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Enzyme-catalyzed Asymmetric Acylation and Hydrolysis of *cis*-2,5-Disubstituted Tetrahydrofuran Derivatives: Contribution to Development of Models for Reactions Catalyzed by Porcine Liver Esterase and Porcine Pancreatic Lipase[1]

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ABSTRACT: Pig liver esterase, lipase from porcine pancreas, lipase from *Pseudomonas sp.* (lipase YS), and lipase from *Candida cylindracea* catalyzed hydrolyses of the *cis*-diacetate **1** and the *trans*-diacetate (±)-**4** to give the *cis*-monoacetate **3** and the *trans*-monoacetate **6** in optically active forms, respectively. Lipase YS-catalyzed acylations of the *cis*-diol **2** and the *trans*-diol (±)-**5** with an acylating agent in cyclohexane yielded (-)-**3** and (-)-**6**, respectively. The group adjacent to the R stereogenic center preferentially reacted in lipase YS-catalyzed hydrolyses of **1** and (±)-**4** and acylations of **2** and (±)-**5**, and the enantioselectivities are rationalized by our rule recently proposed for lipase YS.

INTRODUCTION

Many naturally occurring polyether antibiotics contain substituted tetrahydrofurans as important subunits and several efficient synthetic routes have been developed for the above structural fragments in the literature.[2] We have described the preparation and chiral recognition behaviour of optically active polyethers containing *cis*- and *trans*-2,5-disubstituted tetrahydrofuran derivatives as a building block.[3] Our interest in host molecules containing tetrahydrofuran subunits prompted us to prepare conveniently optically active subunits; tetrahydrofuran derivatives by the enzymatic method, because ready access of large quantities of optically active building blocks is requirement for the preparation of chiral polyethers.

The use of enzymes for the preparation of optically active compounds of synthetic value has been well studied.[4] Especially attractive in this regard are hydrolytic enzymes such as lipases and esterases, which operate without expensive co-enzymes and can function in organic solvents as well as in aqueous solution. Here we report pig liver esterase (PLE), lipase from porcine pancreas (PPL), lipase from *Pseudomonas sp.* (lipase YS), and lipase from *Candida cylindracea* (CCL) catalyzed hydrolyses of *cis*-2,5-bis(acetoxymethyl)tetrahydrofuran (**1**) and *trans*-2,5-bis(acetoxymethyl)tetrahydrofuran (**4**), and lipase YS-mediated acylations of *cis*-2,5-bis(hydroxymethyl)tetrahydrofuran (**2**) and *trans*-2,5-bis(hydroxymethyl)tetrahydrofuran (**5**) with an acylating agent such as vinyl acetate, isopropenyl acetate, and phenyl acetate. In order for an enzyme to be applied widely as a chiral catalyst in organic synthesis, it is desirable that the factors controlling its stereospecificity be understood and rationalised. In this regard, some active site models for hydrolytic enzymes have been reported. We discuss the enantioselectivities of PLE-, PPL-, and lipase YS-mediated reactions examined here on the basis of active site models recently

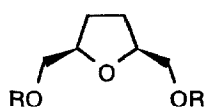
proposed.

RESULTS AND DISCUSSION

The diols 2 and 5 were prepared by the catalytic hydrogenation of 2,5-bis(hydroxymethyl)furan[5] and $LiAlH_4$ reduction of *trans*-2,5-bis(methoxycarbonyl)tetrahydrofuran,[4c] respectively. First, asymmetric hydrolyses of *cis*-2,5-bis(acetoxymethyl)tetrahydrofuran (1) were carried out in phosphate buffer solution using PLE, PPL, lipase YS, and CCL. The progress was monitored by GLC and the reaction was terminated by extraction with $CHCl_3$, when the chemical yield of the monoacetate 3 reached its optimum. Chromatographic separation of the product provided 1 and 3, but the readily water soluble diol 2 could not be extracted from the aqueous solution. The results are recorded in Table 1.

Table 1 Enzyme-catalyzed asymmetric hydrolysis of 1

Enzyme	Reaction temp.	Reaction time (h)	Product	Isolated yield (%)	$[\alpha]_D$ ($CHCl_3$)	Ee (%)
PLE	0 °C	18	(-)-3	65	-4.45	51
PPL	r.t.	305	(-)-3	53	-1.91	22
lipase YS	r.t.	42	(+)-3	42	+2.50	29
CCL	r.t.	96	(+)-3	50	+3.26	38



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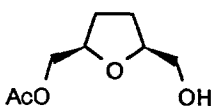
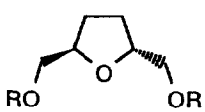
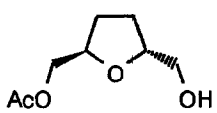
2 R = H



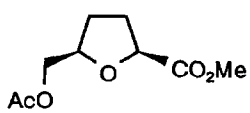
(-)-3



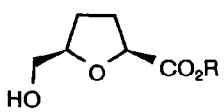
(-)-4 R = Ac
(-)-5 R = H



(-)-6



(-)-7



(-)-8 R = H
(+)-9 R = Me

The conversion rate of hydrolysis of 1 with PLE at room temperature was very high to give a 98:2 mixture of 3:1 after 30 min hydrolysis, but the enantiomeric excess (e.e.) of (-)- 3 isolated was very poor (3%). Therefore the reaction with PLE was carried out at 0 °C and the enantiotopic selectivity was improved to give (-)-3 with moderate e.e. value. The hydrolysis of 1 with lipase YS proceeded at moderate speed, but rate of hydrolysis with PPL and CCL, even at room temperature, was slow. The absolute configuration of (-)-2-(acetoxymethyl)-5-(hydroxymethyl)tetrahydrofuran (3)[6] as 2R,5S was determined by its conversion to (+)-(2S,5R)-*cis*-2-(methoxycarbonyl)-5-(hydroxymethyl)tetrahydrofuran (9) which has been described.[7] Oxidation of (-)-3, $[\alpha]_D$ -4.45 with CrO_3 in acetone followed by treatment with diazomethane gave (-)-7, $[\alpha]_D$ -15.3. Alkaline hydrolysis of (-)-7 yielded (-)- 8, which was converted into (+)-(2S,5R)-9, $[\alpha]_D$ +11.4 by treatment with diazomethane. The absolute value of the specific rotation of (-)- 3 was reconfirmed by its ^1H n.m.r. spectrum in the presence of a chiral shift reagent Eu $(hfc)_3$ and determined to be $[\alpha]_D$ -8.68 ($CHCl_3$).[8]

Recently, Prasad and co-workers have described that in the hydrolysis of *cis*-2,5-bis(butyroxymethyl)-2,5-dimethyltetrahydrofuran, PLE, PPL, and CCL showed the same enantiotopic specificity.[9] On the other hand, Jones and co-workers have described that PLE- and PPL-catalyzed hydrolyses of *cis* -2,5-bis(acetoxymethyl)-3,4-(isopropylidenedioxy)-tetrahydrofuran showed the opposite enantiotopic selectivity with each other.[10] In this study, PLE- and PPL-catalyzed hydrolyses of 1 proceeded in the same enantiotopic selectivity to give (-)-(2R,5S)-3 and the S center selectivity is the same that observed by Prasad[9] and Jones[10] for PLE-mediated hydrolyses of their substrates.

Next, the enantioselective hydrolyses of (±)-3 and (±)-*trans*-2,5-bis(acetoxymethyl)tetrahydrofuran (4) were performed in phosphate buffer solution and terminated at, or close to, the 50%-of-hydrolysis point by extraction with $CHCl_3$. The results are given in Table 2.

Table 2 Enzyme-catalyzed enantioselective hydrolysis of (±)-3 and (±)-4

Substrate	Enzyme	Reaction temp.	Reaction time (h)	Product	Isolated yield	$[\alpha]_D$ ($CHCl_3$)	Ee (%)
(±)-3	PLE	0 °C	17	(-)-3	47	-2.46	30
(±)-3	CCL	r.t.	115	(+)-3	54	+0.31	4
(±)-4	PLE	0 °C	3.5	(-)-4	48	-24.8	58
				(+)-6	47	+22.8	58
(±)-4	lipase YS	r.t.	48	(+)-4	39	+3.19	7
				(-)-6	47	-2.24	6
(±)-4	CCL	r.t.	52.5	(+)-4	38	+0.18	<1
				(-)-6	49	-0.10	<1

As expected from the asymmetric hydrolysis of 1, (2S,5R)-3 and (2R,5S)-3 were preferentially hydrolyzed with PLE and CCL, respectively, but with a lower enantioselectivity.

Treatment of (-)-4, $[\alpha]_D$ -24.8 and (+)-6, $[\alpha]_D$ +22.8 with $LiAlH_4$ gave the known diol (-)-5, $[\alpha]_D$ -24.4 and (+)-5, $[\alpha]_D$ +24.3,[4d] respectively, and these conversions confirmed the absolute configurations and the e.e. values of 4 and 6. The rate of the hydrolysis of (±)-4 with PLE was also very high and even at 0 °C, an about 1:1 mixture of 4: 6 was obtained after 3.5 h hydrolysis. PLE-catalyzed hydrolysis of (±)-4 afforded (-)-(2R,5R)-4 and (+)-(2S,5S)-6 with modest enantioselectivity and the hydrolysis occurred preferentially adjacent to the S stereogenic center of the substrate. The lipase YS- and CCL-mediated reactions of (±)-4 proceeded in the opposite stereochemical sense from the hydrolysis with PLE, but their enantioselectivities were very poor.

Optically active alcohols are obtained either in aqueous solutions by hydrolysis of esters or in organic solvents by acylation of alcohols. The choice of the method depends upon many factors such as yield, purity, reaction rate, and product separation. Next our task was lipase YS-catalyzed asymmetric and enantioselective acylation of tetrahydrofuran derivatives using the acylating agents such as vinyl acetate, isopropenyl acetate, and phenyl acetate in cyclohexane, in which lipase YS was easily dispersed.

The lipase YS-catalyzed acylation of 2 was stopped by filtration of the enzyme, when the chemical yield of 3 reached its optimum. The results of asymmetric acylation of 2 are shown in Table 3. The comparison of the data of Table 3 with the data of Table 1 shows that the reactions in cyclohexane

proceeded more rapidly than those in aqueous solution and improved the e.e. values of (-)-3 isolated. Specially, the acylation with phenyl acetate provided the best method for the preparation of the optically active 3.

Table 3 Lipase YS-catalyzed asymmetric esterification of 2

Acylating agent	Reaction temp.	Reaction time (h)	Product	Isolated yield	$[\alpha]_D$ ($CHCl_3$)	Ee (%)
vinyl acetate	r.t.	2	(-)-3	57	-5.09	59
isopropenyl acetate	r.t.	4	(-)-3	56	-4.86	56
phenyl acetate	r.t.	1.5	(-)-3	53	-6.17	71

The enantioselective acylations of (±)-5 were performed in cyclohexane in the presence of a cosolvent; tetrahydrofuran, which overcame solubility problems of the substrate. When the degree of conversion reached the value of about 50%, the reaction was stopped by filtration of the enzyme. The rate of lipase YS-catalyzed acylation of 5 was very fast and GLC analysis of the reaction mixture showed no remaining starting material after 30 min incubation at room temperature, so the acylations were carried out at 0 °C. The results are given in Table 4. Among the kinetic resolutions of *trans*-substrates (±)-4 and (±)-5, hydrolysis of (±)-4 with PLE at 0 °C gave the best results. However, it was advantage of enzymatic reactions of (±)-5 carried out in organic solvents that the optically active diol 5, which could not be isolated from the enzymatic hydrolysis product in buffer solution, was easily isolated in reasonable yield.

Table 4 Lipase YS-catalyzed enantioselective transesterification of (±)-5

Acylating agent	Reaction temp.	Reaction time (h)	Products	Isolated yield	$[\alpha]_D$ ($CHCl_3$)	Ee (%)
vinyl acetate	0 °C	2.5 h	(-)-4	7	-24.8	58
			(+)-5	50	+10.4	25
			(-)-6	28	-14.7	37
isopropenyl acetate	0 °C	12.5 h	(-)-4	7	-20.8	49
			(+)-5	59	+11.2	26
			(-)-6	30	-17.5	45
phenyl acetate	0 °C	0.5 h	(+)-5	55	+11.0	26
			(-)-6	33	-14.5	37

Recent interest in the synthetic potential of hydrolytic enzymes has led to several models to predict the enantioselectivity and we have also proposed the simple rule based on the sizes of the substituents at the stereocenter to predict which enantiomer of alcohol reacts faster in lipase YS-catalyzed acylation.[11]

As described above, the group adjacent to the R center was preferentially reacted by lipase YS-catalyzed hydrolyses of 1 and (±)-4 and acylations of 2 and (±)-5. In the cases of 2,5-disubstituted tetrahydrofuran derivatives, it is reasonable that the oxygen atom in the tetrahydrofuran ring and the CH_2 group of the framework are regarded as the small polar group and the large and/or hydrophobic group, respectively. Therefore, the enantioselectivities presented in this paper are summarized as shown in Figure 1 and our rule

recently proposed for lipase YS[11] is correctly applicable for hydrolyses and acylations of 2,5-disubstituted tetrahydrofuran substrates.

Fig.1 Simple model for predicting which enantiomer of racemic alcohols and acetates is preferentially reacted in lipase YS-catalyzed reaction.

Recently three papers dealing with the PPL active site model were published.[12] Jones and Hultin[12b] described that their model dose not rationalize well the enantiotopic specificity in PPL-mediated hydrolysis of 1, but their dilemma may result from ambiguous representation of the absolute configuration of 3 described in our previous paper.[6] As can be seen in Table 1, the acetoxymethyl group adjacent to the S center of 1 was rapidly reacted by PPL-catalyzed hydrolysis, so the enantiotopic selectivity of this reaction is well interpreted by Jones' model[12b] and Wimmer's A-model.[12c]

PLE-catalyzed hydrolyses of 1 and (±)-4 gave (-)- 3 and (+)-6, respectively, which resulted from preferential S-center acyl group cleavage and the stereoselectivity is the same that observed by Jones and co-workers for PLE-catalyzed hydrolyses of *cis*-2,5-disubstituted tetrahydrofurans.[7,10] Taking into account the heteroatom effect on stereoselectivity[10] the stereoselectivities of PLE-catalyzed reactions studied here are rationalized by Jones' active site model for PLE.[13]

As mentioned above, PLE- and PPL-catalyzed hydrolyses showed the S center selectivity, and reversals of stereoselectivities were observed for lipase YS and CCL. The stereoselectivities of reactions of *cis*- and *trans*-2,5-disubstituted tetrahydrofurans with PLE, PPL, and lipase YS are well rationalized on the basis of the corresponding active site models reported recently in the literatures.[11,12b,c,13]

EXPERIMENTAL

^{1}H n.m.r. spectra were obtained on a JASCO JNM-MH-270 spectrometer for solutions in $CDCl_3$ with $Si(CH_3)_4$ as internal standard. Elemental analyses were carried out by Yanagimoto CHN-Corder, Type 2. Optical rotations were measured using a JASCO DIP-40 polarimeter and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Gas chromatography was performed on a Simazu GS 8A chromatograph using a SE-52 on Uniport HP, 2m x 2.6mm column and a PEG 20M on Chromosorb W, 2m x 2.6mm column.

PLE (Boehringer Mannheim Gmbh Co.), PPL (Sigma chemical Co.), lipase YS (the Amano phermaceutical Co.), and CCL (Sigma chemical Co.) were used as received without further purification.

***cis*-2,5-Bis(hydroxymethyl)tetrahydrofuran (2)** A mixture of 2,5-bis(hydroxymethyl)furan (38.4 g, 0.300 mol) and ethanol (300 ml) was agitated at 100 °C with Raney-Ni (W-5) under H_2 atmosphere (120 Kg cm^{-2}). After the hydrogen absorption had ceased, the catalyst was filtered off and the solvent was removed. The product was distilled to give 2 (34.6 g, 87% yield), bp 148-150 °C (10 mmHg). Anal. Calc'd for $C_6H_{12}O_3$: C 54.53%, H 9.15%. Found: C 54.56%, H 9.01%.

***trans*-2,5-Bis(hydroxymethyl)tetrahydrofuran (5)** To a suspension of $LiAlH_4$ (3.10 g, 81.6 mmol) in dry diethyl ether (150 ml) was added a solution of *trans*-2,5-bis(methoxycarbonyl)tetrahydrofuran (6.00 g,

31.9 mmol), prepared from *trans*-tetrahydrofuran-2,5-dicarboxylic acid[14] in dry diethyl ether (100 ml). After the mixture was refluxed for 6 h, saturated aqueous solution of NH_4Cl was carefully added to the chilled reaction mixture and an inorganic solid was filtered off. After the filtrate was worked up as usual, the product was distilled to give 5 (3.36 g, 80% yield), bp 118-120 °C (5 mmHg). Anal. Calc'd for $C_6H_{12}O_3$: C 54.53%, H 9.15%. Found C 54.42%, H 8.95%.

***cis*-2,5-Bis(acetoxymethyl)tetrahydrofuran (1)** To a solution of 2 (3.96 g, 30.0 mmol) in pyridine (14.2 g, 180 mmol) was added acetic anhydride (9.19 g, 90.0 mmol) with ice-cooling. After the reaction mixture was stirred for 24 h at room temperature, it was poured onto ice-water and extracted with diethyl ether. After the extract was worked up as usual, the residue was distilled to give 1 (5.30 g, 82% yield), b.p. 105-106 °C (0.5 mmHg), IR (neat film) υ 2960, 2900, 1740, 1240, 1050 cm^{-1}. Anal. Calc'd for $C_{10}H_{16}O_5$, C 55.54%, H 7.46%. Found: C 55.33%, H 7.40%.

***trans*-2,5-Bis(acetoxymethyl)tetrahydrofuran (4)** By the similar manner described above, 5 (4.23 g, 0.0320 mmol) was converted into 4 (5.55 g, 80% yield), bp 98-100 °C (0.2 mmHg), IR (neat film) υ 2950, 2900, 1740, 1240, 1040 cm^{-1}. Anal. Calc'd for $C_{10}H_{16}O_5$: C 55.54%, H 7.46%. Found: C 55.30%, H 7.39%.

(±)-*cis*-2-(Acetoxymethyl)-5-(hydroxymethyl)tetrahydrofura (3) By the similar manner described above, 1 (10.6 g, 80.3 mmol) was treated with acetic anhydride (8.17 g, 80.1 mmol) in pyridine (9.84g, 125 mmol). The product was chromatographed on silica gel and fractions eluted with $CHCl_3$ gave 2 (4.37 g, 25% yield) and fractions eluted subsequently with the same solvent gave 3 (4.60 g, 33% yield), b.p. 91-92 °C (0.3 mmHg), IR (neat film) υ 3400, 1740, 1240, 1040 cm^{-1}. Anal. Calc'd for $C_8H_{14}O_4$: C 67.57%, H 9.93%. Found: C 67.08%, H 9.89%.

Representative Procedure of Asymmetric Hydrolysis of 1 *Using PLE at 0 °C*-To a solution of 1 (4.11 g, 19.0 mmol) in phosphate buffer solution (pH 8.0, 2400 ml) was added PLE (1.20 ml, 1200 U). The mixture was stirred at 0 °C and progress of the reaction was monitored by GLC. After 18 h incubation, the reaction mixture was extracted with $CHCl_3$. The extract was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was chromatographed on silica gel and fractions eluted with CH_2Cl_2 gave 1 (55 mg, 1.3% yield) and fractions subsequently eluted with the same solvent gave a colorless oil, which was distilled to give (-)-3 (2.15g, 65% yield), b.p. 92-92.5 °C (0.3 mmHg); $[\alpha]_D^{21}$ -4.45 (c 4.97, $CHCl_3$).

Representative Procedure of Enantioselective Hydrolysis of (±)-3 *With PLE*-PLE (250 μl, 250 U) was added to a solution of (±)-3 (870 mg, 5.00 mmol) in phosphate buffer solution (pH 8.0, 500 ml) and the mixture was stirred for 17h at 0 °C. The reaction mixture was extracted with $CHCl_3$ and the extract was worked up as usual. Silica gel chromatography of the product gave (-)-3 (409 mg, 47% yield), $[\alpha]_D^{22}$ -2.60 (c 2.50, $CHCl_3$). The hydrolyzed product 2 was not isolated.

Representative Procedure of Enantioselective Hydrolysis of (±)-4 *With lipase YS*-The mixture of (±)-4 (650 mg, 3.01 mmol) and lipase YS (300 mg) in phosphate buffer solution (pH 8.0, 600 ml) was stirred for 48 h at room temperature and extracted with CH_2Cl_2. After a usual work-up, the product was chromatographed on silica gel. The fractions eluted with hexane-30% $CHCl_3$ gave (+)-4 (255 mg, 39% yield), $[\alpha]_D^{20}$ +3.19 (c 1.00, $CHCl_3$) and the fractions eluted with $CHCl_3$ (-)-6 (244 mg, 47% yield), $[\alpha]_D^{20}$ -2.24 (c 0.995 $CHCl_3$).

Representative Lipase YS Catalyzed Asymmetric Acylation of 2 *With Vinyl Acetate as an Acylating Agent*-A mixture of 2 (200 mg, 1.52 mmol), vinyl acetate (400 mg, 4.65 mmol), lipase YS (600 mg), and

dry cyclohexane (50 ml) was stirred at room temperature. Progress of the reaction was monitored by GLC and the enzyme was filtered off after 2 h incubation. The filtrate was concentrated in vacuo and the residue was chromatographed on silica gel. Fractions eluted with hexane gave **1** (70 mg, 21% yield) and fractions eluted with hexane-20% diethyl ether gave (-)-**3** (150 mg, 57% yield), $[\alpha]_D^{26}$ -5.09 (c 0.850, $CHCl_3$); ^1H n.m.r. ($CDCl_3$) δ 1.6-2.0 (4H m $-CH_2-$), 2.07 (3H s $-COCH_3$), 2.41 (1H s -OH), 3.48 (2H dd J=4.6, 11.6Hz $-CH_2-$), 3.74 (2H dd J=3.7, 11.6Hz $-CH_2-$), 4.0-4.2 (2H m -CH-). ^1H n.m.r. ($CDCl_3$, $Eu(hfc)_3$/(-)-**3** = 0.09 molar ratio) δ 5.48 (1H br s -CH-; (+)-enantiomer) and 5.70 (1H br s -CH-; (-)-enantiomer). The spectral data determined the absolute value of the specific rotation of **3** to be $[\alpha]_D$ 8.68 ($CHCl_3$).

Representative Procedure of Lipase YS-catalyzed Enantioselective Acylation of (±)-5 ***With Isopropenyl Acetate as an Acylating Agent***-A mixture of (±)-**5** (509 mg, 3.85 mmol), isopropenyl acetate (1.50 g, 15.0 mmol), lipase YS (500 mg), cyclohexane (14 ml), and THF (7 ml) was stirred at 0 °C. After 12.5 h incubation, the enzyme was filtered off and the solvent was removed in vacuo. The residue was chromatographed on silica gel. Fractions eluted with hexane-30% $CHCl_3$ gave (-)-**4** (58 mg, 7% yield), $[\alpha]_D^{21}$ -20.8 (c 1.10, $CHCl_3$), fractions eluted with $CHCl_3$ provided (-)-**6** (198 mg, 30% yield), $[\alpha]_D^{20}$ -17.5 (c 1.00, $CHCl_3$), and the subsequent fractions eluted with $CHCl_3$-5% methanol provided (+)-**5** (302 mg, 59% yield), $[\alpha]_D^{20}$ +11.2 (c 1.00, $CHCl_3$).

(-)-*cis*-2-Carbomethoxy-5-(acetoxymethyl)tetrahydrofuran (7) To a solution of (-)-**3** (1.76 g, 10.1 mmol), $[\alpha]_D$ -4.45° ($CHCl_3$) in acetone (150 ml) was added excess of Jones reagent with ice-cooling and the mixture was stirred for 3 h at 0-5 °C. After the excess of Jones reagent was destroied with small amount of isopropyl alcohol, the organic layer was decanted and the solvent was removed in vacuo. The residue was extracted with diethyl ether and the extract was washed with saturated aqueous solution of $NaHCO_3$ and water, and dried ($MgSO_4$). After removal of the solvent, the residue was dissolved in diethyl ether and a solution of diazomethane in diethyl ether was added to the ethereal solution with ice-cooling. After standing for 12 h at room temperature, the volatile materials were removed in vacuo and the residue was chromatographed on silica gel. Fractions eluted with benzene gave a colorless oil, which was distilled to give (-)-**7** (905 mg, 44% yield), bp 125-127 °C (7 mmHg); $[\alpha]_D^{22}$ -15.3 (c 2.50, $CHCl_3$), IR (neat film) υ 1740, 1240, 1090, 1040 cm^{-1}. Anal Calc'd for $C_9H_{14}O_5$: C 53.46%, H 6.98%. Found: C 53.45%, H 6.94%.

(+)-*cis*-5-(hydroxymethyl)tetrahydrofuran-2-carboxylic acid (9) A mixture of (-)-**7** (840 mg, 4.16 mmol) and 5% aqueous solution of Na_2CO_3 (50 ml) was stirred for 48 h at room temperature. The reaction mixture was washed with diethyl ether, acidified with hydrochloric acid, and extracted with diethyl ether. Removal of the solvent in vacuo gave (-)-**8** (518 mg), $[\alpha]_D^{23}$ -2.59 (c 3.57, $CHCl_3$) as an oil. To the ethereal solution of (-)-**8** was added a solution of diazomethane in diethyl ether and the volatile matereals were removed in vacuo after standing for 12 h. Silica gel chromatography of the residue gave (+)-**9** (300 mg, 45% yield) (CH_2Cl_2 as eluent), bp 85-88 °C (0.1 mmHg) [lit.[7] bp 80 °C (0.06 mmHg)] , $[\alpha]_D^{25}$ +11.4 (c 1.55, $CHCl_3$) [lit.[7] $[\alpha]_D^{25}$ -9.4 ($CHCl_3$)], IR (neat film) υ 3450, 1735, 1440, 1230, 1100 cm^{-1}. Anal Calc'd for $C_7H_{12}O_4$: C 52.50%, H 7.55%. Found: C 52.30%, H 7.41%.

Conversion of (-)-4 into (-)-5 A solution of (-)-**4**, $[\alpha]_D$ -24.8° (417 mg, 1.93 mmol) in dry diethyl ether (20 ml) was added to a suspension of $LiAlH_4$ (220 mg, 5.79 mmol) in dry diethyl ether (30 ml) and the mixture was gently refluxed for 7 h. After the similar work-up described for the preparation of (±)-**5**, silica gel chromatography of the residue gave (-)-**5** (223 mg, 88% yield) (diethyl ether-5% methanol as eluent), $[\alpha]_D^{24}$ -24.4 (c 1.48, $CHCl_3$).

Conversion of (+)-6 into (+)-5 By the same procedure described above, (+)-6, $[\alpha]_D$ +22.8 (385 mg, 2.21 mmol) was converted into (+)-5 (170 mg, 59% yield), $[\alpha]_D^{24}$ +24.3 (c 1.55, $CHCl_3$).

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