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Stereochemical Features of Baker's Yeast Mediated Transformation of Racemic and Enantiomerically Pure 2-Deutero-3-Chloropropiophenone

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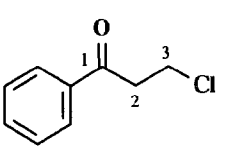
Abstract: Baker's yeast transforms (2*R*) and (2*S*) stereospecifically deuterated 3-chloropropiophenone **11** and **12** into carbinols **14** and **15**, and into propiophenone samples retaining, at position 2, 71% and 84% deuterium, respectively. Analogous experiments with randomly labelled ketone **10** gave rise to a mixture of the carbinols **15** and **14** and to propiophenone, that retained 76% deuterium. The deuterium retention values in propiophenone generated from randomly and stereospecifically labelled substrates **10**, **11** and **12** indicate a non stereospecific elimination, accompanied, however, by a minor pathway in which H_R is preferentially removed.

We recently reported[1] that the baker's yeast (b. y.) reduction of 3-chloropropiophenone **1** to (3*S*)-3-phenyl-3-hydroxy-1-chloropropane **2** is accompanied by the formation of propiophenone **3**.



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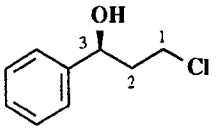

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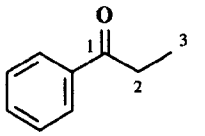

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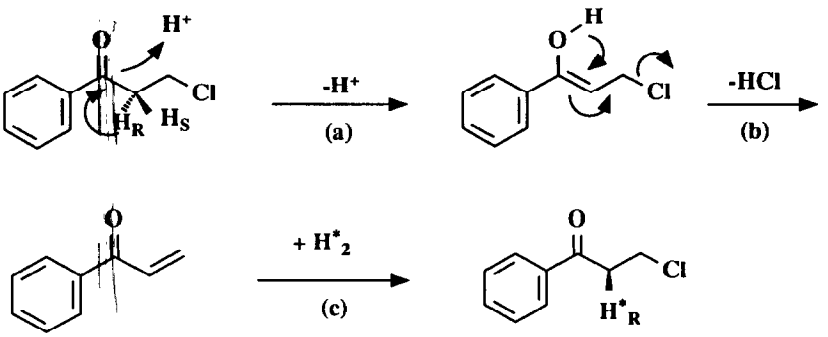
Experiments with 2,2-dideuterated 3-chloropropiophenone indicated that **3** is formed from **1** by (acid-catalyzed) elimination of hydrochloric acid, followed by enzyme-assisted hydrogen addition onto the double bond of the intermediate phenyl vinyl ketone, the hydrogen added at position 2 holding the pro-*R* configuration (Scheme 1).[1] In order to gain information on the mechanism of the elimination step we decided to determinate the fate of the enantiotopic hydrogen atoms at position 2 of **1** during its conversion into **3**. To this end, we submitted to yeast treatment 3-chloropropiophenone **10**, randomly monodeuterated in position 2, and the two pure enantiomeric forms **11** and **12**. The b. y. treatment of **11** and **12** would evidentiate the occurrence of differences in the elimination of hydrochloric acid, the key step for the

formation of propiophenone, between the enantiomers. Furthermore it provides the enantiomerically pure chloroalcohols **14** an **15**, which display in the deuterium spectrum distinct resonances for the deuterium atoms in position 2, thus allowing the evaluation of the enantiomeric purity of the starting materials and the absolute enantioselectivity of the reduction process.

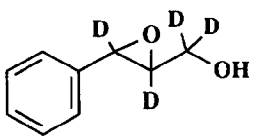


SCHEME 1

Racemic [2-$^2H_1$;3-$^2H_2$] 3-chloropropiophenone **10** was prepared starting from [1-$^2H_2$;2-$^2H_1$;3-$^2H_1$] cinnamyl alcohol. This was obtained, in turn, upon $LiAlD_4$ reduction of ethyl phenylpropiolate followed by D_2O quenching.[2] Deuterated cinnamyl alcohol was converted into (2*R*,*S*) [1-$^2H_2$;2-$^2H_1$;3-$^2H_1$] trans 3-phenyloxiranemethanol **4** upon epoxidation with 3-chloroperbenzoic acid. Regiospecific hydride ring opening, using Red-Al,[3] converted **4** into diol **7**. The latter was subsequently transformed into the desired chloroketone **10** upon sequential treatment with Ph_3P/CCl_4 and MnO_2.[1] NMR studies indicated no significant loss of label from the indicated positions along the synthetic sequence. Product **10** on b. y. treatment at pH 5.5-4 afforded[1] unreacted starting material **1** (ca. 30%), chloroalcohol **2** (40-45%) and propiophenone **3** (20-25%). The deuterium NMR spectra on **10** recovered from the b. y. treatment showed an isotopic content nearly identical to the starting material. The extent of deuterium retained at position 2 of propiophenone **3** formed under these conditions was measured through ^2H and ^{13}C NMR studies (Figure 1 and Table, Entry 1). The observed 76-79% of deuterium retention suggests the occurrence in the elimination process of an isotope effect of ca. 3.2, in agreement with other literature data.[4]

(2*R*) and (2*S*) trideutero chloroketones **11** and **12** were prepared simply applying to deuterated substrates synthetic sequences of known stereochemical courses recently studied in the unlabelled series. The enantiomeric trans-3-phenyloxiranemethanols, **5** and **6**, were prepared by the catalytic Sharpless epoxidation of trideutero cinnamylalcohol.[5] The trideuterated cinnamyl alcohol was obtained upon $LiAlD_4$ reduction of phenyl ethyl propiolate followed in this case by H_2O quenching.[2] From **5** and **6** diols **8** and **9** were prepared,[3] and subsequently converted, as reported above, into the desired chloroketones **11** and **12**. Yeast treatment of (2*R*) [2-$^2H_1$;3-$^2H_2$] 3-chloropropiophenone **11** and of its (2*S*) enantiomer **12** allowed the obtainment, close to unreacted starting materials, of propiophenone samples, showing at NMR studies (Figure 1) the deuterium retention values, relative to position 2, reported in the Table (Entries 2 and 3) and of carbinols **14** and **15**, showing the ^2H NMR spectra reported in Figure 2 (B and C). This experiment which provides from **11** and **12** diastereoisomerically pure reduction products also supports the substantial

enantiomeric purity of the starting α monodeuterated ketones and the enantioselectivity of the yeast reduction.



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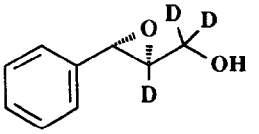

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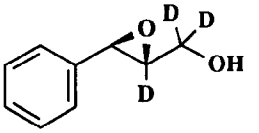

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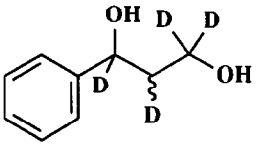

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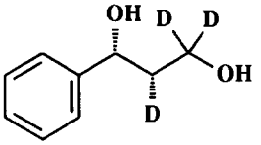

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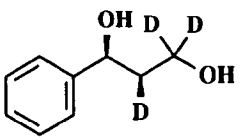

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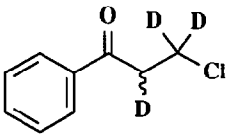

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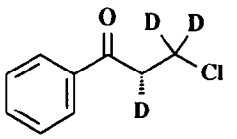

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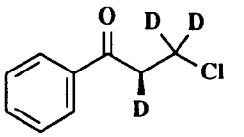

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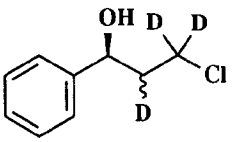

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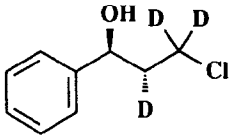

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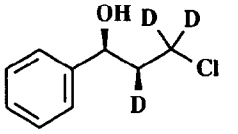

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Seen together, the results of b. y. transformation of racemic and enantiomerically pure deuterated chloroketones **10**, **11** and **12** seem of some interest. Indeed, the conversion of **1** into propiophenone, proceeding in the deuterated series with over 70% retentions of deuterium in α position, suggests as the major pathway, a non stereospecific elimination. However, the modest but definite differences in the deuterium retentions (Table) observed on going from precursors (*R*) **11** to (*R,S*) **10** and to (*S*) **12** is an indication of the minor participation to the overall process of a stereospecific elimination involving the removal from position 2 of H_R.

Furthermore, inspection of the deuterium spectrum of **13** (Figure 2 A) reveals a small difference in the area of the signals relative to the two diastereotopic deuterium atoms at position 2 (2.09 and 2.23 ppm), corresponding to an excess of **15** in the diastereoisomeric mixture formed in the b. y. reduction of randomly labelled **10** (ratio ca. 55:45). The observed excess of the chloroalcohol **15** cannot be a

consequence of the mode of elimination of the elements of hydrochloric acid from **10**. Indeed, in this case one should expect at modest conversion values an enrichment in the survived ketones of the (2*R*) enantiomeric form **11**, which should be transformed at lower rate with respect to **12** because of a deuterium isotopic effect.

Thus this peculiar behaviour must be connected to the complex b. y. reaction medium, where many phenomena may occur, such as an enolisation of **10** involving the preferential removal of D_R with respect to D_S deuterium atom or a kinetic resolution operated by the yeast dehydrogenase complex in the reduction of **10**.

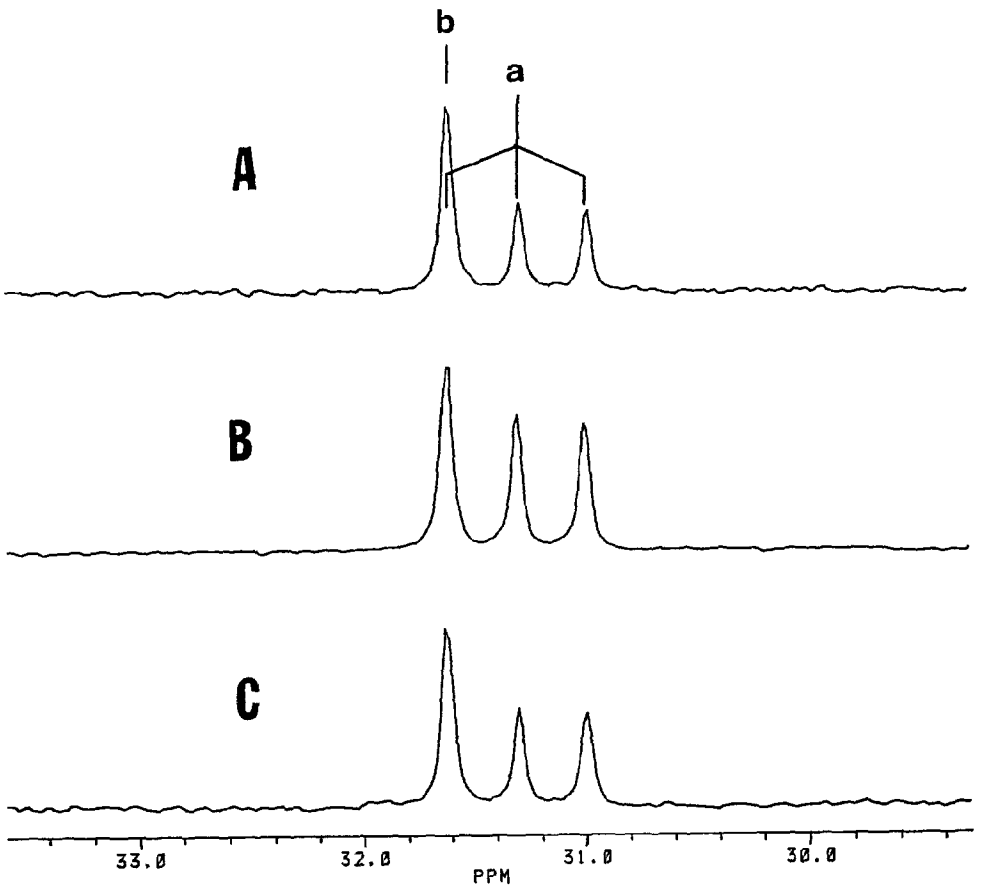

Figure 1. Expanded region of the ^{13}C NMR spectra of propiophenone obtained in the b. y. reduction of [2-$^2H_1$;3-$^2H_2$] 3-chloropropiophenone (A) from (2*R*) **11** (B) from (2*S*) **12** (C) from (2*R,S*) **10**. The peak pattern is formed by the signals of carbon C-2 (a) triplet due to the CHD species (b) singlet due to the CH_2 species. The relative quantities of the two species are reported in the Table.

Table. Extent of monodeuteration at position 2 of [2-$^2H_1$;3-$^2H_2$] propiophenone obtained in b.y. from **10-12** measured by ^{13}C or ^2H NMR.

entry	substrate	% deuterium retention	
		^{13}C	^2H
1	**10**	76	79.6
2	**11**	71	69.5
3	**12**	84	86.4

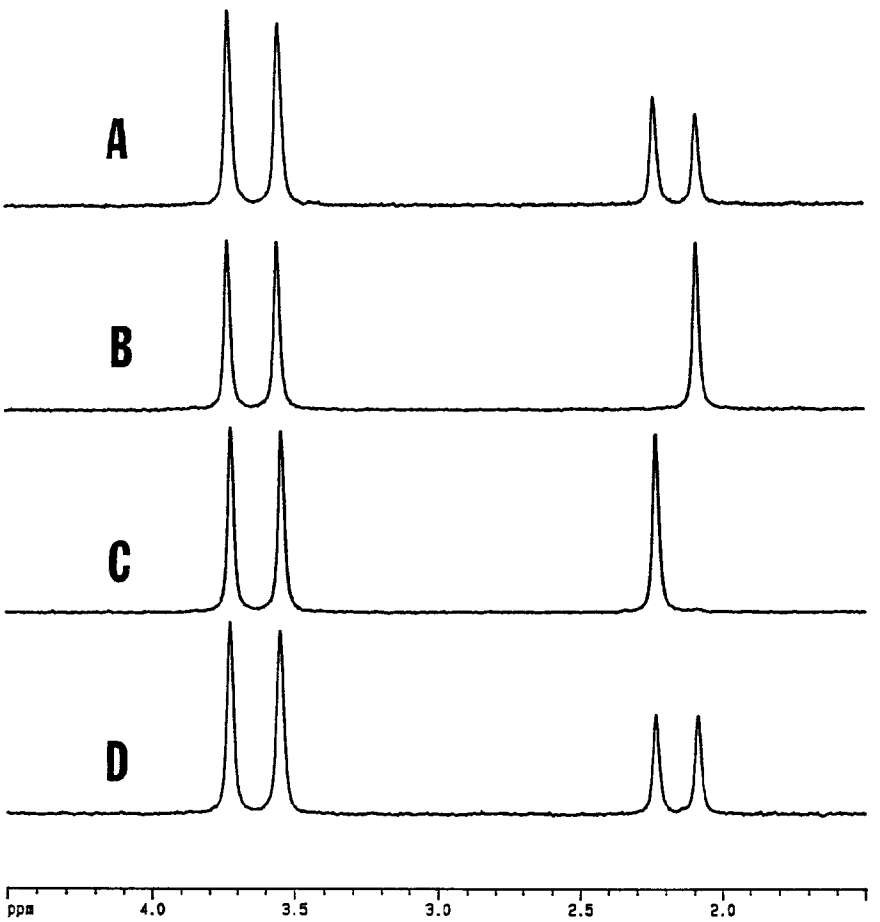

Figure 2. Deuterium NMR spectra of [2-$^2H_1$;3-$^2H_2$] 3-chloro-1-phenylpropan-1-ol obtained in the reduction of [2-$^2H_1$;3-$^2H_2$] 3-chloropropiophenone (A) **13** from the b. y. reduction of (2*R*,*S*) **10** (B) **14** from the b. y. reduction of (2*R*) **11** (C) **15** from the b. y. reduction of (2*S*) **12** (D) reduction of (2*R*,*S*) **10** with the (-)-chlorodiisopinocampheylborane system.

While the origin of the phenomenon is still unclear and await confirmation from the biotransformation of compounds structurally similar to **10**, we studied, for shake of comparison, the stereochemical course of the formation of **2** from **1** by hydride reduction using chiral boron reagents when a deficit of diisopinocampheylchloroborane ($^{d}Ipc_2BCl$)[6] and of the borane/(*S*)-oxazaborolidine system[7] are used to reduce **10**. Treatment of **10** with 0.2 mole of the two reagents was thus performed,[8,9] slowly adding the reductant to the ketone. However, the (3*S*) carbinol obtained in these experiments resulted exactly a 1:1 mixture of **14** and **15**, thus excluding in this case any effect of the stereochemistry of the C-2 deuterium atom on the reduction of racemic **10**. The spectrum of the material obtained with the former reagent is reported in Figure 2, D. These experiments, seen together, support the view that the biological and a-biological reduction of 3-chloropropiophenone, performed using either stoichiometric or catalytic asymmetric reagents, to the corresponding carbinol proceeds with the same extremely high enantioselectivity. Moreover, in the event that the feature thus observed in the b. y. reduction of **10** will be confirmed with a set of different substrates structurally related to **10**, the already rich array of synthetic capacities of b. y. will be further expanded.

Experimental

NMR spectra were run on Bruker CXP 300, AC 250 or AMX 600 spectrometers. The experimental conditions employed in the acquisition of the ^{2}H and ^{13}C NMR spectra used for the quantitative analysis have been carefully chosen to eliminate any contribution to the peak intensities deriving from signal saturation or heteronuclear NOE. Thus all experiments have been collected with a long relaxation delay (5-6 s for the deuterium spectra and 9-10 s for the carbon spectra) to allow a complete relaxation of the spin system and in the gated decoupling mode with the proton decoupler on during the acquisition time and off during the relaxation delay. The signals of the deuterium spectra obtained at 92.125 MHz (Bruker AMX 600 spectrometer, Figure 2) have been deconvoluted and fitted to a lorentzian line shape using the standard Bruker UXNMR program. In this way accurate values of the peak areas can be obtained. The signals of the deuterium nuclei at position 3 of the racemic chloroalcohol **13** (3.55 and 3.72 ppm) display a small but detectable linewidth difference of ca. 0.1 Hz which affects the relative height of the signals. Although such difference was not detected for the two diastereoisomers **14** and **15**, for all samples the areas of the two peaks are strictly comparable. On the contrary the total area of the deuterium nuclei at carbon C-2 is always about 3% less compared with that of deuterium nuclei at carbon C-1. Reasonably this may be due to some wash out of deuterium in the chloroketones **10**, **11** and **12** because of a ketoenolic equilibrium during the bakers' yeast reduction.

GC analyses were performed on a Hewlett-Packard 5890 gaschromatograph equipped with two fused silica capillary columns (DB-1 and DB-1701, J & W, 30 m x 0.25 mm i.d.), mounted in the same injector port, and two flame ionization detectors. Injector (split ratio 50:1) and detector (F.T.D.) point heaters were 280 °C and 300 °C, respectively. Helium carrier gas was used (1 mL min-1) and the temperature program was 50 °C for 3 min, followed by an increase of 5 °C/min to 285 °C for the reminder of the run. The

double column signals were recorded simultaneously and elaborated on a Hewlett-Packard 5890A GC-workstation connected with the gaschromatograph. Linear retention indices of peaks, referred to n-alkanes, were calculated and compared with those of authentic standards chromatographed under identical conditions on DB-1 and DB-1701 columns.

Yeast Transformation of deuterated 3-chloropropiophenone. The b.y. treatment of samples **10**, **11** and **12** was performed exactly as reported for the non-deuterated series.[1] The deuterated chloroalcohols **13**, **14** and **15**, obtained on column chromatography resulted over 99% pure by GC; $[\alpha]_D^{20}$ -24.1 , -23.9 and -24.0 , respectively, (c 1, $CHCl_3$). The purification of propiophenone and of the unreacted chloroketones was performed by bulb-to-bulb vacuum distillation.[1] **13**. δ_H ($CDCl_3$) 2.00 (s 1 H, OH), 2.07 (s broad, 0.55 H, H-2), 2.07 (d, 0.45 H, H-2'), 4.92 (m, 1 H, H-1), 7.2-7.4 (m, % H, C_6H_5). δ_D ($CHCl_3$) 2.09 (0.45 D, D-2), 2.23 (0.54 D, D-2'), 3.55 (1 D, D-3), 3.72 (1 D, D-3'). **14**. δ_H ($CDCl_3$) 1.98 (d broad, 1 H, OH, J(H_2,OH) 3.0 Hz), 2.20 (d, 1 H, H-2, J(H_1,H_2) 8.6 Hz), 4.94 (dd, 1 H, H-1), 7.2-7.4 (m, 5 H, C_6H_5). δ_D ($CHCl_3$) 2.09 (1 D, D-2), 3.55 (1 D, D-3), 3.72 (1 D, D-3'). **15**. δ_H ($CDCL_3$) 2.00 (s broad, 1 H, OH), 2.07 (s broad, 1 H, H-2), 4.92 (d, 1 H, J(H_1H_2) 4.8 Hz), 7.2-7.4 (m, 5 H, C_6H_5). δ_D($CHCl_3$) 2.22 (1 D, D-2), 3.53 and 3.71 (2 D, CD_2Cl) (see Figure 2).

Synthesis of 10, 11 and 12. [1-$^2H_2$;2-$^2H_1$] cinnamyl alcohol and [1-$^2H_2$;2-$^2H_1$;3-$^2H_1$] cinnamyl alcohol were obtained as reported in the non deuterated series.[2] m.p. 33 °C (from petr. ether) (85%). Trideuterated cinnamyl alcohol: δ_H ($CDCl_3$) 1.60 (s broad, 1 H, OH), 6.62 (t, 1 H, H-1, J(H,D) 4.5 Hz), 7.2-7.4 (m, 5 H, C_6H_5). δ_D ($CHCl_3$) 4.29 (2 D, CD_2OH), 6.39 (1 D, D-2). Tetradeuterated cinnamyl alcohol: δ_H ($CDCl_3$) 1.69 (s broad, 1 H, OH), 7.2-7.4 (m, 5 H, C_6H_5). δ_D ($CHCl_3$) 4.29 (2 D, CD_2OH), 6.39 (1 D, D-2), 6.65 (1 D, D-1).

(2R,S-trans) [1-$^2H_2$;2-$^2H_1$;3-$^2H_1$] 3-phenyloxiranemethanol (trans- epoxycinnamyl alcohol) (4). Tetradeuterocinnamyl alcohol, 27.4 g (0.2 mol) in 300 mL of CH_2Cl_2 was treated under stirring at 0 °C with 55% 3-chloroperbenzoic acid, 100 g (ca. 0.3 mol). After 4 h the reaction mixture is filtered, washed with water, $NaHCO_3$ sol., dried and evaporated. The residue is chromatographed on SiO_2 with 20% AcOEt in hexane to give deuterated epoxycinnamyl alcohol, 26 g (85%); δ_H ($CDCl_3$) 2.20 (s broad, 1 H, OH), 7.2-7.4 (m, 5 H, C_6H_5). δ_D ($CHCl_3$) 4.01 (1 D, D-2), 3.77 and 3.21 (2 D, CD_2OH), 3.92 (1 D, D-3).

[1-$^2H_2$;2-$^2H_1$;3-$^2H_1$] 3-phenyl-1,3-dihydroxypropane (7).[3] To a solution of tetradeutero epoxycinnamyl alcohol **4**, 15.3 g (0.1 mol) in 400 mL of dimethoxyethane was added a 3.4 M solution of sodium bis(2- methoxyethoxy)aluminum hydride (Red-Al) in toluene (31 mL, 0.10 mol) dropwise under nitrogen at 0 °C. Usual workup afforded 92% of tetradeutero 3-phenyl-1,3-dihydroxypropane 7; δ_H ($CDCl_3$) 1.91 (s, 1 H, H-2), 2.47 (s broad, 1 H, OH), 3.03 (s broad, 1 H, OH), 7.15-7.40 (m, 5 H, C_6H_5). δ_D ($CHCl_3$) 3.80 (2 D, CD_2OH), 1.98 (1 D, D-2), 4.93 (1 D, D-3).

[2-$^2H_1$;3-$^2H_2$] 3-chloropropiophenone 10. Diol **7** was converted into **10** exactly as reported.[1] δ_H (CDCl3) 3.44 (s broad, H- 2), 7.4-8.0 (m, 5 H, C_6H_5). δ_D ($CHCL_3$) 3.45 (1 D, D-2), 3.89 (2 D, D-3). δ_C ($CDCl_3$) 7.55 (tt, C-3, J(C_3D_3) 20.0 Hz), 31.30 (t, CHD-2) and 31.61 (s, CH_2-2), 127.8, 128.46, 132.79 and 136.79 (aromatic carbons), 200.83 (C=O).

The synthesis of **11** and **12** proceeded in analogous way. (2S-trans) [1-$^2H_2$;2-$^2H_1$] 3-phenyloxiranemethanol **5**, obtained by Sharpless epoxidation of trideuterocinnamyl alcohol,[5] showed

$[\alpha]_D^{20}$ -48.5 (c 2.4 $CHCl_3$). (2R) enantiomer, $[\alpha]_D^{20}$ +48.3 . δ_H ($CDCl_3$) 1.92 (s broad, 1 H, OH), 3.91 s, 1 H, H-1), 7.2-7.4 (m, 5 H, C_6H_5); δ_D ($CHCl_3$) 4.01 (1 D, D-2), 3.77 and 3.21 (2 D, CD_2OH).

(2S,3S) [1-$^2H_2$;2-$^2H_1$] 3-phenyl-1,3-dihydroxypropane **8** and the (2R,3R) enantiomer **9** were obtained, as above, in ca. 20:1 admixture with the 1,2-isomers. The 1,2-diol impurity is removed later in the sequence in the MnO_2 oxidation of the intermediate 1-chloro-3-hydroxy-3-phenylpropane.[1] δ_H ($CDCl_3$) 1.82 (dt, 1 H, H-2, $J(H_1,H_2)$ 3.5, $J(H_1,D_2)$ 1.5 Hz), 3.03 (s broad, 1 H, OH), 3.51 (s broad, 1 H, OH), 4.87 (d, 1 H, H-1), 7.15-7.40 (m, 5 H, C_6H_5); δ_D ($CHCl_3$) 3.80 (2 D, CD_2OH), 1.98 (1 D, D-2).

*Reduction of **10** with (-)-B-chlorodiisopinocampheyl- borane and borane/oxazaborolidine obtained from (S)-(-)-2- (diphenylhydroxymethyl)pyrrolidine and methylboronic acid.* The reduction of **10** with $^dIpc_2BCl$ was performed according to the reported general procedure[8] with the only modification that 0.2 mol borane for 1 mol substrate were added at -78 °C to the solution. The temperature was then raised to -25 °C for the reminder of the experiment. Diethanolamine was omitted in the aqueous workup. The reduction product, isolated in ca. 70% yield upon repeated column chromatography, showed $[\alpha]_D^{20}$ -24.5 (c 1, $CHCl_3$), ^2H NMR spectrum, see Figure D.

Similarly, the reduction of **10** with the borane/oxazaborolidine system[7] was performed as reported,[9] using, again, 0.2 mol reducing agent for mol of ketone. The carbinol isolated under these conditions (80%) showed $[\alpha]_D^{20}$ -24.2 (c 1, $CHCl_3$). The ^2H NMR spectrum of this material is identical to that mentioned above.

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