Determination of pK_a Values for PNAA and PNAA-d_3. The pK_a values of PNAA and PNAA-d_3 were determined spectrophotometrically at 410 nm by using hydroxide concentrations of 2.0×10^{-2} to 2.0 M (μ = 1.0 M with NaCl, except for [OH^-] > 1 M). The dissociation constants were calculated from plots of OD/[PNAA-l_3] vs. 1/[OH^-] according to Hine and Hine.[18] The pK_a's are 13.73 for PNAA and 13.72 for PNAA-d_3 agreeing with previous values for PNAA of 13.6 and 13.8. The isotope effect on the equilibrium constant is $K_a^H/K_a^D = 0.98 \pm 0.04$ and agrees with the previous value[10] of 0.998 (no error limits reported).

Kinetics. Rates of *p*-nitroaniline production were measured spectrophotometrically at 410 nm with automatic data acquisition, with a Cary 16-Hewlett-Packard minicomputer system.[19] Reactions were initiated by the injection of small aliquot (10-50 μL) of a stock solution of the anilide in acetonitrile into a cuvette, usually of 1-cm path length, containing 3.00 mL of hydroxide solution. Reaction temperatures were controlled by a Lauda K4/RD circulating bath connected to the cuvette holder. Temperatures were monitored by a thermistor device with digital readout. For hydroxide concentrations of 0.208 M and greater, rates were determined under first-order conditions. First-order rate constants were obtained by weighted nonlinear least-squares analysis, commonly of 1000 data points. Zero-under conditions were employed for hydroxide concentrations less than 0.208 M. Reaction mixtures containing 1 mM substrate were monitored at 410 nm for 10-60 min, corresponding to less than 0.5% reaction in all cases (absorbance change 0.01-0.05). First-order rate constants were calculated according to

$$k_{obsd} = (dA/dt)/[\text{PNAA-}l_3]\Delta\epsilon_{410}$$

Substrate concentrations were based on gravimetric determinations, after demonstrating spectrophotometrically that the materials were sufficiently pure to allow this. $\Delta\epsilon_{410}$ is the extinction coefficient difference between *p*-nitroacetanilide and *p*-nitroaniline (PNA). This value is independent of isotopic substitution but is dependent on [HO^-] since PNAA, which does not absorb at 410 nm, ionizes to produce a chromophoric anion ($PNAA^-$). Thus

$$\Delta\epsilon_{410} = \epsilon_{PNA} - (\epsilon_{PNAA^-})[K_a/(K_a + [H^+])]$$

where ϵ_{PNA} at 410 nm is 9.20×10^3 M^{-1} cm^{-1}, and ϵ_{PNAA^-} at 410 nm is 4.63×10^3 M^{-1} cm^{-1}. In all studies, rates for protiated and deuterated substrates were measured in alternation.

For runs at very low [HO^-], a special buretting setup was used, which kept the solution under N_2 while it was introduced into a 5-cm cell. Reaction was initiated by injection through a serum cap of 500 μL of stock substrate solution in acetonitrile into 13 mL of base solution. Again, the two isotopic substrates were studied in alternation.

Registry No. *p*-$NO_2C_6H_4NHCOCH_3$, 104-04-1; *p*-$NO_2C_6H_4NHCOCD_3$, 88548-58-7; CH_3COCl, 75-36-5; CD_3COCl, 19259-90-6; *p*-nitroaniline, 100-01-6; deuterium, 7782-39-0.

(18) Hine, J.; Hine, M. *J. Am. Chem. Soc.* **1952**, *74*, 5266.
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Enzymes in Organic Synthesis. 31.[1] Preparations of Enantiomerically Pure Bicyclic [3.2.1] and [3.3.1] Chiral Lactones via Stereospecific Horse Liver Alcohol Dehydrogenase Catalyzed Oxidations of Meso Diols

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Abstract: Preparative-scale horse liver alcohol dehydrogenase catalyzed oxidations of *meso*-1,3-bis(hydroxymethyl)cyclopentyl and -cyclohexyl substrates proceed stereospecifically to give 42-81% yields of the corresponding chiral bridged-bicyclic γ-lactones of 100% ee. For each diol, oxidation of the hydroxymethyl group attached to the *S* center occurs exclusively. The stereospecificites observed are as predicted by the active-site model of the enzyme.

Numerous examples of the broad spectrum of asymmetric synthetic opportunities provided by the use of enzymes as practical chiral catalysts have now been documented.[2] Of particular value in this regard are the abilities of enzymes to induce stereospecific transformations of symmetrical substrates. Meso compounds are one symmetrical-substrate group for which asymmetric enzyme-catalyzed conversions to a range of synthetically useful enantiomerically pure chiral products have been documented.[2] Such reactions require enzymes to discriminate between enantiotopic groups attached to centers of opposite chiralities within the meso substrates. An enzyme that possesses this capability to a synthetically viable degree is horse liver alcohol dehydrogense (HLADH[3]). HLADH is a commercially available NAD-dependent alcohol dehydrogenase that catalyzes CH(OH) $\rightleftharpoons$ C=O oxidoreductions of a wide range of organic chemically significant structures.[1,4,5] In preparative-scale reactions it has been shown to operate stereospecifically on enantiotopic carbonyl groups of meso diones in its reductive mode[5a,b] and to discriminate enantiotopic hydroxyl groups of meso diol substrates under oxidation conditions.[4c,5c] Recently we reported on the ability of HLADH to effect enantiotopically specific oxidations of monocyclic meso *cis*-1,2-diols to chiral lactones.[4c] We have now established that the enzyme remains stereospecific for monocyclic cis-1,3-sub-

(1) Part 30: Jones, J. B.; Takemura, T. *Can. J. Chem.*, in press.
(2) (a) Suckling, C. J.; Suckling, K. E. *Chem. Soc. Rev.* **1974**, *3*, 387. (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; In "Asymmetric Synthesis"; Morrison, J. D., Ed.; Academic Press: New York; Vol. 3 in press. (c) Wong, C.-H.; Whitesides, G. M. *Aldrichimica Acta* **1983**, *16*, 27.
(3) Abbreviations used: HLADH, horse liver alcohol dehydrogenase; NAD, nicotinamide adenine dinucleotide, oxidized form; FMN, flavin mononucleotide (riboflavin phosphate); $Eu(tfc)_3$, tris[((trifluoromethyl)-hydroxymethylene)-(-)-camphorato]europium(III) (optishift I).
(4) (a) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119. (b) Graves, J. M. H.; Clark, A.; Ringold, H. J. *Biochemistry* **1965**, *4*, 2655. (c) Ng, G. S. Y.; Yuan, L.-C.; Jakovac, I. J.; Jones, J. B. *Tetrahedron*, in press and previous papers. Jones, J. B.; Beck, J. F. *Tech. Chem. (N.Y.)* **1976**, *10*, 107. (d) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Suzuki, T.; Iwasaki, M.; Sasaki, Y.; Fujii, T. *J. Org. Chem.* **1981**, *46*, 2726 and previous papers. (e) van Osselaer, T. A.; Lemiere, G. L.; Merckx, E. M.; LePoivre, J. A.; Alderweireldt, F. C. *Bull. Soc. Chim. Belg.* **1978**, *87*, 799. (f) Abril, O.; Whitesides, G. M. *J. am. Chem. Soc.* **1982**, *104*, 1552. (g) MacInnes, I.; NonLebel, D. C.; Orszulik, S. T.; Suckling, C. J., *J. Chem. Soc., Chem. Commun.* **1982**, 121.
(5) (a) Dodds, D. R.; Jones, J. B. *J. Chem. Soc., Chem. Commun.* **1982**, 1080. (b) Nakazaki, M.; Chikamatsu, H.; Taniguchi, M. *Chem. Lett.* **1982**, 1762. (c) Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4659.

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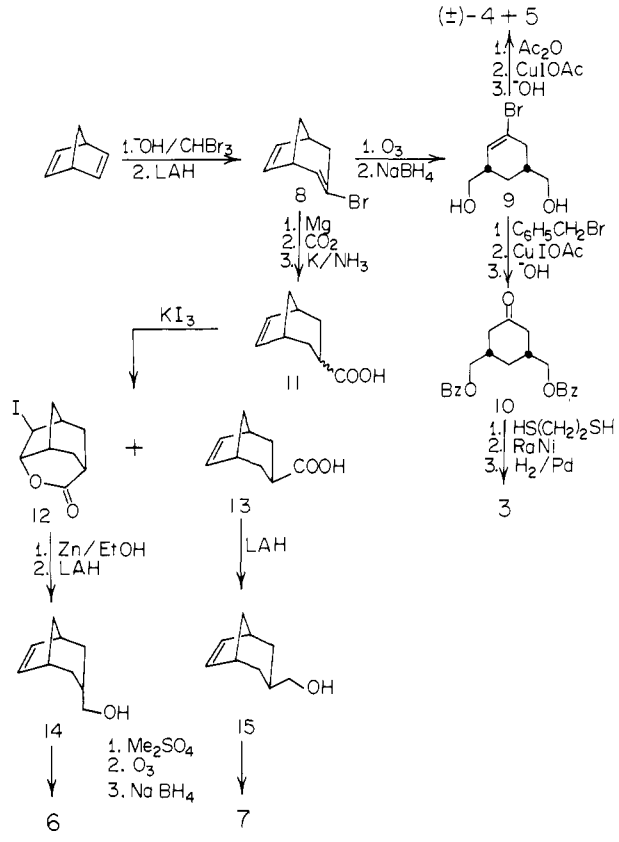
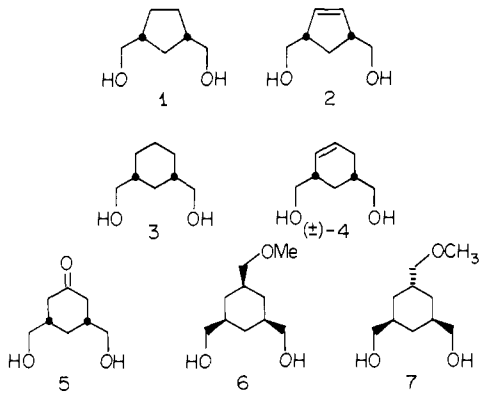
Scheme I



stituted meso diol oxidations, and in this paper, the results of HLADH-catalyzed oxidations of representative members of this group to enantiomerically pure chiral lactones are reported.

Results

Synthesis of Substrates. The substrates evaluated were the cyclopentyl diols **1** and **2** and the cyclohexyl derivatives **3–7**, which,



with the exception of the racemate (±)-**4**, are all meso compounds. The cyclopentyl diol **1** was obtained by ozonolysis, using reductive workup, of norbornene or by hydrogenation of the cyclopentenyl diol **2**, prepared from norbornadiene.[6] The cyclohexyl compounds **3–7** were obtained as outlined in Scheme I, using the literature procedures to **8**[7] **12** and **13**,[8] and **14** and **15**.[9]

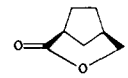
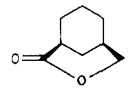
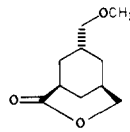
HLADH-Catalyzed Oxidations of 1–7. The rates of the enzyme-catalyzed oxidations of **1–7** relative to those of the standard reference substrate cyclohexanol are given in Table I. Since any alcohol with a oxidation rate >5% of that of cyclohexanol generally

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

substrate	rel rate	substrate	rel rate
cyclohexanol	100	4	64
1	30	5	1.3
2	35	6	40
3	60	7	38

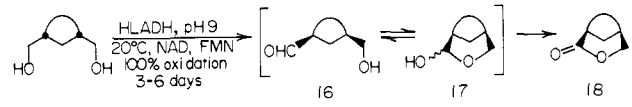
[a] Measured spectrophotometrically at 25 °C in 0.1 M NaOH–glycine buffer (pH 9), with [S] = 10^{-2}–10^{-3} M and [NAD] = 5×10^{-4} M.

Table II. Results of HLADH-Catalyzed Oxidations of Meso Diols **1–5**[a]

substrate	product	yield, %	ee, %[b]
1	 (+)-(1*S*,5*R*)-**19**	81	100[c]
3	 (+)-(1*S*,5*R*)-**20**	76	100[d]
7	 (−)-(1*S*,5*R*,7*R*)-**21**	42	100[d]

[a] Reactions carried out under Scheme II conditions. [b] Error limits ±3%. [c] By NMR. [d] By GLC.

Scheme II



gives satisfactory results on a synthetic scale, all the Table I diols except for **5** appeared to be excellent candidates for preparative HLADH-catalyzed reactions.

Preparative-scale oxidations on **1–4**, **6**, and **7** were performed at pH 9, using FMN to effect recycling[10] of the catalytic quantities of the NAD coenzyme used. For diols **1**, **3**, and **7**, the oxidations were enantiotopically specific for the hydroxymethyl groups attached to the *S*-chiral centers. Under the reaction conditions the initially formed hydroxy aldehyde products **16** then underwent further oxidation via their hemiacetal isomers **17** to give the corresponding lactone products **18** directly. The general reaction pathway is summarized in Scheme II.

The optically active lactones **19–21** were isolated enantiomerically pure and in good yield. Their structures were confirmed by comparison with the racemic materials obtained by silver carbonate[11] oxidation of the corresponding meso diol precursors **1**, **3**, or **7**. The results are summarized in Table II.

The preparative HLADH-catalyzed oxidations of **2**, **4**, and **6** did not prove synthetically viable.

Enantiomeric Excess Determinations. The ee of the cyclopentyl lactone **19** was determined by reaction with methyllithium followed by examination of the ^{1}H NMR behavior of the diastereotopic methyl peaks of the diol obtained in the presence of Eu(tfc)$_3$.[12a] For the cyclohexyl lactones **20** and **21**, the NMR method was not satisfactory. Instead, for these lactones the ee measurements were

(6) Grob, C. A.; Pfaendler, H. R. *Helv. Chim. Acta* **1970**, *53*, 2155.
(7) Moore, W. R.; Moser, W. R.; Laprade, J. E. *J. Org. Chem.* **1963**, *28*, 2200.
(8) Baldwin, J. E.; Fogelsong, W. D. *J. Am. Chem. Soc.* **1968**, *90*, 4303.
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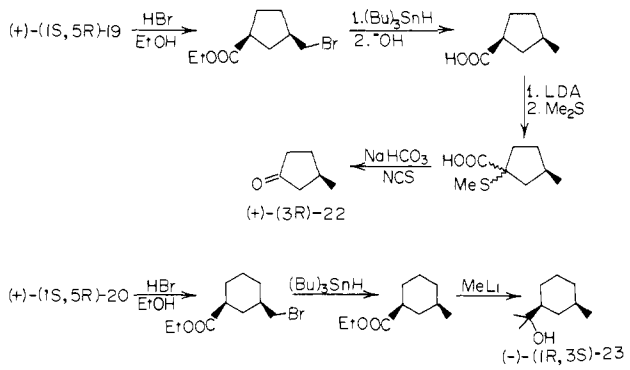
Scheme III



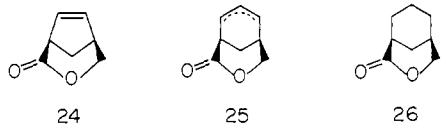
performed by GLC analysis of their ortho esters with (2*R*,3*R*)-butane-2,3-diol.[12b] In each case, parallel analyses were performed on the corresponding racemic lactones in order to provide reference and calibration standards.

Absolute Configuration Determinations. The absolute configurations of the optically pure lactones **19** and **20** of Table II were established by degradations to known compounds viz. (+)-(1*S*,5*R*)-**19** to (+)-(3*R*)-**22**[13] and (+)-(1*S*,5*R*)-**20** to (−)-(1*R*,3*S*)-**23**.[14] The correlation reactions are shown in Scheme III. The configuration of (−)-**21** was assigned as 1*S*,5*R*,7*R* by comparison of its CD spectrum with that of (+)-(1*S*,5*R*)-**20**.

Discussion

The preparations of the cyclopentyl diols **1** and **2** were straightforward. However, the choice of a synthetic approach to the cyclohexyl compounds **3**–**7** was less clear-cut. Several different routes[15-17] were explored before the Scheme II routes were chosen as the most convenient and versatile. The literature[7] conditions for the preparation of the key intermediate **8** were improved considerably by operating at lower temperatures or by employing phase-transfer catalysis. Conversion of **9** to (±)-**4** and **5** by the method shown gave rather low yields, and the preparation of **3** via **10** proved more satisfactory.

As Table I shows, with the exception of **5**, all the diols evaluated were good substrates of HLADH under kinetic analytical conditions. However, the unsaturated diols **2** and (±)-**4** and the all-syn compound **6** did not behave satisfactorily when subjected to preparative-scale oxidations. Interestingly, these inauspicious results are the consequences of chemical factors and do not represent a failure of the enzyme itself. Complex mixtures were produced by HLADH-catalyzed oxidations of both **2** and (±)-**4** owing to base-induced isomerization of the initially formed hydroxy aldehydes, or of the desired lactones **24** and **25**, to their conjugated



24 25 26

isomers and the subsequent reactions of these products under the pH 9 reaction conditions. Similar mixtures were obtained when preparations of the racemic lactones **24** and **25** by silver carbonate oxidations of **2** and (±)-**4** were attempted. A trace of hydroxy aldehyde product was detected during preparative-scale HLADH-catalyzed oxidation of **6**. However, it was unstable and decomposed during the extended (8-day) reaction period applied in an attempt to induce its subsequent transformation to lactone **26**. A similar pattern was observed when silver carbonate oxidation of the diol **6** was attempted. None of the racemic lactone **26** was formed in this reaction either. The failures of both enzymic and chemical methods for the conversion of **6** to **26** are attributed to the steric compression expected between the endo CH_2OCH_3 group at C-3 and the 7-oxygen atom if **26** were formed. Such interactions have been documented previously in bicyclo[3.3.1] systems.[18]

Diols **2**, **4**, and **5** were selected as substrate candidates since their alkene or keto functions would permit facile subsequent extension of functionalization of their lactone products. When it became clear that these diols were not synthetically viable enzyme substrates, alternative ring functionalization was sought. Diols **6** and **7** were selected because of the ease with which their precursors **14** and **15** could be prepared[8,9] from the central intermediate **8** and because of the opportunity they provided to evaluate the effects of both endo- and exo-C-3 substituents[19] on the enzymic reaction.

The HLADH-catalyzed oxidations of the meso diols **1**, **3**, and **7** proceeded smoothly to give good to excellent yields of the corresponding lactones (Table II). The Table II reactions were performed on up to 1 g of substrate. The isolated yields quoted do not represent the maxima that can be achieved. Improved efficiencies will result from optimization of the reaction conditions and by using larger quantities of substrates. Scaling up the reactions to 10 g or more of substrate presents no difficulty if larger quantities of the lactones are required.[20a] All of the lactones **19**–**21** were enantiomerically pure and of the same absolute configuration type. The enzyme is clearly quite stereospecific with respect to oxidation of the *S*-center hydroxymethyl groups.

The normally used NMR ee determination method[12a] worked well when applied to the cyclopentyl lactone **19**. However, the $\Delta\Delta\delta$ shift differences inducible in the attempted analyses of lactones **20** and **21** by NMR were insufficient to permit accurate measurements, and the GLC-dependent ortho ester method[12b] had to be employed instead.

The degradative determinations of the (+)-**19** and (+)-**20** configurations (Scheme III) were straightforward. The absolute configuration of (−)-**21** was assigned on the basis of the positive Cotton effects of the CD spectra of both (+)-**20** and (−)-**21** and their sector rule[21] analyses. The effect on the CD spectra of the methoxyl group of (−)-**21** was insignificant, with the small dichroic influence of the oxygen atom[22] being dissipated by its remoteness from the lactone chromophore.

Cubic Active-Site Section Analysis of Stereospecificity. The active-site model based on cubic space descriptors developed recently[20b] permits the stereospecificity of the Table II reactions and the reasons for the sluggish oxidation rate of the keto diol **5** to be readily interpreted. The analysis for *cis*-1,3-bis(hydroxymethyl)cyclohexane (**3**) oxidation is given in Figure 1. It is representative of all the synthetically viable (Table II) substrates and is unequivocally in agreement with the exclusive *S*-center oxidation observed experimentally.

Cubic section analysis also explains the slow reaction observed for the keto diol **5** (Table I). For this substrate, the binding mode

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(15) Haggis, G. A.; Owen, L. N. *J. Chem. Soc.* **1953**, 399. Armitage, B. J.; Kenner, G. W.; Robinson, M. J. T. *Tetrahedron* **1977**, *30*, 723. McCall, M. A.; Caldwell, J. R.; Moore, H. G.; Beard, H. M. *J. Macromol. Sci., Chem.* **1969**, 911.
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(18) Eakin, M. A.; Martin, J.; Parker, W. *J. Chem. Soc., Chem. Commun.* **1968**, 298.
(19) In addition, preparations of other variously C-3 substituted analogues of **6** and **7** were envisaged, for example, by net reduction or alkylation of the hydroxymethyl groups of **14** and **15**. However, while conversion of **15** to its C-3 methyl derivative via lithium aluminum hydride reduction of the tosylate proceeded smoothly, the *endo*-alcohol **14** underwent predictable rearrangements[8,9] under the same conditions. Accordingly, it was decided to perform this initial enzyme survey on the easily made methyl ethers **6** and **7**.
(20) (a) Jones, J. B.; Jakovac, I. J. *Org. Synth.*, in press (b) *Can. J. Chem.* **1982**, *60*, 19.
(21) Jennings, J. P.; Klyne, W.; Scopes, P. M. *J. Chem. Soc.* **1965**, 7237.
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(23) Donninger, C.; Ryback, G. *Biochem. J.* **1964**, *91*, 11P. Gerlach, H.; Zagalak, B. *J. Chem. Soc., Chem. Commun.* **1973**, 274.

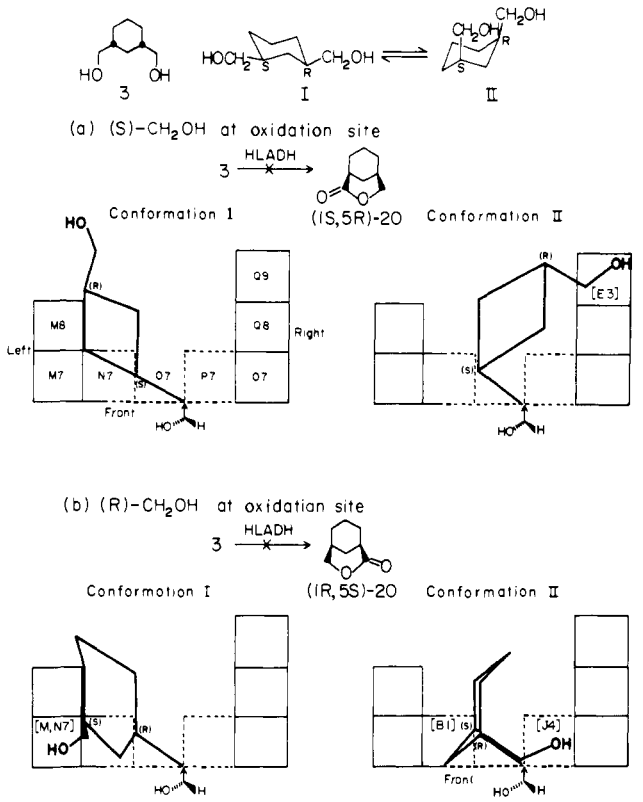


Figure 1. Cubic active-site section analysis of the stereospecificity of HLADH-catalyzed oxidation of the meso 1,3-diol **3**. The model, the analytical procedure employed, and the alphanumeric designations of the cube positions are as described previously.[20b] In this analysis, the top elevation perspective is used to depict the substrate orientations. The orientations shown are the most favored for the reactive ES complex and the least unfavorable for the unreactive orientations. Cubes bounded by solid lines are "forbidden" regions where substrate binding is precluded due to their being occupied by enzymic amino acid residues or by coenzyme. The spaces in front of, underneath, and above the defined[20b] cubic section are forbidden for the same reasons. Cubes bordered by broken lines represent "limited" space where substrate binding is possible but not favored owing to their proximity to forbidden space. The negative effects of limited region binding are synergistic; violation of two limited regions is equivalent to penetration of a forbidden location. The open areas are "allowed" space where substrate is freely accommodated. For oxidation to occur the alcohol group must locate at the oxidation site, identified by the arrow (↑). HLADH-catalyzed oxidations of primary alcohols require abstraction of the *pro-R* hydrogen.[20b,23] This ensures an unique CH_2OH group orientation at the oxidation site. For conformationally mobile substrates, each major conformation must be analyzed separately. In the case of the cyclohexane derivative **3**, the equatorial or axial CH_2OH groups at the *R* or *S* centers of both chair conformations I and II must be positioned in turn at the oxidation site and a separate analysis of the possible binding modes performed for each case. In (a) the oxidations of the CH_2OH group attached to the *S* center are considered. For conformation I, with both substituents equatorial, all of the substrate can be accommodated in allowed regions, as shown. Productive ES complex formation is therefore possible, and oxidation to (1*S*,5*R*)-**20** proceeds readily. In contrast for conformation II, the axial *S*-center CH_2OH group cannot be located at the oxidation site without other parts of the substrate violating forbidden positions, such as E3. Productive ES complex formation is therefore precluded, and no oxidation in this orientation can take place. In (b) orientations required for oxidations of (*R*)-CH_2OH group are depicted. In these cases, no favorable binding modes permitting productive ES complex formation are possible for either conformation. For conformation I the least unfavorable orientation would place the *S*-CH_2OH group in the forbidden M,N7 or above-lattice regions while, for conformation II, the two unavoidable B1 and J4 "limited" region violations are a prohibitive combination.

options are as depicted in Figure 1. However, for the single orientation leading to oxidation in the case of the parent diol (Figure 1a, conformation I), the polar keto group of **5** would intrude into the hydrophobic G4,5:M7,8 intersection region. This electrostatically incompatible juxtaposition eliminates the Figure 1a conformation I orientation as a favored ES complex binding mode for **5**. Rapid oxidation of **5** is thus impossible since there are no favorable substrate orientations.[24]

With chiral lactones being of interest both as synthons and as target molecules, the current results represent a significant addition to asymmetric synthetic methodology. Also, they extend further the exceptional ability of HLADH to control the preparation of chiral molecules with an ease that traditional methods cannot yet match. The approach is generally applicable to five- and six-membered ring meso 1,3-diols, with the limitations in structural acceptability being identifiable in advance using the cubic section model of the active-site region.

Experimental Section

The instrumentation and general purification and analytical procedures employed were as described previously.[5c] NAD was obtained from Kyowa Hakko Kojyo, NY, and FMN and HLADH (EC 1.1.1.1, once recrystallized) from Sigma. The activity of each batch of enzyme was determined[26] prior to use. The amounts of HLADH quoted refer to milligrams of active enzyme. Unless indicated otherwise, IR spectra were measured on films (for liquids) or KBr disks (for solids) and ^1H NMR spectra on $CDCl_3$ solutions.

Preparations of Meso Diols 1–7. *cis*-1,3-Bis(hydroxymethyl)cyclopent-4-ene (2). The unsaturated diol **2** was obtained in 48% yield by ozonolysis of norbornadiene by the method of Grob and Pfaendler:[6] bp 105 °C (0.08 mmHg) (lit.[6] bp 94 °C (0.06 mmHg)); IR 3350 cm^{-1}; ^1H NMR δ 1.44, 2.18 (2 H, AB q of t, d_{AB} = 13.5, $J_t(\delta\ 2.18)$ = 8.5, $J_t(\delta$ 1.44) = 6 Hz), 2.6–3.2 (2 H, m), 3.60 (4 H, d, J = 6 Hz plus 2 H, br s, OH), and 5.67 (2 H, s).

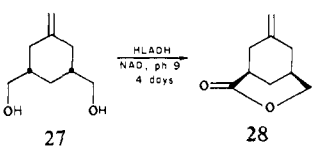
***cis*-1,3-Bis(hydroxymethyl)cyclopentane (1).** This compound was prepared in two ways. (a) Hydrogenation of the above diol **2** (640 mg, 5 mmol) in ethanol (10 mL) in the presence of Raney Ni W-2 (50 mg) at 20 °C under 1 atm of H_2 for 1 h gave, after Kugelrohr distillation, the saturated diol **1** as a colorless viscous oil (638 mg, 98% yield): bp 105 °C (0.05 mmHg); IR 3320 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.6–2.4 (8 H, m), 3.47 (4 H, br d, J = 4 Hz) and 3.8 (2 H, br s, OH). (b) Ozonolysis of norbornene (9.4 g, 0.1 mol) in methanol (100 mL) at −78 °C gave *cis*-1,3-bis(hydroxymethyl)cyclopentane (**1**, 8.9 g, 69% yield) identical in properties with those recorded above.

3-Bromobicyclo[3.2.1]octa-2,6-diene (8). The basic method of Moore et al.[7] was used except that the initial reaction of norbornadiene (92 g, 1 mol) and potassium *tert*-butoxide (134 g, 1.2 mol) in hexane (500 mL) with bromoform (302 g, 1.2 mol) in hexane (250 mL, added dropwise with stirring during 6 h) was performed at −35 °C. The crude dibromide (202 g) obtained was added during 4 h to $LiAlH_4$ (30 g, 0.81 mol) in refluxing ether (500 mL), and the mixture refluxed for 14 h. Workup gave the monobromodiene **8** (99.6 g, 54% yield from norbornadiene): bp 88–97 °C (20 mmHg) (lit.[7] bp 63 °C (5 mmHg)); ^1H NMR δ 1.5–3.0 (6 H, m), 5.82 (1 H, d of d, J = 2.5, 6 Hz), 6.28 (1 H, d of d, J = 2.5, 6 Hz) and 6.38 (1 H, br d, J = 6 Hz).

The use of triethylbenzylammonium chloride as a phase transfer catalyst[27] for norbornadiene–bromoform reactions in EtOH–CH_2Cl_2 solution in the presence of 50% aqueous NaOH also worked well on 0.1-mol scale reactions to give 57% yields of **8**.

***cis*-1,3-Bis(hydroxymethyl)cyclohexane (3).** Ozone was passed through a solution of 3-bromobicyclo[3.2.1]octa-2,6-diene (**8**, 9.25 g, 50 mmol) in methanol (100 mL) at −78 °C until the starting material disappeared (by GLC analysis). The reaction flask was transferred to an ice bath, and sodium borohydride (3.8 g, 100 mmol) was added batchwise during 2 h. The mixture was then warmed to 20 °C and, when

(24) When the direct polar interaction of the keto group at the C-5 position is removed, oxidation can once again proceed as for the parent diol **3**. This is demonstrated by the facile oxidation of **7** for which the CH_2OCH_3 substituent is able to swing into allowed H5,N8 space. Very recently, rapid oxidation has also been observed when the keto function of **5** is replaced by a methylene group, as in **27**, with a good (75%) yield of the corresponding lactone **28** being obtained under preparative-scale conditions.[25]



27 28

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the H_2 evolution ceased, the solvent was rotoevaporated. The residue was dissolved in the minimum of water and the solution saturated with NH_4Cl and continuously extracted with $CHCl_3$ for 24 h. The dried ($MgSO_4$) extract was evaporated and the residue recrystallized from $CHCl_3$ at −20 °C to give *cis*-1-bromo-3,5-bis(hydroxymethyl)cyclohexene (**9**, 8.2 g, 75% yield): mp 81–82 °C, IR 3280 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.6–2.7 (6 H, m), 3.45 (4 H, d, J = 6 Hz), 3.3–3.9 (2 H, br s, OH) and 6.06 (1 H, br s). The bromo diol **9** (2.21 g, 10 mmol) in dry tetrahydrofuran (10 mL) was added to sodium hydride (1.2 g, 25 mmol, from a 50% suspension in oil, prewashed with 3 × 25 mL hexane) in tetrahydrofuran (50 mL) under N_2. The mixture was heated gently under reflux and benzyl bromide (4.28 g, 25 mmol) added. After refluxing for 20 h, when TLC analysis showed no remaining diol, the reaction mixture was cooled and quenched with water (25 mL) and extracted with ether (2 × 50 mL). The ether extracts were washed with water (25 mL), dried ($MgSO_4$), and evaporated to give a yellow oil that was purified by MPLC on silica (ether:hexane (1:9) elution). Kugelrohr distillation gave *cis*-1-bromo-3,5-bis((benzyloxy)methyl)cyclohexene (3.86 g, 96% yield): bp 180 °C (0.07 mmHg); ^1H NMR δ 0.7–3.0 (6 H, m), 3.32 (4 H, br d, J = 6 Hz), 4.46 (4 H, s), 6.0 (1 H, br s) and 7.30 (10 H, s). Acetylation[28] of this material (16.04 g, 0.04 mol) was effected in dry CH_3CN (50 mL) with freshly prepared[29] Cu(I)OAc (15 g, excess) by heating with stirring under reflux for 24 h under N_2. To the cooled (0 °C) mixture was then added 6 N hydrochloric acid (30 mL) and water (50 mL). Extraction with ether (3 × 50 mL) followed by evaporation of the brine-washed, dried ($MgSO_4$), ether solution yielded a yellow oil that was purified by MPLC on silica (ether:hexane (1:4) elution) to give *cis*-1-acetoxy-3,5-bis((benzyloxy)methyl)cyclohexene (10.2 g, 67% yield) as a colorless gum: IR 1740 cm^{-1}; ^1H NMR δ 0.7–3.0 (9 H, m), 3.32 (4 H, br d, J = 6 Hz), 4.5 (4 H, s), 5.4 (1 H, br s) and 7.36 (10 H, s). This acetate (10.1 g, 29 mmol) was dissolved in 5% methanolic NaOH (30 mL) and stirred at 20 °C for 45 min. Water (50 mL) was then added and the mixture extracted with ether (3 × 50 mL). The ether extracts were washed with brine, dried ($MgSO_4$), and rotoevaporated to afford *cis*-3,5-bis((benzyloxy)methyl)cyclohexanone (**10**, 9.05 g, quantitative yield) as a colorless oil: IR 1720 cm^{-1}; ^1H NMR δ 1.0–3.0 (8 H, m), 3.4 (4 H, br d, J = 6 Hz), 4.46 (4 H, s) and 7.29 (10 H, s). The ketone **10** (9.2 g, 27 mmol), 1,2-ethanedithiol (2.82 g, 30 mmol), and toluene-*p*-sulfonic acid (15 mg) in benzene (50 mL) were refluxed for 4 h in a Dean-Stark apparatus. The benzene solution was then evaporated and the residue redissolved in ether (100 mL). The ether solution was washed with 10% aqueous KOH (3 × 20 mL) and then with water (25 mL), dried ($MgSO_4$), and evaporated to give a quantitative yield (11.3 g) of the ethylene thioketal of **10**: ^1H NMR δ 1.0–2.8 (8 H, m), 3.32 (4 H, d, J = 6 Hz), 3.30 (4 H, s), 4.42 (4 H, s) and 7.29 (10 H, s). The crude thioketal (3.6 g, 8.7 mmol) in ethanol (50 mL) was treated with freshly prepared W-2 Raney Ni (15 g, excess) at reflux temperature for 4 h. Evaporative workup gave *cis*-1,3-bis((benzyloxy)methyl)cyclohexane (2.68 g, 95%) as a colorless oil: ^1H NMR δ 0.5–2.1 (10 H, m), 3.24 (4 H, d, J = 6 Hz), 4.4 (4 H, s), and 7.24 (10 H, s). This material (2.67 g, 8.3 mmol) was dissolved directly in methanol (50 mL) and 10% Pd–C (100 mg) was added. After hydrogenation under hydrogen (1 atm) at 20 °C for 48 h the mixture was filtered and rotoevaporated to give *cis*-1,3-bis(hydroxymethyl)cyclohexane (**3**, 1.2 g, quantitative yield) as a white crystalline solid: mp 58 °C, IR ($CHCl_3$) 3600, 3440 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.7–2.0 (10 H, m) and 3.26 (6 H, m, collapsing to 3.24 (4 H, d, J = 6 Hz) with D_2O addition). Anal. Calcd for $C_8H_{10}O_2$: C, 66.63; H, 11.18. Found: 66.44; H, 11.31.

***cis*-3,5-Bis(hydroxymethyl)cyclohexene ((±)-4) and *cis*-3,5-Bis(hydroxymethyl)cyclohexanone (5).** Acetic anhydride (4.5 h, 44 mmol) was added dropwise with stirring at 0 °C to *cis*-1-bromo-3,5-bis(hydroxymethyl)cyclohexene (**9**, 4.42 g, 20 mmol) in dry pyridine (3 mL). After it was kept at 20 °C for 12 h, the mixture was poured onto crushed ice and, after 30 min, extracted with ether (3 × 50 mL). The ether extracts were washed with cold 5% hydrochloric acid (2 × 30 mL), then with water (3 × 30 mL), and finally with brine. The dried ($MgSO_4$) ether solution was evaporated to give *cis*-1-bromo-3,5-bis(acetoxymethyl)-cyclohexene (5.6 g, 92% yield): IR 1740 cm^{-1}; ^1H NMR δ 1.0–2.8 (6 H, m), 2.07 (6 H, s), 3.9 (4 H, d, J = 6 Hz) and 5.92 (1 H, br s). This bromodiacetate (5.55 g, 18 mmol) and freshly prepared Cu(I)OAc[28,29] in acetonitrile (50 mL) were heated under reflux with stirring under N_2 for 24 h. The reaction mixture was then cooled, 3 N hydrochloric acid (80 mL) added, and the whole extracted with ether (3 × 50 mL). The ether solution was washed with water and then with brine, dried ($MgSO_4$), and evaporated. The yellow oil containing two compounds (by TLC) obtained was purified by MPLC on silica (ether:hexane (1:4) elution) to give as the first fraction *cis*-3,5-bis(acetoxymethyl)cyclohexene (1.25 g, 29% yield) [bp 103 °C (4.5 mmHg); IR 1750 cm^{-1}; ^1H NMR δ 1.0–2.8 (6 H, m), 2.07 (6 H, s), 4.02 (4 H, d, J = 6 Hz) and 5.65 (2 H, complex AB q)] and secondly *cis*-1-acetoxy-3,5-bis(acetoxymethyl)cyclohexene (2.6 g, 50% yield) [IR 1750 cm^{-1}; ^1H NMR δ 0.9–2.8 (6 H, n), 2.07 (6 H, s), 2.12 (3 H, s), 4.0 (4 H, n) and 5.32 (1 H, br s)]. These materials were converted directly to (a) (±)-**4** and (b) **5**, respectively.

(a) *cis*-3,5-Bis(acetoxymethyl)cyclohexene (1.25 g, 5.5 mmol) in 5% methanolic NaOH (15 mL) was stirred for 30 min at 20 °C. Water (30 mL) was then added and the solution saturated with NH_4Cl and continuously exracted with ethyl acetate for 24 h. The dried ($MgSO_4$) extract was evaporated and the residue column chromatographed on silica (ethyl acetate elution) to give *cis*-3,5-bis(hydroxymethyl)cyclohexene ((±)-**4**, 780 mg, 95% yield): bp 98 °C (0.6 mmHg); IR 3350 cm^{-1}; ^1H NMR δ 1.0–2.4 (6 H, n), 3.1 (4 H, br d), 3.6 (2 H, br s, OH), and 5.28 (2 H, br s).

(b) *cis*-1-Acetoxy-3,5-bis(acetoxymethyl)cyclohexene (648 mg, 2.6 mmol) in 5% methanolic NaOH (5 mL) was stirred under N_2 at 20 °C for 30 min. Water (10 mL) was then added and the solution saturated with NH_4Cl and continuously extracted with ethyl acetate for 48 h. The dried ($MgSO_4$) extract was decolorized with charcoal and concentrated to a yellow gum (380 mg). This was column chromatographed on silica (acetone:hexane (7:3) elution) to give *cis*-3,5-bis(hydroxymethyl)cyclohexanone (**5**, 292 mg, 63% yield): mp 62 °C; IR 3300, 1720 cm^{-1}; ^1H NMR δ 1.2–2.6 (8 H, m), and 3.3–3.8 (6 H, m, collapsing to 4 H, d, J = 6 Hz with D_2O addition).

***(1R,3S,5s)-all-syn*-1,3-Bis(hydroxymethyl)-5-(methoxymethyl)-cyclohexane (6) and *(1R,3S,5r)-syn-anti*-1,3-Bis(hydroxymethyl)-5-(methoxymethyl)cyclohexane (7).** The title compounds were prepared from the bicyclovinyl bromide **8** as indicated in Scheme I. The conversion of **8** into **12** and **13** was effected by the method of Baldwin and Fogelsong[8] and of **12** and **13** to *endo*- and *exo*-alcohols **14** and **15**, respectively, according to the procedure of Garratt and White.[9]

(a) Preparation of 6 from 14. *endo*-3-(Hydroxymethyl)bicyclo-[3.2.1]oct-6-ene (6.9 g, 50 mmol) in dry tetrahydrofuran (80 mL) was added dropwise with stirring under N_2 at 20 °C to sodium hydride (2.5 g, 50 mmol, from a 50% suspension in oil, prewashed with hexane (2 × 25 mL)). When H_2 evolution had ceased, dimethyl sulfate (6.5 g, 54 mmol) was added and stirring continued for a further 14 h. Water (1 mL) was then added, and after 2 h, the solvent was rotoevaporated. The residue was added to water (50 mL) and extracted with hexane (3 × 25 mL). The hexane solution was washed with brine (25 mL), dried ($MgSO_4$), and evaporated. The colorless oil obtained was dissolved in methanol (50 mL) and the solution ozonized for 2 h at −78 °C, when excess ozone could be detected by starch-iodine paper but not by a blue solution color. The solution was warmed to 0 °C and sodium borohydride (3.8 g, 100 mmol) added slowly in small batches. When gas evolution ceased, the solvent was rotoevaporated, the residue dissolved in water (10 mL), brine (10 mL) added, and the whole continuously extracted with chloroform for 16 h. The dried ($MgSO_4$) chloroform extract was removed to give a syrup that on recrystallization from ether at −20 °C (1*R*,3*S*,5*s*)-*all-syn*-1,3-bis(hydroxymethyl)-5-(methoxymethyl)cyclohexane (1*R*,3*S*,5*S*)-*all-syn*-1,3-bis(hydroxymethyl)-5-(methoxymethyl)-cyclohexane (**6**, 2.26 g, 24% yield) as a microcrystalline solid: mp 81.5–82 °C; IR 3360 cm^{-1}; ^1H NMR δ 0.3–1.0 (3 H, m), 1.1–2.2 (6 H, m), 3.10 (2 H, br s, OH), 3.27 (2 H, d, J = 6 Hz), 3.33 (3 H, s), 3.44 (4 H, d, J = 6 Hz), and 2.95–3.65 (11 H, m). Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.83; H, 10.44. Found: C, 63.54; H, 10.64.

(b) Preparation of 7 from 15. This was effected by the procedure described above for **6**. *exo*-3-(Hydroxymethyl)bicyclo[3.2.1]oct-6-ene (**15**, 1.38 g, 10 mmol) gave (1*R*,3*S*,5*R*)-*syn-anti*-1,3-bis(hydroxymethyl)-5-(methoxymethyl)cyclohexane (**7**, 0.61 g, 29% yield): mp 52–53 °C, IR 3275 cm^{-1}; ^1H NMR δ 0.4–2.8 (9 H, m), 2.80 (2 H, br s, OH), (3 H, s), 3.40 (6 H, d, J = 6 Hz) and 3.44 (3 H, s). Anal. Calcd for $C_{10}H_{20}O_3$: C, 63.83; H, 10.64. Found: C, 64.03; H, 10.75%.

HLADH-Catalyzed Oxidations. The oxidation rates under kinetic conditions of diols **1–7** relative to that of the standard, cyclohexanol, were determined by the general HLADH kinetic assay[4b] as described previously.[5c] The results are recorded in Table I.

Preparative-Scale Reactions. Oxidation of Meso Diol 1 to the (+)-(1*S*,5*R*)-Lactone 19. *cis*-1,3-Bis(hydroxymethyl)cyclopentane (**1**, 195 mg, 1.5 mmol), FMN (2.69 g, 5.2 mmol), and NAD (192 mg, 0.26 mmol) were dissolved in 0.1 M glycine–NaOH buffer (pH 9.0, 150 mL) at room temperature (20–25 °C), and the pH was readjusted to 9.0 with 5 M aqueous NaOH. HLADH (15 mg) was then added and the mixture kept at 20–25 °C. The clear, orange, solution soon turned an opaque, almost black, color as the reaction proceeded, and the pH was readjusted periodically to 9.0 with 5 M aqueous NaOH. The course of the reaction was monitored by GLC analysis, and when no more **1** remained (4 days),

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the mixture was acidified to pH 3.5 with 2 N hydrochloric or sulfuric acid. The solution was then saturated with sodium chloride and continuously extracted with chloroform for 2 days. The dried ($MgSO_4$) chloroform extract was rotoevaporated and the residue column chromatographed on silica (ether:hexane (2:3) elution) to give (+)-(1*S*,5*R*)-3-oxabicyclo[3.2.1]octan-2-one (**19**, 156 mg, 81% yield): mp 97 °C; $[\alpha]^{25}_D$ +4.33° (*c* 1.5, $CHCl_3$); IR ($CHCl_3$) 1740 cm^{-1}; ^1H NMR δ 1.6–2.8 (7 H, m), 3.0 (1 H, m) and 4.3 (2 H, complex AB q). Anal. Calcd for $C_7H_{10}O_2$: C, 66.65; H, 7.99. Found: C, 66.50; H, 8.13.

The other meso diol oxidations were carried out by the same procedure, with the following results:

Oxidation of Meso Diol 3 to the (+)-(1*S*,5*R*)-Lactone 20. *cis*-1,3-Bis(hydroxymethyl)cyclohexane (**3**, 360 mg, 2.5 mmol), FMN (4.86 g, 10.2 mmol), NAD (360 mg, 0.5 mmol), and HLADH (18 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 200 mL) for 3 days at 20–25 °C gave (+)-(1*S*,5*R*)-3-oxabicyclo[3.3.1]nonan-2-one (**20**, 266 mg, 76% yield): mp 127–128 °C; $[\alpha]^{25}_D$ +7.4° (*c* 0.5, $CHCl_3$), CD (*c* 0.0109 M, MeOH, 20 °C) $[\theta]_{240}$ 0, $[\theta]_{211}$ +175000, IR 1726 cm^{-1}; ^1H NMR δ 1.1–2.4 (9 H, m), 2.7–3.0 (1 H, m), and 4.25–4.65 (2 H, m). Anal. Calcd for $C_8H_{12}O_2$: C, 68.54; H, 8.63. Found: C, 68.69; H, 8.57.

Oxidation of Meso Diol 7 to the (−)-(1*S*,5*R*,7*R*)-Lactone 21. (1*R*,3*S*,5*R*)-1,3-Bis(hydroxymethyl)-5-(methoxymethyl)cyclohexane (940 mg, 5 mmol), FMN (9.72 g, 20.3 mmol), NAD (720 mg, 1 mmol), and HLADH (35 mg) in 0.1 M glycine–NaOH buffer (pH 9.0, 400 mL) at 20–25 °C for 6 days yielded (−)-(1*S*,5*R*,7*R*)-7-(methoxymethyl)-3-oxabicyclo[3.3.1]nonan-2-one (**21**, 392 mg, 42% yield): bp 110 °C (0.15 mmHg); $[\alpha]^{25}_D$ −7.33° (*c* 1, $CHCl_3$); CD (*c* 0.0008 M, MeOH, 20 °C) $[\theta]_{240}$ 0, $[\theta]_{211}$ +33120; IR ($CHCl_3$) 1726 cm^{-1}; ^1H NMR δ 1.1–2.4 (8 H, m), 2.7–3.0 (1 H, m), 3.25 (2 H, d, J = 6 Hz), 3.40 (3 H, s), and 4.38 and 4.53 (2 H, br AB q of d, J_{AB} = 12, J_d = 4, 1.5 Hz). Anal. Calcd for $C_{10}H_{16}O_3$: C, 65.22; H, 8.70. Found (on racemate): C, 65.13; H, 8.94.

Preparations of Racemic Lactones (±)-19 to (±)-21. These were required as standards for the enantiomeric excess determinations of the optically active lactones obtained above. They were prepared in 74–94% yields by silver carbonate on Celite oxidations of the corresponding meso diols **1**, **3**, and **7** on the 2–7-mmol scale according to the general procedure of Fetizon et al.[11] Their physical and spectral properties, optical rotations excepted, were identical with those detailed for the optically active lactones **19**, **20**, and **21**, respectively.

Enantiomeric Excess Determinations. The ee of the cyclopentyl lactone (+)-**19** was determined by reacting it with excess methyllithium, and examining the ^1H NMR spectrum of the diastereotopic methyl peaks of the *cis*-1-(hydroxymethyl)-3-(1-hydroxy-1-methylethyl)cyclopentane product in the presence of 0.5 equiv of $Eu(tfc)_3$.[12a] Only one enantiomer was detectable, whereas ΔΔδ methyl separations of 0.1 ppm were observed with the reference diol from (±)-**19**, the ^1H NMR spectrum of which is as follows: δ 0.9–2.0 (8 H, br m), 1.2 (3 H, s), 1.26 (3 H, s), 2.4 (2 H, br s, OH) and 3.44 (2 H, d, J = 6 Hz).

The ees of (+)-**20** and (−)-**21** were established by converting them to their ortho esters with (2*R*,3*R*)-butane-2,3-diol and analyzing for the presence of diastereomers by GLC.[12b] Only one stereoisomer was present, indicating 100% ee. The orthoesters prepared from the racemic lactones on a 0.05-mmol scale[12b] were used as the reference standards. The GLC properties on 3% QFI on Chromasorb columns were as follows: ortho ester from (±)-**20**, two peaks of equal intensity, retention times 3.2, 3.6 min at 170 °C; from (+)-**20**, one peak, retention time 3.2 min; ortho ester from (±)-**21**, two peaks of equal intensity, retention times 10.8, 12.5 min at 160 °C; from (−)-**21**, one peak, retention time 10.8 min.

Absolute Configuration Determinations. The chemical correlations are summarized in Scheme III.

(a) (+)-(1*S*,5*R*)-3-Oxabicyclo[3.2.1]octan-2-one (19). The (+)-(1*S*,5*R*)-lactone **19** (1 g, 8 mmol) in ethanol (10 mL) was cooled to 0 °C and saturated with dry HBr. The solution was stirred at 20 °C for 24 h and then poured into brine and extracted with ether (3 × 50 mL). The ether solution was dried ($MgSO_4$) and rotary evaporated, and the residue was distilled to give ethyl (1*S*,3*R*)-3-(bromomethyl)cyclopentanecarboxylate (830 mg, 50% yield): bp 60 °C (0.75 mmHg) $[\alpha]^{25}_D$ +4.4° (*c* 1, $CHCl_3$); IR 1730 cm^{-1}; ^1H NMR δ 1.2 (3 H, t, J = 8 Hz), 2.0–3.0 (6 H, m), 3.2 (2 H, d, J = 6 Hz) and 4.0 (2 H, q, J = 8 Hz).

This bromo ester (800 mg, 3.4 mmol) and tri-*n*-butyltin hydride (1.4 g, 3.6 mmol) in dry benzene were stirred under N_2 at 20 °C for 8 h and then refluxed for 8 h.[30] The solvent was rotary evaporated and the residue dissolved in 10% ethanolic NaOH (50 mL) and heated under reflux for 3 h. Water (50 mL) was added to the cooled solution, which was then washed with ether (3 × 50 mL). The aqueous solution was acidified carefully with concentrated sulfuric acid and extracted with ether (3 × 50 mL). This latter ether solution was dried ($MgSO_4$), decolorized with charcoal, and rotary evaporated and the residual orange oil distilled to give (1*S*,3*R*)-3-methylcyclopentanecarboxylic acid (348 mg, 80% yield): bp 70 °C (0.05 mmHg) $[\alpha]^{25}_D$ −5.3° (*c* 1, $CHCl_3$) (lit.[31] bp 115 °C (15 mmHg), $[\alpha]_D$ −5.89° (neat)); IR 1710 cm^{-1}; ^1H NMR δ 1.2 (3 H, d, J = 7 Hz), 1.5–2.5 (8 H, m), and 9.8 (1 H, br s).

The above acid (270 mg, 2.1 mmol) in tetrahydrofuran–HMPA (5 mL, 2:1) was added during 3 min with stirring to a 0 °C solution of LDA (prepared from disopropylamine (0.5 g, 5 mmol) and *n*-butyllithium (5 mmol) in tetrahydrofuran (5 mL)). The mixture was stirred at 0 °C for 2 h and dimethyl sulfide (0.47 g, 5 mmol) added.[32] After it was stirred for a further 3 h at 0 °C, the reaction mixture was quenched by pouring into ice water (100 mL) and extracted with ether (2 × 50 mL). The ether solution was extracted with saturated aqueous $NaHCO_3$ (50 mL) and then with water (50 mL). The combined aqueous layers were washed with ether (2 × 30 mL) and the acidified to pH 1 with concentrated hydrochloric acid and extracted with ether (3 × 50 mL). The dried ($MgSO_4$) ether solution was rotary evaporated to give (3*R*)-3-methyl-1-(methylthio)cyclopentanecarboxylic acid (330 mg, quantitative yield): IR 1710 cm^{-1}; ^1H NMR δ 1.0 and 1.08 (3 H, diastereomeric CH_3), 1.4–2.8 (7 H, m), 2.16 and 2.19 (3 H, epimeric SCH_3), and 10.0 (1 H, s). This was employed without further purification. It was dissolved in ethanol (10 mL) and $NaHCO_3$ (0.6 g, 7 mmol) added at 20 °C. The suspension was stirred for 10 min and *N*-chlorosuccinimide (0.66 g, 5 mmol) then added portionwise. CO_2 evolution began immediately, and the solution became warm. The stirring was continued for 2 h, and 1 N hydrochloric acid was then added together with a few drops of saturated aqueous sodium sulfite. After further stirring for 3 h longer, the mixture was diluted with water (50 mL) and extracted with ether (3 × 30 mL). The ether solution was dried ($MgSO_4$) and evaporated to give an oil that was column chromatographed on silica ($CHCl_3$ elution) and distilled to give (3*R*)-3-methylcyclopentanone (**22**, 50 mg, 30% yield): bp 80 °C (13 mmHg) $[\alpha]^{25}_D$ +133.3° (*c* 1, $CHCl_3$) (lit.[13] bp 143–143.5 °C, $[\alpha]_D$ +154.8 (*c* 0.73, $CHCl_3$)); IR 1740 cm^{-1}; ^1H NMR δ 1.2 (3 H, d, J = 6 Hz), 1.5–3.0 (7 H, m).

(b) (+)-(1*S*,5*R*)-3-Oxabicyclo[3.3.1]nonan-2-one (20). The (+)-(1*S*,5*R*)-lactone **20** (50 mg, 0.36 mmol) in ethanol (10 mL) was saturated with dry HBr at 0 °C and then kept at 20 °C for 15 h. The ethanolic solution was then concentrated to 1 mL and ether (30 mL) added. The ethereal solution was washed with saturated aqueous $NaHCO_3$ and then with brine, dried ($MgSO_4$), and evaporated. Distillation of the residue yielded ethyl (1*S*,3*R*)-3-(bromomethyl)cyclopentanecarboxylate (63 mg, 71% yield) $[\alpha]^{25}_D$ +2.27° (*c* 1.5, $CHCl_3$), IR 1740 cm^{-1}; ^1H NMR δ 1.25 (3 H, t, J = 8 Hz), 1.7–2.3 (10 H, m), 3.3 and 3.5 (2 H, d of d, J = 6 Hz), and 4.18 (2 H, q, J = 8 Hz).

To the bromo ester (50 mg, 0.2 mmol) in benzene (10 mL) under N_2 at 20 °C was added tri-*n*-butyltin hydride (75 mg, 0.25 mmol) in benzene (5 mL). The solution was stirred for 20 h at 20 °C and then refluxed for 4 h. After evaporation of the solvent, the oily residue was column chromatographed on silica (CCl_4 elution) to give ethyl (1*S*,3*R*)-3-methylcyclopentanecarboxylate (25 mg, 74% yield): bp 96 °C (15 mmHg) (lit.[33] (±) bp 208–210 °C (760 mmHg)); $[\alpha]^{25}_D$ +1.68 (*c* 1.25, $CHCl_3$); IR 1740 cm^{-1}; ^1H NMR δ 1.0 (3 H, d, J = 3 Hz), 1.25 (3 H, t, J = 8 Hz), 1.35–2.2 (10 H, m), and 4.18 (2 H, q, J = 8 Hz).

The above ester (25 mg, 0.14 mmol) in dry tetrahydrofuran (5 mL) was treated with methyllithium (0.43 mmol, 0.3 mL of 1.5 M ethereal solution) under N_2 at −78 °C. The mixture was then warmed slowly to 20 °C and the excess methyllithium destroyed with solid NH_4Cl. Water (10 mL) was then added and the mixture extracted with ether (3 × 10 mL). The ether solution was washed with brine, then dried ($MgSO_4$), and evaporated to give (1*R*,3*S*)-*m*-menthan-8-ol (18 mg, 74% yield): bp 102–103 °C (20 mmHg) (lit.[33] (±) bp 98–100 °C (25 mmHg); $[\alpha]^{25}_D$ −7.2° (*c* 0.32, C_6H_6 (lit.[14] $[\alpha]_D$ (+)-(1*S*,3*R*) enantiomer +8.38° (C_6H_6)); IR 3330 cm^{-1}; ^1H NMR δ 0.93 (3 H, d, J = 3 Hz), 1.17 (6 H, s), and 1.2–2.25 (10 H + OH, m) ppm.

(c) The absolute configuration of (−)-(1*S*,5*R*,7*R*)-**21** was assigned by comparison of its CD spectrum with that of (+)-(1*S*,5*R*)-**20**, whose configuration was determined chemically above. Both CD curves have a + Cotton effect. Sector rule analysis[21] confirms that their absolute configurations are of the same type.

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Registry No. 1, 3965-56-8; 2, 30213-19-5; 3, 5059-76-7; (±)-4, 88211-17-0; 5, 88211-18-1; 6, 88211-19-2; 7, 88269-11-8; 8, 51788-41-1; (±)-9, 88211-20-5; 10, 88211-21-6; 10 (ethylene thioketal), 88211-28-3; 11, 88211-22-7; 12, 88269-12-9; 13, 10531-02-9; 14, 61597-60-2; 15, 61597-61-3; (+)-19, 88269-13-0; (+)-20, 88211-23-8; (-)-21, 88211-24-9; (±)-24, 88211-25-0; (±)-25, 88314-72-1; alcohol dehydrogenase, 9031-72-5; norbornadiene, 121-46-0; norbornene, 498-66-8; cyclohexanol, 108-93-0; (±)-*cis*-1-bromo-3,5-bis((benzyloxy)methyl)cyclohexene, 88211-26-1; (±)-*cis*-1-acetoxy-3,5-bis((benzyloxy)methyl)cyclohexene, 88211-27-2; *cis*-1,3-bis((benzyloxy)methyl)cyclohexane, 88211-29-4; (±)-*cis*-1-bromo-3,5-bis(acetoxymethyl)cyclohexene, 88211-30-7; (±)-*cis*-3,5-bis(acetoxymethyl)cyclohexene, 88211-31-8; (±)-*cis*-1-acetoxy-3,5-bis(acetoxymethyl)cyclohexene, 88211-32-9.

Molybdenum Sites of *Escherichia coli* and *Chlorella vulgaris* Nitrate Reductase: A Comparison by EXAFS

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Abstract: The molybdenum sites in two different types of nitrate reductase, assimilatory enzyme from *Chlorella vulgaris* and dissimilatory enzyme from *Escherichia coli*, have been investigated and compared by using X-ray absorption edge and EXAFS spectroscopy. The molybdenum environment in *Chlorella* nitrate reductase was found to strongly resemble that in hepatic sulfite oxidase. In the oxidized state of the *Chlorella* enzyme the molybdenum has two terminal oxygens at 1.71 ± 0.03 Å as well as two or three sulfurs at 2.44 ± 0.03 Å. Additional undetected ligands may be present in this and the other structures reported. A single terminal oxygen at 1.67 ± 0.03 Å and a set of sulfurs at 2.37 ± 0.03 Å are found upon full NADH reduction. Data are also presented on dithionite-reduced and cyanide-inhibited forms of the *Chlorella* enzyme. In contrast with all other non-nitrogenase molybdenum enzymes, the *E. coli* nitrate reductase molybdenum appears devoid of oxo groups when fully reduced, although an oxo species appears upon oxidation by nitrate. The reduced *E. coli* enzyme shows evidence for two or three sulfurs at 2.36 ± 0.03 Å and one or two nitrogens and/or oxygens at 2.10 ± 0.03 Å. Furthermore, a feature consistent with a bridged Mo-X interaction at about 2.8 Å is observed. Mechanistic models are proposed that incorporate these results.

Nitrate reductase, sulfite oxidase, xanthine oxidase, and formate dehydrogenase contain a common molybdenum cofactor, "Mo-co", and yet they display significantly different chemical and physical properties.[2] In an effort to understand the structural basis for this diversity, we have undertaken a systematic X-ray absorption study of molybdenum-containing proteins.[3,4] This paper reports results of EXAFS experiments on nitrate reductase from two different sources—*Chlorella vulgaris*[5] and *Escherichia coli*.[6] Nitrate reductase from *Chlorella* is a homotetramer with a molecular weight of about 360000,[7] which contains one molybdenum, one heme, and one FAD prosthetic group per subunit. It is an assimilatory enzyme which reduces nitrate to nitrite, which ultimately is reduced to ammonia for incorporation of the nitrogen into amino acids. In contrast, *E. coli* nitrate reductase is a dissimiliatory enzyme which uses nitrate as a terminal electron acceptor in the absence of O_2. This enzyme is a heterodimer of molecular weight 200000 containing one molybdenum, 16 irons, and 16 acid-labile sulfurs.[6] The latter extrude as four Fe_4S_4 clusters (Adams, M. W. W.; Mortenson, L. E., unpublished results). The EXAFS experiments described in this paper indicate that the molybdenum site of *Chlorella* nitrate reductase is quite similar to that previously observed for sulfite oxidase,[4] whereas the molybdenum site of *E. coli* nitrate reductase exhibits properties different from any molybdenum enzyme yet examined.

Experimental Section

Sample Preparation. ***Chlorella vulgaris*** nitrate reductase was purified as described previously[8] to an A_{413}/A_{280} ratio of better than 0.55 and concentrated in 80 mM pH 7.6 potassium phosphate buffer with an Amicon YM-10 membrane to 34 mg/mL. The molybdenum concentration in the sample was 0.31 mM.[9] The initial NADH:nitrate reductase activity, measured spectrophotometrically,[9] was typically about 80 μmol NADH oxidized per min per mg of protein. NADH-reduced enzyme was prepared by addition of sufficient 0.2 M NADH to generate a 20-fold molar excess of NADH in a deoxygenated sample; cyanide-inactivated (reversible) enzyme was generated by addition of 10 μL of 1.0 M KCN to a 0.3 mL of NADH-reduced sample.[10] Dithionite-reduced enzyme was prepared by addition of a few grains of sodium dithionite to an oxidized sample. After preparation, all samples were frozen in 20 × 10 × 1.5 mm Lucite cuvettes. Enzyme activity was checked after data collection and was typically better than 95% of its initial value.

E. coli nitrate reductase was isolated as described[6] and assayed using reduced methylviologen as an electron donor.[6,11] The specific activity of all preparations was 78-80 μmol of NO_3^- reduced per min per mg of protein. Samples were concentrated to 180 mg/mL in 50 mM pH 7.0 potassium phosphate buffer by Amicon filtration with an XM-100 membrane; the molybdenum concentration was 0.7 mM. Reduced samples were prepared by anaerobic incubation with 5 mM sodium dithionite or H_2/hydrogenase/methylviologen.[6] Ferricyanide-oxidized enzyme was prepared by addition of potassium ferricyanide to a final concentration of 9 mM (under argon), while air-oxidized enzyme was prepared by exposing a concentrated sample to air for several hours with occasional stirring. Nitrate-oxidized enzyme was prepared by treatment of a dithionite-reduced sample with excess nitrate. All samples retained greater than 90% of their nitrate reductase activity after irradiation. The model

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