Enzymes in Organic Synthesis. 16.[1] Heterocyclic Ketones as Substrates of Horse Liver Alcohol Dehydrogenase. Stereospecific Reductions of 2-Substituted Tetrahydrothiopyran-4-ones

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Abstract: Horse liver alcohol dehydrogenase (HLADH) has been shown to have the ability to effect efficient and stereospecific reductions of S-heterocyclic ketone analogues of its good carbocyclic substrates. This extends considerably the asymmetric synthesis potential of the enzyme. Preparative scale (~2 g) HLADH-catalyzed reductions of racemic 2-substituted tetrahydrothiopyran-4-ones are enantioselective. Reductions of each pair of enantiomers occur in the same absolute configuration sense to give the corresponding cis- and trans-2-substituted tetrahydrothiopyran-4-ols of 100% enantiomeric purity and with *S* configurations at each alcohol center. The tolerance by HLADH of the presence of the sulfide function in its substrates adds significantly to the versatility and flexibility of this enzymic synthesis method. For example, enantiomerically pure acyclic secondary alcohols of predetermined absolute configurations cannot be obtained directly by HLADH-mediated reductions of their precursor ketones. This previous disadvantage has now been overcome by exploiting the sulfur atom as a "lock" to maintain a desirable cyclic substrate configuration during the reduction step, followed by Raney nickel removal of the sulfur from the (4*S*)-*cis*- and -*trans*-thiopyranols obtained to give the corresponding acyclic 3*S* alcohols in good yields. The stereospecificities of the reductions, including the influence of the C-2 substituent on the degree of enantioselectivity, are all interpretable in terms of the enzyme's active-site characteristics.

The powerful, and often unique, asymmetric synthesis opportunities provided by the chiral catalytic properties of enzymes have become widely recognized. Many diverse examples of their synthetic applications have been documented, using both native and immobilized enzymes.[2-4] Alcohol dehydrogenases, which catalyze oxidoreductions of the type depicted in eq 1,[5] are currently among the most useful enzymes

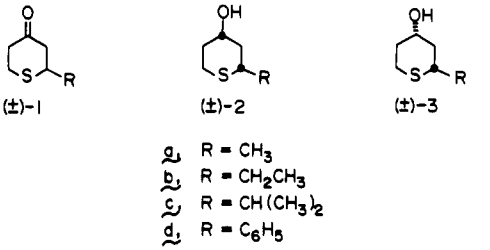
$$\rangle C{=}O + NADH + H^+ \rightleftharpoons \rangle CH(OH) + NAD^+ \quad (1)$$

from the synthetic organic chemical point-of-view. The best documented of these is the commercially available enzyme from horse liver. HLADH[5] is an extremely versatile enzyme with a well-defined and predictable[4b,6] stereospecificity and which accepts a broad structural range of aldehyde, ketone, and alcohol substrates.[1,4b,7-9]

To our knowledge, no attention has yet been devoted to HLADH substrates containing heteroatoms. Accordingly, in view of the widespread synthetic interest in chiral heterocyclic structures, we have initiated a survey of the structural- and stereospecificity of HLADH toward heterocyclic ketones and alcohols.

Sulfur was the heteroatom considered first. This choice was determined by several factors. The enzyme was known to be tolerant of the sulfide functional group.[10] Also, by analogy with the well-documented carbocyclic substrates,[4b] oxido reductions of cyclic sulfur-containing alcohols and ketones were expected to be facile and stereospecific. An important additional benefit of this approach was that subsequent desulfurization of the initial chiral heterocyclic products would provide convenient access to the corresponding stereoisomers of acyclic alcohols and ketones. This feature is of considerable significance since, with the exception of aldehydes and primary alcohols, acyclic compounds are poor substrates of HLADH. Furthermore, the limited number of such oxidoreductions that can be achieved occurs with low stereospecificity at best.[4b] By employing an excisable sulfur atom as a "lock" to maintain the cyclic substrate configurations preferred by HLADH, it was considered that a variety of acyclic asymmetric alcohol and ketone synthons of predeterminable chiralities would become readily accessible via this enzymic approach.[11]

In this paper we report on the stereospecificity of HLADH-catalyzed reductions of racemic 2-substituted thiopyran-4-ones **1a–d** to the corresponding cis and trans alcohols **2a–d** and **3a–d.** The results obtained achieve several of the objectives outlined above.



Results

Preparation of Substrates and Their Racemic Alcohol Products. The racemic substrates **1a–d** were prepared as outlined in Scheme I. The common intermediate, thiopyran-4-one ethylene ketal (**5**), was obtained in 34% overall yield from methyl acrylate. The ketones **1a–d** were obtained in 32–40% yields from **5**. The racemic cis and trans alcohols (±)-**2a–d** and (±)-**3a–d,** respectively, required for reference purposes were prepared by reduction of (±)-**1a–d** with lithium aluminum hydride. The cis–trans (~9:1) mixtures produced were separated by medium pressure liquid chromatography, and the stereoisomers were identified by NMR spectroscopic comparisons of the chemical shifts and half-widths of the C-4 protons[12] of each cis–trans pair.

HLADH-Catalyzed Reductions of (±)-1a–d. The rates of HLADH-catalyzed reductions of **1a–d** relative to that of the standard reference cyclohexanone are recorded in Table I. Each of the 2-alkylthiopyran ketones was an excellent substrate. While **1d** was clearly a much poorer substrate than the others, its rate of reduction was still fast enough to permit a preparative scale reaction to be undertaken with confidence.[4b]

The racemic thiopyranones **1a–d** were each subjected to preparative scale HLADH-mediated reduction using ethanol to effect coupled-substrate recycling[4b,13] of the catalytic

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Scheme I

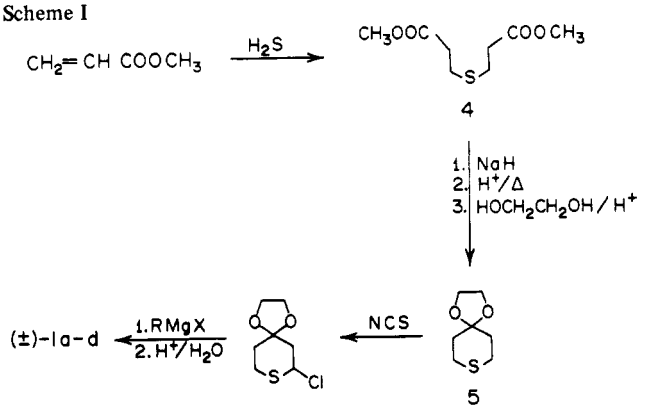


Table I. Relative Rates[a] of HLADH-Catalyzed Reductions of (±)-**1a-d**

substrate	rel rate	substrate	rel rate
cyclohexanone	100	(±)-**1c**	32
(±)-**1a**	45	(±)-**2d**	6
(±)-**1b**	59		

[a] Reduction rates were measured spectrophotometrically at 25 °C in 0.1 M phosphate buffer (pH 7).

amount of the nicotinamide coenzyme used. Each reaction was terminated when GLC analysis showed it to be ~50% complete. The unchanged ketones and product alcohols were easily separated by chromatography. Their structures were confirmed by comparisons with the racemic compounds characterized earlier. The results obtained are summarized in Scheme II.

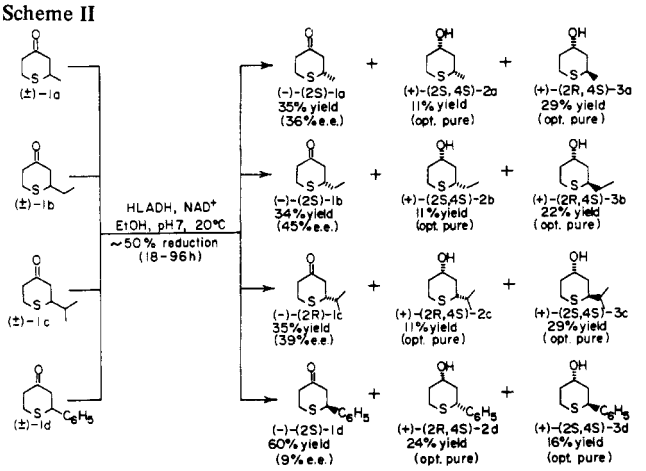
Enantiomeric Excess Determinations. The e.e.'s of the recovered ketones **1a-d** were determined by converting them, in quantitative yields, to the ketals **6a-d** with (−)-(2*R*,3*R*)-2,3-butanediol, followed by ^{13}C NMR examinations[14] of the diastereomeric mixtures obtained from both the racemic and optically active ketones **1a-d.** The $\Delta\delta$ values observed for the various diastereotopic carbon atoms are recorded in Table II. The e.e. values shown in Scheme II for the optically active samples **1a-d** represent the averages of measurements on the ^{13}C resonances of two or more carbon atoms of the corresponding ketals.

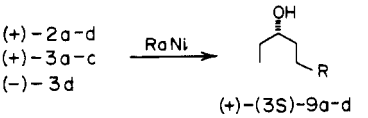
The e.e.'s of the optically active cis and trans alcohols isolated from the enzymic reductions were determined by examination of their ^1H NMR spectra of their MTPA esters **7a-d** and **8a-d,** respectively, in the presence of the shift reagent Eu(fod)$_3$.[15] The methoxyl peak only was monitored. Its $\Delta\Delta\delta$ values for the racemic MTPA esters are recorded in Table III. For the corresponding esters derived from the enzymic reactions, this peak appeared as a singlet, thereby showing the compounds to be optically pure[16] (Scheme II).

Absolute Configuration Determinations. The absolute configurations shown in Scheme II for the optically active ketones recovered following HLADH-catalyzed reductions of (±)-**1a-d** were assigned from octant rule[17] analysis of the Cotton effects observed in their CD spectra. Positive Cotton effects were observed for ethanolic solutions of the recovered ketones **1a-c.** For the preferred chair conformations with equatorially oriented alkyl groups, the C-2 substituents are unequivocally positioned in positive octants for the (2*S*)-**1a,** (2*S*)-**1b,** and (2*R*)-**1c** enantiomers. The Cotton effect for the 2-phenyl ketone **1d** was negative. The phenyl group occupies a negative octant only for the 2*S* enantiomer. The octant rule analyses are not affected by the sulfur,[18] since the heteroatom is in a nodal plane of the carbonyl group and thus does not contribute to the rotatory strength.[17]

The relative configurations of the chiral centers of the cis and trans alcohols **2a-d** and **3a-d,** respectively, were established by NMR spectral comparisons with their racemates. The absolute configurations at C-4 were then determined by desulfurization with Raney nickel to the (+)-3*S* alcohols **9a-d.**[19-23]

Scheme II





Discussion

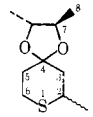
The preparations of 2-substituted tetrahydrothiopyran-4-ones can be achieved via Dieckmann cyclizations of diesters such as **4** appropriately substituted at C-3.[24] However, such syntheses are low yielding and tedious. Accordingly, the generally applicable route depicted in Scheme I was developed. It is convenient and flexible, with all the desired substituents being readily introduced into the common intermediate **5** by a one-pot chlorination-Grignard reaction[25] sequence. Good yields of the desired ketones were obtained in each case.

Lithium aluminum hydride reduction of (±)-**1a-d** was expected to give a mixture of the cis and trans alcohols (±)-**2a-d** and (±)-**3a-d,** with the cis isomer predominating due to delivery of hydride from the axial direction being favored.[26,27] This was found to be so. The identities of the stereoisomers were verified by their ^1H NMR spectra, with the broad (22-28 Hz half-width) bands of the C-4 axial protons of the cis alcohols resonating at higher field by 0.6-0.8 ppm than the narrower (10-12 Hz half-width) peaks of the corresponding equatorial protons of their trans isomers. These results are in accord with the literature data on related carbocyclic and heterocyclic compounds.[12,26b]

Each HLADH-catalyzed reduction of (±)-**1a-d** was performed on a preparatively significant scale (≥2 g of substrate) using very simple reaction conditions. In accordance with our normal practice for racemic substrates,[7] each reaction was terminated after ~50% of reduction had been effected.[28] Workup of the reaction mixtures was also simple, involving only continuous chloroform extraction followed by chromatography. The recovery of material was virtually quantitative by this method, with the total yields of isolated, purified products being 84-95%. For the current thiopyranone substrates the use of ethanol as cosubstrate was found to be more effective as a nicotinamide coenzyme recycling method than the sodium dithionite procedure employed previously.[7a]

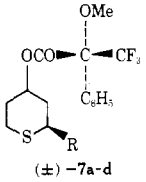
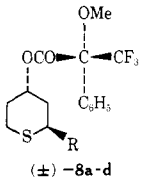
In all cases, direct methods were employed to determine the e.e.'s of the optically active alcohol products and recovered ketones. ^{13}C NMR examination[14] of the ketals **6a-d** provided a flexible method for measuring e.e.'s of the ketones **1a-d,** since

Table II. Enantiomeric Shift Differences in the ^{13}C NMR Spectra of the Diastereomeric Ketals (±)-**6a–d**[a]

ketal structure	compd	Δδ, ppm C-2	C-3	C-4	C-5	C-6	C-7	C-8	other C
 6	**6a**	0.3	1.1	0	1.3[b]	0.3[b]	0.3	0	0 (CH_3)
	6b	0.3[b]	1.1[b]	0	1.2[b]	0.2	0.4	0.05	0 (CH_2CH_3) 0 (CH_2CH_3)
	6c	0.3[b]	1.1	0	1.2[b]	0.2[b]	0.4	0	0 ($CH(CH_3)_2$) 0.4 ($CH(CH_3)_2$)
	6d	0.4	1.1[b]	0	1.3[b]	0.2	0.2	0.05	0–0.1 (C_6H_5)

[a] ^1H noise-decoupled spectra determined in C^2HCl_3. [b] Used for evaluating e.e. differences shown in Scheme II.

Table III. Enantiomeric Shift Differences for the Methoxyl Protons of the Racemic MTPA Esters **7a–d** and **8a–d**[a]

structures	compd	$Eu(fod)_3$, equiv	ΔΔδ, ppm
 (±)-**7a–d**	(±)-**7a**	0.50	0.07
	(±)-**8a**	0.45	0.32
	(±)-**7b**	0.35	0.07
	(±)-**8b**	0.23	0.48
 (±)-**8a–d**	(±)-**7c**	0.43	0.12
	(±)-**8c**	0.24	0.45
	(±)-**7d**	0.17	0.10
	(±)-**8d**	0.20	0.30

[a] Determined at 220 MHz in CCl_4 solutions.

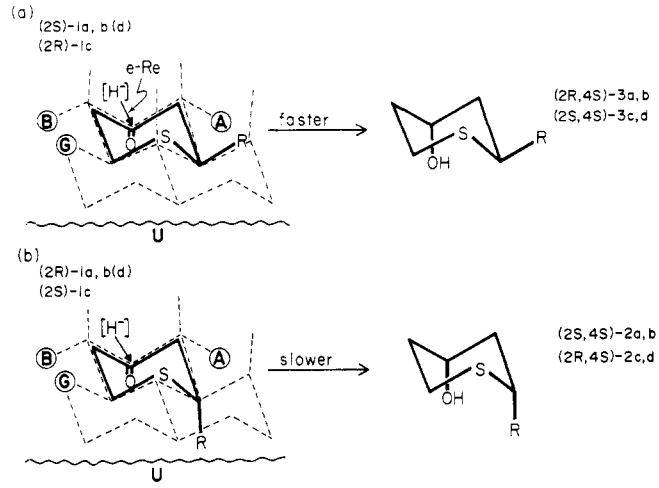


Figure 1. Diamond lattice section[32] analysis of the stereochemical course of HLADH-catalyzed reductions of the enantiomers of **1a–d.** The relevant portions of the lattice (described in detail in ref 4b and 7a), including the forbidden or unfavorable regions **A, B, G,** and **U,** are indicated by the dashed lines. The *R* and *S* enantiomers of **1a–d** are depicted at the active site in the orientations, ensuring delivery of the hydride equivalent from the required *e-Re*[5] direction.[4b] (a) In this orientation, the tetrahydrothiopyranones are in their preferred chair conformations with the R groups equatorially oriented. Unfavorable interactions with forbidden lattice positions are totally avoidable and reduction to the experimentallly observed cis alcohols as shown is a favored process, except for **1d** where R as phenyl is too large (see Discussion). (b) Neither of the chair conformations of these enantiomers can be correctly oriented for reduction without some degree of unfavorable interactions, and reductions are thus generally slower than for a. With the C-2 group equatorial, the R substituent would be forced into the extremely undesirable **G** region. This is strongly disfavored. In the alternative chair form depicted, which leads to the trans alcohols isolated, the axial R group projects toward the **U** region at the bottom of the active site. This region can accommodate moderately sized functions when necessary, but slower reduction rates become inevitable as a result.

the resonances of several pairs of diastereotopic carbon atoms could be utilized within each spectrum. That each of the cis and trans alcohols isolated was optically pure was unequivocally demonstrated by the ^1H NMR spectra of their MTPA esters in the presence of the $Eu(fod)_3$ shift reagent.[15]

The absolute configurations shown in Scheme II were established without difficulty. Octant rule analyses of the CD spectra of the ketones (2*S*)-**1a,b,d** and (1*R*)-**1c** were unambiguous. Assignments of the configurations of the chiral centers of **2a–d** and **3a–d** via their identification as cis or trans isomers, followed by their individual conversion to one of the alcohols (3*S*)-**9a–d,**[29] were also straightforward. Desulfurizations of the (4*S*)-thiopyranols **2** and **3-a–d** were facile, and up to 75% yields of the chiral acyclic alcohols (3*S*)-**9a–d** were isolated.

The efficient and convenient preparation of these alcohols in enantiomerically pure form realizes a major objective of the approach. These results clearly confirm the viability of using the sulfur atom as a "lock" to maintain an enzymically acceptable cyclic substrate configuration when the asymmetric acyclic oxidoreduction desired cannot be induced directly using the open-chain substrates themselves. In the present case, the optically pure alcohols **9a–d** are not obtainable by direct HLADH-catalyzed reductions of the corresponding acyclic ketones. The latter are either not substrates or the reductions are not stereospecific.[4b]

As Scheme II shows, HLADH-mediated reduction of (±)-**1a–c** favors the formation of the trans over the cis alcohols. From (±)-**1d** the proportions of cis and trans products formed are approximately equal. The opposite absolute configurations at C-2 of each pair of cis and trans alcohols show that the alcohol stereoisomers are derived from opposite enantiomers of their racemic ketone precursors. The enzyme is, however, only stereoselective with respect to the enantiomers of the thiopyranones **1a–d.** The degrees of its enantiomeric preferences are reflected by the percentage e.e.'s of the recovered ketones (Scheme II). HLADH exhibits a significant enantioselectivity toward (2*R*)-**1a,b** and (2*S*)-**1c** reductions, but only a marginal partiality toward (2*R*)-**1d.**

It must be stressed that, although the enzyme is only enantioselective with respect to the enantiomers of its **1a–d** substrates, the reduction of each individual enantiomer is in itself completely stereospecific, giving rise to optically pure alcohol products in each case. *The yields of each enantiomerically pure alcohol product of Scheme II could therefore be doubled by permitting the HLADH-catalyzed reductions of* **1a–d** *to proceed to completion.* The overall results obtained parallel those observed with the analogous 3-alkylcyclohexanone substrates.[30,31]

Diamond Lattice Section Analysis. Analysis of alcohol dehydrogenase specificities by the diamond lattice section analytical approach[32] was first conceived and developed by Prelog.[6] The model now used employs an updated system of coordinates incorporating the data of several groups.[4b,7a]

The results observed for the thiopyranone substrates **1a–d** are all interpretable in terms of one basic lattice analysis.[33] This is depicted in Figure 1. The most preferred orientations are

shown in Figure 1a, with the C-2 R group equatorially oriented with respect to the thiopyran ring and with no forbidden regions violated. This avoids all unfavorable lattice interactions as long as R remains reasonably small. This analysis is in accord with the observed stereochemistries of the trans alcohols isolated. The situation depicted in Figure 1b for the opposite enantiomers, while still allowed and predicting the formation of cis alcohols of the correct chiralities, is less favorable since the region **U** is somewhat intolerant of any substituent intrusion.[4b,7a] Thus, the Figure 1a enantiomers would be generally expected to be more readily reduced than those of Figure 1b, as is the case.

This analysis does not hold when the C-2 substituent is large, such as phenyl. In this case unfavorable interactions at the active site become inevitable. For the Figure 1a binding mode, a C-2 phenyl group will begin to intrude seriously on forbidden region **A**. In the Figure 1b situation, bad interactions with region **U** will become unavoidable. The net result predicted is a markedly lower rate of reduction of both enantiomers of **1d** and a narrowing of the enantioselectivity of the enzyme. This is as observed. In fact, there is even a minor reversal of enantiomeric preference, with reduction of (2*R*)-**1d** becoming slightly favored. The diamond lattice based conclusions are further substantiated by the relative rate data of Table I, the almost 1:1 ratio of **2d:3d** obtained, and the low e.e. level of the (2*S*)-**1d** recovered (Scheme II).

The results obtained in this initial study have achieved several of the objectives outlined in the introductory statement. It is clear that incorporation of heterocyclic sulfur into HLADH substrates will increase considerably the versatility and flexibility of this enzymic approach to asymmetric synthesis.[35] Further work along these lines is now in progress and will be reported shortly.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR (as films) and NMR (in C^2HCl_3) spectra were recorded for each compound; the spectra were all in accord with the structures assigned and selected data only are reported. ^1H NMR spectra were recorded in C^2HCl_3 at 60 or 220 MHz on Varian T-60 or HR 220, and ^{13}C NMR on CFT-20, instruments using $(CH_3)_4Si$ as reference. GLC analyses were performed on 3% OV 101 columns with flame ionization detection. Optical rotations were determined in $CHCl_3$ with a Perkin-Elmer 141 polarimeter and CD spectra on a Roussel-Jouan II Dicrographe. The UV absorptions of the enzymic relative-rate studies were monitored using a Unicam SP 1800 spectrophotometer. NAD^+ was purchased from Sigma. HLADH (EC 1.1.1.1) is available from Worthington or Boehringer; the activity of each batch of enzyme was determined[37] prior to use and the amounts of HLADH quoted refer to milligrams of active enzyme.

Preparation of Tetrahydrothiopyran-4-one Ethylene Ketal (5). Methyl acrylate and hydrogen sulfide were reacted as described by Hurd and Gershbein[38] to give dimethyl 3,3-thiodipropionate (**4**, 89% yield), bp 106–116 °C (0.4 mmHg) (lit.[38] bp 161–162 °C (18 mmHg)). The diester **4** was then converted in 41% yield to tetrahydrothiopyran-4-one, mp 59–61 °C (lit.[39] mp 59–60 °C) by the procedure of Fehnel and Carmack.[39]

Tetrahydrothiopyran-4-one (19.1 g, 0.15 mol), ethane-1,2-diol (11.2 g, 0.18 mol), toluene-*p*-sulfonic acid (0.5 g), and benzene (500 mL) were heated under reflux in a Dean-Stark apparatus for 8 h. The benzene was then removed by rotary evaporation and the residue dissolved in ether and washed, first with 10% aqueous sodium hydroxide and then with water. Evaporation of the dried ($MgSO_4$) ether solution followed by distillation gave tetrahydrothiopyran-4-one ethylene ketal (**5**, 24.4 g, 93%): bp 82–83 °C (2.5 mmHg); ^1H NMR δ 1.7–2.0 (4 H, M), 2.6–2.9 (4 H, m), and 3.9 (4 H, s) ppm. Anal. ($C_7H_{12}O_2S$) C, H.

Preparations of Thiopyran-4-one Substrates (±)-1a–d. Each was prepared from the common intermediate **5**.

2-Methyltetrahydrothiopyran-4-one ((±)-1a). *N*-Chlorosuccinimide (9.35 g, 0.07 mol) was added in three portions with stirring during 15 min to a cooled (0 °C) solution of the ethylene ketal **5** (9.6 g, 0.06 mol) in dry benzene (300 mL) under a nitrogen atmosphere.[25] The mixture was stirred for a further 2.5 h. It was then filtered, and the filtrate was added dropwise with stirring under nitrogen to a cooled (0 °C) solution of methylmagnesium iodide (from methyl iodide (34.1 g, 0.24 mol) and magnesium (5.8 g, 0.24 mol)) in dry ether (200 mL). The mixture was stirred for a further 15 h and cold (0 °C), dilute hydrochloric acid added cautiously. The organic layer was separated and washed successively with water, saturated aqueous sodium bicarbonate, and finally with water again. The dried ($MgSO_4$) ether–benzene solution was evaporated, and the residue was dissolved in acetic acid (50 mL) and 2 N aqueous hydrochloric acid (50 mL). After being stirred at 20 °C for 15 h, the mixture was extracted with ether (300 mL) and the ether extract washed with water (2 × 100 mL), 10% aqueous sodium hydroxide (5 × 100 mL), and finally with water once more (2 × 100 mL). The dried ($MgSO_4$) solution was evaporated and the residue chromatographed on silica. Elution with ether/hexane (1:6) gave 2-methyltetrahydrothiopyran-4-one ((±)-**1a**, 2.53 g, 32% yield): bp 79–82 °C (11 mmHg) (lit.[24] mp 119–120 °C); IR 1720 cm^{-1}; ^1H NMR δ 1.3 (3 H, d, J = 7 Hz) ppm. Anal. ($C_6H_{10}OS$) C, H.

The remaining substrates were prepared by the same basic procedure.

2-Ethyltetrahydrothiopyran-4-one ((±)-1b), from ethyl magnesium bromide (0.24 mol): 35% yield (3.0 g); bp 70–72 °C (2 mmHg); IR 1720 cm^{-1}; ^1H NMR δ 1.0 (3 H, t, J = 7 Hz), 1.6 (2 H, m, J = 7 Hz), and 2.4–3.2 (7 H, m) ppm. Anal. ($C_7H_{12}OS$) C, H.

2-Isopropyltetrahydrothiopyran-4-one ((±)-1c), from isopropyl magnesium bromide (0.24 mol): 39% yield (3.7 g); bp (Kugelrohr) 80 °C (6 mm Hg); IR 1725 cm^{-1}; ^1H NMR δ 1.0 (6 H, d, J = 7 Hz), 1.6–2.2 (1 H, m), and 2.4–3.2 (7 H, m) ppm. Anal. ($C_8H_{14}OS$) C, H.

2-Phenyltetrahydrothiopyran-4-one ((±)-1d), from phenyl magnesium bromide (0.24 mol): 40% yield (4.6 g); mp 72–74 °C; IR ($CHCl_3$) 1720 cm^{-1}; ^1H NMR δ 2.7–31 (6 H, m), 4.2 (1 H, m), and 7.3 (5 H, s) ppm. Anal. ($C_{11}H_{12}OS$) C, H.

Preparation of Cis and Trans Alcohols (±)-2a–d and (±)-3a–d. The same procedure was used for each. The appropriate 2-substituted thiopyran-4-one (**1a–d,** 0.01 mol) in dry ether (5 mL) was added dropwise with stirring to a cooled (0 °C) suspension of lithium aluminum hydride (0.01 mol) in dry ether (15 mL). The resulting mixture was stirred for 1 h and water (0.4 mL) was then added carefully, followed by 10% aqueous sodium hydroxide (0.4 mL) and further water (1.2 mL). The mixture was filtered and the filtrate dried ($MgSO_4$), evaporated, and Kugelrohr distilled (100 °C (3 mmHg)). The cis:trans ratios were determined by GLC, and the stereoisomers were separated by medium pressure liquid chromatography on silica (ICN, 0.032–0.063 mm) with ethyl acetate/hexane (1:5) as the eluting solvent. The products obtained were as follows:

***cis*- and *trans*-2-methyltetrahydrothiopyran-4-ol ((±)-2a and (±)-3a):** cis/trans (89:11) mixture (1.24 g, 94% yield); IR 3350 cm^{-1}. Anal. ($C_6H_{12}OS$) C, H. Chromatography of 0.7 g yielded the trans isomer (±)-**3a** (0.07 g) [^1H NMR δ 1.2 (3 H, d, J = 7 Hz) and 4.2 (1 H, m, $W_{1/2}$ = 10 Hz) ppm] and the cis isomer (±)-**2a** (0.54 g) [^1H NMR δ 1.2 (3 H, d, J = 6 Hz) and 3.4 (1 H, M, $W_{1/2}$ = 25 Hz) ppm].

***cis*- and *trans*-2-ethyltetrahydrothiopyran-4-ol ((±)-2b and (±)-3b):** cis/trans (87:13) mixture (1.41 g, 97% yield); IR 3380 cm^{-1}. Anal. ($C_7H_{14}OS$) C, H. Chromatography of 0.8 g gave the trans alcohol (±)-**3b** (0.1 g) [^1H NMR δ 1.0 (3 H, t, J = 6.5 Hz), 1.95 (2 H, q, J = 6.5 Hz), and 4.1 (1 H, m, $W_{1/2}$ = 12 Hz) ppm] and the cis alcohol (±)-**2b** (0.66 g) [^1H NMR δ 1.0 (3 H, t, J = 7 Hz), 1.5 (2 H, q, J = 7 Hz), and 3.5 (1 H, m, $W_{1/2}$ = 28 Hz) ppm].

***cis*- and *trans*-2-isopropyltetrahydrothiopyran-4-ol ((±)-2c and (±)-3c):** cis/trans (86:14) mixture (1.53 g, 96% yield); IR 3350 cm^{-1}. Anal. ($C_8H_{16}OS$) C, H. Chromatography of 1.0 g yielded the trans compound (±)-**3c** (0.14 g) [^1H NMR δ 1.0 (6 H, d, J = 7 Hz) and 4.15 (1 H, m, $W_{1/2}$ = 10 Hz) ppm] and the cis compound (±)-**2c** (0.85 g) [^1H NMR δ 1.0 (6 H, d, J = 7 Hz) and 3.5 (1 H, m, $W_{1/2}$ = 27 Hz) ppm].

***cis*- and *trans*-2-phenyltetrahydrothiopyran-4-ol ((±)-2d and (±)-3d):** cis/trans (88:12) mixture (0.88 g, 93% yield, reaction performed on 5 mmol scale); IR ($CHCl_3$) 3400 cm^{-1}. Anal. ($C_{11}H_{14}OS$) C, H. Chromatography of 0.8 g afforded the trans isomer (±)-**3d** (0.1 g) [^1H NMR δ 4.2 (1 H, m, $W_{1/2}$ = 8 Hz) and 7.3 (5 H, s) ppm] and the cis isomer (±)-**2d** (0.7 g) [^1H NMR δ 3.7 (1 H, m, $W_{1/2}$ = 22 Hz) and 7.3 (5 H, s) ppm].

Relative Rates of HLADH-Catalyzed Reductions of (±)-1a–d.

Assays were performed at 25 °C using 0.1 M potassium phosphate buffer (pH 7) solutions, 1.75×10^{-4} M in NADH. The substrate concentrations were 10^{-2} M for (±)-**1a–c** and 10^{-3} M for (±)-**1d.** For each compound, a reference assay was performed on a solution containing the same concentration of cyclohexanone. The reductions were initiated by adding a 50-μL aliquot of HLADH solution (1 mg/mL) in 0.05 M Tris-HCl buffer (pH 7.4) to make a final volume of 3 mL in a 1-cm pathlength cuvette. The absorbance change at 340 nm was monitored. The relative rate results are recorded in Table I.

HLADH-Catalyzed Reductions of (±)-1a–d. The following general procedure was employed. The substituted thiopyranone (~2 g) was dissolved or suspended in 0.1 M potassium phosphate buffer (pH 7) (1 L) at 20 °C. NAD^+ (~1.5 g) and ethanol (3 mL) were then added, the pH readjusted to 7 with 10 M aqueous potassium hydroxide, and the reduction initiated by the addition of HLADH (80 mg). The extent of reaction was monitored by GLC. After ~50% of reduction had occurred (18–96 h), the mixture was continuously extracted with chloroform for 2 days. The dried ($MgSO_4$) chloroform extract was evaporated, and the residue (quantitative recovery) was chromatographed on silica (100 g). Elution with a solvent gradient of 2–50% ether/hexane effected clean separation of the unchanged optically active ketones **1a–d,** and the optically pure trans alcohols **3a–d** and cis alcohols **2a–d.** The spectral properties of each compound were identical with those of the corresponding racemates characterized earlier.

The individual reactions gave the following results after Kugelrohr distillation of each product (cf. Scheme II).

Reduction of (±)-1a (2.1 g, 16 mmol) with enzyme (80 mg), NAD^+ (1.92 g, 2.9 mmol), and EtOH (3 mL) for 18 h (50% reduction) gave (−)-(2*S*)-**1a** (0.73 g, 36% e.e.) $[[\alpha]^{20}_D$ −5.5° (*c* 1)], (+)-(2*R*,4*S*)-**3a** (0.6 g, opt pure) $[[\alpha]^{20}_D$ +14.8° (*c* 1.1)], and (+)-(2*S*,4*S*)-**2a** (0.22 g, opt pure) $[[\alpha]^{20}_D$ +4.0° (*c* 1)].

Reduction of (±)-1b (2.0 g, 14 mmol) with enzyme (80 mg), NAD^+ (1.69 g, 2.5 mmol), and EtOH (3 mL) for 42 h (50% reduction) yielded (−)-(2*S*)-**1b** (0.67 g, 45% e.e.) $[[\alpha]^{20}_D$ −7.9° (*c* 1)], (+)-(2*R*,4S)-**3b** (0.55 g, opt pure) $[[\alpha]^{20}_D$ +22.3° (*c* 1)], and (+)-(2*S*,4*S*)-**2b** (0.22 g, opt pure) $[[\alpha]^{20}_D$ +15.9° (*c* 1)].

Reduction of (±)-1c (2 g, 12.7 mmol), with enzyme (80 mg), NAD^+ (1.53 g, 2.3 mmol), and EtOH (3 mL) for 68 h (50% reduction) afforded (−)-(2*R*)-**1c** (0.7 g, 39% e.e.) $[[\alpha]^{20}_D$ −0.6° (*c* 1)], (+)-(2*S*,4*S*)-**3c** (0.57 g, opt pure) $[[\alpha]^{20}_D$ +9.0° (*c* 1.1)], and (+)-(2*R*,4*S*)-**2c** (0.2 g, opt pure) $[[\alpha]^{20}_D$ +12.4° (*c* 1)].

Reduction of (±)-1d (2.0 g, 10.4 mmol) with enzyme (80 mg), NAD^+ (1.28 g, 1.9 mmol), and EtOH (3 mL) for 96 h (40% reduction) yielded recovered (−)-(2*S*)-**1d** (1.21 g, 9% e.e.) $[[\alpha]^{20}_D$ −0.5° (*c* 1)], (−)-(2*S*,4*S*)-**3d** (0.31 g, opt pure) $[[\alpha]^{20}_D$ −32.3° (*c* 1)], and (+)-(2*R*,4*S*)-**2d** (0.46 g, opt pure) $[[\alpha]^{20}_D$ +37.4° (*c* 1.1)].

Enantiomeric Excess Determinations of 1a–d Recovered following HLADH-Catalyzed Reductions. Each racemic and optically active ketone **1a–d** (1 mmol) was heated under reflux in a Dean-Stark apparatus with (−)-(2*R*,3*R*)-2,3-butanediol (1.1 mmol), toluene-*p*-sulfonic acid (5 mg), and benzene (20 mL) for 12 h. The benzene solution was concentrated and the residue dissolved in ether (50 mL). It was then washed with 10% aqueous sodium hydroxide (3 × 20 mL) followed by water. The dried ($MgSO_4$) ether solution was evaporated to give a quantitative yield of the corresponding ketal **6a–d.** Each ketal was then Kugelrohr distilled (100 °C (3 mmHg) for **6a–c,** 180 °C (3 mmHg) for **6d**), and its ^1H-decoupled ^{13}C NMR spectra determined. These are recorded below for the racemic ketals. The e.e. values of the ketals derived from the optically active ketones recovered from the enzyme-catalyzed reductions were determined from their corresponding ^{13}C NMR spectra by the procedure of Hiemstra and Wynberg[14] using the enantiomeric shift differences indicated in Table II. The ^{13}C NMR data for each compound are:

2-Methylthiopyran-4-one ketal (±)-6a: δ 16.7 (C-8), 21.1 (C-2 CH_3), 26.3 and 26.6 (C-6), 35.4 and 35.7 (C-2), 36.7 and 37.9 (C-5), 46.6 and 47.7 (C-3), 77.8 and 78.1 (C-7), and 106.9 (C-4) ppm.

2-Ethylthiopyran-4-one ketal (±)-6b: δ 11.4 ($-CH_2CH_3$), 16.85 and 16.9 (C-8), 26.2, and 26.4 (C-6), 28.8 ($-CH_2CH_3$), 37.5 and 38.7 (C-5), 42.7 and 43.0 (C-2), 44.7 and 45.8 (C-3), 78.0 and 78.4 (C-7), and 107.3 (C-4) ppm.

2-Isopropylthiopyran-4-one ketal (±)-6c: δ 17.0 (C-8), 19.5 and 19.9 ($CH(CH_3)_2$), 26.1 and 26.3 (C-6), 32.7 ($CH(CH_3)_2$), 37.6 and 38.8 (C-5), 42.0 and 43.1 (C-3), 48.0 and 48.3 (C-2), 78.0 and 78.4 (C-7), and 107.6 (C-4) ppm.

2-Phenylthiopyran-4-one ketal (±)-6d: δ 16.85 and 16.9 (C-8), 27.7 and 27.9 (C-6), 37.0 and 39.3 (C-5), 45.0 and 45.1 (C-2), 45.0 and 46.1 (C-3), 78.0 and 78.2 (C-7), 107.2 (C-4), and 127.4, 127.5, 128.6, 141.5, and 141.6 (C_6H_5) ppm.

Enantiomeric Excess Determinations of the Enzyme-Derived Alcohols 2a–d and 3a–d. The racemic and optically active alcohols **2a–d** and **3a–d** were converted to their MTPA esters **7a–d** and **8a–d** in quantitative yields by the standard literature procedure[15] using freshly prepared (+)-(2*R*)-α-methoxy-2-trifluoromethylphenyl acetyl chloride: $[\alpha]^{20}_D$ +129.2° (*c* 1, CCl_4) (lit.[15] $[\alpha]_D$ 129.0 ± 0.2° (*c* 5.17, CCl_4)). The enantiomeric purities of the HLADH-derived esters were then determined by 220-MHz ^1H NMR examination of the protons of the methoxyl group in the presence of Eu(fod)$_3$ shift reagent. The MTPA esters of the racemic alcohols were used to provide the necessary reference peaks for determining the $\Delta\Delta\delta$ values for each pair of diastereotopic methoxyl groups.[15] The enantiomeric shift differences used are summarized in Table III. No traces of diastereomeric impurities were detected in any of the spectra of the MTPA esters of the alcohols isolated from the enzymic reactions.[16]

Absolute Configuration Determinations. (1) Of the Optically Active Recovered Ketones 1a–d. These were determined by octant rule analysis of the Cotton effects observed in the CD spectra of the optically active ketones isolated following enzyme-mediated reductions of (±)-**1a–d.** The spectral data used were: (2*S*)-**1a** (*c* 0.004, EtOH, 20 °C) $[\theta]_{320}$ 0°, $[\theta]_{292}$ +211°, $[\theta]_{260}$ 0°; (2*S*)-**1b** (*c* 0.004, EtOH, 20 °C) $[\theta]_{320}$ 0°, $[\theta]_{288}$ +371°, $[\theta]_{288}$ 0°; 2(*R*)-**1c** (*c* 0.004, EtOH, 20 °C) $[\theta]_{320}$ 0°, $[\theta]_{287}$ +454°, $[\theta]_{260}$ 0°; (2*S*)-**1d** (*c* 0.003, EtOH, 20 °C) $[\theta]_{325}$ 0°, $[\theta]_{288}$ −330°, $[\theta]_{260}$ 0°.

(2) Of the HLADH-Derived Alcohols 2a–d and 3a–d. The relative configurations of the cis and trans alcohols were determined by comparison of their ^1H NMR spectra with those of the previously authenticated racemic compounds. The absolute configurations at the C-4 alcohol centers were then established by desulfurization of **2a–d** and **3a–d** with Raney nickel by the following general procedure.

The tetrahydrothiopyranol (150 mg) and freshly prepared Raney nickel (1 g) in ethanol (10 mL) were heated under reflux for 2 days. The cooled mixture was then filtered and concentrated carefully. The acyclic alcohols **9a–d** were isolated in ~75% yields. Their structures were confirmed spectroscopically and their specific rotations then determined. The results obtained are summarized below:

Desulfurization of (+)-*cis*-2a gave (3*S*)-3-hexanol (**9a**): $[\alpha]^{20}_D$ +7.8° (*c* 0.3, EtOH) (lit.[19] for 3*R* enantiomer, $[\delta]_D$ −8.2° (*c* 11.5, EtOH)); ^1H NMR δ 0.9 (6 H, t, *J* = 7 Hz), 1.2–2.0 (7 H, m, includes OH), and 3.5 (1 H, m) ppm. From (+)-*trans*-**3a,** the same alcohol was obtained: $[\alpha]^{20}_D$ +8.0° (*c* 0.6, EtOH).

Desulfurization of (+)-*cis*-2b yielded (3*S*)-3-heptanol (**9b**): $[\alpha]^{20}_D$ +8.5° (*c* 0.8, EtOH) (lit.[21] for 3*R* enantiomer, $[\alpha]_D$ −5.0° (*c* 12, EtOH)); ^1H NMR δ 0.95 (6 H, t, *J* = 7 Hz), 1.2–2.0 (9 H, m, includes OH), and 3.5 (1 H, m) ppm. From (+)-*trans*-**3b,** the (3*S*)-heptanol (**9b**) obtained had $[\alpha]^{20}_D$ +8.1° (*c* 0.9, EtOH).

Desulfurization of (+)-*cis*-2c gave (3*S*)-6-methyl-3-heptanol (**9c**):[29] $[\alpha]^{20}_D$ +7.2° (*c* 0.45, EtOH); ^1H NMR δ 0.9 (6 H, d, *J* = 7 Hz), 0.95 (3 H, t, *J* = 7 Hz), 1.2–1.8 (8 H, m, includes OH), and 3.5 (1 H, m) ppm. From (+)-*trans*-**3c,** the alcohol (3*S*)-**9c**[29] isolated had $[\alpha]^{20}_D$ +5.1° (*c* 0.9, EtOH).

Desulfurization of (−)-*cis*-2d afforded (3*S*)-5-phenyl-3-pentanol (**9d**): $[\alpha]^{20}_D$ +22.2° (*c* 0.9, EtOH) (lit. +26.8°,[22] +31.8°[23] (EtOH)); ^1H NMR δ 0.95 (3 H, t, *J* = 6.5 Hz), 1.2–2.0 (4 H, m), 2.2 (1 H, s, OH), 2.7 (2 H, m), 3.5 (1 H, m), and 7.2 (5 H, s) ppm. The (3*S*)-alcohol **9d** from (+)-*trans*-**3d** had $[\alpha]^{20}_D$ +19.9° (*c* 0.8, EtOH).

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(28) If the enantiomeric specificity of an enzyme is absolute, catalyses of racemic substrates stop automatically after 50% of reaction. Terminating our reactions at this point thus provides an important measure of the enantiomeric selectivity of HLADH toward unnatural substrates such as **1a-d.**
(29) To our knowledge, (3*S*)-6-methylheptan-3-ol (**9c**) has not been reported. However, it is an unexceptional member of the known series of alcohols (+)-3*S*-**9a,b,d,**[19-23] and there is little doubt that the (+) enantiomer of **9c** has the 3*S* configuration.
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(32) Using a C–C bond length of 1.54 Å, a diamond lattice section sufficiently large to encompass the active site is created. The points of the lattice are then used as coordinates to identify and discuss significant regions of the active site. Those areas which are empty, and therefore capable of accommodating part or all of the substrate structure, are designated as "allowed" regions. On the other hand, the regions of the lattice occupied by the atoms of the enzyme or coenzyme will, to a greater or lesser extent, preclude the orientation of the substrate at those general locations. These regions are designated as "forbidden" or "undesirable". The "allowed" orientations of a substrate are determined using Framework Molecular Models of both the lattice and the substrate. For a more complete description of the diamond lattice section approach, and its basis, see ref 6 and 4b, pp 295ff.
(33) Even though the diamond lattice section was developed for use with carbocyclic substrates, it is also well suited to interpreting the behavior of heterocyclic ketones such as **1a-d.** The slight distortion from cyclohexane geometry induced by the ~1.8-Å C–S bond length and ~99° C–S–C bond angle[34] does not affect the validities of the analyses in any way.
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