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Isotopic Labeling for Determination of Enantiomeric Purity by ^{2}H NMR Spectroscopy

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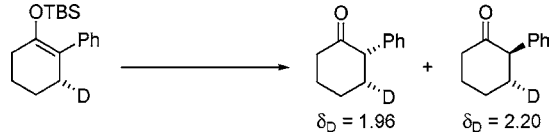
ABSTRACT



The use of ^{2}H NMR spectroscopy as a tool for the analysis of enantiomeric purity is reported. Enantiopure isotopically chiral substrates bearing a monodeuterated methylene unit were prepared; introduction of an additional asymmetric center leads to diastereomers which can be distinguished by ^{2}H NMR on a standard spectrometer. The assays allow for simple semiquantitative analysis of asymmetric transformations.

As screening-based approaches to the discovery and optimization of new catalytic asymmetric processes become more widespread, there has been a concomitant growth in interest in the development of methods for the high-throughput and/or in situ analysis of reaction conversion and enantiomeric purity. Methods which avoid the use of chromatographic techniques include mass spectrometry,[1] IR thermography,[2] UV−visible[3] or fluorescence spectroscopy,[4] and colorimetric[5] and biochemical (antibody[6] or enzyme-based[7]) methods. Given the near-ubiquitous availability of NMR spectrometers to research chemists, new methods based around this technique would be expected to gain widespread uptake;[8−10] in addition, used in conjunction with linked autosamplers, this would facilitate automated parallel screening programmes and the accumulation of real-time kinetic data.

Morken has elegantly demonstrated the use of a ^{13}C-labeled isotopically chiral ketone for direct analysis of asymmetric induction in enantioselective reductions.[8] Initial enantiomeric ratios were determined by a single FT pulse, while more accurate experiments (8 FT pulses) revealed an average error of ±3% by comparison with chiral GC experiment in a data acquisition time of only 4 min. The use of

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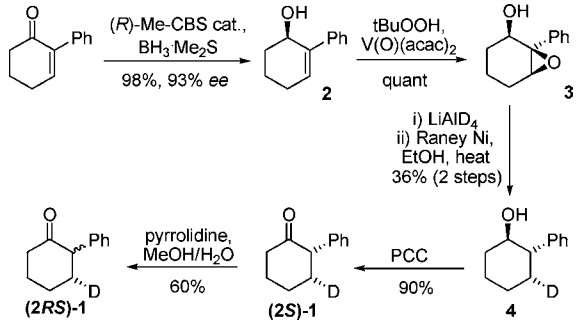
^{13}C labels does, however, require the presence of prochiral carbon substituents (such as a geminal dimethylalkyl group) which necessarily limits the substrate scope of the method. Much greater substrate scope could be achieved by an alternative isotopic substitution, namely that of a deuteron for a proton in a labeled methylene unit. The ^{2}H nucleus is NMR active with spin +1. Moreover, analysis can be performed on standard spectrometers by utilizing the deuterium lock channel as the observation channel; this makes the method available to most workers. Herein we disclose proof-of-principle studies which verify that ^{2}H NMR of isotopically chiral substrates is a viable method for analysis of enantiomeric purity.

To demonstrate the feasibility of the method, we chose the isotopically chiral 2-phenylcyclohexanone **1** as our target system. This was prepared in isotopically enantiopure fashion according to Scheme 1.

Scheme 1. Synthesis of Isotopically Enantiopure Ketone **1**



Known 2-phenylcyclohexenone (prepared according to Wender[11]) was subjected to asymmetric reduction using the CBS catalyst to give alcohol **2** in 98% yield (93% ee). Directed epoxidation of the allylic alcohol with vanadyl acetoacetonate and *tert*-butyl hydroperoxide gave the epoxy alcohol **3** in quantitative yield as a single diastereoisomer. The deuterium label was then introduced by S_N2 ring-opening of the epoxide with lithium aluminum deuteride. This reaction produced an approximately equimolar mixture of regioisomeric ring-opening products that was subjected to stereoselective reduction of the benzylic alcohol according to Sharpless.[12] The desired alcohol **4** was then isolated in 36% yield over two steps. This material (93% ee) could be recrystallized to optical purity from pentane if desired. Finally, oxidation to cyclohexanone (2*S*)-**1** was achieved with PCC in 90% yield. This route gave (2*S*)-**1** in 32% yield over five steps and was found to be reliable on a scale up to 2.5 g.

^{2}H NMR analysis of (2*S*)-**1** in CH_2Cl_2 showed a single signal, as expected, at 1.96 ppm. Conversion of diastereomerically pure **1** to a pseudo-racemic mixture of stereoisomers at the 2-position was achieved by equilibration via the pyrrolidinyl enamine. Analysis by ^{2}H NMR now revealed a second signal at 2.20 ppm, in a 1:1 ratio with the signal at 1.96 ppm, which was assigned as belonging to (2*R*)-**1** (Figure 1a). The absolute chemical shift values were calculated by reference to added C_6D_6, but for most experiments the reference was omitted; although the chemical shifts of the signals drifted slightly between runs, this did not interfere with the crucial data, namely the integration of the signals.

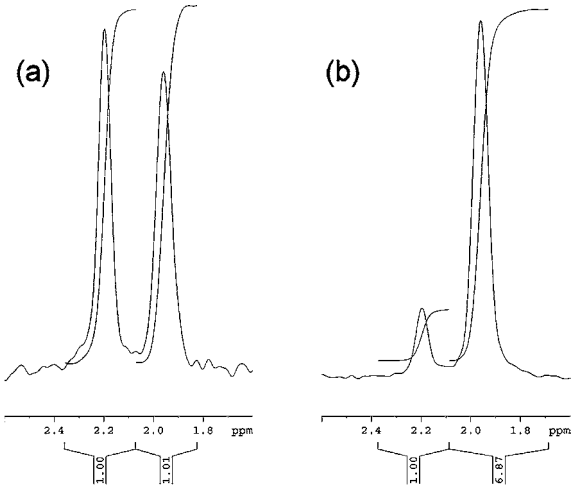


Figure 1. ^{2}H NMR spectra of (a) pseudoracemic (2*RS*) and (b) scalemic (enriched in 2*S*) ketone **1**.

Given that an aim of the study was to develop a method capable of facilitating in situ monitoring of conversion and ee, it was important to demonstrate that the signals could be differentiated in a wide range of solvents. Pleasingly, clear signal separation was observed in acetone, benzene, carbon tetrachloride, chloroform, ether, and THF, with $\Delta\delta_D$ values of between 0.17 and 0.24 ppm.[13] This bodes well for the application of the technique in reaction screening across a range of conditions.

Having demonstrated that differentiation of the two pseudoenantiomers was possible, we next investigated the performance of the assay in a quantitative sense. A series of admixtures was therefore created by mixing different quantities of pseudoracemic (2*RS*)-**1** and stereochemically pure

Table 1. Comparison of HPLC vs ^{2}H NMR for Determination of Enantiopurity of Ketone **1**

entry	% ee (HPLC)	% ee (^{2}H NMR)
1	0.2	0.6
2	17.5	18.8
3	54.5	57.0
4	58.4	67.6
5	71.3	75.0
6	80.9	85.2
7	82.3	87.0
8	83.3	87.0
9	90.0	>95[a]

[a] Minor isotopic diastereomer not detected.

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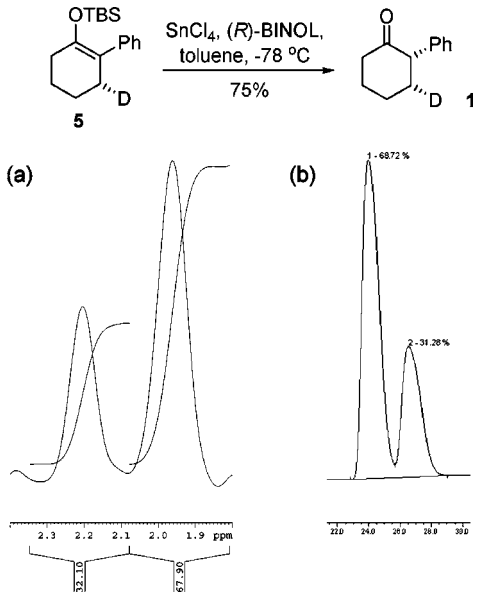
Scheme 2. Assay of Asymmetric Protonation of Silyl Enol Ether 5[a]

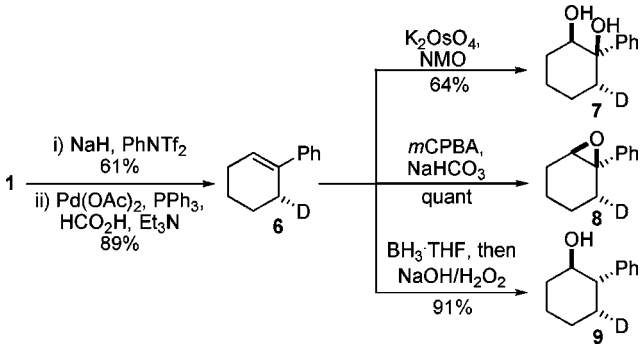


[a] Key: (a) ee by ^{2}H NMR = 38.5% (corrected for 93% ee of **5**); (b) ee by HPLC = 37.4%.

(2*S*)-**1**. These were analyzed by both chiral HPLC and ^{2}H NMR (in CH_2Cl_2) to assess the accuracy of the latter method as a probe of enantiomeric purity (Figure 1b, Table 1). We were gratified to find a good correlation between ee values measured by both techniques, with all but one sample in the range 0−83% ee giving an agreement ±5% ee. For samples of 90% ee and above (i.e., less than 5% of the minor isotopic diastereomer), we were only able to reliably observe single signals, setting an upper boundary to quantitative analysis. Nevertheless, this still represents a qualitative indicator of a highly enantioenriched sample.

We also confirmed that the method could be used to accurately assay the outcome of an asymmetric reaction. Thus, silyl enol ether **5** was prepared and subjected to Yamamoto's asymmetric protonation protocol, utilizing tin tetrachloride/(*R*)-BINOL (Scheme 2).[14] The sample produced was analyzed by both chiral HPLC and ^{2}H NMR, returning

Scheme 3. Synthesis of Additional Substrates for ^{2}H NMR Analysis



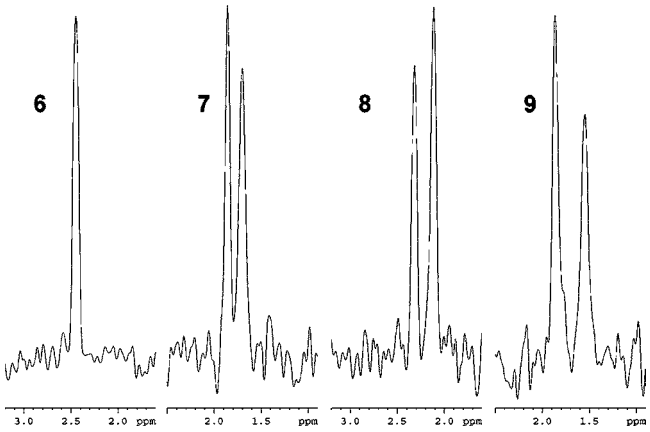
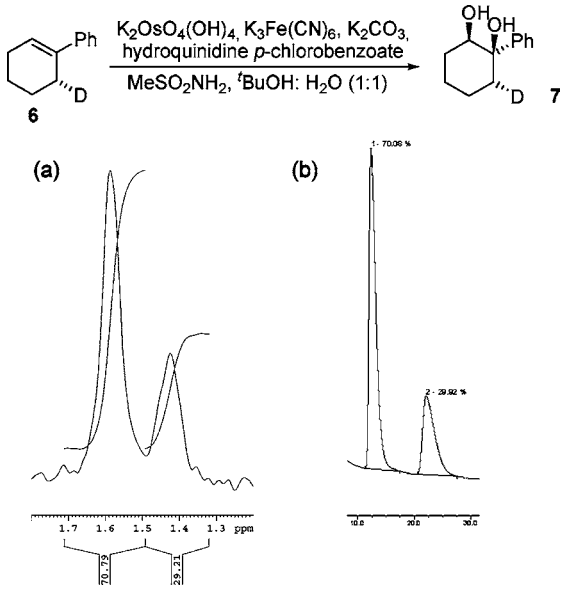


Figure 2. ^{2}H NMR spectra of compounds **6**−**9**.

near identical values for the asymmetric induction. Additionally, the major isomer was identified by ^{2}H NMR as having 2*S* configuration, which is in agreement with the model proposed by Yamamoto. This confirms that any secondary kinetic isotope effect from the neighboring chiral methylene unit is small in magnitude, as expected.

We next sought to broaden the scope of chiral compounds that could be assayed for enantiomeric purity by ^{2}H NMR spectroscopy. Ketone **1** was therefore converted to its enol triflate, then subjected to hydrogenolysis, leading to (isotopically enantiopure) prochiral alkene **6** (Scheme 3). Alkene **6** was then converted by standard methods into pseudoracemic *cis*-diol **7**, epoxide **8** and alcohol **9**: these will be valuable probes given that asymmetric dihydroxylation, epoxidation and hydroboration/oxidation processes are well documented and remain topics of active research.

Scheme 4. Assay of Asymmetric Dihydroxylation of Alkene 6[a]



[a] Key: (a) ee by ^{2}H NMR = 41.6%; (b) ee by HPLC = 40.0%.

Pleasingly, analysis of compounds **7**−**9** by ^{2}H NMR confirmed that in each case the pair of isotopically diastereomeric products could be distinguished with $\Delta\delta_D$ values in the range 0.20−0.31 ppm (Figure 2). Further, since the signals for the diastereomers of **7**−**9** have chemical shifts distinct from that of the starting alkene **6**, the potential exists to use the system for in situ analysis of both the rate and enantioselectivity in new catalytic processes.

Finally, we confirmed that semiquantitative analysis of enantiomeric purity was possible by performing the dihydroxylation of alkene **6** under Sharpless' conditions (Scheme 4).[15] The resulting diol **7** was shown to be of 40.0% ee by chiral HPLC and 41.6% ee by ^{2}H NMR.

In conclusion, we have demonstrated that substrates containing an isotopically chiral methylene unit can be used as convenient probes for enantiomeric purity by ^{2}H NMR on a standard spectrometer. The method is quantitatively accurate for ketone **1** generally ±5% ee up to the mid-80% ee range and is qualitatively useful for higher ee samples. The extension to other substrate classes for asymmetric transformations has also been demonstrated. The method offers the potential to monitor reaction progress in situ and complements the existing methods for nonchromatographic determination of enantiomeric purity.

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Supporting Information Available: Experimental procedures, compound characterization data, and ^{1}H/^{13}C NMR spectra for compounds **1**−**8**, plus ^{2}H NMR/HPLC data for Table 1 and Schemes 2 and 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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