Enzymes in Organic Synthesis. 24.[1] Preparations of Enantiomerically Pure Chiral Lactones via Stereospecific Horse Liver Alcohol Dehydrogenase Catalyzed Oxidations of Monocyclic Meso Diols[2]

Ignac J. Jakovac, H. Bruce Goodbrand, Kar P. Lok, and J. Bryan Jones*

Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1. *Received December 22, 1981*

Abstract: Preparative-scale horse liver alcohol dehydrogenase catalyzed oxidation of monocyclic meso diols provides a direct and convenient one-step route to a broad range of chiral γ-lactones of value as synthons in asymmetric synthesis. The general applicability of the method is demonstrated by oxidations of *cis*-1,2-bis(hydroxymethyl) substrates of the cyclohexyl, cyclohexenyl, cyclopentyl, cyclobutyl, cyclopropyl, and dimethylcyclopropyl series. For each diol, oxidation of the hydroxymethyl group attached to the *S* chiral center occurs exclusively, and the pure γ-lactone products are isolated in high (68–90%) yields and of 100% ee. In contrast, the enzyme does not exhibit significant enantiomeric selectivity in its catalysis of oxidations of the corresponding racemic trans diols. The stereospecificities observed, or lack thereof, are as predicted by the active-site model.

In asymmetric synthesis, reactions leading to racemic compounds are avoided if at all possible since the maximum yield attainable of a desired stereoisomer is 50%. Only in exceptional cases can unwanted enantiomers be reused productively.[4] Accordingly, it is much preferable to employ reagents capable of inducing stereospecific transformations on symmetrical substrates.[6] Enzymes are particularly attractive catalysts for such reactions.[7] One aspect of their stereospecificity in this regard is their ability to discriminate between constitutionally identical enantiotopic groups attached to centers of opposite chiralities in meso compounds.[7a,b,8] Horse liver alcohol dehydrogenase (HLADH[9]), a commercially available NAD^+-dependent alcohol dehydrogenase that catalyzes CH(OH) ⇌ C=O oxidoreductions of a broad spectrum of substrates of organic chemical interest,[7a-d] has been found to possess this meso-compound stereospecificity to a degree that is of considerable asymmetric synthetic value. In this paper, we report on the stereospecificity of HLADH-catalyzed oxidations of a representative structural range of aliphatic monocyclic meso diols and of their racemic trans diol analogues.

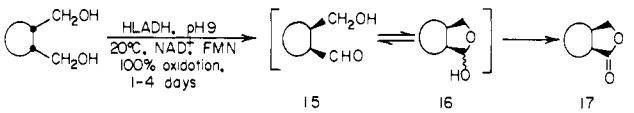
(1) Part 23: Jones, J. B.; Schwartz, H. M. *Can. J. Chem.* **1982**, *60*, 1030.
(2) Abstracted mainly from the Ph.D. Thesis of I.J.J., University of Toronto, 1980. Preliminary communications on some of the results have been published.[3]
(3) (a) Goodbrand, H. B.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1977**, 469. (b) Jakovac, I. J.; Ng, G.; Lok, K. P.; Jones, J. B. *Ibid.* **1980**, 515.
(4) A limited number of examples have been reported where wastage of the "wrong" enantiomer is avoided by recycling.[5]
(5) (a) Tosa, T.; Mori, T.; Fuse, N.; Chibata, I. *Agric. Biol. Chem.* **1969**, *33*, 1047. (b) Terashima, S.; Yamada, S. *Tetrahedron Lett.* **1977**, 1001. (c) Trost, B. M.; Timko, J. M.; Stanton, J. L. *J. Chem. Soc., Chem. Commun.* **1978**, 436. (d) Saddler, J. C.; Donaldson, R. E.; Fuchs, P. L. *J. Am. Chem. Soc.* **1981**, *103*, 2110.
(6) (a) Morrison, J. D.; Mosher, H. S. "Asymmetric Organic Reactions"; American Chemical Society: Washington, D.C., 1976. (b) Scott, J. W.; Valentine, D. *Synthesis* **1978**, 329. (c) ApSimon, J. W.; Geguin, R. P. *Tetrahedron* **1979**, *35*, 2797. (d) Kagan, H.; Fiaud, J. C. *Top. Stereochem.* **1978**, *10*, 175.
(7) (a) Jones, J. B.; Beck, J. F. *Tech. Chem. (N.Y.)* **1976**, *10*, 107. (b) Jones, J. B. In "Enzymic and Nonenzymic Catalysis"; Dunnill, P., Wiseman, A., Blakeborough, N., Eds.; Ellis Horwood/Wiley: Chichester/New York: 1980; pp 54–83. (c) Suckling, C. J.; Suckling, K. E. *Chem. Soc. Rev.* **1974**, *3*, 387. (d) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Suzuki, T.; Iwasaki, M.; Sasaki, Y.; Fujii, T. *J. Org. Chem.* **1981**, *46*, 2726. (e) Mosbach, K., Ed. *Methods Enzymol.* **1977**, *44*, 717–856. (f) Martinek, K.; Berezin, I. V. *J. Solid-Phase Biochem.* **1978**, *2*, 343. (g) Chibata, I.; Tosa, T. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 197. (h) Abbott, B. J. *Dev. Ind. Microbiol.* **1979**, *20*, 345. (i) May, S. W. *Enz. Microb. Technol.* **1979**, *1*, 15.
(8) Alworth, W. L. "Stereochemistry and its Application in Biochemistry"; Wiley-Interscience: New York, 1972.
(9) Abbreviations used: HLADH, horse liver alcohol dehydrogenase; NAD^+, nicotinamide adenine dinucleotide, oxidized form; FMN, flavin mononucleotide (riboflavin phosphate); ee, enantiomeric excess; $Eu(tfc)_3$, tris-[(trifluoromethyl)hydroxymethylene-(−)-camphorato]europium(III) (optishift I).

Table I. Relative Rates[a] of HLADH-Catalyzed Oxidations of Diols 1–14

substrate	rel rate	substrate	rel rate
cyclohexanol	100		
1	<1	(±)-8	1
2	80	(±)-9	75
3	85	(±)-10	80
4	60	(±)-11	30
5	92	(±)-12	82
6	47	(±)-13	44
7	41	(±)-14	53

[a] Oxidation rates were measured spectrophotometrically at 25 °C in 0.1 M NaOH–glycine buffer (pH 9), with [S] = 10^{-2}–10^{-4} M and [NAD^+] = 5×10^{-4} M.

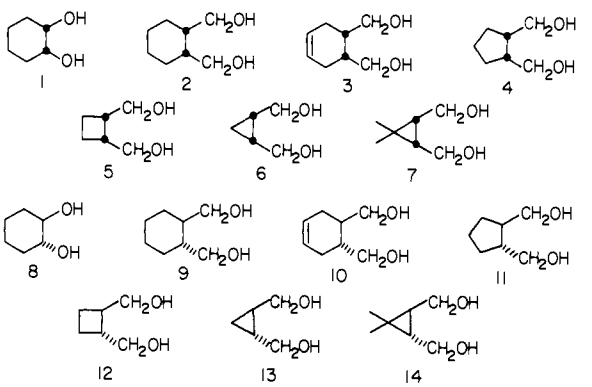
Scheme I



15 16 17

Results

Synthesis of Substrates. The substrates evaluated were the meso diols **1–7** and the racemic trans diols (±)-**8–14**. They were



1 2 3 4 5 6 7 8 9 10 11 12 13 14

prepared by literature methods or by unexceptional routes that are described in detail in the Experimental Section.

Relative Rates of HLADH-Catalyzed Oxidations of 1–14. The rates of HLADH-catalyzed oxidations of **1–14** relative to that of the standard reference substrate cyclohexanol are recorded in Table I. With the exception of *cis*-cyclohexane-1,2-diol (**1**) and its racemic trans isomer (±)-**8**, which were nonsubstrates, the rates of oxidation of each meso diol and its racemic trans analogue were

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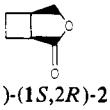
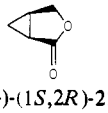
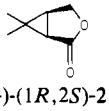
Table II. Results of HLADH-Catalyzed Oxidations of Meso Diols 2-7[a]

substrate	product	yield, %	ee, %[b]
2	 (+)-(1*S*,2*R*)-**18**	79	100
3	 (−)-(1*S*,6*R*)-**19**	80	100[13]
4	 (+)-(1*S*,2*R*)-**20**	72	100
5	 (+)-(1*S*,2*R*)-**21**	90	100
6	 (−)-(1*S*,2*R*)-**22**	68	100
7	 (−)-(1*R*,2*S*)-**23**	71	100

[a] Reactions carried out under Scheme I conditions. [b] Error limit ±3%.[14]

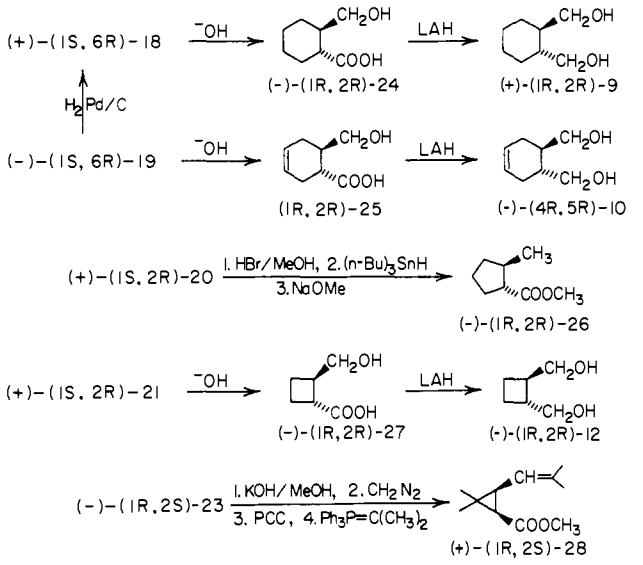
sufficiently fast for preparative-scale reactions to be carried out effectively.[10]

Preparative-Scale Oxidations of the Meso Diols 2–7. The meso diols **2–7** were each subjected to preparative-scale enzyme-mediated oxidation at pH 9, using FMN to effect recycling[11] of the catalytic amount of the NAD^+ coenzyme used. In each case, enantiotopically stereospecific oxidation of the hydroxymethyl group attached to the *S* chiral center occurred. Under the reaction conditions the intermediate hydroxy aldehyde products **15** formed initially underwent further in situ HLADH-catalyzed oxidation via their hemiacetals[12] **16** to give the lactone products **17** directly, all in excellent yield, enantiomerically pure, and of the same general absolute configuration type. The general reaction pathway followed is summarized in Scheme I. The results for each individual reaction are recorded in Table II. The structures of the optically active lactone products **18–23** were confirmed by comparison with authentic racemic compounds obtained by silver carbonate oxidation or sodium borohydride reduction, respectively, of the corresponding precursor meso diol or anhydride.

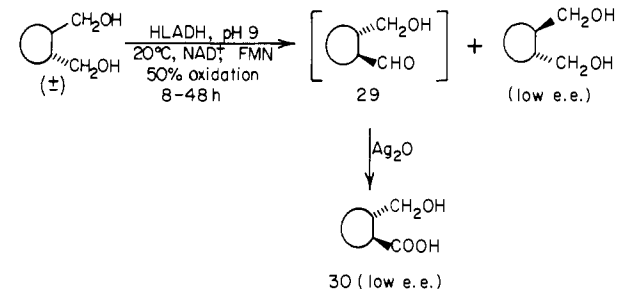
Enantiomeric Excess Determinations. The ee's of the Table II lactones were determined by first reacting them with methyllithium and then examining the ^1H NMR behavior of the diastereotopic methyl peaks of their diol products in the presence of $Eu(tfc)_3$.[14] Excellent $\Delta\Delta\delta$ separations of 0.12–0.3 ppm were observed for the methyl peaks of the diols obtained from the racemic lactones (±)-**18–23** used as the reference standards, thereby permitting the complete enantiomeric purities of the enzymically derived lactones to be unambiguously established.

Absolute Configuration Determinations of 18–23. The absolute configurations of lactones **18–21** and **23** were established by their

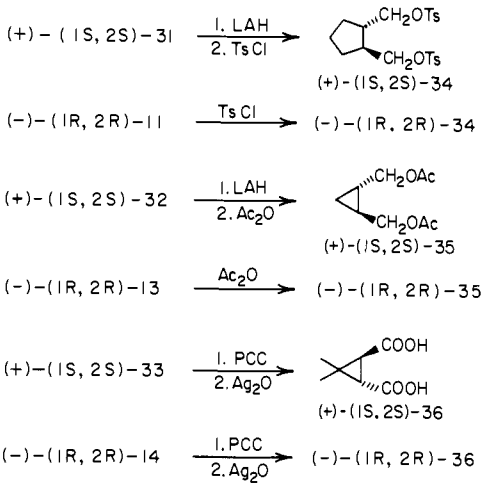
Scheme II



Scheme III



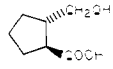
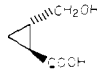
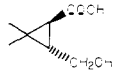
Scheme IV



degradations to the known compounds (1*R*,2*R*)-**9**,[15] (4*R*,5*R*)-**10**,[16] (1*R*,2*R*)-**26**,[17] (1*R*,2*R*)-**12**,[18] and (1*R*,2*R*)-**28**,[19] respectively, by the pathways depicted in Scheme II. The configuration of the remaining lactone, **22**, was assigned by comparison of its CD

(10) Rates of oxidation >5% that of cyclohexanol generally guarantee good preparative-scale results.[7a]
(11) Jones, J. B.; Taylor, K. E. *Can. J. Chem.* **1976**, *55*, 2969.
(12) (a) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1977**, *99*, 556. (b) Jones, J. B.; Lok, K. P. *Can. J. Chem.* **1979**, *57*, 1025.
(13) The 85% optical purity reported initially for this compound[3a] was based on optical rotation data. None of the opposite enantiomer was detectable when the ee was redetermined by the direct NMR method developed subsequently.[14]
(14) Jakovac, I. J.; Jones, J. B. *J. Org. Chem.* **1979**, *44*, 2165.
(15) Nagai, U.; Shishido, T.; Chiba, R.; Mitsuhashi, H. *Tetrahedron* **1965**, *21*, 1701.
(16) Walborsky, H. M.; Barash, L.; Davis, T. C. *Tetrahedron* **1963**, *19*, 2333.
(17) Hill, R. K.; Foleg, P. J., Jr.; Gardella, L. A. *J. Org. Chem.* **1967**, *32*, 2330.
(18) Windhorst, J. C. A. Ph.D. Thesis, Rijksuniversiteit Te Leiden, The Netherlands, 1975, p 28.
(19) Cocker, W.; Lauder, H. S. J. *J. Chem. Soc., Perkin Trans. 1*, 1975, 332.

Table III. Results of HLADH-Catalyzed Oxidations of Trans Diols (±)-9-14[a]

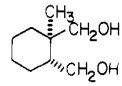
substrate	product isolated[b] + recovered substrate	yield, %	ee, %
(±)-9	[(1*R*,2*R*)-24][c,d]	30	8
	+		
	(−)-(1*S*,2*S*)-9	37	16
(±)-10	[25][c,d]	50	<1
	+		
	(−)-(4*R*,5*R*)-10	31	1
(±)-11		23	2
	(+)-(1*S*,2*S*)-31[d]		
	+		
	(−)-(1*R*,2*R*)-11	57	4
(±)-12	(+)-(1*S*,2*S*)-27[d]	28	2
	+		
	(−)-(1*R*,2*R*)-12	39	4
(±)-13		44	2
	(+)-(1*S*,2*S*)-32[d]		
	+		
	(−)-(1*R*,2*R*)-13	31	2
(±)-14		57	<1
	(+)-(1*S*,2*S*)-33[e]		
	+		
	(−)-(1*R*,2*R*)-14[e]	20	1

[a] Reactions carried out under Scheme VI conditions. [b] The initially formed hydroxy aldehydes were chemically oxidized to the corresponding hydroxy acids during workup. [c] Significant lactonization of this acid occurred during workup. [d] Characterized by $LiAlH_4$ reduction to the corresponding diol. [e] Characterized by oxidation to the corresponding diacid.

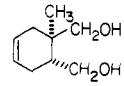
spectrum with that of (−)-(1*R*,2*S*)-**23**.

Preparative-Scale Oxidations of the Trans Diols (±)-9–14. The racemic trans-diol substrates were exposed to the same enzyme-catalyzed oxidation conditions as the meso diols, except that the reactions were terminated at the 50% oxidation stage.[20] All reactions proceeded in the same basic manner to give trans hydroxy aldehydes **29** initially as expected. These initial products were not isolated directly but were oxidized with silver oxide to their trans hydroxy acids **30**. The general reactions are summarized in Scheme III. The hydroxy acids **30** were generally reduced to the corresponding trans diols for complete characterization.

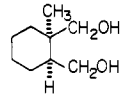
In contrast to the high stereospecificity manifest by HLADH toward the meso-diol substrates, the enzyme exhibited marginal enantiomeric selectivity at best toward their racemic trans-diol counterparts. The details of the stereoselectivity of each individual reaction are recorded in Table III. The ee values cites are based on optical rotation data. They were not verified further by a direct NMR procedure since the lack of enantiomeric selectivity clearly eliminates trans diol oxidation from any asymmetric synthetic consideration. The optically active compounds of Table III whose absolute configurations were not available from the Scheme II data were assigned by the straightforward correlations of (+)-**31** and (−)-**11** with (+)- and (−)-**34**,[18,21] (+)-**32** and (−)-**13** with (+)- and (−)-**35**,[22] and (+)-**33** and (−)-**14** with (+)- and (−)-**36**,[23] respectively. The data are summarized in Scheme IV. In order to ascertain whether the enantiomeric specificity of HLADH toward trans diol substrates could be amplified by the introduction of additional substituents, we prepared the methyl derivatives **37** and **38** of **9** and **10**, respectively. Disappointingly, neither was a substrate of the enzyme. Their cis-diol analogues **39** and **40** were also nonsubstrates.



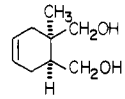
(±)-37



(±)-38



(±)-39



(±)-40

Discussion

The preparative-scale HLADH-catalyzed oxidations were carried out on up to 2 g of substrate; GLC was used to monitor the extent of reaction. Scaling up of the reactions to 10 g or more presents no problem.[24] The experimental procedure is very simple, involving reaction in Erlenmeyer flasks and workup by continuous chloroform extraction, first at basic and then at acidic pH's, generally followed by Kugelrohr distillation. The oxidations of the meso diols **2–7** were continued until all starting material had been consumed. Those of the racemic trans diols **9–14** were terminated as close to ~50% reaction as possible.[20] The yields of the lactones **18–23** (Table II), and of the combined trans acid product plus recovered trans diol starting material (Table III), were routinely excellent. For all reactions the yields quoted represent the isolated amounts of purified compounds.

As Table II shows, the chiral lactones obtained from the meso diol oxidations were all enantiomerically pure. Furthermore, their absolute configurations are all of the same type. The enzyme is remarkable in both the degree and the constancy of its enantiotopic specificity toward only the hydroxymethyl groups attached to *S* centers over a considerable range of structural variations in the monocyclic meso 1,2-diol substrates.

The method is clearly of general asymmetric synthetic value, particularly with lactones being such useful synthetic intermediates. Traditional chemical approaches do not at present provide ready access to the range of chiral lactones illustrated in Table II. Such compounds are attractive synthons of considerable current interest. For example, the cyclobutyl lactone **21** has been converted into grandisol,[24] and the dimethylcyclopropyl lactone **23**, readily transformed[25] into (+)-(1*R*,2*S*)-*cis*-methylchrysanthemate (**28**) (Scheme II), leads easily into the pyrethroids.[26a]

The behaviors of the racemic trans diols **9–14** under preparative-scale HLADH-catalyzed oxidation conditions were evaluated mainly for comparison purposes and in the hope that additional insights into the topography of the active site would emerge. The marginal enantiomeric distinctions observed were disappointing in preparative terms. Furthermore, the enzyme was found to be fickle in its enantiomeric preferences, with the absolute configuration types of the hydroxy acid products of Table III changing back and forth as the ring structures are varied.

Cubic Active-Site Section Analysis of Stereospecificity. Despite the advances made in the kinetic analyses and X-ray structure determinations of HLADH, the active-site representations derived from such studies[27] are not yet sufficiently refined to enable specificity analyses of the range required by organic chemists[27b] to be carried out easily. Accordingly, simple structural models

(20) Enzyme-catalyzed transformations of racemates are traditionally terminated after 50% of reaction since this is the point at which optically pure products are obtained if the stereospecificity is absolute. The trans diol oxidations were therefore stopped as close to this stage as possible in order to provide a consistent measure of the degree of enantiomeric selectivity achievable.

(21) Collet, A.; Brienne, M. J.; Jacques, J. *Bull. Soc. Chim. Fr.* **1972**, 32.

(22) Inamasu, S.; Horiike, M.; Inouye, Y. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 1393.

(23) Crombie, L.; Doherty, C. F.; Pattenden, G. *J. Chem. Soc. C* **1970**, 1076.

(24) Jones, J. B.; Jakovac, I. J.; Finch, M. A. W. *Can. J. Chem.*, in press.

(25) Severin, M.; Hevesi, L.; Krief, A. *Tetrahedron Lett.* **1976**, 3915.

(26) (a) Elliott, M.; Janes, N. F. *Chem. Soc. Rev.* **1978**, *7*, 473. (b) *Tetrahedron* **1969**, *25*, 1727.

(27) (a) Brändén, C-I. In "Pyridine Nucleotide-Dependent Dehydrogenases"; Sund, H., Ed.; Walter de Gruyter: Berlin, 1977; p 325. (b) Dutler, H.; Brändén, C-I. *Biorg. Chem.* **1981**, *10*, 1.

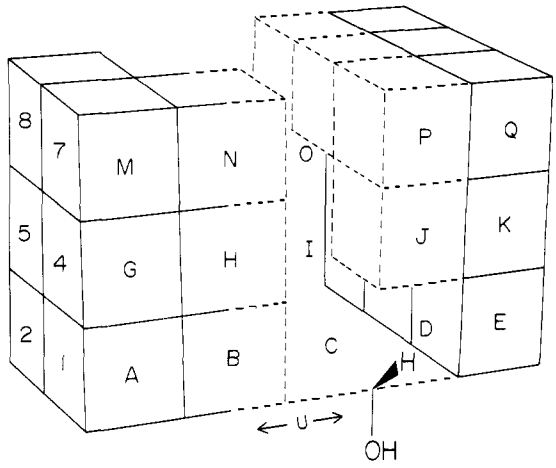


Figure 1. Cubic-space section of the active-site region of HLADH. The front faces of the cubes, each of which has a 1.3-Å edge, are labeled alphabetically, and the side faces numerically. Not all cubes of the section are shown. Future needs have been anticipated, and the total section specified[28b] exceeds the volume currently required. The hydride equivalent is delivered to or removed from the coenzyme at the oxidation site at the bottom of the C1, D1 intersection. The OH bond of an alcohol substrate projects downward from this point to coordinate with the active-site Zn^{2+} atom. The cubes bounded by solid lines are designated as "forbidden" because they are occupied by enzymic amino acid residues, as is the underneath region, U. The front of the section is occupied by coenzyme and is also forbidden. The broken-line regions are "limited", with substrate binding possible, but not favored due to their proximity to active-site amino acid residues. The open spaces of the model are the "allowed" regions, where substrate can be readily accommodated. For specificity analyses, the model and alcohol or carbonyl substrate are built to the same scale (Framework Molecular models are convenient). The CH(OH) group is positioned at the C1, D1 oxidation site, and the various orientations of the substrate in the cubic section are compared to identify which, if any, will permit binding in allowed, or, less desirably, in limited, regions. If location of a group in a forbidden cube is required to fit the substrate, a productive ES complex cannot form and oxidation will be precluded. A complete description of the model and its application is given in ref 28b.

that can be applied quickly and accurately remain preferable for predicting and interpreting the enzyme's stereospecificity. The diamond lattice section initially formulated by Prelog[28a] served admirably in this role for many years. However, it is no longer adequate for the full scope of analyses now encountered, and a new active-site section using cubic-space descriptors was formulated recently.[28b] The working model of the cubic-space active-site section is shown in Figure 1. All of the stereochemical results reported in this paper are in accord with the cubic model predictions. The oxidations of the meso diols are interpretable by the same basic approach. The analysis for the cyclobutyl meso diol **5** depicted in Figure 2 is representative.[29]

The lack of significant enantiomeric discrimination in the trans diol series is also rationalizable by the cubic model. Both enantiomers of each racemate **9–14** can be fitted adequately in allowed regions of the active site. Thus each stereoisomer should be a substrate, and only low enantiomeric selectivity in the oxidation of the racemates should be observed. The experimental results (Table III) are in accord with this prediction. Furthermore, use of the cubic model shows that the lack of substrate activity of the diols **37–40** is due to the fact that, with the additional angular methyl groups present, none of the stereoisomers can be oriented satisfactorily for oxidation without violating one or more forbidden cubes. Similarly, for the *cis*- and *trans*-cyclohexane-1,2-diols (**1** and (±)-**8**), both of which are inactive as substrates, all active-site orientations leading to oxidation would require the positioning of one or more groups in forbidden cubes or would place a polar

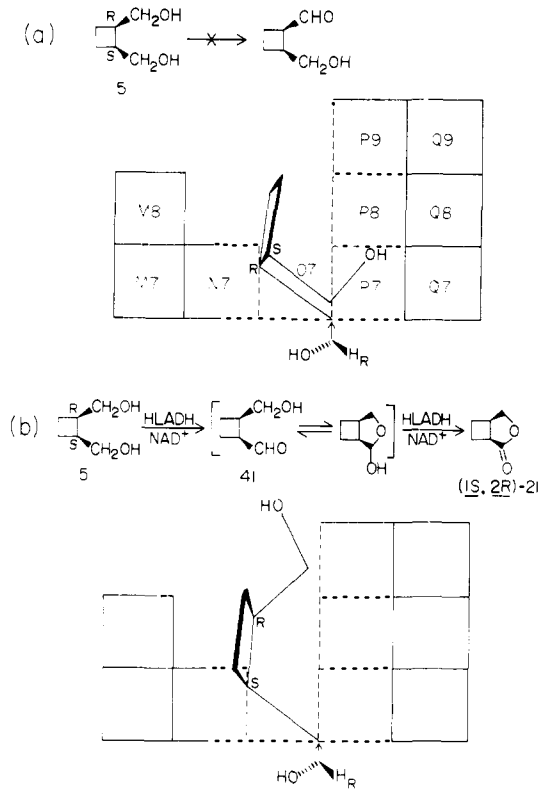


Figure 2. Cubic model analysis of the stereospecificity of HLADH-catalyzed oxidation of the cyclobutyl mexo diol **5**. The substrate orientations are depicted from a top perspective[28b] viewpoint of the cubic section defined in Figure 1. (a) In HLADH-mediated primary alcohol oxidations, the pro-*R* hydrogen is always abstracted.[28] For the 1(*R*)-hydroxymethyl group to be oxidized via H_R abstraction would require C-3 and C-4 of the cyclobutyl group to take up positions in the forbidden U region of the section or the hydrophilic hydroxyl group of the 2*S*-CH_2OH group to locate in the hydrophobic limited cube(s) P4 (or P7), as shown. ES complex formation is thus precluded and oxidation does not occur. (b) With HLADH-catalyzed removal of H_R of the 2(*S*)-hydroxymethyl group, the substrate can be accommodated in fully allowed active-site regions, as shown. ES complex formation is therefore favored, and oxidation in this orientation can take place readily. The hemiacetal form of the initially formed hydroxy aldehyde **41** can also bind favorably and is thus further oxidized under the reaction conditions to give (1*S*,2*R*)-**21** directly.

hydroxyl group in the hydrophobic limited region of H4. The formation of a favorable ES complex is therefore precluded for each stereoisomer, and HLADH-catalyzed oxidation cannot occur.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 237 or Pye Unicam SP3-200 spectrophotometer; spectra of solids were determined in $CHCl_3$ solutions and liquids as neat films unless otherwise specified. NMR spectra (Me_4Si internal standard) were performed in $CDCl_3$ (unless otherwise indicated) on a Varian T-60 instrument. Optical rotations were measured on a Perkin-Elmer 141 polarimeter and CD spectra on a JASCO J-41A spectropolarimeter. All compounds were purified at least until no impurities could be detected by GLC. GLC analyeses were performed on a Hewlett-Packard 5710A instrument (flame ionization detector) with 3% QF1 on Chromasorb G columns. Relative substrate-oxidation rates were monitored by UV spectroscopy with a Unicam SP1800 spectrophotometer. NAD^+ was purchased from Kyowa Hakko Kogyo Co., Ltd., and FMN from Sigma Chemical Co. HLADH, prepared by the method of Roy and Nishikawa,[30] was a gift from Hoffmann-La Roche, Inc., Nutley, NJ, or was purchased from Sigma (>98% protein). The activity of each batch of enzyme was determined[31] prior to use, and the amounts of HLADH quoted refer to milligrams of active enzyme. The structures of all previously known compounds were in total accord with their IR and NMR spectra.

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Preparations of Meso Diols 1–7 and Cis Diols (±)-39 and (±)-40. *cis*-3,3-Dimethyl-1,2-bis(hydroxymethyl)cyclopropane (7). A solution of diphenylethylsulfonium tetrafluoroborate[32] (11.6 g, 38.7 mmol) and dichloromethane (3.30 g, 38.7 mmol) in dry dimethoxyethane (200 mL) was cooled to −78 °C under dry O_2-free N_2 and treated with a cold (−78 °C) solution of LDA (42.6 mmol, prepared from *n*-butyllithium (28.4 mL of a 1.5 M solution) and diisopropylamine (4.60 g, 45.0 mmol)). The resultant bright yellow solution was stirred for 30 min at −78 °C and then treated with methyl iodide (5.80 g, 40.6 mmol). The mixture was maintained at −78 °C for 2 h. Cold (−78 °C) LDA (42.6 mmol) was again added and the orange mixture stirred for 1 h at −78 °C.[33] 4-Oxacyclopenten-3-one (Δ^2-butenolide)[34] (3.30 g, 38.7 mmol) was then added and the mixture stirred for a further 2 h at −78 °C. After the addition of water (100 mL), the mixture was extracted with ether (3 × 50 mL). The aqueous solution was acidified and extracted again with ether (2 × 50 mL). The combined ether extracts were dried ($MgSO_4$) and rotoevaporated. The reddish-brown residue was chromatographed on silica gel (75 g, (1:5) ethyl acetate–hexane elution) and then Kugelrohr distilled to yield (±)-*cis*-6,6-dimethyl-3-oxabicyclo[3.1.0]hexan-2-one[35] (**23**, 2.68 g, 55% yield): bp 100 °C (10 mmHg); IR 1770 and 1761 cm^{-1}; ^1H NMR δ 1.10 (6 H, s), 1.76–2.20 (2 H, m), 3.90–4.40 (2 H, m). The lactone (±)-**23** (2.1 g, 16.7 mmol) in THF (50 mL) was added to $LiAlH_4$ (0.63 g, 16.7 mmol) in THF (50 mL). The mixture was stirred for 4 h at 20 °C. Water (2 mL) was then added carefully, followed by 15% aqueous NaOH (2 mL) and then more water (5 mL). The mixture was filtered and the residue washed with THF (100 mL). Evaporation of the solvent followed by Kugelrohr distillation gave *cis*-3,3-dimethyl-1,2-bis(hydroxymethyl)cyclopropane (**7**, 2.05 g, 94% yield): bp 90 °C (1 mmHg) (lit.[36] bp 123–124 °C (8 mmHg)); IR, 3378 cm^{-1}; ^1H NMR, δ 0.94–1.20 (2 H, m), 1.04 (6 H, d, J = 2 Hz), 3.18–4.20 (6 H, m).

The other diols were prepared in the reported, or superior, yields by the literature procedures cited, or by straightforward modifications of the published routes, as follows: *cis*-cyclohexane-1,2-diol (**1**), mp 97–98 °C (lit.[37a] mp 96–98 °C); *cis*-1,2-bis(hydroxymethyl)cyclohexane (**2**), mp 42–43 °C (lit.[38] mp 42–43 °C), by $LiAlH_4$ reduction of *cis*-cyclohexane-1,2-dioic acid anhydride (from Aldrich); *cis*-4,5-bis(hydroxymethyl)cyclohexene (**3**),[39] bp 115 °C (0.2 mmHg) (lit.[39] bp 112–114 °C (0.2 mmHg)); *cis*-1,2-bis(hydroxymethyl)cyclopentane (**4**), bp 100–101 °C (0.75 mmHg) (lit.[40] bp 103 °C (1.5 mmHg)), by $LiAlH_4$ reduction of *cis*-cyclopentane-1,2-dioic acid anhydride;[40] *cis*-1,2-bis(hydroxymethyl)cyclobutane (**5**), bp 85 °C (0.5 mmHg) (lit.[41] bp 111–113 °C (2.7 mmHg), by $LiAlH_4$ reduction of *cis*-cyclobutane-1,2-dioic acid anhydride (from Aldrich); *cis*-1,2-bis(hydroxymethyl)cyclopropane (**6**), bp 90 °C (1 mmHg) (lit.[42] bp 96–102 °C (1 mmHg)), by $LiAlH_4$ reduction of dimethyl *cis*-cyclopropanedioate obtained by MPLC separation (ethyl acetate–*n*-hexane (1:20) elution) of the cis diester, ^1H NMR δ 1.0–1.8 (2 H, m), 2.08 (2 H, t of d, J = 7 Hz), 3.64 (6 H, s), from the more slowly eluted trans diester, ^1H NMR[43] δ 1.44 (2 H, d of d, J = 7 Hz), 2.20 (2 H, d of d, J = 7 Hz), 3.64 (6 H, s), using the 60:40 cis:trans mixture obtained by the condensation of methyl acrylate and methyl chloroacetate according to the method of McCoy;[44] *cis*-1-methyl-1,2-bis(hydroxymethyl)cyclohexane ((±)-**39**), bp 128 °C (0.15 mmHg) (lit.[45] bp 131 °C (2 mmHg)), by catalytic (5% Pd/C) hydrogenation of *cis*-4-methyl-4,5-bis(hydroxymethyl)cyclohexene ((±)-**40**), bp 100 °C (0.1 mmHg) (lit.[46] bp 108 °C (0.1 mmHg)).

Preparations of Trans Diols (±)-8–14, 37, and 38. (±)-*trans*-1,2-Bis(hydroxymethyl)cyclobutane (12). To *cis*-cyclobutane-1,2-dicarboxylic acid anhydride (from Aldrich, 5.0 g, 39.7 mmol) in methanol (60 mL) was added concentrated sulfuric acid (1 mL). The mixture was refluxed for 6 h and the methanol then removed by rotoevaporation. The residue was dissolved in ether (50 mL), washed with water (25 mL) and then with saturated aqueous sodium hydrogen carbonate (25 mL), and then dried (K_2CO_3). The ether solution was evaporated and the residue refluxed in methanol (50 mL) containing sodium methoxide (2.25 g, 41.7 mmol) for 12 h. The cooled mixture was then neutralized with 2 N aqeuous hydrochloric acid and extracted with ether (3 × 50 mL). The ethereal extract was washed with water (2 × 25 mL) and then with aqueous 1 N sodium carbonate (2 × 25 mL), dried (K_2CO_3), and evaporated. Kugelrohr distillation of the residue yielded dimethyl *trans*-cyclobutane-1,2-dicarboxylate (3.2 g, 50% yield), bp 90 °C (10 mmHg) (lit.[48] bp 75 °C (2.25 mmHg)). The trans diester was reduced with $LiAlH_4$ in the usual way to give *trans*-1,2-bis(hydroxymethyl)-cyclobutane ((±)-**12**, 1.9 g, 88% yield), bp 100 °C (1 mmHg) (lit.[47] bp 111–114 °C (3 mmHg)).

(±)-*trans*-1,2-Bis(hydroxymethyl)-3,3-dimethylcyclopropane (14). In an analogous manner to that detailed for the cis diol **7**, diethyl fumarate (3.44 g, 0.02 mol) was treated with diphenylsulfonium isopropylide (0.02 mol) according to the procedure of Corey and Jautelat[33] to give diethyl 3,3-dimethyl-1,2-cyclopropandioate (3.3 g, 77% yield), bp 120 °C (25 mmHg) (lit.[47] bp 240–241 °C (760 mmHg)). This diester was then reduced with $LiAlH_4$ in THF by the standard procedure described above to give *trans*-3,3-dimethyl-1,2-bis(hydroxymethyl)cyclopropane ((±)-**14**, 1.2 g, 72% yield), bp 140 °C (35 mmHg) (lit.[36] bp 123–124 °C (8 mmHg)).

The remaining trans diols were prepared in good yields by the literature procedures unless otherwise indicated: *trans*-cyclohexane-1,2-diol ((±)-**8**), mp 103–104 °C (lit.[37b] mp 101.5–103 °C); *trans*-1,2-bis(hydroxymethyl)cyclohexane ((±)-**9**), mp 54–55 °C (lit.[38] mp 51–52 °C), by $LiAlH_4$ reduction of *trans*-cyclohexane-1,2-dicarboxylic acid (from Aldrich); *trans*-4,5-bis(hydroxymethyl)cyclohexene ((±)-**10**), bp 110 °C (0.1 mmHg) (lit.[46] bp 108 °C (0.1 mmHg)); *trans*-1,2-bis(hydroxymethyl)cyclopentane ((±)-**11**) by $LiAlH_4$ reduction of *trans*-cyclopentane-1,2-dicarboxylic acid;[49] *trans*-1,2-bis(hydroxymethyl)cyclopropane ((±)-**13**), bp 100 °C (1 mmHg) (lit.[50] bp 95–103 °C (1 mmHg)), by $LiAlH_4$ reduction of dimethyl *trans*-cyclopropanedioate (obtained above in preparation of *cis*-diol **6**); *trans*-4-methyl-4,5-bis-(hydroxymethyl)cyclohexene ((±)-**38**), bp 100 °C (0.1 mmHg) (lit.[46] bp 100 °C (0.1 mmHg)); *trans*-1-methyl-1,-bis(hydroxymethyl)cyclohexane ((±)-**37**), bp 128 °C (1.5 mmHg) (lit.[45] bp 141.5–143 °C (2.0 mmHg)), by catalytic (5% Pd/C) hydrogenation of (±)-**38** above.

Preparations of Racemic Lactones (±)-18–23. Lactones (±)-**18–21** were obtained in 65–85% yields by reduction of the corresponding anhydrides with $NaBH_4$, using the general procedure of Bailey and Johnson:[51] (±)-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**18**), bp 90–91 °C (1 mmHg) (lit.[38] bp 72–77 °C (0.5 mmHg)), from *cis*-cyclohexane-1,2-dioic acid anhydride (from Aldrich); (±)-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**19**), bp 80 °C (0.05 mmHg) (lit.[38] bp 85 °C (0.1 mmHg)), from *cis*-cyclohex-4-ene-1,2-dioic acid anhydride;[39] (±)-*cis*-3-oxabicyclo-[3.3.0]octan-2-one (**20**), bp 130–133 °C (20 mmHg) (lit.[52] bp 127–128 °C (19 mmHg)), from *cis*-cyclopentane-1,2-dioic acid anhydride;[40] (±)-*cis*-3-oxabicyclo[3.2.0]heptan-2-one (**21**), bp 100–103 °C (10 mmHg) (lit.[46] bp 109–113 °C (15 mmHg)), from *cis*-cyclobutane-1,2-dioic acid anhydride (from Aldrich). (±)-*cis*-3-Oxabicyclo[3.1.0]hexan-2-one (**22**), bp 100 °C (10 mmHg) (lit.[42] bp 55–65 °C (1 mmHg)), was obtained by silver carbonate on Celite oxidation of *cis*-bis(hydroxymethyl)cyclobutane (**6**) according to the general procedure of Fetizon.[53] (±)-*cis*-6,6-dimethyl-3-oxabicyclo[3.1.0]hexan-2-one (**23**) was prepared as described above as an intermediate in the synthesis of the meso diol **7**.

Relative Rates of HLADH-Catalyzed Oxidations of Diols 1–14 and 37–40. The relative oxidation rates were determined by monitoring the change in 340-nm NAD^+ absorption by using the general HLADH kinetic assay method.[54,55] The assays were performed at 25 °C at pH 9.0 with the following stock solutions: HLADH, 1 mg mL^{-1} in 0.05 M Tris-HCl buffer, pH 7.4; NAD^+, 10 mg mL^{-1} in 0.1 M glycine–NaOH buffer, pH 9.0; substrates, 2 × 10^{-2} M solutions in 0.05 M glycine–NaOH buffer, pH 9.0. For each diol substrate a reference assay was performed under the same conditions with a solution of cyclohexanol at

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the same concentration. The rates observed, relative to cyclohexanol = 100, are recorded in Table I. Those of **37–40** were immeasurably slow under the assay conditions.

Preparative-Scale HLADH-Catalyzed Oxidations of Meso Diols 2–7. Oxidation of Meso Diol 2 to the (+)-(1*S*,2*R*)-Lactone 18. *cis*-1,2-Bis(hydroxymethyl)cyclohexane (**2**, 2 g, 13.8 mmol), NAD^+ (720 mg, 1.1 mmol), and FMN (9.72 g, 20.3 mmol) were dissolved in 0.1 M glycine–NaOH buffer (pH 9.0, 400 mL) in a 1 L Erlenmayer flask. The pH of the mixture was readjusted to 9 with 15% aqueous NaOH. HLADH (40 mg) was then added, and the mixture was kept at room temperature (20–25 °C), with periodic adjustment of the pH to 9. The mixture turned from its initial clear orange to an opaque almost-black color as the reaction proceeded. The course of the oxidation was monitored by GLC analysis of $CHCl_3$ extracts of small aliquots. When the reaction was complete (2–3 days), the pH was raised to 12 with 15% aqueous NaOH and the mixture continuously extracted for 12 h with $CHCl_3$, mainly to remove traces of unreacted diol. This extract was eventually discarded. The aqueous mixture was then acidified to pH 3 with dilute hydrochloric acid and reextracted continuously with $CHCl_3$ for 24 h. The $CHCl_3$ extract was dried ($MgSO_4$) and rotoevaporated and the residue Kugelrohr distilled to give (+)-(1*S*,6*R*)-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**18**, 1.6 g, 80% yield, 100% ee): bp 86 °C (2 mmHg) (lit.[38] (±) bp 72–77 °C (0.5 mmHg)); $[\alpha]^{25}_D$ +48.8° (*c* 0.5, $CHCl_3$); IR 1770 cm^{-1}; ^1H NMR δ 0.7–2.9 (10 H, m), 3.8–4.5 (2 H, m).

The oxidations of the other meso diols **3–7** were carried out by similar experimental procedures, as follows:

Oxidation of Meso Diol 3 to the (−)-(1*S*,6*R*)-Lactone 19. *cis*-4,5-Bis(hydroxymethyl)cyclohexene (**3**, 2 g, 13.9 mmol), NAD^+ (720 mg, 1.1 mmol), FMN (9.72 g, 20.3 mmol), and HLADH (40 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 400 mL) at 20 °C for 2 days gave (−)-(1*S*,6*R*)-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**19**, 1.65 g, 83% yield, 100% ee): bp 90 °C (0.15 mmHg) (lit.[38] (±) bp 85 °C (0.1 mmHg)); $[\alpha]^{25}_D$ −67.1 °C (*c* 1, $CHCl_3$), −71.0° (*c* 0.9, MeOH); IR 1770 cm^{-1}; ^1H NMR δ 1.9–3.0 (6 H, m), 3.95–4.5 (2 H, m) 5.75 (2 H, m).

Oxidation of Meso Diol 4 to the (+)-(1*S*,5*R*)-Lactone 20. *cis*-1,2-Bis(hydroxymethyl)cyclopentane (**4**, 1.95 g, 15 mmol), NAD^+ (720 mg, 1.1 mmol), FMN (9.72 g, 20.3 mmol), and HLADH (50 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 600 mL) at 20 °C for 3 days gave (+)-(1*S*,5*R*)-*cis*-3-oxabicyclo[3.3.0]octan-2-one (**20**, 1.43 g, 72% yield, 100% ee): bp 60 °C (0.25 mmHg) (lit.[52] (±) bp 127–128 °C (19 mmHg)); $[\alpha]^{25}_D$ +96.9° (*c* 1, $CHCl_3$); IR 1775 cm^{-1}; ^1H NMR δ 1.3–2.3 (6 H, m), 2.8–3.2 (2 H, m), 3.9–4.7 (2 H, m).

Oxidation of Meso Diol 5 to the (+)-(1*S*,5*R*)-Lactone 21. *cis*-1,2-Bis(hydroxymethyl)cyclobutane (**5**, 2.0 g, 17.2 mmol), NAD^+ (720 mg, 1.1 mmol), FMN (9.7 g, 20.2 mmol), and HLADH (40 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 600 mL) at 20 °C for 4 days yielded (+)-(1*S*,5*R*)-*cis*-3-oxabicyclo[3.2.0]heptan-2-one (**21**, 1.75 g, 90% yield, 100% ee): bp 100 °C (10 mmHg) (lit.[46] (±) bp 109–113 °C (15 mmHg)); $[\alpha]^{25}_D$ +118.7° (*c* 10, $CHCl_3$); IR 1770 cm^{-1}; ^1H NMR δ 1.80–2.80 (4 H, m), 2.85–3.50 (2 H, m), 4.0–4.5 (2 H, m).

Oxidation of Meso Diol 6 to the (−)-(1*S*,5*R*)-Lactone 22. *cis*-1,2-Bis(hydroxymethyl)cyclopropane (**6**, 1.0 g, 9.8 mmol), NAD^+ (720 mg, 1.1 mmol), FMN (9.72 g, 20.3 mmol), and HLADH (35 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 400 mL) at 20 °C for 5 days afforded (−)-(1*S*,5*S*)-*cis*-3-oxabicyclo[3.1.0]hexan-2-one (**22**, 0.66 g, 68% yield, 100% ee): bp 100 °C (10 mmHg) (lit.[42] (±) bp 55–65 °C (1 mmHg)); $[\alpha]^{25}_D$ −61.8° (*c* 6, $CHCl_3$); IR 1770 cm^{-1}; ^1H NMR δ 0.90 (1 H, m), 1.38 (1 H, m), 2.20 (2 H, m), 4.30 (2 H, m).

Oxidation of Meso Diol 7 to the (−)-(1*R*,5*S*)-Lactone 23. *cis*-3,3-Dimethyl-1,2-bis(hydroxymethyl)cyclopropane (**7**, 2.0 g, 15.4 mmol), NAD^+ (720 mg, 1.1 mmol), FMN (9.72 g, 20.3 mmol), and HLADH (35 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 300 mL) at 20 °C for 5 days gave (−)-(1*R*,5*S*)-*cis*-6,6-dimethyl-3-oxabicyclo[3.1.0]hexan-2-one (**23**, 1.38 g, 71% yield, 100% ee): bp 100 °C (10 mmHg) ((±) bp 100 °C (10 mmHg); see above); $[\alpha]^{25}_D$ −36.6° (*c* 1.4, $CHCl_3$); spectral properties were identical with those of (±)-**23**[35] described above in preparation of **7**.

Preparative-Scale HLADH-Catalyzed Oxidations of Trans Diols 9–14. Oxidation of Trans Diol (±)-9. *trans*-1,2-Bis(hydroxymethyl)cyclohexane ((±)-**9**, 900 mg, 6.3 mmol), NAD^+ (144 mg, 0.2 mmol), and FMN (2.0 g, 4.1 mmol) were dissolved in 0.1 M glycine–NaOH buffer (pH 9.0, 300 mL) in a 1-L Erlenmeyer flask. The pH was readjusted to 9 with 15% aqueous NaOH and HLADH (35 mg) added. The mixture was kept at 20–25 °C, with periodic adjustment of the pH to 9. The color turned from clear orange to opaque near-black as the oxidation proceeded. The course of reaction was monitored by GLC analysis of chloroform extracts of small aliquots. After ~50% reaction (2 days), the mixture was saturated with NaCl and continuously extracted with $CHCl_3$ for 12 h. The $CHCl_3$ extract was dried ($MgSO_4$) and rotoevaporated. The residue (a mixture of the hydroxy aldehyde, hemiacetal, and starting diol) was dissolved in ethanol–water (1:5, 25 mL) and $AgNO_3$ (1.02 g, 6 mmol), after which NaOH (3.5 g, 12.5 mmol) was added. The mixture was stirred for 12 h at 20 °C and filtered, and the ~pH 12 aqueous layer was continuously extracted for 12 h (*basic extract*). The aqueous layer was acidified to pH 3 with 6 N hydrochloric acid and reextracted continuously with $CHCl_3$ (*acidic extract*). The dried ($MgSO_4$) *basic extract* was evaporated, Kugelrohr distilled, and column chromatographed (silica gel, ether elution) to give the (−)-(1-*S*,2*S*)-**9** (330 mg, 37% yield, 16% ee): mp 60–62 °C, $[\alpha]^{25}_D$ −3.7° (*c* 3.3, C_6H_6) (lit.[15] (+) mp 60–63 °C, $[\alpha]^{17}_D$ +22.2° (*c* 3.4, C_6H_6)); IR 3400–3600 cm^{-1}; ^1H NMR δ 0.9–2.0 (1 H, m), 3.6 (2 H, br s), 3.6–4.3 (4 H, m).

Evaporation of the dried ($MgSO_4$) *acidic extract* gave a mixture (293 7g, 30% yield) of trans hydroxy acid **24** and its lactone. This was reduced directly with $LiAlH_4$ (100 mg, 2.6 mmol) in ether (50 mL) for 12 h to give, after usual workup, the (+)-(1*R*,2*R*) trans diol **9** (230 mg, 26% yield, 8% ee): mp 58 °C; $[\alpha]^{25}_D$ +1.9° (*c* 2.3, C_6H_6); spectral properties as for (−)-**9** above.

The other trans diol oxidations were carried out in a parallel manner. The results are summarized below, and in Table III.

Oxidation of *trans*-4,5-bis(hydroxymethyl)cyclohexene ((±)-10) (1.18 g, 8.3 mmol) for 2 days gave recovered diol (−)-(4*R*,5*R*)-**10** (367 mg, 31% yield, 0.7% ee): $[\alpha]^{25}_D$ −0.5° (*c* 3.7, $CHCl_3$) (lit.[16] $[\alpha]^{22}_D$ −70.4° (*c* 3, $CHCl_3$)); ^1H NMR δ 1.5–2.15 (6 H, m), 3.3–3.9 (4 H, m), 4.2 (2 H, s), 5.65 (2 H, m) and a mixture of trans hydroxy acid **25** and its lactone,[56] which gave the racemic diol (±)-**9** on reduction with $LiAlH_4$.

Oxidation of *trans*-1,2-bis(hydroxymethyl)cyclopentane ((±)-**11**) (700 mg, 5.4 mmol) for 8 h yielded starting diol (1*R*,2*R*)-**11** (400 mg, 57% yield), which was treated directly with toluene-*p*-sulfonyl chloride in pyridine to give the ditosylate (−)-(1*R*,2*R*)-**34** (950 mg, 67% yield, 4% ee): mp 66–67 °C; $[\alpha]^{25}_D$ −1.3° (*c* 0.1, EtOH) (lit.[21] mp 62–64 °C, $[\alpha]^{25}_D$ −31.9° (EtOH)); ^1H NMR δ 1.20–2.20 (8 H, m), 2.58 (6 H, s), 3.98 (4 H, m), 7.38 (4 H, d, *J* = 8 Hz), 7.80 (4 H, d, *J* = 8 Hz). The acidic extract gave the *trans*-hydroxy acid (1*S*,2*S*)-**31** (200 7g, 23% yield), characterized after reduction and tosylation as the ditosylate (+)-(1*S*,2*S*)-**34** (247 mg, 43% yield, 2% ee): mp 65–67 °C, $[\alpha]^{25}_D$ + 0.54° (*c* 1, EtOH).

Oxidation of *trans*-1,2-bis(hydroxymethyl)cyclobutane ((±)-**12**) (700 mg, 6 mmol) for 8 h yielded recovered diol (−)-(1*R*,2*R*)-**12** (276 mg, 39% yield, 4% ee): bp 100 °C (1 mmHg) (lit.[41] (±) bp 111–114 °C (3 mmHg), lit.[18] (−) bp 90 °C (0.2 mmHg)); $[\alpha]^{25}_D$ −0.15° (*c* 2.8, EtOH) (lit.[18] $[\alpha]^{25}_D$ −4.3° (*c* 0.85, EtOH)); IR 3390 cm^{-1}; ^1H NMR δ 1.00–2.58 (6 H, m), 3.60 (4 H, m), 3.90 (2 H, broad s). The acidic extract gave *trans*-2-hydroxymethylcyclobutanoic acid ((1*S*,2*S*)-**27**, 200 mg, 28% yield), characterized as the trans diol (+)-(1*S*,2*S*)-**12** (178 mg, 87% yield, 2% ee), $[\alpha]^{25}_D$ +0.07° (*c* 0.9, EtOH).

Oxidation of *trans*-1,2-bis(hydroxymethyl)cyclopropane ((±)-**13**) (600 mg, 5.9 mmol) for 8 h produced recovered diol **13** (187 mg, 31% yield): bp 100 °C (1 mmHg) (lit.[50] (±) bp 95–103 °C (1 mmHg)); IR 3390 cm^{-1}; ^1H NMR δ 0.40 (2 H, t, *J* = 5 Hz), 0.64–1.32 (2 H, m), 3.10 (2 H, t, *J* = 10 Hz), 3.58–4.20 (2 H, m), 4.60 (2 H, broad s). This was treated with acetic anhydride in pyridine to give the diacetate (−)-(1*R*,2*R*)-**35** (188 mg, 55% yield, 2% ee): bp 90 °C (10 mmHg), $[\alpha]^{25}_D$ −0.37° (*c* 1.45, EtOH) (lit.[22] bp 130–132 °C (25 mmHg), $[\alpha]^{25}_D$ −17.75° (*c* 2, EtOH)); IR 1736 cm^{-1}; ^1H NMR δ 0.60 (2 H, m), 1.00 (2 H, m), 2.00 (6 H, s), 3.88 (2 H, d, *J* = 6 Hz). The pH 3 extract gave *trans*-2-(hydroxymethyl)cyclopropanoic acid (**31**, 300 mg, 44% yield): ^1H NMR δ 0.80–2.00 (4 H, m), 3.4–4.0 (2 H, m), 6.24 (2 H, s). This was reduced with $LiAlH_4$ and then acetylated as above to give the diacetate (+)-(1*S*,2*S*)-**35** (270 mg, 64% yield, 2% ee), $[\alpha]^{25}_D$ +0.31° (*c* 2.35, EtOH).

Oxidation of *trans*-1,2-bis(hydroxymethyl)-3,3-dimethylcyclopropane ((±)-**14**) (1.0 g, 7.7 mmol) for 4 h gave recovered diol **14** (195 mg, 20% yield): bp 140 °C (35 mmHg) (lit.[36] (±) bp 123–124 °C (8 mmHg)); IR 3378 cm^{-1}; ^1H NMR δ 0.80 (2 H, m), 1.10 (6 H, s), 2.84 (2 H, br s), 3.30–3.98 (2 H, m). This was oxidized with pyridinium chlorochromate in pyridine to (−)-(1*R*,2*R*)-*trans*-caronic acid (**36**, 88 mg, 48% yield, 1% ee): $[\alpha]^{25}_D$ −0.34° (*c* 0.88, EtOH) (lit.[23] $[\alpha]^{25}_D$ −32.96° (EtOH)); IR 3704–2500, 1712 cm^{-1}; ^1H NMR δ 1.30 (6 H, s), 2.18 (2 H, s), 10.13 (2 H, br s). The acidic extract yielded *trans*-3,3-dimethyl-2-hydroxymethylcyclopropanoic acid (630 mg, 57% yield), which was oxidized without purification with pyridinium chlorochromate followed by silver oxide to (+)-(1*S*,2*S*)-*trans*-caronic acid (**36**, 428 mg, 62% yield, 0.6% ee), $[\alpha]^{25}_D$ + 0.21° (*c* 0.97, EtOH).

Enantiomeric Excess Determinations. The ee's of the cis lactones **18–23** were determined by reacting each with methyllithium and examining of the ^1H NMR spectra of the diastereotopic methyl peaks of the resulting diols in the presence of 0.1–0.4 equiv of $Eu(tfc)_3$.[14] ΔΔδ sep-

(56) Partial epimerization to the (±)-*cis*-lactone **19** was also detected.

arations of 0.12–0.3 ppm were observed with the reference diols obtained from racemic cis lactones. Only one enantiomer was detectable when the HLADH-derived cis lactones were used (Table II). The optical purities of the products of trans diols **9–14** HLADH-catalyzed oxidations were too low to justify extensive efforts to determine the ee's by direct methods. The ee levels cited (Table III) are based on optical rotation data.

Absolute Configuration Determinations. (A) The correlations for the cis lactones **18–23** are summarized in Scheme II.

(+)-(1*S*,2*R*)-*cis*-3-Oxabicyclo[4.3.0]nonan-2-one (18). The (+)-(1*S*,2*R*)-lactone **18** (1.49 g, 10.6 mmol) was placed in a 12 × 2.5 cm stainless steel tube fitted with a Teflon-lined screw cap together with NaOH (4.24 g, 106 mmol) in water (20 mL). The sealed vessel was heated at 130 °C for 8 days and the cooled reaction mixture then neutralized with saturated aqueous oxalic acid and continuously extracted with $CHCl_3$ for 24 h. The dried ($MgSO_4$) extract was evaporated and the residue recrystallized from ether to give (−)-(1*R*,2*R*)-*trans*-2-(hydroxymethyl)cyclohexanoic acid (**24**, 578 mg, 34% yield): mp 106.5–107.5 °C (lit.[57] (±)mp 106–109 °C); $[\alpha]^{25}_D$ −50.0° (*c* 0.54, MeOH); IR (KBr) 3600, 3400–2500, 1715 cm^{-1}; ^1H NMR δ 0.95–1.2 (10 H, m), 3.65 (2 H, br d), 6.9 (2 H, br s)). This hydroxy acid (578 mg, 3.6 mmol) was dissolved in dry ether (50 mL) and $LiAlH_4$ (417 mg, 10.9 mmol) in dry ether (100 mL) added dropwise with stirring. The mixture was then refluxed for 12 h and the cooled reaction mixture quenched with aqueous THF (1:1, 100 mL). The resulting mixture was continuously extracted with $CHCl_3$ for 24 h and the $CHCl_3$ solution dried ($MgSO_4$) and evaporated. Recrystallization of the product from benzene–hexane gave (+)-(1*R*,2*R*)-*trans*-1,2-bis(hydroxymethyl)cyclohexane (**9**, 405 mg, 80% yield): mp 62–63 °C; $[\alpha]^{17}_D$ +22.1° (*c* 1.7, C_6H_6) (lit.[15] mp 60–63 °C, $[\alpha]^{17}_D$ +22.2° (*c* 3.89, C_6H_6); spectral properties were identical with those of **9** above.

(−)-(1*S*,6*R*)-*cis*-3-Oxabicyclo[4.3.0]non-7-en-2-one (19). (a) The (−)-(1*S*,6*R*) lactone **19** (1.20 g, 8.7 mmol) was epimerized with NaOH (3.5 g, 87 mmol) in water (20 mL) as described above for (+)-**18**. The material isolated (1.05 g) was a mixture of the trans hydroxy acid (1*R*,2*R*)-**25** and its lactone; it was reduced directly in dry ether (50 mL) with $LiAlH_4$ (1.78 g, 46.8 mmol) in dry ether (100 mL) under reflux for 12 h and then worked up as described above. Distillation of the product gave (−)-(4*R*,5*R*)-*trans*-4,5-bis(hydroxymethyl)cyclohexene (**10**, 600 mg, 49% overall yield): bp 148 °C (2 mmHg); $[\alpha]^{22}_D$ −55.5° (*c* 0.38, $CHCl_3$) (lit.[16] bp 101–102 °C (0.2 mmHg), $[\alpha]^{22}_D$ −70.4° (*c* 3, $CHCl_3$)); spectral properties were identical with those of **10** above.

(b) Hydrogenation over 10% Pd/C of (−)-**19** (275 mg, 2 mmol) in methanol (50 mL) gave (+)-(1*S*,6*R*)-**18** (237 mg, 86% yield), $[\alpha]^{25}_D$ + 38.8° (*c* 2.37, $CHCl_3$).

(+)-(1*S*,2*R*)-*cis*-3-Oxabicyclo[3.3.0]octan-2-one (20). A solution of (+)-(1*S*,2*R*)-lactone **20** (1.0 g, 7.1 mmol) in dry methanol (10 mL) was saturated with dry HBr at 0 °C and the solution then stirred for 12 h at 20 °C. Saturated aqueous NaCl (50 mL) was then added and the mixture extracted with ether to give methyl (+)-(1*S*,2*R*)-*cis*-2-(bromomethyl)cyclopentanoate (920 mg, 63% yield): bp 100 °C (5 mmHg); IR 1735 cm^{-1}; ^1H NMR δ 1.4–4.6 (10 H, m), 3.7 (3 H, s). Anal. ($C_8H_{13}O_2Br$) C, H. The above bromo ester (900 mg, 4.1 mmol) and tri-*n*-butyltin hydride (1.45 g, 5 mmol) in dry benzene (30 mL) was stirred under N_2 at 20 °C for 12 h and then refluxed for 4 h. The benzene was then distilled off carefully and the residue distilled to give methyl (+)-(1*S*,2*R*)-*cis*-2-methylcyclopentanoate (422 mg, 72% yield): bp 80 °C (28 mmHg); $[\alpha]^{25}_D$ +21.8° (*c* 1, $CHCl_3$); ^1H NMR δ 0.9 (3 H, d, *J* = 6 Hz), 1.1–2.0 (8 H, m), 3.65 (3 H, s). Anal. ($C_8H_{14}O_2$) C, H. This methyl ester (400 mg, 2.8 mmol) in methanolic sodium methoxide (1% solution, 15 mL) was heated at 80° C for 8 h. Aqueous acetic acid (1% solution, 30 mL) was then added to the cooled reaction mixture. Extraction with ether yielded a 1:1 mixture of the cis and trans esters (280 mg, 70% yield), bp 98 °C (25 mmHg), from which GLC purification[17] gave a sample of methyl (−)-(1*R*,2*R*)-2-methylcyclopentanoate: $[\alpha]^{25}_D$ −46.2° (*c* 1, CCl_4) (lit.[17] $[\alpha]^{25}_D$ −57.5° (*c* 0.2, CCl_4)); IR 1736 cm^{-1}; ^1H NMR δ 1.1 (3 H, d, *J* = 5 Hz), 1.2–2.1 (8 H, m), 3.65 (3 H, s).

(+)-(1*S*,2*R*)-*cis*-3-Oxabicyclo[3.2.0]heptan-2-one (21). The lactone **21** (500 mg, 5.3 mmol) was isomerized with NaOH (3.1 g, 7.8 mmol) in water (10 mL) as described for **18** above to give (−)-(1*R*,2*R*)-*trans*-2-(hydroxymethyl)cyclobutanoic acid (**27**, 360 mg, 52% yield) as a viscous oil: $[\alpha]^{25}_D$ −32.4° (*c* 0.55, $CHCl_3$); IR 3704–2500, 1712 cm^{-1}; ^1H NMR δ 1.44–2.20 (4 H, m), 2.40–3.24 (2 H, m), 3.40–3.80 (2 H, m), 6.78 (2 H, s). This was reduced without purification with $LiAlH_4$ in the usual way to yield (−)-(1*R*,2*R*)-*trans*-1,2-bis(hydroxymethyl)cyclobutane (**12**, 308 mg, 97% yield): bp 100 °C (1 mmHg); $[\alpha]^{25}_D$ −4.8° (*c* 0.38, EtOH) (lit.[18] bp 90 °C (0.2 mmHg), $[\alpha]^{25}_D$ −4.3° (*c* 0.85, EtOH)); spectral properties were identical with those of **12** above.

(−)-(1*R*,2*S*)-*cis*-6,6-Dimethyl-3-oxabicyclo[3.1.0]hexanon-2-one (23). The (−)-lactone **23** (500 mg, 3.97 mmol) was treated with KOH (335 mg, 5.95 mmol) in methanol (3 mL), followed by ethereal diazomethane, according to the method developed by Krief et al.[25] for the racemic compounds. The unpurified methyl (−)-(1*R*,3*S*)-*cis*-2,2-dimethyl-3-(hydroxymethyl)cyclopropanoate (363 mg, 58% yield) obtained had $[\alpha]^{25}_D$ −15.1° (*c* 3.6, $CHCl_3$); IR 1736 cm^{-1}; ^1H NMR δ 1.2 (6 H, d, *J* = 1.5 Hz), 1.30–1.70 (2 H, m), 2.40 (1 H, s), 3.60 (3 H, s), 3.90 (2 H, d, *J* = 7 Hz). This hydroxy ester (360 mg, 2.3 mmol) was oxidized with pyridinium chlorochromate[58] to give methyl (−)-(1*R*,3*S*)-2,2-dimethyl-3-formylcyclopropanoate (316 mg, 89% yield): $[\alpha]^{25}_D$ −23.1° (*c* 3.2, $CHCl_3$); IR 1736, 1706 cm^{-1}; ^1H NMR δ 1.22 (3 H, s), 1.68 (3 H, s), 1.60–2.20 (2 H, m), 3.62 (3 H, s), 9.10 (1 H, d, *J* = 6 Hz). Without further purification, this aldehyde ester (178 mg, 1.8 mmol) was treated with triphenylphosphonium isopropylide (2.7 mmol) according to the method of Krief and co-workers[25] to afford methyl (+)-(1*R*,2*S*)-*cis*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanoate (methyl (+)-*cis*-chrysanthemate (**28**), 150 mg, 46% yield): bp 90 °C (10 mmHg); $[\alpha]^{25}_D$ +27.8° (*c* 0.67, $CHCl_3$) (lit.[59] (1*S*,2*R*)bp 85 °C (10 mmHg), $[\alpha]^{25}_D$ −30° (*c* 1.5, $CHCl_3$); IR 1736 cm^{-1}; ^1H NMR[19,26b] 1.20 (6 H, d, *J* = 1.5 Hz), 1.30–1.70 (2 H, m), 2.40 (1 H, s), 3.60 (3 H, s), 3.90 (2 H, d, *J* = 7 Hz).

(−)-(1*S*,2*R*)-*cis*-3-oxabicyclo[3.1.0]hexan-2-one (22). The absolute configuration of (−)-(1*S*,2*R*)-**22** was assigned by sector rule[60] analysis and comparison[61] of its CD spectrum, $[\theta]_{250}$ 0, $[\theta]_{217}$ −22342 (*c* 0.0111 M, MeOH, 20 °C), with that of (−)-(1*R*,2*S*)-**23** assigned chemically above, $[\theta]_{250}$ 0, $[\theta]_{218}$ −7168 (*c* 0.0113 M, MeOH, 20 °C).

(B) The absolute configurations of the trans diols **9–14** were assigned by using the trans diol data of Scheme II together with the correlations summarized in Scheme IV. Full details have already been included in the experimental descriptions of the individual enzyme-mediated trans-diol oxidations.

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Registry No. 1, 1792-81-0; **2**, 15753-50-1; **3**, 20141-17-7; **4**, 75658-83-2; **5**, 54445-65-6; **6**, 2345-68-8; **7**, 67528-55-6; (±)-**8**, 54383-22-1; (±)-**9**, 76155-27-6; (−)-(1*S*,2*S*)-**9**, 3205-34-3; (+)-(1*R*,2*R*)-**9**, 65376-05-8; (±)-**10**, 56084-96-6; (−)-(4*R*,5*R*)-**10**, 15679-28-4; (±)-**11**, 82442-56-6; (−)-(1*R*,2*R*)-**11**, 57287-24-8; (±)-**12**, 82442-57-7; (−)-(1*R*,2*R*)-**12**, 55659-54-6; (−)-(1*S*,2*S*)-**12**, 82442-58-8; (±)-**13**, 82442-59-9; (−)-(1*R*,2*R*)-**13**, 52745-75-2; (±)-**14**, 82442-60-2; (−)-(1*R*,2*R*)-**14**, 61228-54-4; (±)-**18**, 82390-70-3; (+)-(1*S*,6*R*)-**18**, 82442-61-3; (±)-**19**, 82391-71-4; (−)-(1*S*,6*R*)-**19**, 82442-62-4; (±)-**20**, 82442-63-5; (+)-(1*S*,5*R*)-**20**, 75658-84-3; (±)-**21**, 82442-64-6; (±)-**22**, 82442-65-7; (±)-**23**, 62222-77-9; (−)-(1*R*,2*R*)-**24**, 65376-04-7; (−)-(1*R*,2*R*)-**27**, 82390-72-5; (+)-(1*R*,2*S*)-**28**, 55701-01-4; (−)-(1*R*,2*R*)-**34** ditosylate, 36405-94-4; (+)-(1S,2*S*)-**34** ditosylate, 70723-28-3; (−)-(1*R*,2*R*)-**35**, 25144-34-7; (+)-(1*S*,2*S*)-**35**, 53166-30-6; (−)-(1*R*,2*R*)-**36**, 1701-82-2; (+)-(1*S*,2*S*)-**36**, 3966-58-3; (±)-**37**, 82442-66-8; (±)-**38**, 82390-73-6; (±)-**39**, 82442-67-9; (±)-**40**, 64149-65-1; methyl (+)-(1*S*,2*R*)-*cis*-2-(bromomethyl)cyclopentanoate, 82390-74-7; methyl (+)-(1*S*,2*R*)-cis-2-methylcyclopentanoate, 82442-68-0; methyl (−)-(1*R*,2*R*)-2-methylcyclopentanoate cyclopentanoate, 13012-50-5; methyl (-1-((1*R*,3*S*)-*cis*-2,2-dimethyl-3-hydroxymethylcyclopropanoate, 82442-69-1; methyl (−)-(1*R*,2*S*)-2,2-dimethyl-3-formylcyclopropanoate, 55701-075-5; cyclohexanol, 108-93-0; 4-oxacyclopenten-3-one, 22929-52-8; *cis*-cyclobutane-1,2-dioic acid anhydride, 4462-96-8; dimethyl *cis*-cyclopropanedioate, 826-34-6; dimethyl (±)-*trans*-cyclobutane-1,2-dicarboxylate, 82442-70-4; diethyl 3,3-dimethyl-(±)-*trans*-1,2-cyclopropandioate, 67776-64-1; (±)-*trans*-cyclohexane-1,2-dicarboxylic acid, 610-10-6; *cis*-cyclohexane-1,2-dioic acid anhydride, 13149-00-3; 3,3-dimethyl-2-hydroxymethylcyclopropanoic acid, 82442-71-5; alcohol dehydrogenase, 9031-72-5; (+)-(1*S*,5*R*)-**21**, 75658-85-4; (−)-(1*S*,5*R*)-**22**, 75658-86-5; (−)-(1*R*,5*S*)-**23**, 82442-72-6.

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