= 17.6, 8.8, 6.0 Hz), 2.41 (ddd, 1 H, J = 8.8, 8.6, 6.6 Hz), 2.36 (m, 1 H), 2.24–2.10 (m, 3 H), 2.10 (s, 3 H, $COCH_3$), 1.93 (ddd, 1 H, J = 14.1, 8.3, 6.0 Hz), 1.84 (ddd, 1 H, J = 7.1, 4.4, 2.7 Hz), 1.75 (tt, 1 H, J = 13.1, 3.8 Hz), 1.50 (dddd, 1 H, J = 14.1, 8.6, 6.6, 4.7 Hz), 1.36 (m, 2 H). IR (CH_2Cl_2): 3000 (broad —OH), 2940, 2870, 1710 cm^{-1}. EIMS m/e (rel intensity): 226 (4), 208 (50). HRMS calcd for $C_{12}H_{18}O_4$ 226.120, found 226.120. Anal. Calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found: C, 63.69; H, 8.17.

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Registry No. **2**, 40958-79-0; **3**, 119297-21-1; (±)-(*E*)-**4**, 119297-22-2; (±)-(*Z*)-**4**, 119364-69-1; (±)-**5** (isomer 1), 119297-23-3; (±)-**5** (isomer 2), 119364-70-4; (±)-**5** (isomer 3), 119364-71-5; (±)-**5** (isomer 4), 119364-72-6; (±)-**6** (isomer 1), 119297-24-4; (±)-**6** (isomer 2), 119364-73-7; (±)-**6** (isomer 3), 119364-75-9; (±)-**6** (isomer 4), 119364-76-0; (±)-(*E*)-**7**, 119297-25-5; (±)-(*Z*)-**7**, 119364-74-8; (±)-**8**, 119297-26-6; (±)-**9**, 119297-27-7; (±)-**10**, 119297-28-8; $CH_2(TMS)_2$, 2117-28-4; $(Ph)_2POCH(CH_3)(OMe)$, 64304-77-4; pyrrolidinocyclohexene, 1125-99-1; *cis*-1,4-dichloro-2-butene, 1476-11-5; methyl 2-[3-[3-(diphenylphosphinyl)-2-hydroxy-3-methoxybutyl]-2-[(trimethylsilyl)methylene]cyclohexyl]acetate, 119297-29-9.

Enzymes in Organic Synthesis. 46.[1] Regiospecific and Stereoselective Horse Liver Alcohol Dehydrogenase Catalyzed Reductions of *cis*- and *trans*-Bicyclo[4.3.0]nonanones

Andrzej R. Krawczyk[†] and J. Bryan Jones*[,‡]

Departments of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1, and Warsaw University, 02093 Warsaw, Poland

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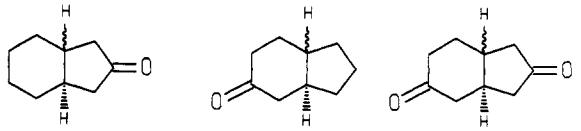
Further evidence for the preference of horse liver alcohol dehydrogenase for six-membered rather than five-membered ring ketone substrates is presented. This chemospecificity can be exploited preparatively, as illustrated by the HLADH-catalyzed reductions of (±)-*cis*- and -*trans*-bicyclo[4.3.0]nonane-3,8-diones. In these reactions, the chemospecificity of the enzyme is accompanied by diastereotopic face specificity and enantiomeric selectivity, resulting in good yields of keto alcohol products and recovered diketones of up to 94% enantiomeric excess. The results are fully in accord with the predictions of the cubic space-section model of the enzyme's active site. Enzyme-controlled combinations of specificity of this type have considerable asymmetric synthetic potential and cannot yet be duplicated in a single-step reaction by nonenzymic catalysts.

In recent years, the versatility of enzymes as chiral catalysts for organic synthesis has been widely recognized.[2] Among the major advantages of enzymes as catalysts is their ability to combine several different specificities in a single catalytic step reaction. In this paper, we report on some horse liver alcohol dehydrogenase (HLADH[3]) catalyzed reductions in which regiospecificity, enantioselectivity, and stereoheterotopic selectivity are controlled concurrently.

HLADH is a commercially available, NAD/H-coenzyme dependent, enzyme that catalyzes a broad range of CH(OH) ⇌ C=O oxidoreductions.[1,2,4] The ability of HLADH to combine regio- and stereospecificity in its catalyses has previously been demonstrated for diol oxidations.[5] In order to ascertain if similar discriminations could be exploited in the reduction mode, we have investigated the HLADH-catalyzed reductions of the *cis*- and *trans*-bicyclo[4.3.0]nonane mono- and diketones (±)-**1**–**3a,b**.



(±)-1a,b (±)-2a,b (±)-3a,b

a, C-6α-H, *cis*-series
b, C-6β-H, *trans*-series

[†] Warsaw University.
[‡] University of Toronto.

Results

Preparations of Substrates. The cis substrates (±)-**1**–**3a** were prepared from *cis*-bicyclo[4.3.0]non-3-en-8-one (**4a**),[6] and the trans isomers (±)-**1**–**3b** from *trans*-4,5-bis(hydroxymethyl)cyclohexene[6] as shown in Scheme I.

HLADH-Catalyzed Reductions. The rates of HLADH-catalyzed reductions of (±)-**1**–**3a,b** relative to that of the standard reference substrate cyclohexanone under the same conditions are recorded in Table I, together with the results of the preparative-scale reduction experiments, which were carried out on (±)-**2,3a,b** with ≈1 g of substrate

(1) Part 45. Bur, D.; Luyten, M. A.; Wynn, H.; Provencher, L. R.; Gold, M.; Friesen, J. D.; Clarke, A. R.; Holbrook, J. J.; Jones, J. B. *Can. J. Chem.*, submitted for publication.

(2) (a) Jones, J. B.; Beck, J. F. *Tech. Chem. (NY)* **1976**, *10*, 107. Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (b) Wong, C. H.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. Akiyama, A.; Bednarski, M.; Kim, M. J.; Simon, E. S.; Waldmann, H.; Whitesides, G. M. *Chem. Br.* **1987**, *23*, 645. (c) Klibanov, A. M. *Science* **1983**, *219*, 722; *Chem. Tech.* **1986**, 354. (d) *Enzymes in Organic Synthesis*; Ciba Foundation Symposium 111; Porter, R., Clark, S., Eds.; Pitman: London, 1985. (e) *Biocatalysis in Organic Synthesis*; Tramper, J., von der Plas, H. C., Linko, P., Eds.; Elsevier: Amsterdam, 1985. (f) *Enzymes as Catalysts in Organic Synthesis*; Schneider, M., Ed.; Reidel: Dordrecht, Netherlands, 1986.

(3) Abbreviations used: HLADH, horse liver alcohol dehydrogenase; NAD/H, oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide; MTPA, (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl; $Eu(fod)_3$, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate)europium (III).

(4) Dodds, D. R.; Jones, J. B. *J. Am. Chem. Soc.* **1988**, *110*, 577.

(5) Irwin, A. J.; Jones, J. B. *J. Am. Chem. Soc.* **1977**, *99*, 1625.

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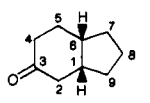
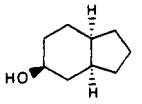
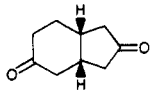
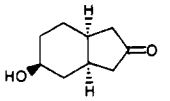
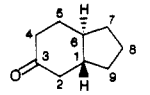
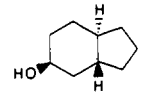
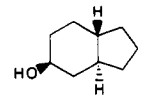
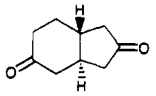
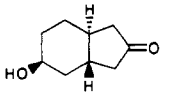
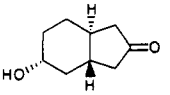
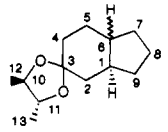
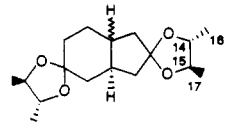
0022-3263/89/1954-1795$01.50/0

Table I. HLADH-Catalyzed Reductions of (±)-1-3ab[a]

substrate (rel rate[b])	products [% yield,[c] % ee[d]]		
(±)-**1a** (11)	–		–
(±)-**2a** (227)	 (+)-(1*R*,6*S*)-**2a** [40, 20]		 (−)-(1*S*,3*S*,6*R*)-**5a** [37, 12]
(±)-**3a** (130)	 (−)-(1*S*,6*R*)-**3a** [25, 69]		 (+)-(1*R*,3*S*,6*S*)-**8a** [55, 60]
(±)-**1b** (2)	–		–
(±)-**2b** (190)	 (−)-(1*R*,6*R*)-**2b** [40, 11]	 (−)-(1*R*,3*S*,6*R*)-**6b** [17, 51]	 (−)-(1*S*,3*S*,6*S*)-**5b** [16, 94]
(±)-**3b** (120)	 (−)-(1*R*,6*R*)-**3b** [47, 76[e]]	 (+)-(1*S*,3*S*,6*S*)-**7b** [42, 94]	 (1*S*,3*R*,6*S*)-**8b** [trace[f], 95[g]]

[a] At pH 8, 20 °C. [b] Relative to cyclohexanone = 100. Reduction rates were measured spectrophotometrically at 25 °C in aqueous 0.1 M phosphate buffer (pH 7) containing 5% MeOH with [S] = 2×10^{-2} M and [NADH] = 1.67×10^{-4} M. [c] Isolated yields. [d] Error limits ±3%. [e] ±10%. [f] ≈5% by GLC. [g] Estimated.

Table II. Enantiomeric Shift Differences in the ^{13}C NMR Spectra of the Ketals of (±)-2,3a,b[a]

ketal	C-6 H config	Δδ, ppm C-2	C-3	C-4	C-5	C-6	C-7
 **9**	α			0.56		0.11	
	β	0.40	1.13		0.91	0.53	
 **10**	α	0.66		1.02			0.48
	β	0.37	1.14		1.06	0.38	

[a] ^1H Noise decoupled spectra determined in C^2HCl_3.

and with ethanol as the coupled-substrate for recycling[7,8] of the catalytic amount of NAD-coenzyme used. The course of each reaction was monitored by GLC and the reactions terminated at the ≈50%-of-reduction point.

Enantiomeric Excess Determinations. The ee's of the enzymically derived Table I products were established by NMR. The recovered mono- or diketones **2a,b** and **3a,b** were converted, in quantitative yields, to their corresponding monoketal (**9a,b**) or diketal (**10a,b**) derivatives with (−)-(2*R*,3*R*)-2,3-butanediol, followed by ^{13}C NMR examination.[9] The ketals derived from (±)-**2,3a,b** were used as the reference standards. The ee's of the hydroxy products (−)-**5a** and (+)-**8a** were determined in the same way following their oxidations to (−)-**3a**, respectively, prior to ketal formation. The Δδ values for the diastereotopic carbon atoms used in these ee determinations are recorded in Table II.

Table III. Enantiomeric Shift Differences for Diastereotopic Methoxyl Protons of MTPA Esters of (±)-5,6b[a]

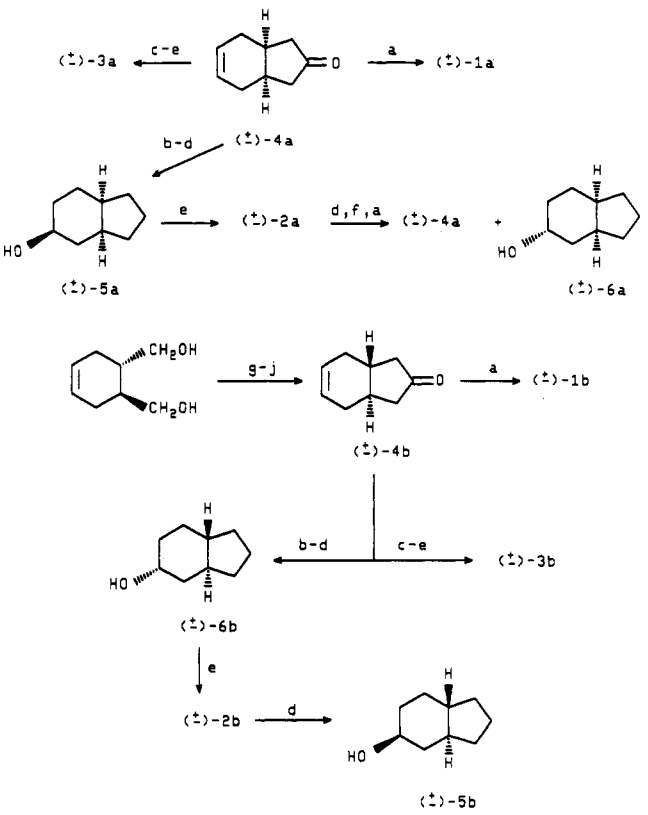
derivative	$Eu(fod)_3$, equiv	(Δ)Δδ of CH_3O, ppm
(±)-**5b**-MTPA	0	0.015
	0.25	0.0
(±)-**6b**-MTPA	0	0.02
	0.23	0.38

[a] ^1H NMR spectra recorded in $[^2H_8]$toluene.

The ee's of the trans product alcohols **5**–**8b** of Table I were established by ^1H NMR inspection[10] of the methoxyl protons of the MTPA derivatives of **5b** and **6b** and of the corresponding alcohols derived from **7b** and **8b** by

(7) Zagalak, B.; Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. *J. Biol. Chem.* **1966**, *241*, 3028.
(8) Davies, J.; Jones, J. B. *J. Am. Chem. Soc.* **1979**, *101*, 5405.
(9) (a) Hiemstra, H.; Wynberg, H. *Tetrahedron Lett.* **1977**, *25*, 2183. (b) Lemiere, G. L.; Demmisse, R. A.; Le Poivre, J. A.; Alderweireldt, F. C.; Hiemstra, H.; Wynberg, H.; Jones, J. B.; Toone, E. J. *J. Am. Chem. Soc.* **1987**, *109*, 1363.
(10) Dale, J.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

Scheme I[a]



[a] Reagents: (a) H_2/Pt; (b) NH_2NH_2, ^-OH; (c) *m*-CPBA; (d) $LiAlH_4$; (e) CrO_3; (f) Na/EtOH; (g) TsCl; (h) NaCN; (i) KOH; (j) $Ba(OH)_2$, Δ.

Wolff–Kishner reduction. The Δδ or (Δ)Δδ values, with and without added Eu(fod)$_3$, respectively, observed for the racemic MTPA esters used as the reference standards are recorded in Table III. ^{1}H NMR examination of the methyl ether derivatives of (−)-**5a,b**, (−)-**6b**, and (+)-**8a** was not found suitable for ee evaluation in this study, although the approach had proven successful in related work.[4]

Relative and Absolute Configuration Determinations. The relative configurations at C-3 of (−)-**5a** and of (+)-**8a** via its reduction to (−)-**5a** were assigned by comparisons of the ^{1}H NMR spectra of the MTPA esters of (−)-**5a** with those prepared from authentic samples of (±)-**5a** and (±)-**6a** of established[11] C-3δ and C-3α configurations, respectively. The relative configurations of the hydroxyl-bearing centers of (−)-**5b** and (−)-**6b**, and of (+)-**7b** and **8b** following their reductions to (−)-**6b** and (+)-**5b**, respectively, were assigned by establishing the axial or equatorial orientations of the C-3 protons by ^{1}H NMR spectroscopy[12,13] with use of the chemical shift values for the Scheme I derived racemic standards (±)-**5b** (axial C-3 H, 3.75 ppm) and (±)-**6b** (equatorial C-3 H, 4.1 ppm). While the axial C-3 proton peak of **5b** has the usual broader shape than the less highly split equatorial C-3 H of **6b**, their chemical shift values represent another example of a reversal[12b] of the normal[12a] axial-higher-than-equatorial pattern.

The absolute configuration assignments of the cis compounds of Table I were made by optical rotation comparisons with the known,[14] closely analogous, (+)-(1*R*,6*S*)-6-methyl derivative of **2a**, which correlated (+)-**2a** with (+)-(1*R*,6*R*)-methyl-**2a**, (−)-**5a** with (−)-**2a**, (+)-**8a** with (−)-**5a**, and (−)-**3a** with the (+)-**3a** obtained from the oxidation of (+)-**8a**. In the trans series, the absolute configurations were also designated by direct or indirect optical rotation correlations, in this case with the established[15] (+)-(1*S*,6*S*)-**2b** structure, as follows: (−)-**2b** as the 1*R*,6*R* stereoisomer, (−)-**5b** to (+)-**2b** and (−)-**6b** to (−)-**2b**, (+)-**7b** to (−)-**6b**, **8b** to (−)-**2b**, and (−)-**3b** as the 1*R*,6*R* stereoisomer from the oxidation of (+)-(1*S*,3*S*,6*S*)-**7b** to (+)-**3b**.

Discussion

From the relative rates of HLADH-catalyzed reductions recorded in Table I, it is evident that, in both the cis and trans bicyclic series, a six-membered ring ketone function is reduced far more readily than is a cyclopentanone moiety. This is in total accord with the data for monocyclic cyclohexanone and cyclopentanone substrates.[2a,16] In fact, each of the preparative-scale substrates (±)-**2,3a,b** is transformed into alcohol products even more rapidly than the cyclohexanone standard (Table I). The six-ring ketone preference is due partly to its better fit at the active site[17] and partly to its inherently greater susceptibility to reduction by a hydride equivalent.[16] The preparative-scale reductions of (±)-**2,3a,b** proceeded smoothly, but more slowly than expected from the Table I relative rate measurements, perhaps due to product inhibition.

For the cis compounds, the assignment of the relative configurations at C-3 of the enzymically derived hydroxy compounds (−)-**5a** and (+)-**8a** rested on comparisons with the known C-3β and C-3α epimers (±)-**5a** and -**6a**, respectively. Reference samples of (±)-**5a** were obtained from (±)-**4a** via epoxidation and reduction (Scheme I) or by hydrogenation of (±)-**2a**.[11a,c] For (±)-**6a**, the mixture of **5a** and **6a** (62:38) obtained by Na–EtOH reduction[11a,b] of (±)-**2a** was employed. These C-3 epimers could not be separated by column chromatography nor by GLC, nor were their NMR spectra significantly different. However, the ^{1}H NMR spectra of their MTPA esters were unequivocally diagnostic in the methoxyl ether proton regions. For (±)-**5a**-MTPA and (±)-**6a**-MTPA the chemical shifts of the methoxyl protons, at 3.47 and 3.40 ppm, respectively, were distinctive and permitted and relative hydroxyl configurations shown for (−)-**5a** and (+)-**8a** in Table I to be assigned unambiguously. Only the δ 3.47 ppm peak was observed in the enzyme-derived (−)-**5a**-MTPA spectrum with the absence of the δ 3.40 ppm resonance being verified by the addition of a small sample of (±)-**6a**-MTPA to the NMR tube. The methyl group difference between (+)-**2a** and its (+)-(1*R*,6*S*)-6-methyl analogue[14] used as the basis for the absolute configuration correlations is considered unimportant since its introduction at the C-6 position will induce no change in the

(11) (a) Kimoto, K.; Leong, T.-Y.; Imagawa, T.; Kawanisi, M. *Can. J. Chem.* **1972**, *50*, 3805. (b) Dauben, W. G.; Jin, J. *J. Am. Chem. Soc.* **1954**, *76*, 4426. (c) Granger, R.; Chapat, J. P.; Crassous, J.; Girard, J. P.; Contastin, A. *Bull. Soc. Chim. Fr.* **1971**, 2662. Granger, R.; Girard, J. P.; Rossi, J. C.; Boussinesq, J. *Ibid.* **1967**, 4035. Granger, R.; Girard, J. P. *Ibid.* **1965**, 1704.

(12) (a) Jackman, L. M. *Applications of NMR Spectroscopy in Organic Chemistry*; Pergamon: New York, **1959**; p 116. (b) Williamson, K. L.; Johnson, W. S. *J. Am. Chem. Soc.* **1961**, *83*, 4623. Wellman, K. M.; Bordwell, F. G. *Tetrahedron Lett.* **1963**, 1703. Nickon, A.; Castle, M. A.; Harada, R.; Berkoff, C. E.; Williams, R. O. *J. Am. Chem. Soc.* **1963**, *85*, 2185.

(13) Granger, R.; Chapat, J. P.; Crassous, J.; Girard, J. P.; Constatin, A. *Bull. Soc. Chim. Fr.* **1971**, 2662. Granger, R.; Girard, J. P.; Rossi, J. C.; Boussinesq, J., *Ibid.* **1967**, 4035. Granger, R.; Girard, J. P. *Ibid.* **1965**, 1704.

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(15) Bourn, P. M.; Klyne, W. *J. Chem. Soc.* **1960**, 2044.

(16) Van Osselaer, T. A.; Lemiere , G. L.; Lepoivre, T. A.; Alderweireldt, F. C. *Bull. Soc. Chim. Belg.* **1978**, *87*(2), 153–4.

(17) (a) Jones, J. B.; Jakovac, I. J. *Can. J. Chem.* **1982**, *60*, 19. (b) Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4659.

sign of optical rotation.[18] In the trans series, the availability of the two C-3-epimers (±)-**5b** and -**6b** from the Scheme I reactions enabled assignments of the relative configurations at C-3 of (−)-**5b**, (−)-**6b**, and (+)-**7b** to be made without difficulty.

The absolute configuration and enantiomeric excess determinations were straightforward in both series. In view of the high ee's of the product alcohol **7b** from HLADH-catalyzed reduction of (±)-**3b**, the 76% ee value recorded for the recovered diketone (−)-**3b** is lower than expected. This is attributable in part to the fact that the ^{13}C NMR integration for the diektal **10b** was possible for only one carbon atom (C-3) rather than the several preferred for this ee evaluation method.[9] Based on the value of the specific rotation of (−)-**3b** of −110.7°, compared with that (+135°) of (+)-**3b** derived from (+)-**7b** of 94% ee, an optical purity of 83% is indicated.

The current results, together with the previous data on bicyclic diketones[4] and monocyclic diols,[5] demonstrate that combining regiospecificity with enantiomeric and/or stereoheterotopic selectivity is a generally exploitable synthetic property of HLADH in both reduction and oxidation modes. As in the previous cases, the specificity is predictable using the cubic active-site section model[17] of the enzyme.

Cubic Active-Site Section Model Analysis of Stereospecificity. The structural- and stereospecificity of HLADH are readily interpreted by using an active-site model, based on cubic space descriptors, of the volumes available for substrate binding and their relative orientations. Despite the advances that have been made in protein X-ray crystallography, including the excellent HLADH structures available,[19] at this time simple active-site models of this type remain the most reliable for interpreting and predicting enzyme specificity for the unnatural structures of most interest in asymmetric synthesis. The cubic section active-site model of HLADH is now very comprehensive. It is easy to use, and its application has been fully described.[17a] The most recent update[17b] has been employed in the following analyses.

Cis Series Analyses. In evaluating the possible enzyme–substrate (ES) complexes for cis bicyclic substrates such as **2a** and **3a**, their conformational mobility must be taken into account, and the active site binding of all preferred chair cyclohexane conformations considered, with both faces of the carbonyl group presented in turn to the NADH hydride delivery direction.[17] When this is done for all possibilities for (+)- and (−)-**2a** and -**3a**, only two favorable binding modes are found. These are depicted in Figure 1. For all other binding orientations, adverse steric interactions preclude the formation of the productive ES complexes required for reduction to occur.

Both binding modes of Figure 1 avoid undesirable interactions. Each involves delivery of the hydride equivalent to the carbonyl group from the equatorial direction with respect to the cyclohexane chair preferred by the enzyme,[17a] giving the kinetically controlled products (−)-**5a** and (+)-**8a** with axially oriented hydroxyl groups. These then become predominantly equatorial in the thermodynamically preferred conformations of the products. The enantiomeric preference for diketone (+)-**3a** reduction (Figure 1(i)) is the result of the influence of slight steric interactions of the C-8 carbonyl group with the forbidden E4 region for the other enantiomer (−)-**3a**, thereby disfavoring its reduction via the Figure 1(ii) complex. For the monoketone enantiomers, (−)- and (+)-**2a**, the influence of the C-8 carbonyl group is removed, and the reduction rates of both enantiomers via both Figure 1(i) and Figure 1(ii) complexes become similar, resulting in the much lower product ee's observed experimentally.

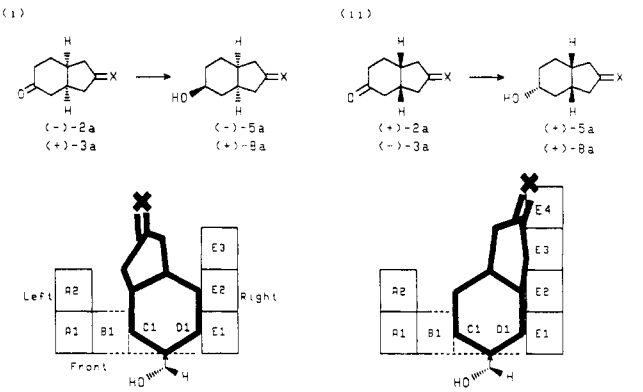

Figure 1. Cubic active-site section analysis of the substrate activity and product stereochemistry for the HLADH-catalyzed reductions of (±)-**2a** and -**3a**. The model, the α-numeric descriptions of the cube locations, and the analytical procedures are as specified previously.[17] Analyses are depicted from the top-elevation[17] perspective. The arrow at the C1,D1 intersection identifies the oxidoreduction site, where the carbonyl group must locate for reduction, via hydride delivery from the equatorial direction with respect to the cyclohexane ring, to occur. Alcohol-like structures are employed because these are considered to resemble the transition state of the reaction. The cubes bounded by solid lines are "forbidden" regions where binding is precluded due to their being occupied by enzymic amino acid residues or by coenzyme. Cubes bounded by broken lines are "limited" regions where substrate binding is possible, but not favored, as a result of their proximity to active-site residues. The open areas are "allowed" space where substrate can be readily accommodated. Only the binding modes shown in (i) and (ii) can meet these criteria[17] without violating forbidden or limited binding regions of the active site. Reductions of (+)- and (−)-**1a** and -**2a** thus occur exclusively via these enzyme substrate complexes, with those of (i) being somewhat more favored because of the proximity of the cyclopentanone moiety to forbidden region E4 in the (ii)-binding modes.

Trans Series Analyses. The analyses for reductions of (±)-**2b** and -**3b** are depicted and discussed in Figure 2. As indicated, the only binding modes leading to allowed, productive, ES complexes, are those of Figure 2(iv) for **2,3b** and Figure 2(i) for **2b**. The absolute configurations of the products observed experimentally (Table I) are in accord with the model's predictions, except that the trace of (1*S*,3*R*,6*S*)-**8b** formed from the reduction of (±)-**3b** indicates that the C-8 carbonyl group may distort the Figure 2(iii) picture to a very limited extent. Nevertheless it remains a disfavored pathway, compared with that of Figure 2(iv). The difference in the enantiomeric selectivity of HLADH toward the monoketone (±)-**2b** and the diketone (±)-**3b** demonstrates the powerful influence exerted by the C-8 keto group in this regard. The basis for this influence, i.e. that the "extra" oxygen atom of (1*R*,6*R*)-**3b** at C-8 would have to violate forbidden space in M8, is clearly identified by the model (Figure 2(i)). For both cis and trans series, the slow rates of cyclopentanone moiety reduction are due to bad interactions of the hydrogen atoms of the carbons α to the C-8 carbonyl group with forbidden region E1 and limited region B1.

Experimental Section

The equipment and analytical procedures used were as described previously.[4] Unless noted otherwise, all NMR spectra were in C^2HCl_3, IR's as films, and optical rotations in EtOH.

(18) Brewster, J. H. *J. Am. Chem. Soc.* **1959**, *81*, 5475. (b) Djerassi, C.; Marshall, D.; Nakaano, T. *J. Am. Chem. Soc.* **1958**, *80*, 4853.
(19) Plapp, B. V.; Eklund, H.; Bränden, C.-I. *J. Mol. Biol.* **1978**, *122*, 23 and previous papers.

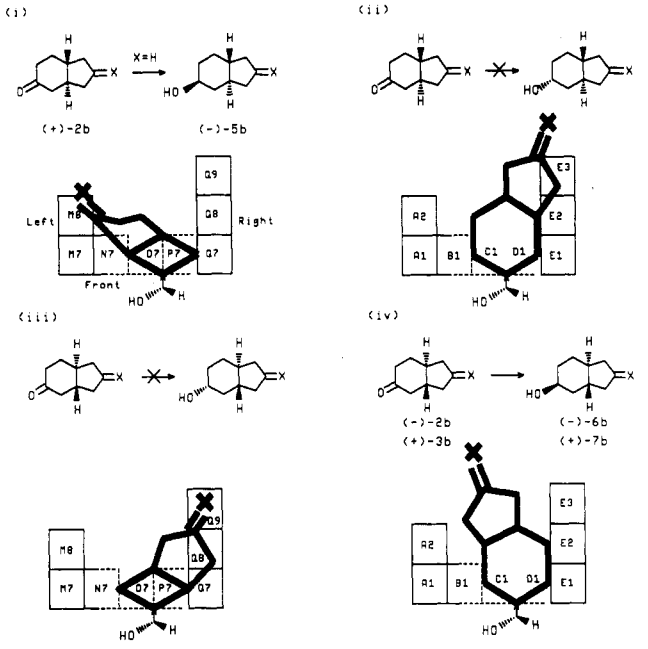

Figure 2. Cubic active-site section analysis of the substrate activity and product stereochemistry for the HLADH-catalyzed reductions of (±)-**2b** and -**3b**. The criteria applied are as outlined for Figure 1, again from a top-elevation model perspective. Comparison of the various binding modes leads to the following conclusions: (i) Only for (1*S*,6*S*)-**2b**, where X = H_2, can the substrate occupy allowed space. This leads to the (−)-**5** product observed. Equivalent binding of the corresponding dione (1*R*,6*R*)-**3b**, where X = O, is precluded by the fact that the C-8 C=O function would have to penetrate forbidden region M8. Thus its reduction to (1*R*,3*S*,6*R*)-**7b** does not occur. (ii) This is not an allowed pathway because C-9 would have to locate in highly forbidden region E3 for both substrates. (iii) These reduction complexes are disfavored because of the need to locate the substrate in forbidden regions Q7–Q9. (iv) In this orientation there are no forbidden interactions. Both X = O and X = H_2 substrates can occupy fully allowed space, and reduction to the experimentally observed alcohols shown is a favored process.

HLADH (EC 1.1.1.1., 1.6 units/mg) was purchased from Sigma, and NAD from Kyowa Hakko Kogyo, New York.

Preparations of Substrates. Cis Series. *cis*-Bicyclo[4.3.0]nonan-8-one ((±)-1a). *cis*-Bicyclo[4.3.0]non-3-en-8-one[6] ((±)-**4a**, 1.2 g, 8.0 mmol) in EtOH (12 mL) containing 5% Pt on Al_2O_3 (0.067 g, catalyst selected to avoid ring-junction isomerization[6]) was hydrogenated under H_2 (1 atm) at 20 °C to give, after Kugelrohr distillation, *cis*-bicyclo[4.3.0]nonan-8-one ((±)-**1a**, 1g, 85%) bp 60 °C (0.3 mmHg) (lit.[20] bp 127–8 °C (45 mmHg)); IR 1745 cm^{-1}; ^1H NMR δ 1.55 (8 H, broad m), 2.25 (6 H, broad m).

***cis*-Bicyclo[4.3.0]nonan-3-one ((±)-2a).** *cis*-Bicyclo[4.3.0]-non-3-en-8-one ((±)-**1**, 4.2 g, 31 mmol)8 KOH (5.6 g, 100 mmol), and hydrazine hydrate (100%, 5.6 mL, 120 mmol) in $HOCH_2C-H_2OH$ (60 mL) were heated at 130 °C for 2 h. The bath temperature was then raised slowly to 220 °C to remove the product by a pseudo-steam-distillation process. The reaction mixture was then cooled to 80 °C, water (20 mL) was added, and the pseudo-steam-distillation process was repeated. The combined distillates were extracted with diethyl ether; the ether extract was dried ($MgSO_4$) and evaporated. After distillation, the residual oil yielded *cis*-bicyclo[4.3.0]non-3-ene (3.1 g, 80%); bp 173 °C, 30 °C (0.2 mmHg) (lit.[21] bp 165–70 °C, 175–7 °C); IR 3020, 1660 cm^{-1}; ^1H NMR δ 1.6 (6 H, broad m), 1.96 (6 H, broad m), 5.60 (2 H, broad m).

The above bicyclononene (3.1 g, 25.4 mmol) in CH_2Cl_2 (75 mL) was added dropwise to a stirred solution of *m*-CPBA (6.5 g, 32.0 mmol) in CH_2Cl_2 (150 mL) at 20 °C. After stirring for 1 h, $Ca(OH)_2$ (10 g, 135 mmol) was added, and the resulting mixture was stirred for an additional 1 h. After filtering off the solid and solvent rotary evaporation, 3,4-epoxy-*cis*-bicyclo[4.3.0]nonane (3.5 g, 90%) was obtained: IR 810 cm^{-1}; ^1H NMR δ 1.51 (6 H, broad m), 1.85 (6 H, broad m), 3.07 (2 H, broad m).

This epoxide (3.5 g, 23.0 mmol) in THF (100 mL) was added dropwise with stirring at 20 °C to $LiAlH_4$ (1.5 g, 40 mmol) in THF (50 mL), and the mixture was refluxed for 5 h. Workup with Rochelle salt (saturated aqueous potassium hydrogen tartrate, 12 mL) followed by the evaporation of the dried ($MgSO_4$) solvent afforded *cis*-bicyclo[4.3.0]nonan-3β-ol[11] ((±)-**5a**, 3.6 g, quant): IR 3360 cm^{-1}; ^1H NMR δ 1.20–2.10 (14 H, m), 2.30 (1 H, broad s, D_2O exchangeable), and 3.70 (1 H, m). (−)-MTPA-(±)-**5a**: ^1H NMR ($[^2H_8]$toluene) δ 1.20–1.80 (14 H, m), 3.47 (3 H, s), 5.05 (1 H, broad s), 7.10 (3 H, m), 7.60 (2 H, m).

The above alcohol ((±)-**5a**, 3.3 g, 23.0 mmol) in acetone (100 mL) was oxidized with Jones reagent to give, after Kugelrohr distillation, *cis*-bicyclo[4.3.0]nonan-3-one ((±)-**2a**, 2.7 g, 80%); bp 40 °C (0.25 mmHg) (lit.[22] bp 106 °C (13 mmHg)); IR 1720 cm^{-1}; ^1H NMR δ 1.25–2.10 (8 H, m), 2.10–2.45 (6 H, m).

***cis*-Bicyclo[4.3.0]nonane-3,8-dione ((±)-3a).** *cis*-Bicyclo-[4.3.0]non-3-en-8-one ((±)-**4a**, 4.76 g, 35.0 mmol) in CH_2Cl_2 (80 mL) was added dropwise with stirring at 20 °C to *m*-CPBA (8.24 g, 41 mmol) in CH_2Cl_2 (120 mL). The mixture was stirred for 2 h, $Ca(OH)_2$ (10 g, 135 mmol) was added, and stirring was continued for an additional 1 h. After filtration and solvent evaporation, 3,4-epoxy-*cis*-bicyclo[4.3.0]nonan-8-one (5.1 g, 96%) was obtained: IR 1740, 1240, 800 cm^{-1}; ^1H NMR δ 1.80–2.50 (10 H, m), 3.20 (2 H, broad s).

This epoxide (5 g, 33.0 mmol) in THF (100 mL) was added dropwise with stirring at 20 °C to $LiAlH_4$ (3.5 g, 90.1 mmol) in THF (90 mL). The mixture was refluxed for 4 h. Workup with Rochelle salt gave a *cis*-bicyclo[4.3.0]nonane-3,8-diol mixture (4.4 g, 85%); IR 3340 cm^{-1}; ^1H NMR ($[^2H_6]$acetone) δ 1.00–2.10 (12 H, m), 3.50–3.80 (3 H, m, 2H D_2O exchangeable), 4.33 (1 H, broad m).

This diol mixture (4.0 g, 25.6 mmol) in acetone (100 mL) was oxidized with Jones reagent to yield *cis*-bicyclo[4.3.0]nonane-3,8-dione ((±)-**3a**, 2.7 g, 60%): bp 80 °C (0.5 mmHg); IR 1750, 1715 cm^{-1}; ^1H NMR δ 1.70–2.90 (broad m). Anal. Calcd for $C_9H_{12}O_2$: C, 71.05; H, 7.90. Found: C, 70.13; H, 8.04.

Preparations of MTPA Esters of Reference 3β- and 3α-Alcohols (±)-5a,6a.[11] (±)-5a-MTPA. *cis*-Bicyclo[4.3.0]nonan-3β-ol ((±)-**5a**) from the epoxide route above, or from hydrogenation of (±)-**2a**,[11a,c] was converted quantitatively the method of Mosher and co-workers[10] to give (±)-**5a**-MTPA: bp 140 °C (0.5 mmHg); ^1H NMR ($[^2H_8]$toluene) δ 1.20–1.80 (14 H, m), 3.47 (3 H, s), 5.00 (1 H, m), 7.10 (3 H, m), and 7.60 (2 H, m).

(±)-6a-MTPA. *cis*-Bicyclo[4.3.0]nonan-3-one ((±)-**2a**) was reduced with Na–EtOH to give, in 83% yield, a mixture of 3β-alcohol ((±)-**5a**, 38%) and 3α-alcohol ((±)-**6a**, 62%).[11a,b] This was transformed[10] quantitatively into MTPA esters: bp 140 °C (0.5 mmHg); ^1H NMR ($[^2H_8]$toluene) δ 1.20–1.80 (14 H, m), 3.40 and 3.47 (3 H, 2 s, ratio 62(3α)/38(3β)), 4.50 (1 H, m), 7.10 (3 H, m), and 7.60 (2 H, m).

Trans Series. *trans*-Bicyclo[4.3.0]non-3-en-8-one ((±)-4b). *trans*-4,5-Bis(hydroxymethyl)cyclohexene[6] (75 g, 0.53 mol) in pyridine (250 mL) was added dropwise with stirring to *p*-toluenesulfonyl chloride (300 g, 1.6 mol) in pyridine (500 mL) at 0 °C. After being stirred for 3 h, the mixture was poured into water (1 L) and filtered, and the solid was dried and recrystallized from methanol to give the bis(toluene-*p*-sulfonate) (205 g, 85%): mp 93–95 °C; ^1H NMR δ 1.90 (6 H, m), 2.40 (6 H, s), 3.92 (4 H, broad d, J = 3.5 Hz), 5.49 (2 H, m), 7.30 (4 H, d, J = 8 Hz) and 7.73 (4 H, d, J = 8 Hz). This ditosylate (205 g, 0.45 mol) and NaCN (71 g, 1.5 mol) in dry EtOH (1.6 L) were heated under reflux for 3 days. The mixture was cooled, water (230 mL) was added, and the EtOH was removed by distillation. The cooled residue recrystallized and was filtered, dried, and then taken up into $CHCl_3$ (4 × 500 mL). The $CHCl_3$ solution was dried ($MgSO_4$) and rotary evaporated to give *trans*-4,5-bis(cyanomethyl)cyclohexene (73 g, quant): IR ($CHCl_3$) 3040, 2260, 2180, and 1640 cm^{-1}; ^1H NMR δ 2.18 (6 H, broad m), 2.49 (4 H, broad d, J = 4 Hz), and 5.61 (2 H, m).

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(22) Granger, R.; Nau, P.; Nau, J. *Bull. Soc. Chim. Fr.* **1958**, *25*, 531.

The above dicyanide (73 g, 0.45 mol) in aqueous KOH (400 mL of 33% solution) was refluxed for 1 day (until the evolution of NH_3 ceased) and then cooled. Aqueous 85% phosphoric acid (400 mL) was added at 0 °C, the mixture was filtered, and the residue was washed with warm water (4 × 30 mL) and then dried. Recrystallization from 25% aqueous MeOH yielded *trans*-4,5-bis(carboxymethyl)cyclohexene (60 g, 66%): mp 189–191 °C; ^{1}H NMR (DMSO-d_6) δ 1.93 and 2.16 (10 H, broad s and d, J = 6 Hz), 5.57 (2 H, m), and 12.06 (2 H, broad s). This diacid (30 g, 0.15 mol), $Ba(OH)_2$ (4 g, 0.025 mol), and powdered Fe (30 g) were mixed in a 10-mL round-bottomed flask and heated with an open flame. The product distilled and solidified in the receiver. It was then purified by steam distillation to give, after filtration and drying, *trans*-bicyclo[4.3.0]non-3-en-8-one ((±)-**4b**, 17.1 g, 42%): mp 60–62 °C; IR ($CHCl_3$) 1745 and 1640 cm^{-1}; ^{1}H NMR δ 1.63–2.70 (10 H, m) and 5.75 (2 H, m). Anal. Calcd for $C_9H_{12}O$: C, 79.41; H, 8.82. Found: C, 78.99; H, 8.79.

***trans*-Bicyclo[4.3.0]nonan-8-one ((±)-1b).** *trans*-Bicyclo[4.3.0]non-3-en-8-one ((±)-**4b**, 2g, 14.7 mmol) in EtOH (20 mL) containing 5% Pt on Al_2O_3 (0.19 g, catalyst selected to avoid ring-junction isomerization[6]) was hydrogenated under H_2 (1 atm) at 20 °C to give, after a Kugelrohr distillation, *trans*-bicyclo[4.3.0]nonan-8-one ((±)-**1b**, 1.7 g, 84%): bp 60 °C (0.5 mmHg) (lit.[23] bp 88–89 °C (10 mmHg)); IR 1740 cm^{-1}; ^{1}H NMR δ 1.20–2.55 (broad).

***trans*-Bicyclo[4.3.0]nonan-3-one ((±)-2b).** *trans*-Bicyclo[4.3.0]non-3-en-8-one ((±)-**4b**, 1.38 g, 10.0 mmol), KOH (1.8 g, 32.0 mmol), and hydrazine hydrate (100%, 1.7 mL, 32.0 mmol) in $HOCH_2CH_2OH$ (25 mL) were heated at 130 °C for 2 h. The bath temperature was then raised slowly to 220 °C to remove the product by a pseudo-steam-distillation process. The mixture was then cooled, water (5 mL) was added, and the pseudo-steam-distillation process was repeated. The organic layer of the combined distillate was extracted with diethyl ether, and the ether extract was dried ($MgSO_4$) and evaporated. A Kugelrohr distillation of the residual oil yielded *trans*-bicyclo[4.3.0]non-3-ene (1.1 g, 90%): bp 40–50 °C (1 mmHg); IR 3040, 1645 cm^{-1}; ^{1}H NMR δ 1.00–2.03 (10 H, broad m), 2.15–2.40 (2 H, broad m), and 5.63 (2 H, broad d, J = 3 Hz). *m*-Chloroperbenzoic acid (6.5 g, 32.2 mmol) in CH_2Cl_2 (200 mL) was added dropwise with stirring at 20 °C to the above bicyclonon-3-ene (3.2 g, 26.2 mmol) in CH_2Cl_2 (50 mL). After the mixture was stirred for 1 h, $Ca(OH)_2$ (10 g, 135.1 mmol) was added, and stirring was continued for a further 1 h. The mixture was filtered, and the solution was rotary evaporated and then Kugelrohr distilled to give 3,4-epoxy-*trans*-bicyclo[4.3.0]nonane (3.3 g, 92%): bp 60 °C (0.4 mmHg); ^{1}H NMR δ 0.95–2.00 (10 H, broad m), 2.20–2.53 (2 H, broad m), and 3.17 (2 H, d, J = 4 Hz).

This epoxide (3.3 g, 24.0 mmol) in THF (100 mL) was added dropwise with stirring at 20 °C to $LiAlH_4$ (2.5 g, 65.8 mmol) in THF (70 mL). The mixture was refluxed for 4 h and then kept at 20 °C for 12 h. Workup with saturated aqueous potassium hydrogen tartrate (Rochelle salt, 20 mL) followed by evaporation of the dried ($MgSO_4$) THF solution and a Kugelrohr distillation afforded *trans*-bicyclo[4.3.0]nonan-3α-ol[13] ((±)-**6b**, 2.95 g, 90%): bp 60 °C (0.4 mmHg); mp ≈20 °C; IR 3350 cm^{-1}; ^{1}H NMR δ 1.00–2.10 (14 H, broad m), 1.54 (1 H, s, D_2O exchangeable), and 4.10 (1 H, broad s, equatorial[12] C-3 H).

The above 3α-alcohol ((±)-**6b**, 2.7 g, 19.3 mmol) in acetone (75 mL) was oxidized with Jones reagent to yield *trans*-bicyclo[4.3.0]nonan-3-one ((±)-**2b**, 2.0 g, 75%): bp 65 °C (0.35 mmHg) (lit.[15] bp 100 °C (14 mmHg)); IR 1720 cm^{-1}; ^{1}H NMR δ 1.05–2.00 (10 H, broad m), 2.23–2.60 (4 H, broad m).

***trans*-Bicyclo[4.3.0]nonane-3,8-dione ((±)-3b).** *m*-Chloroperbenzoic acid (6.14 g, 30.2 mmol) in CH_2Cl_2 (100 mL) was added dropwise with stirring at 20 °C to *trans*-bicyclo[4.3.0]non-3-en-8-one ((±)-**4b**, 3.7 g, 27.2 mmol) in CH_2Cl_2 (40 mL). The mixture was then stirred for a further 2 h, $Ca(OH)_2$ (10 g, 135.1 mmol) was added, and the mixture was filtered and rotoevaporated to give 3,4-epoxy-*trans*-bicyclo[4.3.0]nonan-8-one (3.9 g, 95%): mp 46–48 °C; IR ($CHCl_3$) 1750, 990, and 870 cm^{-1}; ^{1}H NMR δ 1.80 (6 H, broad m), 2.40 (4 H, broad m), and 3.23 (2 H, d, J = 4 Hz).

This epoxide (3.9 g, 25.6 mmol) in THF (100 mL) was added dropwise with stirring to $LiAlH_4$ (2.7 g, 70.9 mmol) in THF (70 mL) and then refluxed for 4 h. Workup with aqueous Rochelle salt as described above gave a mixture of 3- and 4-hydroxy-*trans*-bicyclo[4.3.0]nonan-8-ol (4.1 g, quant): IR 3320 cm^{-1}; ^{1}H NMR δ 1.10–2.20 (1i H, broad m), 2.70 (2 H, broad s, D_2O exchangeable), 4.10 (1 H, broad s), and 4.35 (1 H, broad s). This diol mixture (4 g, 25.9 mmol) in acetone (150 mL) was oxidized with Jones reagent to yield *trans*-bicyclo[4.3.0]nonane-3,8-dione ((±)-**3b**, 2.4 g, 60%): bp 80–90 °C (0.5 mmHg); mp ≈20 °C; IR ($CHCl_3$) 1745 and 1710 cm^{-1}; ^{1}H NMR δ 1.75–2.90 (broad m). Anal. Calcd for $C_9H_{12}O_2$: C, 71.05; H, 7.90. Found: C, 70.43; H, 8.01.

Preparations of Reference 3β- and 3α-Alcohols (±)-5b and 6b. *trans*-Bicyclo[4.3.0]nonan-3α-ol ((±)-**6b**) was obtained as described above in the route to (±)-**2b**.

***trans*-Bicyclo[4.3.0]nonan-3β-ol ((±)-5b).** *trans*-Bicyclo[4.3.0]nonan-3-one ((±)-**2b**, 270 mg, 1.96 mmol) in THF (10 mL) was reduced with $LiAlH_4$ (70 mg, 1.84 mmol) followed by Rochelle salt workup as above to give *trans*-bicyclo[4.3.0]nonan-3β-ol[12] ((±)-**5b**, 270 mg, 98%): bp 60 °C (0.4 mmHg); IR 3350 cm^{-1}; ^{1}H NMR δ 0.95–1.40 (6 H, broad m), 1.40–2.30 (8 H, broad m), 2.75 (1 H, s, D_2O exchangeable), and 3.75 (1 H, m, axial[12] C-3 H).

Relative Rates of HLADH-Catalyzed Reductions of (±)-1–3a,b. Assays were performed spectrophotometrically under the Table I conditions with cyclohexanone as the reference standard. The reductions were initiated by adding a 50-μL aliquot of HLADH solution 1 mg of HLADH/1 mL of buffer) in 0.1 M phosphate buffer (pH 7.0) to make a final volume of 3 mL in a 1-cm pathlength cuvette. The NADH/NAD absorbance change at 340 nm was monitored. The results are recorded in Table I.

Cis Series. Preparative-Scale HLADH-Catalyzed Reductions of (±)-2,3a. The following general procedure was used. The racemic ketone (≈1–1.5 g) was suspended in 0.1 M potassium phosphate buffer (pH 8.0, 1.5 L) containing EtOH (≈8–10 mL) at 20 °C, NAD (7-8 g) was added, and the pH was readjusted to 8.0 with 10 M aqueous KOH. The reduction was then initiated by the addition of HLADH (≈400 mg). The extent of reaction was monitored by GLC (5% QF-1 column). At the 50%-of-reduction point (5–7 days), the mixture was continuously extracted with $CHCl_3$ for 2 days, and the dried ($MgSO_4$) $CHCl_3$ solution was evaporated. The products, whose physical and spectral properties were identical with those of their racemates, were separated by chromatography on neutral alumina (grade III) with a hexane–benzene–chloroform gradient elution. The individual reactions gave the following results, as summarized in Table I.

Reduction of (±)-2a (1.486 g, 10.8 mmol) with enzyme (374 mg) and NAD (6.927 g, 10.2 mmol) for 7 days gave (1*R*,6*S*)-*cis*-bicyclo[4.3.0]nonan-3-one (**2a**, 586 mg, 39.5%, 20% ee), $[\alpha]^{25}_D$ +9.1° (*c* 8.0) (lit.[14] (1*R*,6*S*)-6-methyl, $[\alpha]^{25}_D$ +15.2°), and (1*S*,3*S*,6*R*)-*cis*-bicyclo[4.3.0]nonan-3-ol (**5a**, 552 mg, 37%, 12% ee), $[\alpha]^{25}_D$ −4.8° (*c* 8.0).

Reduction of (±)-3a (1.7 g, 11.2 mmol) with enzyme (433 mg) and NAD (8.07 g, 11.8 mmol) for 5 days gave (1*S*,6*R*)-*cis*-bicyclo[4.3.0]nonane-3,8-dione (**3a**, 401 mg, 25%, 69% ee), $[\alpha]^{25}_D$ −19.6° (*c* 5.6), and 1*R*,3*S*,6*S*)-3-hydroxy-*cis*-bicyclo[4.3.0]nonan-8-one (**8a**, 883 mg, 55%, 60% ee), $[\alpha]^{25}_D$ + 47.7° (*c* 12.6): IR 3400, 1735 cm^{-1}; ^{1}H NMR ($[^2H_8]$toluene) δ 1.20–1.65 (6 H, broad m), 1.80–2.05 (6 H, m), 2.55 (1 H, broad s, D_2O exchangeable), 3.50 (1 H, broad m).

Enantiomeric Excess Determinations. Of (+)-2a and (−)-3a. Each racemic and optically active (di)ketone **2a** and **3a** (1 mmol) and (−)-(2*R*,3*R*)-butanediol (1.5 equiv) were converted as described previously[8] to the corresponding (di)ketals **9a** and **10a**. The ee values were calculated from the ^{13}C NMR spectra[9] using the diastereomeric chemical shift differences recorded in Table II. The properties of the ketals from the racemic (di)ketones are as follows. **(±)-2a-Ketal (9a)**: bp 50–60 °C (0.2–0.25 mmHg); IR 1100 cm^{-1}; ^{1}H NMR δ 1.15 (6 H, d, J = 5 Hz), 1.60 (12 H, m), 2.00 (2 H, m), 3.58 (2 H, m); ^{13}C NMR δ 16.82 (C-12,13), 22.18 and 22.29 (C-8), 24.34 and 24.45 (C-7), 27.36 and 27.45 (C-9), 30.82 (C-5), 31.80 and 32.36 (C-4), 37.40, 37.68, 37.75 and 38.07 (C-1,2,6), 77.55 (C-10,11) and 108.41 (C-3). **(±)-4-Diketal (10a)**: bp 100–110 °C (0.3 mmHg); IR 1100 cm^{-1}; ^{1}H NMR δ 1.17 (12 H, d, J = 6 Hz), 1.65 (6 H, broad m), 1.90 (6 H, broad m), 3.57 (4 H, m); ^{13}C NMR δ 16.99 (C-12,13,16,17), 24.23 and 24.71 (C-7), 31.94 and

(23) Van Tamelen, E. E.; Shamma, M.; Aldrich, P. *J. Am. Chem. Soc.* **1956**, *78*, 4628.

32.44 (C-9), 35.61 (C-6), 36.30 (C-1), 37.17 and 37.35 (C-5), 41.13 and 42.15 (C-4), 44.10 and 44.76 (C-2), 77.61 and 77.71 (C-14), 77.79 (C-10,11), 78.18 and 78.59 (C-15), 108.00 and 108.15 (C-3), 116.95 and 117.03 (C-8).

Of (-)-5a. The alcohol (-)-**5a** was oxidized with Jones reagent, the ketone obtained was transformed into its corresponding ketal **9a**, and the ee value was calculated from ^{13}C NMR spectrum as above.

Of (+)-8a. The hydroxy ketone (+)-**8a** was reduced to (-)-**5a** as described previously[18b] and analyzed as above.

Relative Configuration Determinations of (-)-5a and (-)-8a. The samples of (-)-**5a** obtained directly from HLADH-catalyzed reduction of (±)-**2a**, and from reduction of (-)-**8a** above, were converted to their Mosher esters.[10] The MeO peaks of their ^1H NMR spectra were at δ 3.47 ppm, identical with those of the authentic reference (±)-**5a**-MTPA esters characterized above.

Absolute Configuration Determinations. Of (+)-(1*R*,6*S*)-2a: $[\alpha]^{25}_D$ +9.1° (*c* 8.0) (lit.[14] (1*R*,6*S*)-6-methyl $[\alpha]_D$ +15.2°).

Of (-)-(1*S*,3*S*,6*R*)-5a: by oxidation with Jones reagent in 85% yield to (-)-**2a**, $[\alpha]^{25}_D$ -9.9° (*c* 4.0).

Of (+)-(1*R*,3*S*,6*S*)-8a: by Wolff–Kishner reduction to give (1*S*,3*S*,6*R*)-**5a** in 68% yield, $[\alpha]^{25}_D$ -14.3° (*c* 5.5).

Of (-)-(1*S*,6*R*)-3a: by comparison of its $[\alpha]^{25}_D$ -19.6° (*c* 5.6) with that of (+)-(1*R*,6*S*)-**4**, $[\alpha]^{25}_D$ +16.0° (*c* 0.9) obtained in 84% yield from Jones oxidation of (+)-(1*R*,3*S*,6*S*)-**8a**.

Trans Series. Preparative-Scale HLADH-Catalyzed Reductions of 2,3b. The general procedure described above for the cis series was followed. The results, summarized in Table I, were as follows:

Reduction of (±)-2b (1.09 g, 7.9 mmol) with enzyme (226 mg) and NAD (5.04 g, 7.4 mmol) for 7 days gave (1*R*,6*R*)-*trans*-bicyclo[4.3.0]nonan-3-one (**2b**, 390 mg, 40%, 11% ee), $[\alpha]^{25}_D$ -6.7° (*c* 3.9) (lit.[15] (1*S*,6*S*), $[\alpha]^{21}_D$ +68°); (1*R*,3*S*,6*R*)-*trans*-bicyclo-[4.3.0]nonan-3α-ol (**6b**, 162 mg, 17%, 51% ee), $[\alpha]^{25}_D$ -4.0° (*c* 2,3); and (1*S*,3*S*,6*S*)-*trans*-bicyclo[4.3.0]nonan-3β-ol (**5b**, 157 mg, 16%, 94% ee), $[\alpha]^{25}_D$ -7.1° (*c* 2.2).

Reduction of (±)-3b (1.26 g, 8.3 mmol) with enzyme (270 mg) and NAD (6.0 g, 8.8 mmol) for 7 days yielded (1*R*,6*R*)-*trans*-bicyclo[4.3.0]nonane-3,8-dione (**3b**, 593 mg, 47%, 76% ee), $[\alpha]^{25}_D$ -110.7° (*c* 2.4), and (1*S*,3*S*,6*S*)-3-hydroxy-*trans*-bicyclo[4.3.0]-nonan-8-one (**7b**, containing ≈5% by GLC of (1*S*,3*R*,6*S*)-**8b**[24a]), 524 mg, 42%, 94% ee), $[\alpha]^{25}_D$ +158.2° (*c* 2,1).

Enantiomeric Excess Determinations. Of (-)-2b and (-)-3b. Each racemic and optically active (di)ketone **2b** and **3b** (1 mmole and (-)-(2*R*,3*R*)-butanediol (1.2 equiv) were reacted as described previously[8] to give quantitative yields of the corresponding (di)ketals **9b** and **10b**. The ee values were calculated from the ^{13}C NMR spectra[9] with the diastereomeric chemical shift differences recorded in Table II. The properties of the ketals from the racemic (di)ketones are as follows. **(±)-2b-Ketal (9b):** bp 60–70 °C (0.3 mmHg); IR 1100 cm^{-1}; ^1H NMR δ 1.00–1.51 with doublet (J = 6 Hz) at 1.23 (12 H, m), 1.55–2.10 (8 H, m), and 3.60 (2 H, m); ^{13}C NMR δ 16.54, 16.86 and 16.95 (C-12,13), 22.64 (C-8), 27.64 and 28.04 (C-5), 30.10 (C-7), 30.74 (C-9), 36.29 and 37.40 (C-4), 41.80 and 42.71 (C-2), 43.64 and 44.19 (C-1), 45.68 (C-6), 77.69, 77.80 and 77.99 (C-10,11), and 109.24 (C-3). **(±)-3b-Diketal (10b):** bp 100–110 °C (0.25 mmHg), IR 1100 cm^{-1}; ^1H NMR δ 1.03–2.10 (12 H, m), 1.23 (12 H, d, J = 6 Hz), and 3.60 (4 H, m); ^{13}C NMR δ 16.63, 16.80 and 17.23 (C-12,13,16,17), 27.25 and 27.62 (C-5), 36.03 and 37.17 (C-4), 41.01 and 42.07 (C-2), 41.15 and 41.53 (C-1), 43.34 (C-6), 44.75 (C-7), 45.27 (C-9), 77.88, 78.16 (C-10,11,14,15), 108.23 (C-3), and 116.39 (C-8).

Of (-)-5b, (-)-6b, and (+)-7b. The racemic and optically active alcohols **5b,6b** were converted to their MTPA esters in quantitative yields by the standard literature method[10] by using freshly prepared (-)-(2*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The ee's were then determined by 80-MHz ^1H NMR examination of the methoxyl protons, both in the presence and absence of Eu(fod)$_3$ (recorded in [$^2H_8$]toluene). The ee of the keto alcohol (+)-**7b** was determined in the same way, following its Wolff–Kishner reduction (described below in the absolute configuration section) to (-)-**6b**. The $\Delta\delta$ or $(\Delta)\Delta\delta$ values used are recorded in Table III.

The properties of the MTPA esters from the racemic alcohol standards were as follows.

(±)-5b-MTPA: bp 130–140 °C (0.2 mmHg); ^1H NMR δ 1.03–2.30 (14 H, m), 3.53 (3 H, d, J = 2 Hz), 5.00 (1 H, broad s), and 7.37 (5 H, m).

(±)-6b-MTPA: bp 120–130 °C (0.35 mmHg); ^1H NMR δ 0.80–2.30 (14 H, m), 3.56 (3 H, d, J = 2 Hz), 5.40 (1 H, broad s), and 7.41 (5 H, m).

Relative Configuration Determinations of (-)-5,6b and (+)-7,8b. The hydroxy ketones (+)-**7b** and (+)-**8b** were reduced to (-)-**6b** and (+)-**5b**, respectively. Their C-3 configurations, and those of the other enzyme-derived (-)-**5b** and (-)-**6b**, were established by ^1H NMR comparisons[12] of the C-3 H peaks with those of authentic[13] **(±)-5,6b.**

Absolute Configuration Determinations. Of (-)-(1*R*,6*R*)-2b: $[\alpha]^{25}_D$ -6.7° (*c* 3.9, EtOH) (lit.[13] (1*S*,6*S*) $[\alpha]_D$ +68° (EtOH)).

Of (-)-(1*S*,3*S*,6*S*)-5b: by oxidation with Jones reagent (56% yield after Kugelrohr distillation) to (+)-**2b**, $[\alpha]^{25}_D$ +54.2° (*c* 0.5).

Of (-)-(1*R*,3*S*,6*R*)-6b: by oxidation with Jones reagent in 66% yield (after Kugelrohr distillation) to (-)-**2b**, $[\alpha]^{25}_D$ -32.3° (*c* 0.6).

Of (+)-(1*S*,3*S*,6*S*)-7b: by Wolff–Kishner reduction (using the procedure described above in the (±)-**4b** to (±)-**2b** section) to give an 82% yield of (1*R*,3*S*,6*R*)-**6b**, which after careful chromatographic purification[24b] had $[\alpha]^{25}_D$ -8.6° (*c* 1.2).

Of (-)-(1*R*,6*R*)-3b: by comparison of its $[\alpha]^{25}_D$ -110.7° (*c* 2.1) with that of (+)-(1*S*,6*S*)-**3b**, $[\alpha]^{25}_D$ +135° (*c* 0.6), obtained in 75% yield after chromatography and Kugelrohr distillation from Jones oxidation of (+)-(1*S*,3*S*,6*S*)-**7b**.

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Registry No. (±)-**1a**, 5689-04-3; (±)-**1b**, 16484-17-6; (±)-**2a**, 96308-51-9; (+)-**2a**, 119677-48-4; (±)-**2b**, 119594-92-2; (-)-**2b**, 119677-50-8; (±)-**3a**, 119594-91-1; (-)-**3a**, 119677-49-5; (±)-**3b**, 119594-93-3; (-)-**3b**, 119677-51-9; (+)-**4**, 119678-60-3; (±)-**4a**, 25886-63-9; (±)-**4b**, 60844-23-7; (-)-**5a**, 119677-44-0; (±)-**5a**, 119595-05-0; (±)-**5a**-MTPA isomer 1, 119594-98-8; (±)-**5a**-MTPA isomer 2, 119619-04-4; (-)-**5b**, 119677-46-2; (±)-**5b**-MTPA isomer 1, 119637-76-2; (±)-**5b**-MTPA isomer 2, 119595-08-3; (±)-**6a**, 119595-06-1; (±)-**6a**-MTPA isomer 1, 119594-99-9; (±)-**6a**-MTPA isomer 2, 119595-07-2; (-)-**6b**, 119677-45-1; (±)-**6b**, 119595-02-7; (±)-**6b**-MTPA isomer 1, 119595-10-7; (±)-**6b**-MTPA isomer 2, 119595-09-4; (+)-**7b**, 119594-95-5; (+)-**8a**, 119594-94-4; **8b**, 119594-96-6; **9a** isomer 1, 119595-03-8; **9a** isomer 2, 119677-54-2; **9b** isomer 1, 119677-52-0; **9b** isomer 2, 119677-56-4; **10a** isomer 1, 119595-04-9; **10a** isomer 2, 119677-55-3; **10b** isomer 1, 119677-53-1; **10b** isomer 2, 119677-57-5; HLADH, 9031-72-5; bicyclo[4.3.0]non-3-ene, 22981-96-0; 3,4-epoxy-*cis*-bicyclo-[4.3.0]nonane, 6784-63-0; 3,4-epoxy-*cis*-bicyclo[4.3.0]nonan-8-one, 119594-97-7; *cis*-bicyclo[4.3.0]nonane-3,8-diol, 92464-20-5; *trans*-4,5-bis(hydroxymethyl)cyclohexene, 56084-93-6; *trans*-4,5-bis(hydroxymethyl)cyclohexene, bis(toluene-*p*-sulfonate), 60894-68-0; *trans*-4,5-bis(cyanomethyl)cyclohexene, 119595-00-5; *trans*-4,5-bis(carboxymethyl)cyclohexene, 119595-01-6; *trans*-bicyclo[4.3.0]non-3-ene, 60844-61-3; 3,4-epoxy-*trans*-bicyclo-[4.3.0]nonane, 3716-43-6; 3,4-epoxy-*trans*-bicyclo[4.3.0]nonan-8-one, 119677-47-3; (-)-(2*R*,3*R*)-butanediol, 24347-58-8; (-)-(2*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 39637-99-5.

(24) (a) The structure of this compound was established during the absolute configuration determination of (+)-**7b**. (b) From this stage, careful chromatography eventually enabled a small amount (≈10 mg) of (+)-**5b**, derived from the trace of **8b** formed enzymically, to be accumulated to establish its C-3α configuration by ^1H NMR, its ee via the MTPA ester method, and its 1*S*,6*S* absolute configuration from its oxidation to (-)-**2b**.