.4 (c 1.46, MeOH), >99% ee.

The above recovered acetate (0.7 g, 56%) upon hydrolysis (KOH/MeOH)

furnished (+)-alcohol (0.55 g, 95% yield), $[\alpha]_D^{22}$ + 29.6 (c 1.63, MeOH).

Determination of enantiomeric purity:

Mosher's ester of (±)-1d: ^{1}H NMR (200 MHz): δ 1.20-2.30 (m, 8H), 2.56-2.78 (m, 1H), 3.18 and 3.28 (two singlets, 3H), 3.78 and 3.80 (two singlets, 3H) 5.10-5.34 (m, 1H), 6.71-7.30 (m, 9H).

The two singlets with almost equal integration appeared at δ 3.18 and 3.28 (OMe protons), indicate the presence of two diastereomers in 1:1 ratio.

Mosher's ester of (-)-1d: The ^{1}H NMR spectrum of this compound contained only one singlet at δ 3.18 establishing the enantiomeric purity of (-)-1d to be >99%.

Mosher's ester of (+)-1d: The ^{1}H NMR spectrum of this compound contained both the singlets (δ 3.18 & 3.28) in the ratio of 3.4:11.6, establishing the enantiomeric purity of (+)-1d to be 55%.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(4-bromophenyl)cyclohexane (2e) :

Hydrolysis of (±)-2e (1.49 g, 5 mM) with CCLE (1 g) in 12 days afforded (-)-alcohol and unhydrolyzed acetate in 28:72 ratio.

(-)-Alcohol : yield : 0.28 g (22%); m.p. 121-122°C; $[\alpha]_D^{22}$ - 26.2 (c 1.67, $CHCl_3$), >99% ee.

The above recovered acetate (0.91 g, 61% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.75 g ,96% yield), $[\alpha]_D^{22}$ + 11.3 (c 1.42, $CHCl_3$).

Determination of enantiomeric purity:

Mosher's ester of (±)-1e : ^{1}H NMR (200 MHz): δ 1.20-2.42 (m, 8H), 2.60-2.85 (m, 1H), 3.22 and 3.38 (two singlets, 3H), 5.10-5.39 (m, 1H), 6.90-7.64 (m, 9H).

The two singlets with almost equal integration appeared at δ 3.22 and 3.38 (OMe protons) indicate the presence of two diastereomers in equal ratio.

Mosher's ester of (-)-1e: The ^{1}H NMR spectrum of this compound contained only one singlet at δ 3.22 establishing the enantiomeric purity of (-)-1e to be >99%.

Mosher's ester of (+)-1e : The ^{1}H NMR (200 MHz) spectrum of this compound contained both the singlets (δ 3.22 & 3.38) in the ratio of 4.3:10.6, establishing the enantiomeric purity of (+)-1e to be 42%.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(2,4,6-trimethylphenyl)-cyclohexane (2f):

Hydrolysis of (±)-2f (1.30 g, 5 mM) with CCLE (1 g) in 12 days

afforded (-)-alcohol and unhydrolyzed acetate in 25:75 ratio.
(-)-Alcohol : 0.23 g (21%); m.p. 81-82°C; $[\alpha]_D^{22}$ - 32.4 (c 1.26, MeOH), >99% ee.

The above recovered acetate (0.85 g, 65% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.67 g, 95% yield), $[\alpha]_D^{22}$ + 10.2 (c 1.40, MeOH).

Determination of enantiomeric purity:
Mosher's ester of (±)-1f: ^{1}H NMR (100 MHz): δ 1.08-2.60 (m, 17H), 3.00-3.48 (m, 4H), 5.60-5.98 (m, 1H), 6.56-7.44 (m, 7H).
^{1}H NMR (100 MHz) analysis in presence of Eu(hfc)$_3$:

The ^{1}H NMR (100 MHz) spectrum of Mosher's ester of (±)-**1f** (5 mg), recorded in presence of Eu(hfc)$_3$ (30 mg), revealed that the original singlet at δ 3.20 (merged with multiplet) arising from OMe group, shifts and splits into two distinct singlets with almost equal integration indicating that the Mosher's ester is a 50:50 mixture of two diastereomers.
Mosher's ester of (-)-1f: The ^{1}H NMR spectrum of this compound, recorded in presence of Eu(hfc)$_3$, revealed that the original singlet at δ 3.20 shifts but remains intact as singlet establishing the enantiomeric purity of (-)-**1f** to be >99%. Similar analysis established the enantiomeric purity of (+)-**1f** to be 30%.

Enzymatic hydrolysis of (±)-*trans*-1-acetoxy-2-(2-methylphenyl)cyclohexane (2g) :

Hydrolysis of (±)-**2g** (1.16 g, 5 mM) with CCLE (1 g) in 10 days afforded (-)-alcohol and unhydrolyzed acetate in 28:72 ratio.
(-)-Alcohol: yield : 0.21 g (22%); $[\alpha]_D^{22}$ - 63.9 (c 1.45, $CHCl_3$), 90% ee (lit.[18] $[\alpha]_D^{26}$ - 71.1 (c 10.0, $CHCl_3$), 100% ee).

The above recovered acetate (0.69 g, 59% yield) upon hydrolysis (KOH/MeOH) furnished (+)-alcohol (0.53 g, 95% yield), $[\alpha]_D^{22}$ + 24.5 (c 2.52, $CHCl_3$), 34% ee (lit.[18] $[\alpha]_D^{26}$ + 70.6 (c 10.0, $CHCl_3$), 100% ee).

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