Enzymes in organic synthesis. 28.[1] Reinvestigation of the horse liver alcohol dehydrogenase-catalyzed reductions of 2-alkylcyclohexanones. Identification of *cis*-alkylcyclohexanols as minor products

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The formation of *cis*-2-alkylcyclohexanol products from horse liver alcohol dehydrogenase-catalyzed reductions of 2-alkylcyclohexanones has been detected for the first time. The *cis*-alcohols are minor (< 4%) products, with the isomeric *trans*-products being heavily predominant under all conditions. *cis*-Alcohol production can be suppressed by suitable manipulation of the reaction conditions. Exclusive formation of the *trans*-alcohols is favoured by operating in an appropriate buffer such as Tris-HCl at the lowest [E]/[S] and pH conditions possible, and by minimizing the reaction time. The results parallel the *cis*-alcohol formation observed previously with the analogous 3-alkyltetrahydrothiopyran-4-one substrates.

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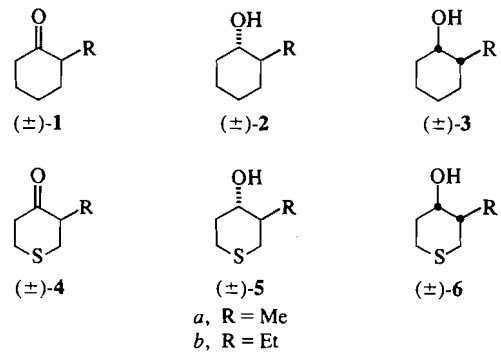
On a décelé pour la première fois la formation d'alkyl-2 cyclohexanols-*cis* lors des réductions d'alkyl-2 cyclohexanones catalysées par la déshydrogénase de l'alcool du foie de cheval. Les alcools *cis* sont des produits secondaires (<4%) tandis que les produits *trans* sont fortement majoritaires dans toutes les conditions. On peut éviter la formation d'alcool *cis* en jouant convenablement avec les conditions de réaction. On favorise la formation exclusive d'alcools *trans* en travaillant avec un tampon convenable tel le Tris-HCl aux plus faibles conditions possibles de [E]/[S] et de pH et en minimisant le temps de réaction. Les résultats sont identiques à ceux obtenus antérieurement lors de la formation d'alcools *cis* à partir des substrats analogues du type alkyl-3 tétrahydrothiopyranones-4.

[Traduit par le journal]

Horse liver alcohol dehydrogenase (HLADH)[3] is a commercially available NAD/H-dependent oxidoreductase that catalyzes highly stereoselective C=O ⇌ CH(OH) transformations for a broad structural range of aldehyde, ketone, and alcohol substrates (1, 2–9). As a result it is currently one of the most versatile and useful enzymes from the asymmetric synthetic point of view and many illustrations of its potential for the preparation of useful chiral synthons have been documented (2–9). Reductions of 2-alkylcyclohexanones were among the first of the preparative-scale HLADH-catalyzed reactions to be studied. Reductions of 2-methyl- and 2-ethylcyclohexanones ((±)-**1***a*,*b*) were reported to be stereospecific, with the (2*S*)-ketones being converted to the corresponding *trans*-alcohols (1*S*,2*S*)-**2***a*,*b* of 100% e.e., and the unreactive ketone enantiomers (2*R*)-**1***a*,*b* being recovered virtually optically pure (2–6). Subsequent work confirmed these results (5, 6). However, it was noted that in the reduction of 2-fluorocyclohexanone, a small (4%) amount of *cis*-2-fluorocyclohexanol of unrecorded chirality was formed in addition to the usual, predominant (96%), *trans*-product (6). Very recently, HLADH-catalyzed reductions of the related 3-alkyltetrahydrothiopyranone substrates (±)-**4***a*,*b* to the *trans*-alcohols **5***a*,*b* were also found to produce minor (2–3%) amounts of *cis*-alcohols **6***a*,*b*.



The formation, to whatever degree, of *cis*-alcohols during HLADH-mediated reductions of ketones such as **1** and **4** is of considerable interest with respect to delineating the possible active site orientations of such substrates (9*c*). Accordingly, we decided to reinvestigate the reductions of (±)-**1***a*,*b* since trace proportions of *cis*-alcohol products could well have escaped detection during the earlier studies. The results reported in this

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[3]Abbreviations used: HLADH, horse liver alcohol dehydrogenase; NAD^+, oxidized and reduced forms respectively of nicotinamide adenine dinucleotide; MTPA, (+)-2-methoxy-2-trifluoromethylphenylacetyl; $Eu(fod)_3$, tris(6.6.7.7.8.8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III).

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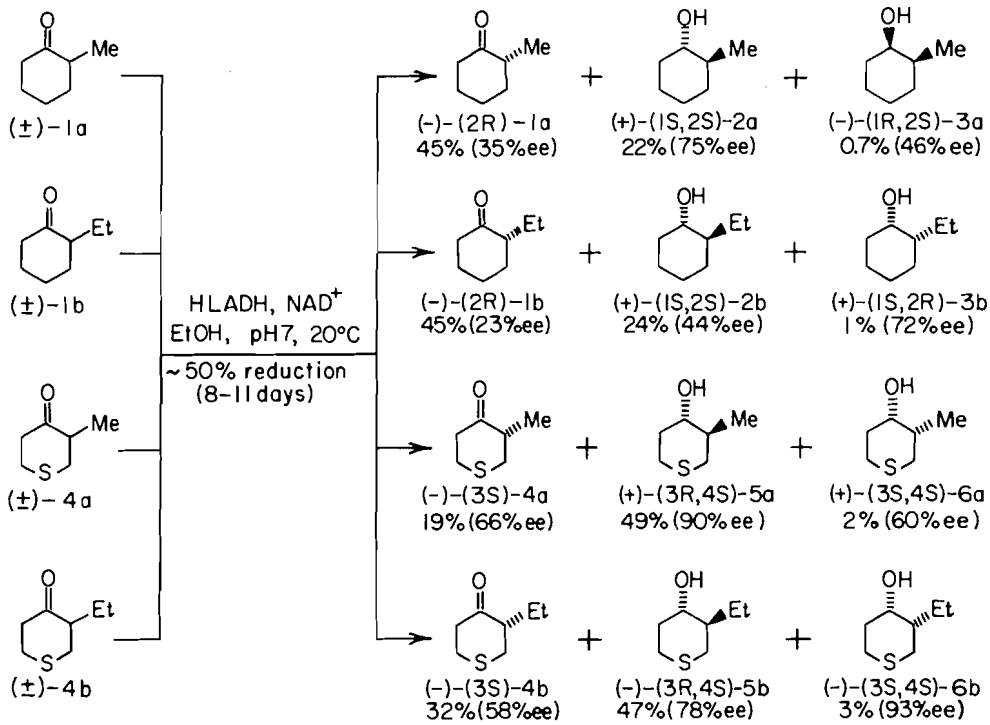


SCHEME 1

paper confirm that *cis*-alcohols can be produced in minor amounts during the reductions of all 2-substituted cyclohexanone and related substrates studied to date. Furthermore, the extent to which the *cis* compounds are formed is found to reflect the nature of the reaction conditions used. With the appropriate controls the formation of the *cis* by-products can be completely suppressed.

Results

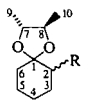
The racemic substrates and potential products **1**–**3***a*,*b* were all known compounds and were either purchased or prepared according to published procedures. Preparative-scale HLADH-catalyzed reductions of the alkylcyclohexanones (±)-**1***a*,*b* were carried out in phosphate buffer pH 7 on a 4-g scale. The results obtained are recorded in Scheme 1 together with the data obtained previously (1) on the 3-alkyltetrahydrothiopyranones (±)-**4***a*,*b*.

The unreacted ketones **1***a*,*b* and the product alcohols **2**,**3***a*,*b* were separated by mplc and their identities confirmed by comparison with their racemic counterparts. The e.e.'s of the ketones (−)-**1***a*,*b* were determined by their quantitative conversion to the corresponding ketals **7***a*,*b* with (−)-(2*R*,3*R*)-2,3-butanediol followed by ^{13}C nmr examinations of the diastereomeric ketal mixtures obtained (10). The corresponding ketals from the racemic ketones were used as reference standards. The $\Delta\delta$ values observed for the individual diastereotopic carbon atoms are recorded in Table 1. The e.e. values shown for (−)-**1***a*,*b* in Scheme 1 represent the averages of measurements on ^{13}C resonances of at least four different carbon atoms of **7***a*,*b*. The optical purities of the *trans*- and *cis*-alcohols **2**- and **3***a*,*b* of Scheme 1 were evaluated by ^1H nmr examination in the presence of Eu(fod)$_3$ of the methoxyl proton resonances of their MTPA-esters, **8***a*,*b* and **9***a*,*b* respectively (11). The $\Delta\Delta\delta$ values of the methoxyl peaks of the corresponding esters of the racemic alcohols used for comparison purposes are recorded in Table 2.

The absolute configurations of (−)-**1***a*, (+)-**2***a*, and (−)-**3***a* were assigned from the literature data (5, 12–14) and that of (−)-**1***b* by octant rule analysis (12, 15) of the Cotton effect of its cd spectrum. The absolute configurations of the *cis*- and *trans*-alcohols (+)-**3***b* and (+)-**2***b* were then established by their oxidation with pyridinium chlorochromate under neutral conditions (16) to (−)-(2*R*)-**1***b* and (+)-(2*S*)-**1***b* respectively.

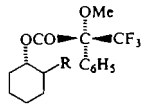
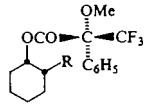
The degree to which *cis*-alcohols are produced was found to be influenced by the reaction conditions and was studied in detail using (±)-**1***b* as the

TABLE 1. Enantiomeric shift differences in the ^{13}C nmr spectra of the diastereomeric ketals 7*a*,*b*[a]

Ketal structure	Compound	Δδ, ppm										
		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Other C
 7	7*a*	0	0.78[b]	0.09	0	0.42[b]	0.69[b]	0.30	0.69[b]	0.14	0	0.08 (CH_3)
	7*b*	0.27[b]	0.67[b]	0.07	0	0.49[b]	0.67[b]	0.13	0.33[b]	0	0	0.09 (CH_2CH_3)
												0 (CH_2CH_3)

[a]^1H noise-decoupled spectra determined in C^2HCl_3. See ref. 1 for data on ketals of ketones 4*a*,*b*.
[b]Used for determining e.e. values shown in Scheme 1.

TABLE 2. Enantiomeric shift differences for the methoxyl protons of the MTPA esters 8,9*a*,*b*[a] from (±)-2,3*a*,*b*

Structures	Compound	$Eu(fod)_3$, equiv.	ΔΔδ, ppm
 8	8*a*	0.29	0.86
		0.58	1.25
	8*b*	0.30	1.22
		0.75	1.80
 9	9*a*	0.23	0.35
		0.55	0.50
	9*b*	0.27	0.78
		0.65	0.98

[a]Determined at 60 MHz in CCl_4 solution. See ref. 1 for data on MTPA esters of 5,6*a*,*b*.

representative substrate. As shown in Table 3, the proportions of *cis*-alcohol **3***b* formed during HLADH-catalyzed reduction of 2-ethylcyclohexanone ((±)-**1***b*) can be minimized by working at lower pH's, and by appropriate choice of the buffer. Tris-HCl is a more suitable buffer in this regard than phosphate. The rate of the reaction is also an important parameter (Table 4). The amount of *cis*-alcohol formed increases steadily as the reaction period is extended and selection of a suitably short reaction time ensures that the *trans*-alcohol is the exclusive product. Formation of the *cis*-alcohol can also be suppressed by using a low [E]/[S] ratio. The yields of *cis*-alcohol isolated rise monotonically as the proportions of enzyme in the reaction mixture are increased, as illustrated by Table 5.

TABLE 3. Influence of buffer and of pH on *cis*-alcohol formation in HLADH-catalyzed reduction of (±)-**1***b*[a]

pH	Buffer	Alcohol products (%)[b]	
		trans (**2***b*)	*cis* (**3***b*)
6	Phosphate	13.2	0
8	Phosphate	53.8	3.4
7	Tris-HCl	28.6	<0.1
8	Tris-HCl	54.8	0.6
9	Tris-HCl	54.2	1.5

[a]Reactions carried out for 3 days at 20°C with [E]/[S] 3.1×10^{-4} in 0.4 *M* buffer solutions.
[b]By glc.

Discussion

The preparative-scale HLADH-catalyzed reductions summarized in Scheme 1 proceeded smoothly. Ethanol was used as the coupled substrate to recycle the catalytic quantity of the expensive NAD^+ coenzyme employed (17). The reductions of (±)-**1***a*,*b* were performed on a 4-g scale in order to ensure that sufficient quantities to permit complete and unequivocal characterization

TABLE 4. Effect of reaction time on *cis*-alcohol formation during HLADH-catalyzed reduction of (±)-**1***b*[a]

Reaction period (h)	Alcohol products (%)[b]	
	trans (**2***b*)	*cis* (**3***b*)
17	33	0
43	44.5	2.8
332	74	4.0

[a]In 0.4 *M* phosphate buffer pH 8, with [E]/[S] = 3.1×10^{-4}.
[b]By glc.

TABLE 5. Influence of [E]/[S] ratio on *cis*-alcohol formation in HLADH-catalyzed reduction of (±)-**1***b*[a]

[E]/[S] (× 10^4)	Reaction period	Extent of reduction %	Alcohol products (%)[b]	
			trans (**2***b*)	*cis* (**3***b*)
4.9	26 h	48	47.3	0.7
3.3	28 h	43	42.5	0.5
0.65	9 days	47	46.6	0.4
0.44	9 days	49	48.9	0.1

[a]In 0.4 *M* Tris-HCl buffer pH 8, 20°C.
[b]By glc.

of the minor *cis*-alcohol products **3***a*,*b* were isolable from each reaction.

The direct ^{1}H- and ^{13}C-nmr based measurements of the e.e.'s of the alcohol products and recovered ketones isolated from the enzymic reactions presented no problems and the values cited in Scheme 1 are considered accurate to within ±3%. The peak assignments (Experimental section) and Δδ values recorded for the ^{13}C resonances of the ketals **7***a*,*b* (Table 1) are in excellent agreement with those published recently by Meyers *et al.* (18), with the exception of the C-3 and C-4 assignments which are reversed. Our assignments are based on the substituent-induced shifts observed between the 2-methyl- and 2-ethyl-derivatives, and their analogy with the corresponding shifts in methyl and ethylcyclohexane (19). The low e.e.'s of the recovered ketones **1***a*,*b* are largely attributable to nonenzymic epimerization at C-2 during the lengthy reaction period.[4]

The absolute configuration determinations of the Scheme 1 cyclohexane derivatives were also straightforward, with the configurations of **1***a*, **2***a*, and **3***a* having been established previously (5, 9–11). The octant rule analysis (15) of the cd spectrum of (−)-**1***b* used to assign its C-2 chirality followed the well documented approach developed on the 2-methyl analogue (12). The configurations of (+)-**2***b* and (+)-**3***b* were then confirmed by their oxidation to (+)- and (−)-**1***b* respectively.

In the earlier studies on HLADH-catalyzed reductions of 2-alkylcyclohexanones, *trans*-alcohol products were obtained exclusively (5, 6). The absence of *cis*-alcohols in these previous reactions is in marked contrast to the present results. However, the Zürich (2, 3, 5) and Antwerp (6) groups effected their transformations under reaction conditions somewhat different from those employed in the Scheme 1 reactions. Recognition of this prompted an evaluation of the influence of various experimental parameters on *cis*-alcohol formation. As the data recorded in Tables 3–5 show, the reaction conditions employed are of prime importance in controlling the degree of *cis*-alcohol content.

If suppression of *cis*-alcohol production is desired, operating in Tris-HCl (6) rather than phosphate buffer and at pH's no higher than 7 is recommended (Table 3). While the use of phosphate buffer alone at pH > 7 does permit *cis*-alcohol formation, substitution of a glycine–arsenate-modified phosphate buffer of pH 8 once again appears to preclude the production of these minor by-products (5, 9*a*). Exclusive reduction to the *trans*-alcohol products can also be ensured by keeping the reaction time short (Table 4) and by operating with the [E]/[S] ratio at a minimum (Table 5). In the HLADH-mediated reductions of 2-alkylcyclohexanones performed by the Zürich and Antwerp groups, in which no *cis*-alcohols were produced, the [E]/[S] ratios employed were even lower, by factors of ~5 (5) and ~10 (6) respectively, than the lowest level of Table 5.

Both the diamond lattice (2, 4) and the cubic-section (9*c*) active-site models developed for predicting the stereochemistry of HLADH-catalyzed oxidoreductions are valid for kinetically controlled reactions only. Under such conditions, virtually exclusive *trans*-alcohol formation is predicted (2, 4, 9*a*, *c*). The Table 4 and 5 data support this view, with short reaction times and very high excesses of [S] over [E] favouring *trans*-, and minimizing *cis*-, alcohol accumulation.

For good substrates, HLADH-catalyzed reductions are rapid and no special precautions are needed to ensure that only the kinetically favoured products are formed (2, 4, 9). However, poor substrates are reduced slowly. As a result lengthy reaction periods are often required, thereby permitting products of pathways other than that of the initial, kinetically determined, direction to build up. The 2-alkylcyclohexanone **1***a*,*b* and 3-alkyl-

[4]The variations in the optical purities of the *trans*-alcohols **3***a*,*b* and **6***a*,*b* contrast the 100% e.e. levels cited previously for such HLADH-derived products (5, 6). These e.e. variations have now been found to be markedly influenced by the reaction conditions. A systematic study of the factors controlling product e.e.'s has been undertaken and will be reported shortly.

thiopyranone **4***a*,*b* reductions are in this latter category. In these cases, the *trans*-alcohol products of kinetic control are also thermodynamically favoured, having both substituents equatorial in the preferred chair conformations. However, in the presence of HLADH, the *trans*-alcohols **2***a*,*b* and **5***a*,*b* are also oxidized back to the parent ketones much more rapidly than the slowly accumulating *cis*-alcohols **3***a*,*b* and **6***a*,*b* (1). The reverse oxidation reaction can occur because, owing to the limitations of the NADH–coenzyme recycling procedure, there is always a small proportion of NAD^+ available to participate in an alcohol oxidation step. Accordingly, because the *trans*-alcohols are present in much higher concentrations, and are oxidized much faster than their *cis*-isomers, any *cis*-alcohols that do form accumulate.

The cubic-section model of the active-site region (9*c*) is in accord with the more rapid oxidation of the *trans*- than the *cis*-alcohols of a given series. Furthermore, it predicts that the *cis*-alcohols formed preferentially in the reduction mode will predominate in the enantiomers possessing the (1*S*) (or (4*S*) for the thioketones) absolute configurations. This is seen to be correct for (+)-**3***b*, (+)-**6***a*, and (+)-**6***b*. Disappointingly, favouring of the opposite (1*R*,2*S*) configuration is manifest in (−)-**3***a*.[5] However, since the model was formulated only for predicting the stereochemical outcome of kinetically controlled, preferred, oxidoreductions, this is not a significant discrepancy. Furthermore, use of the enzyme is clearly not preparatively viable for the preparation of *cis*-alcohols. In contrast, HLADH-catalyzed reduction of 2-alkylcyclohexanones and related substrates remains an excellent route to the corresponding optically active *trans*-alcohols, with pure products to be expected if phosphate buffer is avoided and the minimum amount of enzyme is used in solutions of the lowest practicable pH.

[5]The preferred stereochemistry of reduction of 2-alkylcyclohexanones has been analyzed in detail in cubic-section terms (9*c*). Only the (2*S*)-enantiomer of **1***a*,*b* (or (3*R*)-**4***a*,*b*) can form an allowed ES complex to lead to *trans*-(1*S*,2*S*)-**2***a*,*b* (or (3*R*,4*S*)-**5***a*,*b*). The allowed oxidation reactions follow the microscopic reverse paths. On the other hand, neither enantiomer of any *cis*-alcohol of Scheme 1 can bind to form an ES complex without the alkyl substituent violating either cube **E1** (e.g. 1*R*,2*S*-**3***b*) or cube **B1** (e.g. 1*S*,2*R*-**3***b*). All of **E1**, and half of **B1** are forbidden regions, and will not accommodate a substrate group. Formation of the *cis*-products is thus strongly disfavoured. However, the remaining half of cube **B1** will accept a small group and is thus less intolerant than **E1**. The configurations reflecting positioning of the alkyl substituents of the (2*R*)-**1** and (3*S*)-**4** ketones adjacent to, and in, the **B1** region should thus dominate any *cis*-alcohols that are formed. This agrees with the experimental data for (1*S*,2*R*)-**3***b* and (3*S*,4*S*)-**6***s*,*b*, but not for (1*R*,2*S*)-**3***a*. The formation of the latter stereoisomer cannot be explained at the current level of sophistication of the model. Interestingly, reduction of (±)-2-fluorocyclohexanone gives the highest proportion (4%) of *cis*-alcohol (of unspecified configuration) yet reported (6). This would be expected in terms of the above analysis since fluorine is a very small substituent and should, on steric grounds, be more easily accommodated in the open half of cube **B1** than any alkyl group.

Experimental

The instrumentation and general purification and analytical procedures used were as described previously (9*d*). HLADH (EC 1.1.1.1) was 1× crystallized material purchased from Sigma. The activity of each batch of enzyme was determined (20) prior to use; amounts of HLADH quoted refer to milligrams of active enzyme. NAD^+ was obtained from Kyowa Hakko Kogyo, New York. The mplc separations were performed on silica (ICN 0.032–0.63 mm) with ethyl acetate – hexane (1:6) elution. Unless indicated otherwise, ir spectra were determined in CCl_4 and nmr spectra in C^2HCl_3. The Scheme 1 data on the tetrahydrothiopyrans **4***a*,*b*, **5***a*,*b*, and **6***a*,*b* are from ref. 1.

Preparations of racemic ketone substrates and alcohol products

2-Methylcyclohexanone ((±)-1a) was purchased from Aldrich and purified by Kugelrohr distillation, bp 162–163°C (lit. (21) bp 165–166°C); ir: 1714 cm^{-1}; ^1H nmr δ: 1.02 (3H, d, J = 6 Hz) and 1.15–2.8 (9H, m) ppm.

cis- *and* trans-*2-Methylcyclohexanol* were prepared by $LiAlH_4$ reduction of 2-methylcyclohexanone. Separation of the mixture by mplc gave *cis*-2-methylcyclohexanol ((±)-**3***a*, 25% yield) bp 70°C (20 Torr) (lit. (13) bp 77–78°C (20 Torr)); ir (film): 3400 cm^{-1}; ^1H nmr δ: 0.97 (3H, d, J = 6 Hz), 1.1–2.3 (9H, m), 1.86 (OH, s), and 3.80 (1H, m, $W_{\frac{1}{2}}$ = 8 Hz) ppm and *trans*-2-methylcyclohexanol ((±)-**2***a*, 44% yield) bp 74°C (27 Torr) (lit. (13) bp 78–79°C (20 Torr)); ir: 3640, 3380 cm^{-1}; ^1H nmr δ: 0.7–2.2 (12H, m), 2.70 (OH, s), and 3.10 (1H, m, $W_{\frac{1}{2}}$ = 20 Hz) ppm.

2-Ethylcyclohexanone ((±)-1b) was obtained in 90% yield by Jones oxidation of 2-ethylcyclohexanol (*cis*/*trans* mixture, purchased from Aldrich) bp 70°C (10 Torr) (lit. (22) bp 110°C (36 Torr)); ir: 1720 cm^{-1}; ^1H nmr δ: 0.9 (3H, t, J = 7 Hz) and 1.1–2.5 (11H, m) ppm.

cis- *and* trans-*2-Ethylcyclohexanol*

The mplc separation of the commercial mixture (Aldrich) gave *cis*-2-ethylcyclohexanol ((±)-**3***b*, 29% yield) bp 82°C (10 Torr) (lit. (23) bp 180–182°C); ir: 3625, 3480 cm^{-1}; ^1H nmr δ: 0.92 (3H, t, J = 6 Hz), 0.9–2.1 (11H, m), 1.77 (OH, s), and 3.92 (1H, m, $W_{\frac{1}{2}}$ = 8 Hz) ppm and *trans*-2-ethylcyclohexanol ((±)-**2***b*, 36% yield) bp 82°C (10 Torr) (lit. (24) bp 87–91°C (25 Torr)); ir: 3640, 3440 cm^{-1}; ^1H nmr δ: 0.87 (3H, t, J = 6 Hz), 0.9–2.25 (11H, m), 2.10 (OH, s), and 3.15 (1H, m, $W_{\frac{1}{2}}$ = 20 Hz) ppm.

HLADH-catalyzed reductions of (±)-1a,b

The general procedure described previously (1, 9*e*) was used to reduce the ketone (4.0 g, ~35 mmol) with HLADH (300 mg), NAD^+ (6.0 g, 9.1 mmol), EtOH (11 mL) in 0.1 *M* potassium phosphate buffer pH 7 (1 L) at 20°C. The reactions were monitored by glc and terminated at the ~50% reduction point. The unchanged ketones and product alcohols were separated by mplc. The spectral properties of each compound were identical with those of the corresponding racemates characterized above. The individual reactions gave the following results after Kugelrohr distillation of each chromatographically purified product.

Reduction of (±)-1a (44% conversion) yielded (−)-2-methylcyclohexanone ((2*R*)-**1***a*, 1.8 g, 45% yield, 35% e.e.) bp 59°C (22

Torr) (lit. (12) bp 59–60°C (20 Torr)), $[\alpha]_D^{25}$ −3.39° (*c* 2.2, EtOH) (lit. (5) (2*S*)-**1***a* $[\alpha]_D^{25}$ +14.4° (*c* 1.03, EtOH)); (+)-*trans*-2-methylcyclohexanol ((1*S*,2*S*)-**2***a*, 881 mg, 22% yield, 75% e.e.), bp 70°C (15 Torr) (lit. (13) bp 78°C (20 Torr)), $[\alpha]_D^{25}$ +31.0° (*c* 2.2, EtOH) (lit. (5) $[\alpha]_D^{25}$ +40.8° (*c* 0.7, EtOH); and (−)-*cis*-2-methylcyclohexanol ((1*R*,2*S*)-**3***a*, 30 mg, 0.7% yield, 46% e.e.), bp 72°C (21 Torr) (lit. (13) bp 78–79°C (29 Torr)), $[\alpha]_D^{25}$ −10.2° (*c* 0.3, EtOH) (lit. (5) (1*S*,2*R*)-**3***a* $[\alpha]_D^{25}$ +15.7° (*c* 3.4, EtOH).

Reduction of (±)-*1*b (44% conversion gave (−)-2-ethylcyclohexanone ((2*R*)-**1***b*, 1.81 g, 45% yield, 23% e.e.), bp 82–85°C (22 Torr), $[\alpha]_D^{25}$ −4.75° (*c* 2.2, EtOH) (lit. (5) 18° (*c* 1.8, EtOH)); (+)-*trans*-2-ethylcyclohexanol ((1*S*,2*S*)-**2***b*, 960 mg, 24% yield, 44% e.e.), bp 80°C (18 Torr), $[\alpha]_D^{25}$ +26.6° (*c* 2.1, EtOH) (lit. (5) $[\alpha]_D$ 59.2° (*c* 1.45, EtOH); and (+)-*cis*-2-ethylcyclohexanol ((1*S*,2*R*)-**3***b*, 47 mg, 1.2% yield, 72% e.e.), bp 80°C (21 Torr), $[\alpha]_D^{25}$ +15.5° (*c* 0.54, EtOH).

Enantiomeric excess determinations

1. *Of ketones* **1**a,b

Each racemic (for reference) and optically active ketone **1***a*,*b* (1 mmol) was converted in quantitative yield to its ketal **7***a*,*b* with (−)-(2*R*,3*R*)-2,3-butanediol (1.3 mmol) as described previously (1). The e.e. values of the ketals from the optically active ketones of Scheme 1 were determined from the ^{1}H-decoupled ^{13}C nmr spectra of **7***a*,*b* by the method of Hiemstra and Wynberg (10). The Δδ values used are recorded in Table 1. The bp's (Kugelrohr) and nmr resonances of the ketals from the racemic ketones are recorded below.

2-Methylcyclohexanone ketal **7**a, bp 75°C (1 Torr), ^{1}H nmr δ: 0.85 and 0.9 (3H, d of d, J = 6 Hz), 1.25 (6H, d of d, J = 6 Hz), 1.4–2.4 (9H, m), and 3.65 (2H, m, $W_{\frac{1}{2}}$ = 12 Hz) ppm; ^{13}C nmr δ: 14.40 and 14.48 (C-2 CH_3), 16.16 and 16.30 (C-9), 17.82 (C-10), 23.84 and 24.26 (C-5), 25.00 (C-4), 32.17 and 32.26 (C-3), 36.59 and 37.28 (C-6), 39.73 and 40.51 (C-2), 77.48 and 77.78 (C-7), 79.14 and 79.83 (C-8), and 110.07 and 110.34 (C-1) ppm.

2-Ethylcyclohexanone ketal **7**b, bp 80°C (1.2 Torr), ^{1}H nmr δ: 0.9 (3H, t, J = 6 Hz), 1.2 (6H, d of d, J = 4 Hz), 0.6–2.4 (11H, m), and 3.60 (2H, m, $W_{\frac{1}{2}}$ = 12 Hz) ppm; ^{13}C nmr δ: 12.00 (CH_2CH_3), 16.28 (C-9), 17.89 (C-10), 20.85 and 20.94 (CH_2CH_3), 24.16 and 23.67 (C-5), 24.84 (C-4), 28.19–28.26 (C-3), 36.62 and 37.29 (C-6), 46.79 and 47.46 (C-2), 77.51 and 77.64 (C-7), 79.15 and 79.48 (C-8), and 110.07 and 110.34 (C-1) ppm.

2. *Alcohols* **2**a,b *and* **3**a,b

The racemic (for reference) and optically active alcohols **2**,**3***a*,*b* (20–30 mg) were each converted to their MTPA esters **8**,**9***a*,*b* in quantitative yields by the literature (1, 8) procedure using freshly prepared (+)-(2*R*)-2-methoxy-2-trifluoromethylphenylacetyl chloride (100 mg, ~2 equiv.), $[\alpha]_D^{25}$ 131.9°C (*c* 1, CCl_4) (lit. (8) $[\alpha]_D^{25}$ 129.0 ± 0.2° (*c* 5.17, CCl_4). The e.e.'s of the MTPA esters of the Scheme 1 alcohols were then determined from the 60 MHz ^{1}H nmr methyl resonances in the presence of Eu(fod)$_3$. The bp's (Kugelrohr) and spectral data of the MTPA esters of the racemic alcohols **2**,**3***a*,*b* used to establish the ΔΔδ values summarized in Table 2 are as follows.

trans-*2-Methylcyclohexanol MTPA ester* **8**a, bp 90°C (0.2 Torr), ir: 1757 cm^{-1}; ^{1}H nmr (CCl_4) δ: 0.73 and 0.96 (3H, d of d, J = 6 Hz), 1.0–2.5 (9H, m), 3.53 (3H, s), 4.60 (1H, m, $W_{\frac{1}{2}}$ = 20 Hz), and 7.2–7.8 (5H, m) ppm.

cis-*2-Methylcyclohexanol MTPA ester* **9**a, bp 95°C (0.25 Torr), ir: 1762 cm^{-1}; ^{1}H nmr (CCl_4) δ: 0.75 and 0.95 (3H, d of d, J = 6 Hz), 1.05–2.40 (9H, m), 3.56 (3H, s), 5.12 (1H, m, $W_{\frac{1}{2}}$ = 8 Hz), and 7.15–7.75 (5H, m) ppm.

trans-*2-Ethylcyclohexanol MTPA ester* **8**b, bp 90°C (0.2 Torr), ir: 1757 cm^{-1}; ^{1}H nmr (CCl_4) δ: 0.50–2.33 (14H, m), 3.56 (3H, s), 4.74 (1H, m, $W_{\frac{1}{2}}$ = 20 Hz), and 7.2–7.8 (5H, m) ppm.

cis-*2-Ethylcyclohexanol MTPA ester* **9**b, bp 95°C (0.25 Torr), ir: 1765 cm^{-1}; ^{1}H nmr (CCl_4) δ: 0.90 (3H, t, J = 5.5 Hz), 1.05–2.35 (11H, m), 3.50 (3H, s), 5.25 (1H, m, $W_{\frac{1}{2}}$ = 8 Hz), and 7.15–7.75 (5H, m) ppm.

Absolute configuration determinations

1. *Of the enzyme-derived ketones* **1**a,b

The (2*R*)-configuration of (−)-**1***a* was assigned from the literature data (5, 12–14). The configuration of (−)-**1***b* was established from octant rule analysis (12, 15) of its cd spectrum: cd (*c* 0.0149, EtOH, 20°C): $[\theta]_{329}$ 0°; $[\theta]_{292}$ −1350°; $[\theta]_{240}$ 0°, and comparison with that of the known (12) (−)-(2*R*)-**1***a*.

2. *Of the HLADH-derived alcohols* **2**,**3**a,b

The configurations of (+)-(1*S*,2*S*)-**2***a* and (−)-(1*R*,2*S*)-**3***a* were assigned from the literature data (5, 12–14). For the ethylcyclohexanols **2**,**3***b*, the relative configurations at C-1 and C-2 were established by their identification as *cis*- or *trans*-isomers from their ^{1}H nmr spectra. The chiralities of the C-2 centres were then determined by oxidation of (+)-**2***b* and (+)-**3***b* to (+)-(2*S*)-**1***b* and (−)-(2*R*)-**1***b* respectively, and by comparison of the Cotton effect of the cd spectra of (+)-(2*R*)-**1***b* with that of (+)-(2*S*)-**1***a* established previously (12).

Oxidation of trans-*alcohol (+)-***2**b *to (+)-(2*R*)-***1**b

(+)-*trans*-2-Ethylcyclohexanol (**2***b*, 90 mg, 0.7 mmol, $[\alpha]_D^{25}$ +55.4° (*c* 0.9, EtOH)), pyridinium chlorochromate (335 mg, 1.55 mmol), and sodium acetate (17 mg, 0.21 mmol) in CH_2Cl_2 (5 mL) was stirred at 20°C for 1 day (16). Base-free work-up and purification gave 2-ethylcyclohexanone ((2*R*)-**1***b*, 70 mg, 79% yield) bp 80°C (7 Torr), $[\alpha]_D^{25}$ +21.3° (*c* 0.6, EtOH); cd (*c* 0.0123, EtOH, 20°C): $[\theta]_{330}$ 0°; $[\theta]_{293}$ +1870°; $[\theta]_{240}$ 0°.

Oxidation of cis-*alcohol (+)-***3**b *to (−)-(2*S*)-***1**b

Oxidation of (+)-*cis*-2-ethylcyclohexanol (**3***b*, 98 mg, 0.76 mmol, $[\alpha]_D^{25}$ +1.60° (*c* 1.2, EtOH)) with pyridinium chlorochromate (360 mg, 1.67 mmol) and sodium acetate (19 mg, 0.23 mmol) in CH_2Cl_2 (5 mL) for 12 h at 20°C, followed by base-free work-up, gave 2-ethylcyclohexanone ((2*S*)-**1***b*, 72 mg, 74% yield) bp 40°C (3 Torr), $[\alpha]_D^{25}$ −2.2° (*c* 1, EtOH).

Influence of reaction conditions on cis-*alcohol formation during HLADH-catalyzed reduction of* (±)-***1**b

The effects of buffer and pH were surveyed by following (by glc) the course of reduction of (±)-**1***b* (51 mg) with enzyme (10 mg), NAD^+ (79 mg), and EtOH (0.1 mL) in 0.4 *M* potassium phosphate, or Tris-HCl, buffer (10 mL) of pH 6 and 8, or 7, 8, and 9 respectively, at 20°C. The [E]/[S] ratio was 3.1 × 10^{-4} for these studies. The results are summarized in Table 3. The dependence of *cis*-alcohol formation on reaction time was monitored in the same way under the same conditions in 0.4 *M* potassium phosphate buffer pH 8. The results are recorded in Table 4.

The influence of changes in the [E]/[S] ratio within the range 0.44–5.0 × 10^{-4} was evaluated under the above conditions in 0.4 *M* Tris-HCl pH 8 (100 mL) using (±)-**1***b* (500 mg, 4 mmol), HLADH (14–159 mg, 0.175–2.00 μmol), NAD^+ (700 mg, 1.06 mmol), and EtOH (1 mL). The yields of *trans*- and *cis*-alcohols **2**,**3***b* observed are recorded in Table 5.

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