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Horse Liver Alcohol Dehydrogenase-catalyzed Enantioselective Reduction of Cyclic Ketones: The Effect of the Hydrophobic Side Chain of the Substrate on the Stereoselectivity of the Reaction

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Abstract: Horse liver alcohol dehydrogenase (HLADH)-catalyzed enantioselective reductions of alkyl 3-oxocyclopentanecarboxylates, *endo*-5-acyloxybicyclo[2.2.1]heptan-2-ones and *exo*-5-acyloxybicyclo[2.2.1]heptan-2-ones gave the corresponding homochiral alcohols and ketones and the interaction between the hydrophobic side chain of the substrate and the hydrophobic zone in the active site played an important role in the specificity of the reduction. The stereoselectivities of the reactions were interpreted on the basis of the cubic space section model and a new rule, which contributes to development of a specificity analysis on the basis of the model, is introduced.

The application of oxidoreductases in the asymmetric and enantioselective synthesis of homochiral compounds is well-documented.[1] One of the most versatile enzymes in this regard is horse liver alcohol dehydrogenase (HLADH), which is a commercially available nicotinamide cofactor dependent enzyme and has become a powerful tool for the preparation of homochiral alcohols and ketones. In order for enzymes to be applied as a chiral catalyst in organic synthesis, it is desirable that the factors controlling its stereoselectivity are rationalised. In this regard, some rules for predicting acculately the stereochemistry of a product obtained by the HLADH-catalyzed oxidoreduction have been proposed[2] and one of the most successful, the so called 'cubic space section model' has been introduced by J. B. Jones.[3] G. L. Lemiere and co-workers have also proposed their model which showed that the stereoselectivity of the HLADH-catalyzed reduction of ketones having a hydrophobic substituent was affected by interactions between a hydrophobic substituent of a substrate and hydrophobic zones of the enzyme.[4] Herein we report the enantioselective reduction of racemic ketones having a hydrophobic side chain; alkyl 3-oxocyclopentanecarboxlates **1a-1f**, *endo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **4a-4f**, and *exo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **6a-6e** demonstrating that the interaction between the hydrophobic side chain of the substrate and the hydrophobic zone in the active site affected the selectivity of the reaction. The observed enantioselectivities are interpreted in terms of the active site model; the cubic space section model and further we introduce a new rule which contributes to development of a specificity analysis on the basis of the cubic space section model.

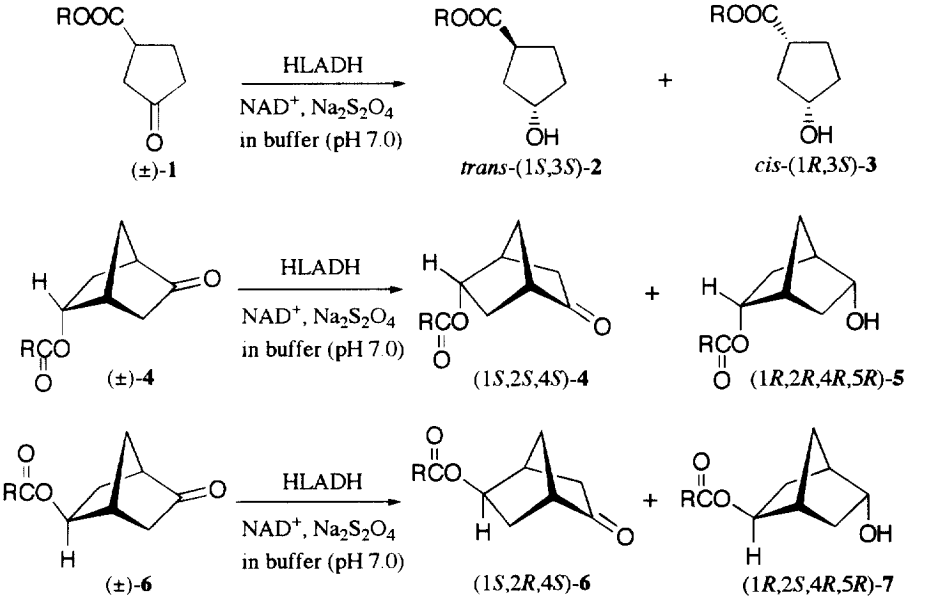
RESULTS AND DISCUSSION

Enantioselective reductions of racemic ketones mediated by HLADH were carried out with NAD^+ and $Na_2S_2O_4$ in 1/15 M Sφrensen phosphate buffer (pH 7.0) and the progress of the reaction was monitored by GLC. The reactions were terminated at, or closs to, 50% reduction point by extraction with diethyl ether

and the products were separated on thin layer chromatography (TLC). The absolute configurations and e.e. values of the products were confirmed as follows.

After the mixture of alkyl *trans*-3-hydroxycyclopentanecarboxylate **2** and alkyl *cis*-3-hydroxycyclopentanecarboxylate **3** was converted into the mixture of methyl *trans*-3-hydroxycyclopentanecarboxylate and its *cis*-isomer by hydrolysis followed by treatment with diazomethane, determination of the **2**/**3** ratios and e.e. values of the products was performed by HPLC analysis of the mixture of methyl *trans*-3-(*p*-nitrobenzoyloxy)cyclopentanecarboxylate and its *cis*-isomer. The absolute configurations of products were confirmed by comparison of their retention times (Rt) in HPLC with those of the authentic samples derived from known 3-oxocyclopentanecarboxylic acid.[5] The e.e. values and the absolute configurations of the recovered ketones **1a-1f** were also determined by HPLC analysis of the corresponding 2,4-dinitrophenylhydrazones.

Recovered *endo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **4a-4f** were hydrolyzed to give *endo*-5-hydroxybicyclo[2.2.1]heptan-2-one, e.e. value of which was determined by HPLC analysis of its benzoate, and the absolute configurations of the keto esters **4a-4f** were confirmed by conversion into known (1*S*,2*S*,4*S*,5*S*)-*endo*,*endo*-bicyclo[2.2.1]heptane-2,5-diol.[6] Treatment of *endo*,*endo*-5-acyloxybicyclo[2.2.1]heptan-2-ols **5a-5f** with $LiAlH_4$ gave (1*R*,2*R*,4*R*,5*R*)-*endo*,*endo*-bicyclo[2.2.1]heptane-2,5-diol, e.e. value of which was determined by HPLC analysis of its bis(3,5-dichlorobenzoate). Hydrolysis of recovered *exo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **6a-6e** gave known (1*S*,2*R*,4*S*)-*exo*-5-hydroxybicyclo[2.2.1]heptan-2-one,[6] e.e. value of which was determined by HPLC analysis of its *p*-nitrobenzoate. The absolute configurations of *exo*,*endo*-5-acyloxybicyclo[2.2.1]heptan-2-ols **7a-7e** were confirmed by correlation with known (1*R*,2*S*,4*R*)-*exo*-5-acetoxybicyclo[2.2.1]heptane-2-one[6] and e.e. values of the alcohols **7a-7e** were determined by HPLC analysis of *exo*,*endo*-2,5-bis(*p*-nitrobenzoyloxy)bicyclo[2.2.1]heptane.



It has been described that the HLADH-catalyzed reduction of cyclopentanone was very slow;[7] however, alkyl 3-oxocyclopentanecarboxylates **1a**-**1f** were smoothly reduced and especially the rate of the reduction of the ketones **1c** and **1d** having the long hydrophobic side chain was higher than that of cyclohexanone. J. J. Willaert and co-workers have also reported that the HLADH-catalyzed reduction of pentyl 3-oxocyclohexanecarboxylate, that is, cyclohexanone having the long hydrophobic side chain was roughly sixteen times faster than that of methyl 3-oxocyclohexanecarboxylate.[8] The accelerations are assumed to be due to that the attractive interaction between the hydrophobic side chain of the substrates **1c** and **1d** and the hydrophobic zone in the active site promoted the formation of a productive ES complex.

The reductions of the substrates **1a**-**1f** occurred with high stereoselectivity to convert (*S*)-**1** and (*R*)-**1**, respectively, into (1*S*,3*S*)-*trans*-**2** of >99% e.e. and (1*R*,3*S*)-*cis*-**3** of >85 % e.e. (except **3d**); hence, the ketones **1a**-**1f** were recovered in poor enantiomeric purity. The results are given in Table 1.

Table 1 Enantioselective reductions of alkyl 3-oxocyclopentanecarboxylates **1a**-**1f** mediated by HLADH

Substrate		Relative	Ketone **1**			Alcohols **2** and **3**			
	R	rate[a]	*R*/*S*	% yield	%e.e.	Total yield (%)	**2** / **3**	(1*S*,3*S*)-**2**	(1*R*,3*S*)-**3**
1a	CH_3	2	*R*	13	10	62	51 / 49	>99 %e.e.	96 %e.e.
1b	C_4H_9	5	*S*	37	2	52	45 / 55	>99	88
1c	C_6H_{13}	174	*S*	31	5	50	47 / 53	>99	89
1d	C_8H_{17}	117	*S*	41	15	45	31 / 69	>99	69
1e	$C(CH_3)_3$	3	*R*	34	8	49	54 / 46	>99	85
1f	$CH_2C(CH_3)_3$	4	*R*	37	9	53	50 / 50	>99	85

[a]Relative rate values given are relative to the rate for cyclohexanone=100.

Next we interpret the observed enantioselectivities on the basis of the cubic space section model; substrate orientations at the active site 1a, 1b, 1c, and 1d are illustrated according to the literature.[3] In the case of the reduction of (*S*)-**1**, the orientation 1a, where (*S*)-**1** is correctly oriented at the active site without the side chain violating any forbidden positions, is favorable; the orientation 1b is excluded because it requires the side chain to be positioned in the forbidden position U(E3).[9] The obvious difference in the stability between these orientations resulted in the exclusive formation of (1*S*,3*S*)-*trans*-**2** from (*S*)-**1**. In the case of the reduction of (*R*)-**1**, it is obvious that the orientation 1d is unfavorable because of the intrusion of the side chain at the (*R*)-stereogenic center into the forbidden position E3. On the other hand, the orientation 1c, where the cyclopentane framework is flattened, shows that the side chain violates the forbidden position U(C3);[3] however, (1*R*,3*S*)-*cis*-**3** was formed in rather high enantiomeric purity and about in the same amount as that of (1*S*,3*S*)-*trans*-**2**. On the basis of the observations, we estimate that the carbon framework of **1** in the transition state is in an envelope conformation with the *pseudo*-equatorial side chain which violates no forbidden position or the attractive interaction between the hydrophobic side chain of the substrate and the hydrophobic zone in the active site overcomes the unfaborable interaction between the side chain and the position U(C3) leading to a productive ES complex.

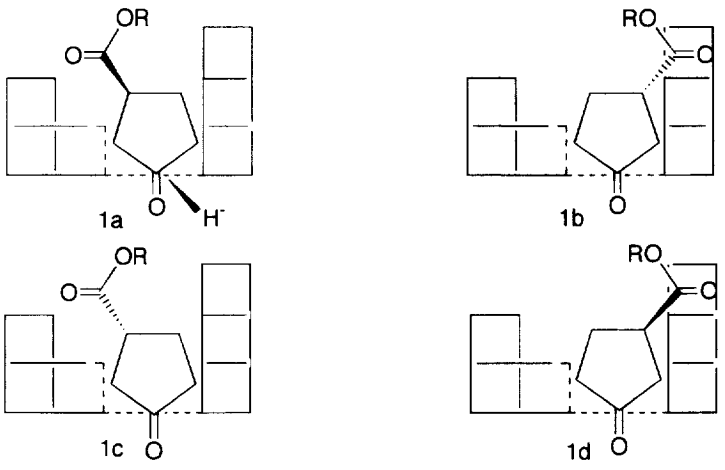


Figure 1. Top perspective view of the substrate orientation at the active site. (1a) and (1b); Substrate orientation of (*S*)-**1**, (1c) and (1d); substrate orientation of (*R*)-**1**.

Table 2 Enantioselective reductions of *endo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **4a-4f** and *exo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **6a-6e** mediated by HLADH

Substrate	R	Relative rate[a]	Ketone		Alcohol	
			isolated yield (%)	%e.e.	isolated yield (%)	%e.e.
4a	CH_3	2.5	43	90	42	95
4b	C_2H_5	2.8	48	94	51	96
4c	C_3H_7	6.4	42	84	37	97
4d	C_4H_9	8.1	46	83	36	97
4e	C_5H_{11}	17.1	45	81	38	96
4f	$CH_2C(CH_3)_3$	8.4	50	80	40	95
6a	CH_3	7.0	31	64	30	91
6b	C_3H_7	14.5	46	15	40	7
6c	C_4H_9	25.7	42	4	44	3
6d	C_5H_{11}	40.9	51	10	33	6
6e	$CH_2C(CH_3)_3$	27.1	28	49	52	37

[a]Relative rate values given are relative to the rate for cyclohexanone=100.

The HLADH-catalyzed reductions of *endo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **4a-4f** gave the recovered (1*S*,2*S*,4*S*)-ketones **4a-4f** and (1*R*,2*R*,4*R*,5*R*)-5-acyloxybicyclo[2.2.1]heptan-2-ols **5a-5f** in high enantiomeric purity. On the other hand, the reductions of *exo*-5-acyloxybicyclo[2.2.1]heptan-2-ones **6a-6e** proceeded faster than those of the *endo*-isomers **4a-4f** but e.e. values of the recovered (1*S*,2*R*,4*S*)-

ketones **6b-6d** and (1*R*,2*S*,4*R*,5*R*)-5-acyloxybicyclo[2.2.1]heptan-2-ols **7b-7d**; that is, the products with the long hydrophobic side chain were extremely poor as can be seen in Table 2. The facts led us to estimate that the hydrophobic side chain of the substrates **4a-4f** and **6a-6e** affected significantly the stereoselectivity of the reduction.

A. J. Irwin and J. B. Jones have described that the HLADH-catalyzed reduction of (±)-bicyclo[2.2.1]heptan-2-one gave (-)-(1*R*,4*S*)-bicyclo[2.2.1]heptan-2-one of 46% e.e. and (+)-(1*S*,2*R*,4*R*)-*endo*-bicyclo[2.2.1]heptan-2-ol of 64 % e.e.[10] In this case, the orientation 2a is illustrated for the favorable one leading to the (+)-(1*S*,2*R*,4*R*)-*endo*-alcohol.

The stereoselectivities of the reductions of (±)-**4a-4f** are discussed in terms of the orientations 2b and 2c, where the orientation of the carbon framework of the ketone **4** is the same as that of bicyclo[2.2.1]heptan-2-one, because the configuration of the hydroxyl group of the resulting alcohols **5a-5f** was the same as that of (+)-*endo*-bicyclo[2.2.1]heptan-2-ol. The facts that the (1*S*,2*S*,4*S*,5*S*)-alcohols **5a-5f** were little formed are reasonable because the orientation 2c, where the side chain at the (2*S*)-stereogenic center of the (1*S*,2*S*,4*S*)-ketones **4a-4f** violates the forbidden position U(D3), is unfavorable. On the other hand, the orientation 2b shows that the side chain at the (2*R*)-stereogenic center of the (1*R*,2*R*,4*R*)-ketones **4a-4f** violates the position U(C3); however, the (1*R*,2*R*,4*R*,5*R*)-alcohols **5a-5f** were almost exclusively formed demonstrating that 2b is the favorable orientation leading to a productive ES complex. From these observations together with the stereoselectivities of the reductions of **1a-1f**, we estimate that U(C3) is the limited position rather than the forbidden position and the disadvantage be resulted from the intrusion of the side chain of the substrate into the limited position can be overcome by the attractive interaction between the hydrophobic side chain and the hydrophobic zone.

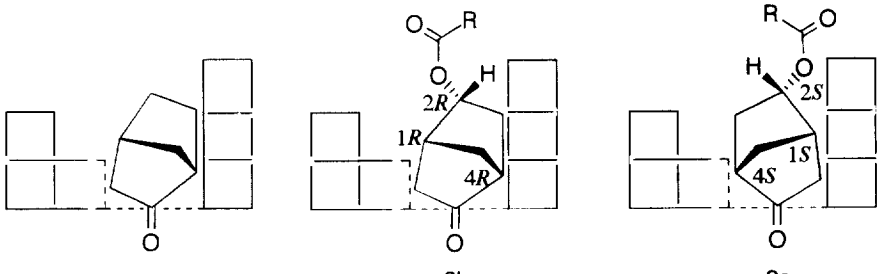


Figure 2. (2a); Substrate orientation of (1*S*,4*R*)-bicyclo[2.2.1]heptan-2-one at the active site, (2b) and (2c); substrate orientation of (1*R*,2*R*,4*R*)-**4** and (1*S*,2*S*,4*S*)-**4** at the active site, respectively.

The enantioselectivity of the reduction of *exo*-5-acetoxybicyclo[2.2.1]heptan-2-one **6a** was higher than that of bicyclo[2.2.1]hepatn-2-one; however, the reductions of the ketones **6b-6d** having the longer alkyl side chain gave the (1*S*,2*R*,4*S*)-ketones **6b-6d** and the (1*R*,2*S*,4*R*,5*R*)-alcohols **7b-7d** in rather low enantiomeric purity suggesting that the long hydrophobic side chain of these substrates reduced the stereoselectivity of the reduction. The orientations 3a and 3b are illustrated for (1*S*,2*R*,4*S*)-**6** and (1*R*,2*S*,4*R*)-**6**, respectively; the former is more stable than the latter. The observed low stereoselectivities are assumed to be due to that the attractive interaction between the long hydrophobic side chain and the hydrophobic zone made the orientation 3a and 3b stable reducing the difference in the stability between them.

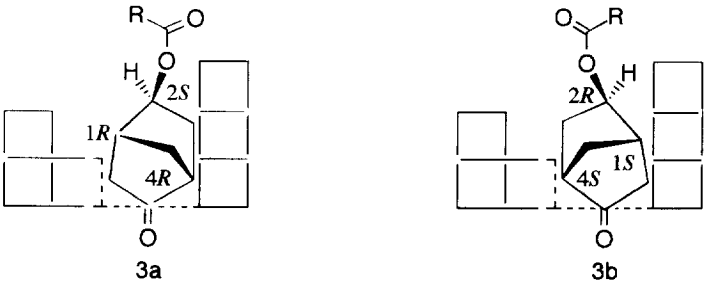


Figure 3. (3a) and (3b); Substrate orientation of (1*R*,2*S*,4*R*)-**6** and (1*S*,2*R*,4*S*)-**6** at the active site, respectively.

The results mentioned here demonstrated that, in estimation of the stereoselectivity of the HLADH-catalyzed reduction of ketones having a hydrophobic side chain in terms of the cubic space section model, it is necessary to take into account an interaction between a hydrophobic side chain of a substrate and the hydrophobic zone which is situated at the rear of the active site. The attractive hydrophobic interaction makes the transition state stable and would allow a substrate to intrude into one of the limited positions leading to a productive ES complex.

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 XC (Waters) or Chiralpak AD (Daicel), 250 mm x 4.6 mm. Horse liver alcohol dehydrogenase was purchased from Boehringer (Mannheim) as a crystalline suspension in phosphate buffer containing 10% ethanol. NAD^+ was obtained from Kohjin Co., Ltd., Tokyo.

General Procedure for HLADH-catalyzed Reduction of Alkyl 3-Oxocyclopentanecarboxylate (±)-1. A solution of hexyl (±)-3-oxocyclopentanecarboxylate **1c** (40 mg, 0.19 mmol), HLADH (3 mg), NAD^+ (15 mg, 0.021 mmol), and $Na_2S_2O_4$ (366 mg, 2.08 mmol) in 1/15 M Sφrensen phosphate buffer (pH 7.0, 60 cm^3) was stirred at 30 °C and the progress of the reaction was monitored by GLC. After stirring for 10 days, the solution was saturated with NaCl and then extracted with diethyl ether. The extract was washed with water and dried ($MgSO_4$). After removal of the solvent, the products were separated by TLC on silica gel to give the mixture of hexyl *trans*-3-hydroxycyclopentanecarboxylate **2c** and its *cis*-isomer **3c** (20 mg, 50% yield) and (-)-**1c** (13 mg, 32%); $[\alpha]_D^{26}$ -0.82 (c 0.625, MeOH).

General Procedure for Determination of E.e. Value and the Absolute Configuration of Alkyl 3-Hydroxycyclopentanecarboxylates 2 and 3. A solution of the mixture of **2c** and **3c** (10 mg, 0.047 mmol) in 5% aqueous solution of NaOH (5 cm^3) was stirred for 24h at room temperature and then it was extracted with diethyl ether. The extract was immediately treated with an excess of a solution of diazomethane in diethyl ether to give the mixture of methyl *trans*-3-hydroxycyclopentanecarboxylate and its *cis*-isomer. To a solution of the mixture of the esters in pyridine (2 cm^3) was added *p*-nitrobenzoyl chloride (20 mg, 0.12 mmol) and then it was stirred for 24h at room temperature. After addition of dil. HCl, the mixture was extracted with diethyl ether and the extract was worked up as usual. The products were separated by TLC on silica gel to give the mixture of methyl *trans*-3-(*p*-nitrobenzoyloxy)cyclopentanecarboxylate and

its *cis*-isomer. The **2c**/**3c** ratio and e.e. values of the products were determined by HPLC analysis (Chiralpak AD, hexane/ethanol 98/2 eluent, 0.96 cm^3 min^{-1}) of the mixture of the *p*-nitrobenzoates to show four peaks; Rt (min): 25 for (1*S*,3*R*)-*cis*-isomer, 29 for (1*R*,3*S*)-*cis*-isomer, 37 for (1*R*,3*R*)-*trans*-isomer, and 50 for (1*S*,3*S*)-*trans*-isomer.

General Procedure for Determination of E.e. Value and the Absolute Configuration of Alkyl 3-Oxocyclopentanecarboxylate 1. A reagent (5 cm^3), which was prepared from 2,4-dinitrophenylhydradine (100 mg), conc. H_2SO_4 (0.8 cm^3), ethanol (7.5 cm^3), and water (25 cm^3) was added to **1c** (2 mg) and then the mixture was stirred for 1h at room temperature. It was extracted with diethyl ether and the extract was washed with water and dried ($MgSO_4$). After removal of the solvent, the residue was purified by TLC on silica gel to give the mixture of diastereoisomeric 2,4-dinitrophenylhydrazones. HPLC analysis (Opti-Pak XC, hexane/*i*-propylalcohol 9/1 eluent, 1.2 cm^3 min^{-1}) of the hydrazones showed four peaks; Rt (min): 36 for (*S*)-isomer, 39 for (*R*)-isomer, 49 for (*R*)-isomer, and 69 for (*S*)-isomer.

General Procedure for HLADH-catalyzed Reduction of *endo*-5-Acyloxybicyclo[2.2.1]heptan-2-one (±)-4. A mixture of (±)-*endo*-5-hexanoyloxybicyclo[2.2.1]heptan-2-one **4e** (36 mg, 0.16 mmol), HLADH (2 mg), NAD^+ (44 mg, 0.062 mmol), and $Na_2S_2O_4$ (1.08 g, 6.15 mmol) in 1/15 M Sϕrensen phosphate buffer (pH 7.0, 40cm^3) was stirred for 22h at 30 °C. After the same work up as described for the reduction of **1c**, the products were separated by TLC on silica gel to give (-)-**4e** (16 mg, 45%); $[\alpha]_D^{23}$ -12.6 (c 0.810, $CHCl_3$) and (+)-*exo,endo*-5-hexanoyloxybicyclo[2.2.1]heptan-2-ol **5e** (14 mg, 38%); $[\alpha]_D^{23}$ +20.8 (c 0.680, $CHCl_3$).

General Procedure for Determination of E.e. Value and the Absolute Configuration of *endo,endo*-5-Acyloxybicyclo[2.2.1]heptan-2-ol 5. A mixture of (+)-**5e** (19 mg, 0.066 mmol) and $LiAlH_4$ (10 mg, 0.26 mmol) in dry diethyl ether (10 cm^3) was gently refluxed for 2h. After a usual work up, the products were purified by TLC on silica gel to give known (+)-(1*R*,2*R*,4*R*,5*R*)-*endo,endo*-bicyclo[2.2.1]heptane-2,5-diol (6 mg, 60%); $[\alpha]_D^{22}$ +16.1 (c 0.318, $CHCl_3$),[6] which was dissolved in pyridine (1 cm^3). To the solution was added 3,5-dichlorobenzoyl chloride (21 mg, 0.10 mmol) and then the mixture was sitirred for 12h at room temperature. After the reaction mixture was neutralized with dil. HCl, it was extracted with diethyl ether. The extract was washed with aqueous solution of $NaHCO_3$ and water and then dried ($MgSO_4$). After removal of the solvent, the residue was purified by TLC on silica gel to give *endo,endo*-2,5-bis(3,5-dichlorobenzoyloxy)bicyclo[2.2.1]heptane (5 mg), HPLC analysis (Opti-Pak XC, hexane/*i*-propylalcohol 99/1 eluent, 0.1 cm^3 min^{-1}) of which showed two peaks; Rt (min): 51 for (1*R*,2*R*,4*R*,5*R*)-isomer and 59 for (1*S*,2*S*,4*S*,5*S*)-isomer.

General Procedure for Determination of E.e. Value and the Absolute Configuration of *endo*-5-Acyloxybicyclo[2.2.1]heptan-2-one 4. A mixture of (-)-**4e** (30 mg, 0.13 mmol) and 10% methanolic solution of KOH (5 cm^3) was gently refluxed for 2h. After a usual work up, the products were separated by TLC on silica gel to give *endo*-5-hydroxybicyclo[2.2.1]heptan-2-one (10 mg, 60%), which was treated with benzoyl chloride (17 mg, 0.12 mmol) in pyridine (1 cm^3) to provide *endo*-5-benzoyloxybicyclo[2.2.1]heptan-2-one. HPLC analysis (Opti-Pak XC, hexane/*i*-propylalcohol 99/1 eluent, 0.1 cm^3 min^{-1}) of the benzoate showed two peaks; Rt (min): 126 for (1*S*, 2*S*, 4*S*)-isomer and 140 for (1*R*,2*R*,4*R*)-isomer. A mixture of *endo*-5-hydroxybicyclo[2.2.1]heptan-2-one (8 mg, 0.063 mmol) and $LiAlH_4$ (5 mg, 0.26 mmol) in dry diethyl ether (5 cm^3) was refluxed for 3h. After a usual work up, TLC of the products on silica gel gave (-)-(1*S*,2*S*,4*S*,5*S*)-bicyclo[2.2.1]hepane-2,5-diol (5 mg, 62%); $[\alpha]_D^{26}$ -13.6

(c 0.310, $CHCl_3$).

General Procedure for HLADH-catalyzed Reduction of *exo*-5-Acyloxybicyclo[2.2.1]heptan-2-one (±)-6. A mixture of (±)-*exo*-5-acetoxybicyclo[2.2.1]heptan-2-one **6a** (568 mg, 3.39 mmol), HLADH (7 mg), NAD^+ (122 mg, 0.170 mmol), and $Na_2S_2O_4$ (2.68 g, 17.0 mmol) in 1/15 M Sφrensen phosphate buffer (pH 7.0, 150cm^3) was stirred for 7 days at 30 °C. After the same work up as described for the reduction of **1c**, the products were separated by TLC on silica gel to give (-)-**6a** (187 mg, 33%); $[\alpha]_D^{20}$ -21.4 (c 0.610, MeOH)[6] and *exo,endo*-5-acetoxybicyclo[2.2.1]heptan-2-ol **7a** (233 mg, 41%).

General Procedure for Determination of E.e. Value and the Absolute Configuration of *exo*-5-Acyloxybicyclo[2.2.1]heptan-2-one 6. A solution of (-)-**6a** (173 mg, 1.00 mmol) and 5% metanolic solution of KOH (5 cm^3) was refluxed for 1.5h. After a usual work up, the products were separated by TLC on silica gel to give (1*S*,2*R*,4*S*)-*exo*-5-hydroxybicyclo[2.2.1]heptan-2-one (101 mg, 80%); $[\alpha]_D^{20}$ -9.5 (c 0.622, MeOH).[6] HPLC analysis (Chiralpak AD, hexane/ethanol 97/3 eluent, 1.5 cm^3 min^{-1}) of *exo*-5-(*p*-nitrobenzoyloxy)bicyclo[2.2.1]heptan-2-one showed two peaks; Rt (min): 80 for (1*R*,2*S*,4*R*)-isomer and 120 for (1*S*,2*R*,4*S*)-isomer.

General Procedure for Determination of E.e. Value and the Absolute Configuration of *exo,endo*-5-Acyloxybicyclo[2.2.1]heptan-2-ol 7. A mixture of **7a** (120 mg, 0.710 mmol) and pyridinium chlorochromate (0.30 g, 1.4 mmol) in methylene dichloride (6 cm^3) was stirred at room temperature for 4h. After addition of diethyl ether to the reaction mixture followed by filtration of the solid, chromatography of the products on silica gel gave known (1*R*,2*S*,4*R*)-*exo*-5-acetoxybicyclo[2.2.1]heptan-2-one (101 mg, 85%); $[\alpha]_D^{24}$ +24.6 (c 0.800, MeOH).[6] After treatment of (1*R*,2*S*,4*R*,5*R*)-**7a** (50 mg, 0.29 mmol) with 5% methanolic solution of KOH (5 cm^3), TLC of the products on silica gel gave (1*R*,2*S*,4*R*,5*R*)-*exo,endo*-bicyclo[2.2.1]heptane-2,5-diol (28 mg, 76%); $[\alpha]_D^{20}$ -7.2 (c 0.710, MeOH). The absolute configurations of *exo*-2-acyloxybicyclo[2.2.1]heptan-5-ols **7b-7e** were confirmed by conversion into *exo,endo*-bicyclo[2.2.1]heptane-2,5-diol, e.e. value of which was determined by HPLC analysis (Chiralpak AD, hexane/ethanol 95/5 eluent, 1.0 cm^3 min^{-1}) of *exo,endo*-2,5-bis(*p*-nitrobenzoyloxy)bicyclo[2.2.1]heptane to show two peaks; Rt (min): 90 for (1*R*,2*S*,4*R*,5*R*)-isomer and 110 for (1*S*,2*R*,4*S*,5*S*)-isomer.

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