DOI: 10.1002/ejoc.201000442

Stereochemical Analysis of the Enzymic Reduction of the Double Bond of α- and β-Substituted Nitrostyrenes and α-Ethoxycinnamaldehyde through Deuterium Labelling Experiments

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Keywords: Isotopic labelling / Enzyme catalysis / Reduction

^{2}H NMR studies and comparison with authentic labelled reference compounds prepared from the (*Z*)-acetamidocinnamic acid **10** and from (*Z*)-2-ethoxy-3-(4-methoxyphenyl)prop-2-en-1-ol (**14**), respectively, by catalytic *syn* reduction with deuterium gas show that the baker's yeast-mediated saturation in the presence of deuterated water of the double bond of (*E*)-α-(hydroxymethyl)nitrostyrene (**2**) and of the (*Z*)-α-ethoxycinnamaldehyde (**3**) leads to (2*R*,3*R*)-[2,3-$^2H_2$]-3-(4-methoxyphenyl)-2-nitropropan-1-ol (**5**) and to (1*R*,2*S*,3*R*)-[1,2,3-$^3H_2$]-2-ethoxy-3-(4-methoxyphenyl)propan-1-ol (**6**), respectively, thus supporting an *anti* mode of hydrogen addition across the activated double bond, with hydride delivery in β-position respect to the activating group from the upper side of the molecule.

Introduction

For some years now we have been using baker's yeast-mediated transformations of compounds containing a carbonyl-activated double bond as a method for the preparation of enantiomerically enriched compounds,[1] including materials that are chiral due to isotopic hydrogen substitution. In this context, the cryptic stereochemistry of the reduction of the double bond of α,β-unsaturated aldehydes,[2,3] ketones[4–7] and δ- and γ-lactones[8,9] has been examined in some detail by means of deuterium labelling.

The enzyme-mediated saturation of the activated double bond is thought to occur by addition in β-position of an hydrogen atom from the reduced cofactor, followed by incorporation in the α-place of a proton from the solvent water. By performing the baker's yeast reduction in the presence of D_2O – where exchange of the hydrogen atoms of the reduced cofactor(s)[10] with those of the solvent takes place – and/or by using specifically labelled substrates, it has been possible to obtain stereochemical information relative to the reduction of unsaturated materials belonging to quite different structural classes. In this context, it has been shown that in the case of perillaldehyde, a chiral cyclic terpenic aldehyde, the double bond of the cyclohexene ring, in α position to the formyl group, is reduced with *anti* stereochemistry in the case of one enantiomer, but in *syn*-fashion in the other one.[3] The observation, acquired in the whole cell system, has been subsequently confirmed using NADPH and Ltb4 dehydrogenase derived from mouse as a catalyst.[11] Also, cinnamaldehyde is transformed into 3-phenylpropan-1-ol by *anti* formal addition of hydrogen to the double bond,[2] while the opposite is true when 4-(4-hydroxyphenyl)buten-2-one is converted into 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone).[7]

Currently, the reductive transformation of a wide set of substrates, already performed partially with baker's yeast and other microbial systems, is being studied using purified enzymes, obtaining in some instances outstanding improvements in terms of selectivity.[12] Reportedly,[13] the added value of the enzymatic reduction of activated olefins is represented by the *anti* stereochemistry of hydrogen addition, which is complementary to the *syn* addition promoted by transition metal based homogeneous catalysts. The differentiation between the two modes of addition can be achieved using a tetrasubstituted olefin as a substrate. However, to the best of our knowledge, the only two examples studied in this field are the baker's yeast reductions of (*Z*)-3-phenyl-2-nitro-2-butene[14] and of (*E*)-2,3-dimethylcinnamaldehyde,[15] both leading to mixtures of diastereoisomers in which the *syn* addition products prevail. This means that of the rich array of enzymes presiding over the reductive manipulation of activated double bonds in baker's yeast those particularly active towards the two mentioned materials operate in *syn* fashion.

In this context it seemed of interest to develop an analytical procedure enabling the determination of the hidden

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201000442.

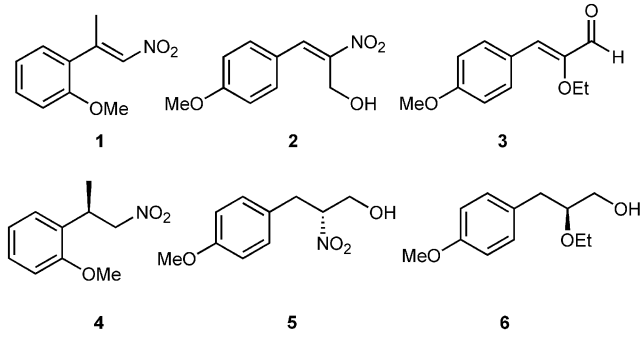
stereochemistry of the enzymatic reduction of trisubstituted olefins. This would be a useful tool to investigate those bioreductions in which *syn* or *anti* hydrogen addition can afford the two possible enantiomers of a chiral product. For example it could be applied to elucidate the mode of action of the two sets of enzymes which are described to transform (*E*)-1-phenyl-2-nitropropene into the two enantiomers of 1-phenyl-2-nitropropane,[16,17] providing that the substrate we choose as a model (see below) is transformed by the same enzymes.

The goal could be formally achieved only through isotopic hydrogen substitution experiments and we now report on a study showing that satisfactory results can be obtained by performing the reductive transformations in the presence of deuterated water, thus avoiding the troublesome synthesis of precursors which are regiospecifically labelled with isotopic hydrogen.

At this exploratory stage, the enzymatic reactions were performed in fermenting baker's yeast, since this whole cell system contains all the three required enzymatic capacities, consisting in the ability of *a*) exchanging the hydrogen atoms of the reduced cofactor(s) with the deuterium atoms of the solvent water,[10] *b*) saturating the double bond of the activated olefin and *c*) reducing an aldehyde to the corresponding alcohol, respectively.

Results and Discussion

Substrates of choice for this investigation were nitrostyrenes **1**[17] and **2**[18] and the structurally similar α-ethoxycinnamaldehyde **3**[19] (Scheme 1), not only because they are efficiently transformed by baker's yeast into (*R*)-**4**, (*R*)-**5** and (*S*)-**6** of fair or good enantiomeric purity, respectively, but, most important, because products **4**–**6** display the ^{2}H NMR signals of the side chain hydrogen atoms with the dispersion which is required to allow a proper analysis.

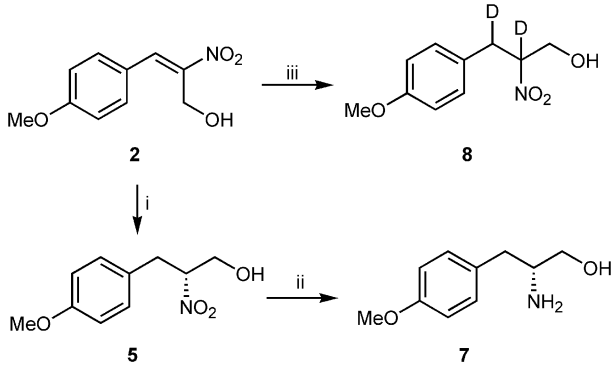


Scheme 1. Baker's yeast-mediated asymmetric reduction of compounds **1**–**3** to **4**–**6**.

First, the (*E*) stereochemistry of the α-(hydroxymethyl)-nitrostyrene **2** was firmly established through a NOE experiment showing that the hydroxymethylene protons interact strongly with the *ortho* position of the phenylene ring, while they do not display any contact with the vinylic hydrogen. Substrate **2** was adsorbed on nonpolar resins (XAD 1180), and incubated with baker's yeast actively fermenting on D-glucose. Derivative **5** was obtained as a unique transformation product, shown to be a 85:15 mixture of two enantiomers by HPLC analysis. However, conversion was too low to allow the recovery of a suitable quantity of compound **5** for deuterium NMR analysis.

When incubation was repeated on the free substrate, added to fermenting baker's yeast in ethanol solution, conversion was complete, but the enantiomeric excess of the resulting derivative **5** was found to be rather low (*ee* = 40%), anyway enough to perform deuterium analysis. The enantiomeric excess is often a function of dilution: if a lot of substrate is available in solution it can be transformed by competing enzymes working with lower stereoselectivity.

Compound **5** was assigned (*R*) absolute configuration because it was converted by Zn/H_2SO_4/EtOH reduction[20] into (*R*)-**7** (Scheme 2), whose absolute configuration was known.[21] In a separate experiment, product **2** was submitted to catalytic hydrogenation with deuterium gas (AcOEt, moist 5% Pd/C) to provide the [2,3-$^2H_2$] derivative **8**.



Scheme 2. i. Fermenting baker's yeast + 1% D_2O; ii. Zn/30% H_2SO_4/ethanol, 40–50 °C; iii. deuterium gas/10% Pd/C/ethyl acetate, room temp.

The natural abundance ^{2}H NMR spectrum of **5** (Figure 1, a) shows separate signals for all the five hydrogen atoms of the side chain. Subsequently, the exchangeability of the hydrogen atoms of **5** with the solvent hydrogen atoms was determined submitting the synthetic racemic material to incubation at room temp. for three days with fermenting baker's yeast in the presence of ca. 1% deuterated water. In this time interval, the pH of the mixture remained around 7. The spectrum of **5** recovered from the experiment (Figure 1, b) indicates, as judged from the increase of the signal at δ = 4.36 ppm respect to that at δ = 3.39 ppm, relative to the three deuterium atoms of the *O*-methyl group assumed as internal standard (see Figure 1, a), incorporation of deuterium from the solvent in position α to the nitro group. This result qualitatively confirms what observed when 2-nitro-1-phenylpropane was incubated with deuterated water.[16]

The baker's yeast reduction of **2** was then performed in the presence of ca. 1% deuterated water, isolating labelled **5** with an *ee* value of ca. 40%. The ^{2}H NMR spectrum of **5** obtained in these conditions (Figure 1, c) shows incorporation of deuterium at positions 2 and 3 of the side chain.

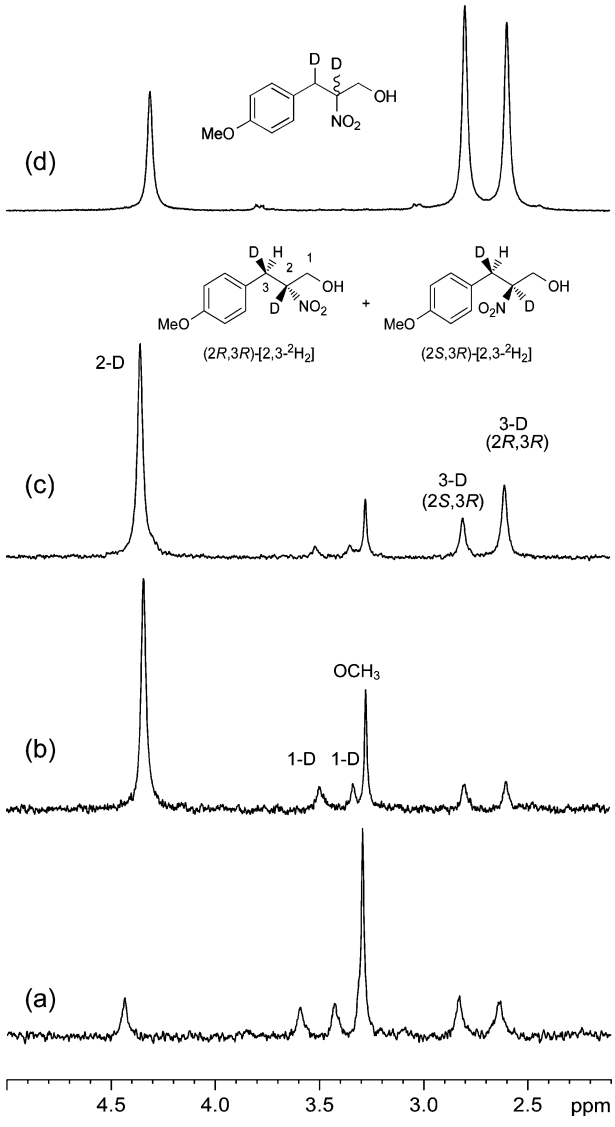
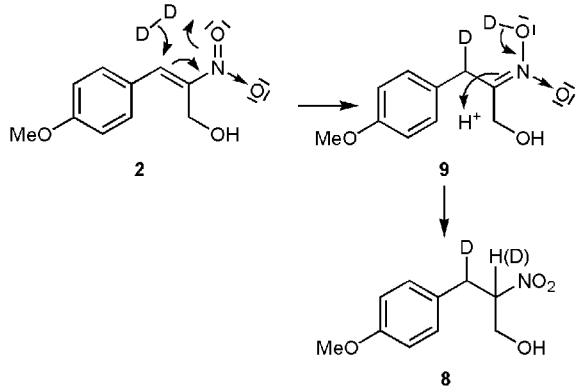


Figure 1. Deuterium NMR spectra in C_6H_6 of (a) unlabelled **5** (deuterium natural abundance spectrum) (b) **5** after incubation with B. Y. in H_2O/D_2O (1%) (c) **5** obtained from **2** by B. Y. reduction in H_2O/D_2O (1%) (d) **8** obtained from **2** by catalytic reduction (D_2-Pd/C). The deuterium chemical shifts of **5** in benzene are slightly dependent on the sample concentration and thus the signals show some variability throughout the spectra (within 0.1 ppm).

However, the signals at δ = 2.62 and 2.81 ppm, respectively, relative to the diastereotopic hydrogen atoms in position 3, appear in ca. 7:3 ratio, once depurated from the contribution to the peak intensity of the natural abundance signal. This contribution was evaluated from the peak areas of the nuclei at C-1 and of the methoxy group. As expected, the intensity of the deuterium atom signal at δ = 4.36 ppm (position 2) is much higher than those at δ = 2.62 and 2.81 ppm (position 3), in agreement with the fact that this hydrogen atom is thought to be delivered from the solvent water in the reduction step and, moreover, it is incorporated from the medium in **5** once the reduction has taken place (see Figure 1, c). It is instead reasonable to expect a lower incorporation of deuterium in benzylic position as a consequence of multiple isotope effects. Indeed, this species is depleted because it derives from the reduced cofactor which has been deuterium labelled through an enzyme-controlled exchange with diluted deuterated water.[10] We were confident to be able to assign the deuterium benzylic signals of **5** comparing its ^{2}H NMR spectrum (Figure 1, c) with that of the chemically identical material produced from **2** by hydrogenation with deuterium gas in the presence of Pd/C in the expectation of a *syn* process.[13] However, the ^{2}H NMR spectrum (Figure 1, d) of [2,3-$^2H_2$] 3-(4-methoxyphenyl)-2-nitropropan-1-ol (**8**, Scheme 2), produced from **2** by treatment with deuterium gas in AcOEt in the presence of moist Pd/C, shows, in addition to the signal at δ = 4.36 ppm (position 2), two peaks of equal intensity at δ = 2.62 and 2.81 ppm, respectively, for the two diastereotopic hydrogen atoms in position 3. Also, there is a dramatic difference in the extent of the labelling of the two positions (Figure 1, d), specifically inverse respect to the one observed in compound **5** bio-generated in baker's yeast (Figure 1, c).

The labelling pattern of **8** could be the consequence of a two steps mechanism, involving 1,4-deuterium addition onto **2** to yield (Scheme 3) the nitronic acid derivative **9**. Subsequently, the sp^2 carbon atom at position 2 of the latter is protonated without steric control with the prevalent incorporation of ^{1}H species from the medium. This hypothesis explains both the lack of stereospecificity in the hydrogenation and the difference in the deuterium incorporation at the two sites and might be of relevance for the elucidation of the mechanism of the enzymatic reduction of nitro alkenes.[16,22,23]



Scheme 3. Steps in the chemical reduction with deuterium gas of **2** to provide randomly deuterated **8**.

Thus, to unambiguously establish the relative stereochemistry of the deuterium signals in positions 2 and 3 of (*R*)-**5** obtained in deuterated water, the ^{2}H NMR spectrum of the derived amine **7** (Figure 2, a) was acquired and compared with that (Figure 2, b) of the chemically identical synthetic (2*SR*,3*SR*)-[2,3-$^2H_2$] amine **13**. The latter was prepared (Scheme 4) from the (*Z*)-acetamido-cinnamic acid derivative **10**[24,25] by catalytic hydrogenation with deuterium gas in the presence of 10% Pd/C. The resulting (2*SR*,3*SR*)-

[2,3-$^2H_2$] derivative **11**, via the ester hydrochloride **12** and $LiAlH_4$ reduction, provided the required stereospecifically *syn* labelled amine **13**.

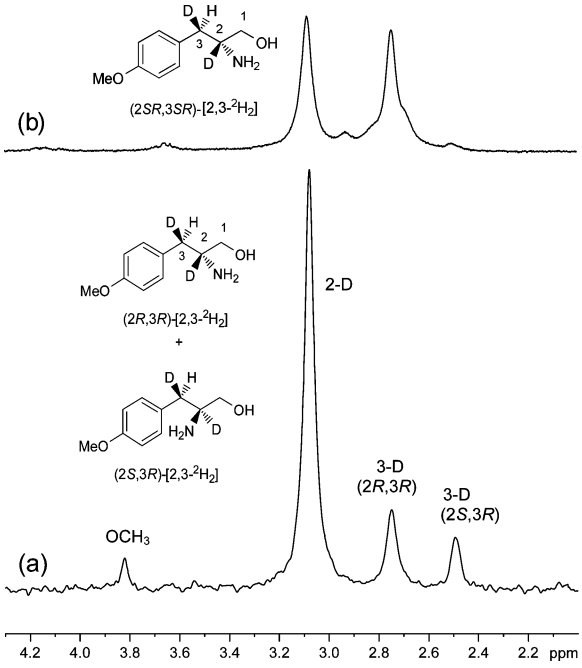
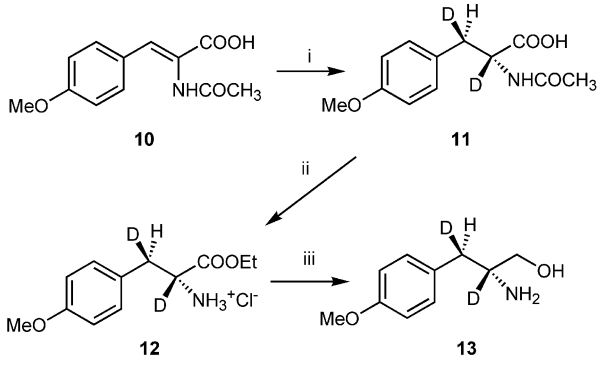


Figure 2. Deuterium NMR spectra in $CHCl_3$ of (a) **7** obtained from **5** by reduction with Zn/H_2SO_4 (b) **13** synthesized starting from **10** (see Scheme 4).



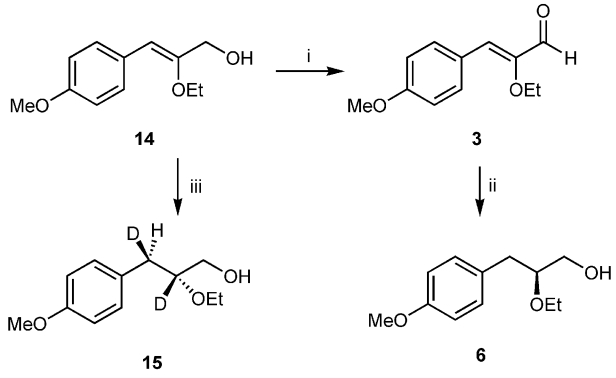
Scheme 4. i. deuterium gas, 10% Pd/C, ethanol, room temp.; ii. HCl (gas)/ethanol, reflux, 10 h; iii. aqueous $NaHCO_3/Et_2O$ extraction, then $LiAlH_4$ reflux, 12 h.

It thus clearly appears from the inspection of spectra in parts a and b of Figure 2 that **7** is composed by the (2*R*,3*R*)-[2,3-$^2H_2$] diastereoisomer in ca. 7:3 mixture with the (2*S*,3*R*)-[2,3-$^2H_2$] material.

Going back from **7** to **5** and to the (*E*)-unsaturated precursor **2** it follows that the enzymatic reduction of **2** to **5** involved the addition in position β respect to the nitro group of an hydrogen atom delivered from the upper face of the molecule, while the protonation in α occurred prevalently from the opposite site to yield ca. 70% of the (*2R*) material.

A further confirmation of the utility of ^2H NMR studies on the starting materials obtained in baker's yeast-mediated reductions performed in the presence of D_2O as an analytical tool for the determination of the stereochemical course of the process arose from experiments on the aldehyde (*Z*)-**3**, which shows some structural similarity with nitrostyrene derivative **2**. The material, when administered to baker's yeast loaded onto an adsorption resin under conditions assuring a very low concentration of substrate in the fermentation medium, is converted into enantiomerically pure (2*S*)-**6** (*ee* = 99%).[19] In the present work, using a reduced volume due to the presence of deuterated water, at higher substrate concentration, the reduced material **6** with *ee* = 92% was isolated.

Thus, aldehyde **3**, prepared by MnO_2 oxidation of allylic alcohol **14**,[19] administered to actively fermenting baker's yeast in the presence of ca. 4% deuterated water, afforded labelled [1,2,3-$^3H_2$]-**6**, containing 96% (HPLC) of the (*S*)-enantiomer (Scheme 5). In a separate experiment, allylic alcohol **14** was converted into (2*SR*,3*SR*)-[2,3-$^2H_2$]-**15** on hydrogenation with deuterium gas in ethyl acetate in the presence of wet 10% Pd/C.



Scheme 5. i. MnO_2, CH_2Cl_2, reflux, 3 h; ii. baker's yeast/deuterated water; iii. deuterium gas, ethyl acetate, 10% Pd/C, room temp.

The deuterium NMR spectrum of **6** (Figure 3, a) shows significant signals for three of the five hydrogen atoms of the side chain at δ = 3.52, 3.46 and 2.81 ppm, respectively. Minor signals are at δ = 3.61 and 2.70 ppm. Comparison of the spectrum of biogenerated **6** with that of *syn*-2,3-dideuterated **15** (Figure 3, b) clearly indicates that the two materials are diastereoisomers. Thus, in **6** the deuterium atom at position 3 is in *anti* relationship with that in 2. This means that the biological hydrogenation of the double bond of **3** involves the addition of an hydrogen atom in position β respect to the activating carbonyl group from the upper part of the molecule, while α-protonation took place by 96% in *anti* manner to yield (*S*)-**6**. The spectrum of **6** shows deuterium labelling also in position 1, as expected for an alcohol generated in baker's yeast from an aldehyde like **3**.

In order to simplify the assignment and the quantitative evaluation of the signals relative to position 1, the alcohol **6** was converted into the corresponding acetyl derivative and its ^2H NMR spectrum is reported in Figure 3 (c). The signals for the two diastereotopic deuterium atoms in position 1 of the derivative of **6** appear at δ = 4.02 and 4.13 ppm in ca. 95:5 ratio, as that of the two deuterium atoms at

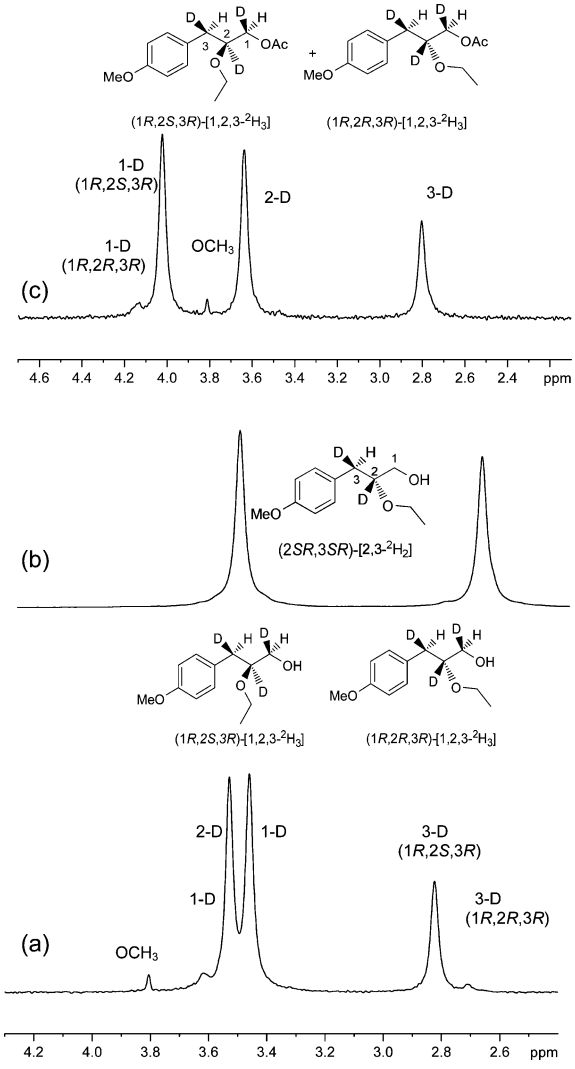
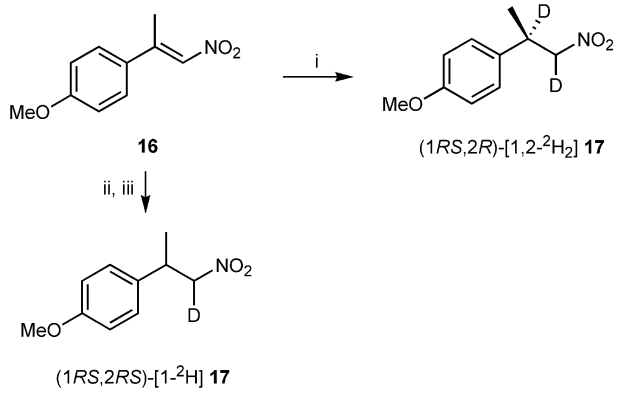


Figure 3. Deuterium NMR spectra in $CHCl_3$ of (a) **6** obtained by B. Y. reduction of the aldehyde **3** in H_2O/D_2O (4%) (b) **15** obtained from the allylic alcohol **14** by catalytic reduction (D_2-Pd/C) (c) acetyl derivative of **6**.

position 3. If we consider reasonable that the mode of yeast reduction of the carbonyl of the substituted cinnamaldehyde **3** is the same of cinnamaldehyde, i.e., addition onto the carbonyl C atom of a *pro-R* deuterium atom mediated by yeast alcohol dehydrogenase,[2] we can depict labelled **6** obtained in the baker's yeast reduction of **3** in the presence of deuterated water as substantially composed by the (1*R*,2*S*,3*R*)-[1,2,3-$^2H_3$] diastereoisomer. In this instance, the stereopecific reduction of the aldehydic group of **3** by yeast alcohol dehydrogenase with hydride delivery from deuterated reduced cofactor serves is an internal probe of the stereospecificity of the saturation of the double bond.

The attention was finally drawn on the mode of reduction of **1** by baker's yeast in the presence of deuterated water. The choice of **1** as a substrate was dictated from the circumstance that the reported[17] spectrum of the saturated racemic material **4** shows a signal pattern amenable for a full assignment. In fact the two methylene protons H-1a (δ = 4.47 ppm) and H-1b (δ = 4.68 ppm) of **4** show the following homo- and heteronuclear vicinal coupling constants: $J(H_{1a},H_2)$ = 8.7, $J(H_{1b},H_2)$ = 6.0, $J(H_{1a},^{13}CH_3)$ = 3.3, $J(H_{1b},^{13}CH_3)$ = 5.3 Hz. Such values unequivocally indicate that the most populated conformational state of the molecule is that with the aromatic ring and the nitro group *trans*-oriented where the protons H-1a and H-1b can be assigned the *pro-S* and *pro-R* configuration, respectively.

Luckily enough, product **1**, which was poorly reduced to racemic **4** with *Clostridium sporogenes* extract,[17] in baker's yeast and D_2O rapidly afforded **4**, shown by HPLC analysis to be a 98:2 mixture of two enantiomers. This material was tentatively assigned the (*R*)-configuration because of the order of elution on chiral HPLC, identical to that of (2*R*)-**17** prepared from **16** with fermenting baker's yeast in the presence of D_2O (Scheme 6). ^2H NMR studies show that the recovered material (Figure 4, a) is (1*RS*,2*R*)-[1,2-$^2H_2$]-**4** since there are two deuterium signals of equal intensity at δ = 4.70 and 4.49 ppm relative to the hydrogen atoms in position 1 and a signal at δ = 3.96 ppm for the deuterium atom in position 2.



Scheme 6. i. Baker's yeast/deuterated water; ii. $NaBH_4$, EtOH; iii. Baker's yeast deuterated water.

In order to confirm these results, the reduction product obtained from nitrostyrene **16** was submitted to ^2H NMR analysis, and it was found to be (1*RS*,2*R*)-[1,2-$^2H_2$] **17** (Figure 4, b) as indicated from the signals of equal intensity at δ = 4.52 ppm and 4.47 ppm (position 1) and at δ = 3.60 ppm (position 2).

Finally, a blank experiment was performed, incubating synthetic **17** with baker's yeast in the presence of deuterated water under the conditions used in the reduction of **1** and **16**. The ^2H NMR spectrum (Figure 4, c) of the recovered product indicates that it is randomly labelled [1-^2H]-2-(4-methoxyphenyl)-1-nitropropane (**17**) with signals at δ = 4.52 and 4.47 ppm (position 1). Thus, no mechanistic conclusions can be drawn from the mode of deuterium labelling at position 1 of the two nitro alkanes **4** and **17** generated in the presence of deuterated water from **1** and **16**, also at the light of the results of the exchange experiments performed early on synthetic racemic **5** (Figure 1, b) incubated with deuterated water.

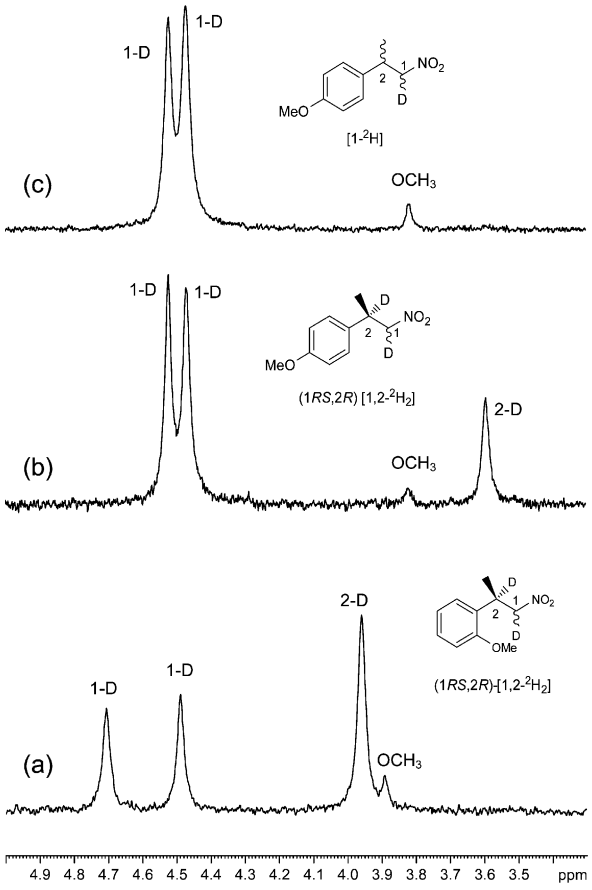


Figure 4. Deuterium NMR spectra of (a) **4** obtained from **1** by B. Y. reduction in H_2O/D_2O (1%) (b) **17** obtained from **16** by B. Y. reduction in H_2O/D_2O (1%) (c) **17** recovered after incubation of unlabelled 2-(4-methoxyphenyl)-1-nitropropane with B. Y. in H_2O/D_2O (1%).

Conclusion

The present results demonstrate that ^2H NMR studies on materials obtained in the presence of deuterated water in the enzymatic reduction of activated trisubstituted olefins represent a direct analytical tool for the determination of the steric course of the reduction process. Synthetic deuterated materials obtained by *syn* metal-catalysed reduction with deuterium gas were employed for comparison purposes. A relevant prerequisite of the method is a dispersion of the ^2H NMR spectra signals of the relevant hydrogen atoms large enough as to allow a proper analysis. For practical reasons, the procedure has been here applied using baker's yeast due to the presence in the whole cell system of the capacity to exchange of an hydrogen of the reduced cofactor for a deuterium atom. The operation with isolated purified reducing enzymes would require the addition of the reduced deuterated cofactor or the presence of an enzymatic machinery suitable for its generation. Finally, as far as the stereochemistry of the transformations here investigated is concerned it is worth to mention that in baker's yeast **2** and **3**, trisubstituted analogs of the tetrasubstituted materials observed to be prevalently reduced in *syn* fashion,[14,15] are converted into (*R*)-**5** and (*S*)-**6** of good enantiomeric purity through *anti* addition of hydrogen atoms across the double bond, with formal hydride delivery in position β with respect to the activating group from the "upper" side of the molecule.

Experimental Section

General Procedure for the Baker's Yeast Reduction: In an open jar a mixture was made up composed of baker's yeast (200 g), D-glucose (50 g), tap water (1 L) and 99% deuterated water (20 mL). Under mechanical stirring, at 35 °C, the indicated substrate (typically 1 g) dissolved in the minimum amount of ethanol was added dropwise within 10 min. After 24–48 h stirring at room temp., acetone, 0.5 L was added at once. The mixture was stirred for 30 min and then extracted three times with a total of 1.2 L of ethyl acetate/hexane, 9:1. The organic phase was evaporated to remove most of the acetone and washed twice with brine. The residue obtained upon evaporation of the dried (Na_2SO_4) organic phase was column chromatographed with increasing amounts of ethyl acetate in hexane. Typically, the recovery was 60–70%. When non-polar resins were used, the substrate was dissolved in diethyl ether in 1:15 w/w ratio with XAD-1180. The solvent was removed under reduced pressure and the residue was added to fermenting Baker's yeast.

(2*R*)-3-(4-Methoxyphenyl)-2-nitropropan-2-ol (5): Yeast reduction of **2** yields **5** (60%) as a yellowish oil, which solidifies on standing. ^1H NMR (400 MHz, C_6D_6): δ = 6.80 (m, 2 H, aromatic H), 6.66 (m, 2 H, aromatic H), 4.37 (m, 1 H, 2-H), 3.55 (dd, J = 12.2, 7.5 Hz, 1 H, 1-H), 3.40 (dd, J = 12.2, 3.4 Hz, 1 H, 1-H), 3.31 (s, 3 H, OCH_3), 2.85 (dd, J = 14.1, 7.6 Hz, 1 H, 3-H), 2.64 (dd, J = 14.1, 6.9 Hz, 1 H, 3-H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 158.7, 129.8, 128.7, 114.1, 90.4, 62.4, 55.1, 34.9 ppm. ^2H NMR (76.7 MHz, C_6H_6): δ = 4.36 (2-D), 3.29 (OCH_2D), 2.82 (3-D), 2.61 (3-D) (see Figure 1, c) ppm. $[a]_{20}^{D}$ = +35.1 (c = 2.05, $CHCl_3$), *ee* (HPLC) = 70%. HRMS (ESI) 211.0849. $C_{10}H_{13}NO_4$ requires 211.0844.

[2,3-$^2H_2$]-3-(4-Methoxyphenyl)-2-nitropropan-2-ol (8): Product **2** (2 g, 10 mmol), in ethyl acetate (50 mL) was treated at room temp. with deuterium gas in the presence of wet 5% Pd/C (150 mg). The reaction was interrupted after the absorption of ca. 0.5 mol. equiv. of gas. The residue obtained upon evaporation of the filtered solution was chromatographed, as above, to afford dideuterated **8** in 30% yield. ^1H NMR (400 MHz, C_6D_6): δ = 6.78 (m, 2 H, aromatic H), 6.76 (m, 2 H, aromatic H), 4.30 (m, 0.8 H, 2-H), 3.45 (m, 1 H, 1-H), 3.31 (m, 1 H, 1-H), 3.28 (s, 3 H, OCH_3), 2.81 (m, 0.7 H, 3-H), 2.59 (m, 0.7 H, 3-H) ppm. ^2H NMR (76.7 MHz, C_6H_6): δ = 4.28 (2-D), 2.79 (D-3), 2.58 (3-D) (see Figure 1, d) ppm.

(2*R*)-2-Amino-3-(4-methoxyphenyl)propan-1-ol (7): (2*R*)-3-(4-Methoxyphenyl)-2-nitropropan-1-ol (**5**) obtained in the yeast reduction of **2** (0.6 g, 3 mmol), in ethanol (50 mL), in the presence of Zn powder (5 g) under mechanical stirring was treated dropwise with 30% sulfuric acid (10 mL).[20] After 12 h stirring, the mixture was filtered with suction and the solid washed repeatedly with ethanol. The organic phase was concentrated to a small volume, diluted with ice-water and extracted twice with ethyl acetate (discarded). The cold aqueous phase was made strongly alkaline with 30% NaOH and extracted with ethyl acetate (3 × 100 mL). The residue obtained upon evaporation of the dried organic extract was dissolved in ethyl acetate (5 mL), and treated with dry HCl in ethanol (2 mL). After 24 h at 4 °C, upon filtration, the crystalline hydrochloride of (2*R*)-2-amino-3-(4-methoxyphenyl)propan-1-ol (**10**) was obtained (0.42 g, 68%): ^1H NMR (400 MHz, [D_6]DMSO): δ = 7.18

(m, 2 H, aromatic H), 6.86 (m, 2 H, aromatic H), 3.73 (s, 3 H, OCH_3), 3.50 (dd, J = 11.6, 3.5 Hz, 1 H, *CH*HOH), 3.38 (dd, J = 11.6, 5.9 Hz, 1 H, *CH*HOH), 3.23 (m, 1 H, *CH*NH_2), 2.89 (dd, J = 13.3, 5.3 Hz, ArC*H*H), 2.75 (dd, J = 13.3, 9.1 Hz, ArC*H*H) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]DMSO) δ = 157.9, 130.1, 128.3, 113.8, 59.4, 54.9, 53.9, 33.7 ppm. $[\alpha]_{20}^{D}$ = –32.0 (c = 2, HCl 1 N) [lit. for (*S*)-7 $[\alpha]_{20}^{D}$ = +48.8 (c = 2, aq. HCl)].[21] In the deuterated series from yeast, the free amine **7** for the ^{2}H NMR studies was obtained as follows. The crude ethyl acetate extract of the Zn reduction, dissolved in the minimum amount of ethyl acetate was treated with an equal weigh of oxalic acid dissolved in ethyl acetate. The mixture was left aside 24 h at room temp. The white precipitate was collected and treated in a two phase system CH_2Cl_2/water with an excess of aqueous potassium carbonate. The organic phase was separated, dried and evaporated to furnish the amine **7**. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.11 (m, 2 H, aromatic H), 6.86 (m, 2 H, aromatic H), 3.80 (s, 3 H, OCH_3), 3.63 (dd, J = 10.7, 4.0 Hz, 1 H, 1-H), 3.37 (dd, J = 10.7, 7.3 Hz, 1 H, 1-H), 3.08 (m, 1 H, 2-H), 2.74 (dd, J = 13.7, 5.4 Hz, 1 H, 3-H), 2.48 (dd, J = 13.7, 8.6 Hz, 1 H, 3-H), 1.70 (s br, 1 H, OH) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$): δ = 3.82 (OCH_2D), 3.08 (2-D), 2.75 (3-D), 2.49 (3-D) (see Figure 2, a) ppm.

(2*SR*,3*SR*)-[2,3-$^2H_2$]-2-Amino-3-(4-methoxyphenyl)propan-1-ol (13): The acetamido-cinnamic acid **10** was obtained[24] from acetyl glycine and anisaldehyde as sparingly soluble brownish solid. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.59 (m, 2 H, aromatic H), 7.23 (s, 1 H, C*H*=C), 6.96 (m, 2 H, aromatic H), 3.79 (s, 3 H, OCH_3), 1.99 (s, 3 H, NHCO*CH*$_3$) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]-DMSO) δ = 168.8, 166.4, 159.8, 131.4, 126.1, 124.9, 116.9, 113.9, 55.1, 51.1, 22.3 ppm. A suspension of **10** (4.7 g, 50 mmol), in ethyl acetate (50 mL) was treated whilst stirring at room temp. with deuterium gas until the gas absorption ceased. The filtered solution was taken to dryness and the residue was crystallized from ethanol/ethyl acetate to afford 2,3-dideuterated 2-(acetylamino)-3-(4-methoxyphenyl)propionic acid (**11**) quantitatively. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10 (m, 2 H, aromatic H), 6.86 (m, 2 H, aromatic H), 5.83 (s br, 1 H, NH), 4.82 (m, 0.6 H, 2-H), 3.80 (s, 3 H, OCH_3), 3.18 (m, 0.65 H, 3-H), 3.10 (m, 1 H, 3-H), 2.00 (s, 3 H, $COCH_3$) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 4.82 (2-D), 3.18 (3-D) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, the molecule was partially deuterated and thus the signals of some C atoms are split in a few peaks due to the deuterium isotope effect) δ = 173.2, 169.2, 157.9 130.0, [129.52, 129.49, 129.47 (C-1 aromatic quaternary C atom)], 113.6, 54.9, [53.73, 53.68, 53.43 (t, J = 22.0 Hz, C-2)], [35.97, 35.89, 35.61 (m br, C-3)], 22.3 ppm. Product **11** was treated at reflux under mechanical stirring with ethanol saturated with HCl gas (50 mL). After 10 h the reaction mixture was evaporated to dryness and the residue was crystallized from hot ethanol to provide ethyl [2,3-$^2H_2$]-2-amino-3-(4-methoxyphenyl)propionate hydrochloride (**12**) (3.2 g, 65% from **10**). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.70 (s br, 3 H, NH_3^+), 7.23 (m, 2 H, aromatic H), 6.84 (m, 2 H, aromatic H), 4.32 (m, 0.6 H, 2-H), 4.16 (q, J = 7.1 Hz, 2 H,OCH_2), 3.76 (s, 3 H, OCH_3), 3.39 (m, 1 H, 3-H), 3.31 (m, 0.75 H, 3-H), 1.21 (t, J = 7.1 Hz, 3 H, CH_3) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 4.32 (2-D), 3.32 (3-D) ppm. ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, the molecule was partially deuterated and thus the signals of some C atoms are split in a few peaks due to the deuterium isotope effect) δ = 168.9, 158.5, 130.5, [126.49, 126.45 (C-1 aromatic quaternary C atom)], 113.9, 61.4, 55.0, [53.36, 53.31, 53.06 (t, J = 22.0 Hz, C-2)], [35.02, 34.94, 34.67 (m br, C-3)], 13.8 ppm. Product **12** (2.6 g, 10 mmol), was suspended in diethyl ether/water, 1:1 (100 mL) and treated whilst stirring with satd. $NaHCO_3$ solution at alkaline pH. The organic phase was washed with brine, dried (Na_2SO_4) and added dropwise to $LiAlH_4$ (4 g), in refluxing diethyl ether (100 mL) under mechanical stirring in a N_2 atmosphere. After 10 h under these conditions the cooled reaction mixture was treated with caution with a satd. solution of potassium sodium tartrate. The residue obtained upon evaporation of the washed and dried organic phase in a small volume of ethyl acetate was treated with oxalic acid (1 g) in ethyl acetate. The separated crystals collected by filtration were treated in CH_2Cl_2/water, as above, with potassium carbonate to provide, on evaporation of the solvent, (2*SR*,3*SR*)-[2,3-$^2H_2$]-2-amino-3-(4-methoxyphenyl)propan-1-ol (**13**), thick oil, 0.5 g (27%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.11 (m, 2 H, aromatic H), 6.86 (m, 2 H, aromatic H), 3.80 (s, 3 H, OCH_3), 3.64 (m, 1 H, 1-H), 3.38 (m, 1 H, 1-H), 3.09 (m, 0.6 H, 2-H), 2.74 (m, 0.7 H, 3-H), 2.49 (m, 1 H, 3-D) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$): δ = 3.09 (2-D), 2.74 (3-D) (see Figure 2, b) ppm.

(2*S*)-2-Ethoxy-3-(4-methoxyphenyl)propan-1-ol (6): Yeast reduction of **3** provided **6** as a yellow oil, in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.12 (m, 2 H, aromatic H), 6.83 (m, 2 H, aromatic H), 3.78 (s, 3 H, OCH_3), 3.61 (dd, J = 11.0, 3.8 Hz, 1 H, H-1), 3.57–3.49 (m, 3 H, H-2 + OCH2), 3.45 (dd, J = 11.0, 6.2 Hz, 1 H, H-1), 2.81 (dd, J = 13.5, 5.9 Hz, 1 H, H-3), 2.70 (dd, J = 13.5, 6.9 Hz, 1 H, H-3), 2.33 (s br, 1 H, OH) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 3.79 (OCH_2D), 3.61 (1-D), 3.52 (2-D), 3.46 (1-D), 2.81 (3-D), 2.71 (3-D) (see Figure 3, a) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 158.0, 130.2, 130.1, 113.7, 81.1, 65.2, 63.6, 55.2, 36.4, 15.5 ppm. $[\alpha]_D^{20}$ = +3.8 (c = 3.1, $CHCl_3$), *ee* (HPLC) = 92%; lit. ref. [19]: $[\alpha]_D^{20}$ = +4.0 (c = 3.5, $CHCl_3$) for (*S*)-**6** with *ee* (HPLC) = 99%. This material (0.3 g) was treated with acetic anhydride (2 mL) and pyridine (2 mL) overnight at room temp. The reagents were taken to dryness at the rotary evaporator at 50 °C. The oily residue was kept under high vacuum for 10 h to provide the acetyl derivative used for the ^{2}H NMR studies. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.12 (m, 2 H, aromatic H), 6.81 (m, 2 H, aromatic H), 4.12 (dd, J = 11.5, 4.1 Hz, 1 H, 1-H), 4.00 (dd, J = 11.5, 5.7 Hz, 1 H, 1-H), 3.79 (s, 3 H, OCH_3), 3.63 (m, 1 H, 2-H), 3.58–3.41 (m, 2 H, OCH_2), 2.80 (dd, J = 13.9, 7.0 Hz, 1 H, 3-H), 2.74 (dd, J = 13.9, 5.9 Hz, 1 H, 3-H), 2.06 (s, 3 H, $COCH_3$), 1.12 (t, J = 7.1 Hz, CH_3) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$): δ = 4.13 (1-D), 4.00 (1-D), 3.78 (OCH_2D), 3.62 (2-D), 2.81 (3-D) (see Figure 3, c) ppm.

(2*SR*,3*SR*)-[2,3-$^2H_2$]-2-Ethoxy-3-(4-methoxyphenyl)propan-1-ol (15): 2-Ethoxy-3-(4-methoxyphenyl)-2-propen-1-ol (**14**) (0.4 g, 2 mmol) in ethyl acetate (40 mL) was treated with deuterium gas at room temp. in the presence of wet 10% Pd/C (100 mg). At the end of the hydrogenation the reaction mixture was filtered through a Celite® pad to provide on evaporation of the solvent the desired compound quantitatively. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.12 (m, 2 H, aromatic H), 6.84 (m, 2 H, aromatic H), 3.79 (s, 3 H, OCH_3), 3.63–3.42 (m, 4.2 H, 2-H, CH_2OH, OCH_2), 2.82 (m, 1 H, 3-H), 2.69 (m, 0.2 H, 3-H), 2.13 (s br, 1 H, OH), 1.18 (t, J = 7.1 Hz, CH_3) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$): δ = 3.53 (2-D), 2.70 (3-D) (see Figure 3, b) ppm.

(2*R*)-2-(2-Methoxyphenyl)-1-nitropropane (4):[17] The product was obtained in baker's yeast from **1** in 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.26 (m, 1 H, aromatic H), 7.17 (m, 1 H, aromatic H), 6.94 (m, 1 H, aromatic H), 6.90 (m, 1 H, aromatic H), 4.69 (dd, J = 11.9, 6.0 Hz, 1 H, 1-H), 4.47 (dd, J = 11.9, 8.7 Hz, 1 H, 1-H), 3.96 (m, 1 H, 2-H), 3.87 (s, 3 H, OCH_3), 1.39 (d, J = 7.1 Hz, CH_3) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 4.70 (1-D), 4.49 (1-D), 3.96 (2-D), 3.89 (OCH_2D) (see Figure 4, a) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 157.1, 128.8, 128.5, 127.4, 120.9, 110.9, 80.5, 55.3, 33.5, 17.1 ppm. $[\alpha]_{20}^{D}$ = +6.4 (c = 2.1, $CHCl_3$); *ee* (HPLC) = 96%.

(2*R*)-2-(4-Methoxyphenyl)-1-nitropropane (17):[17] The material obtained in baker's yeast from **16**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.15 (m, 2 H, aromatic H), 6.88 (m, 2 H, aromatic H), 4.51 (dd, J = 11.8, 7.1 Hz, 1 H, 1-H), 4.46 (dd, J = 11.8, 8.0 Hz, 1 H, 1-H), 3.80 (s, 3 H, OCH_3), 3.60 (sextet, J = 7.2 Hz, 1 H, 2-H), 1.37 (d, J = 7.1 Hz, 3 H, CH_3) ppm. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 4.52 (1-D), 4.47 (1-D), 3.81 (OCH_2D), 3.60 (2-D) (see Figure 4, b) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 158.9, 132.9, 127.9, 114.4, 82.1, 55.3, 37.9, 18.8 ppm. $[a]_{20}^{D}$ = +60.5 (c = 1.2, $CHCl_3$), *ee* (HPLC) = 88%; ref.[17]: $[a]_{20}^{D}$ = +66.2 (c = 0.99, $CHCl_3$, 30 °C) for (*R*)-**17** *ee* = 97%.

(2*RS*)-2-(4-Methoxyphenyl)-1-nitro propane (17):[17] This material, prepared as reported,[17] was recovered in 70% yield from the incubation in fermenting baker's yeast in the presence of deuterated water. ^{2}H NMR (76.7 MHz, $CHCl_3$) δ = 4.52 (1-D), 4.47 (1-D), 3.81 (OCH_2D), 3.60 (2-D) (see Figure 4, c) ppm. The ^{1}H and ^{13}C NMR spectra were in accordance with those of compound (2*R*)-**17**.

Supporting Information (see also the footnote on the first page of this article): General experimental methods; acquisition of the ^{2}H spectra; spectroscopic data of compounds **2**, **3**, **1** and **16**.

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Received: April 1, 2010
Published Online: July 26, 2010

