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Routine Use of Natural Abundance Deuterium NMR in a Polypeptidic Chiral Oriented Solvent for the Determination of the Enantiomeric Composition of Chiral Building Blocks

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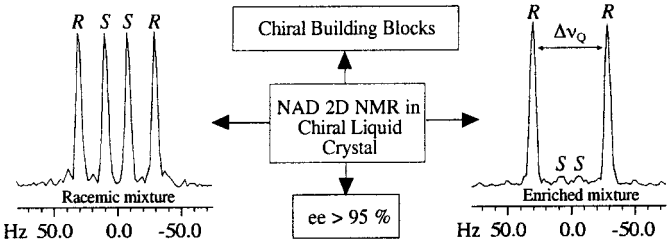
ABSTRACT



Natural abundance deuterium 2D NMR spectroscopy in chiral liquid crystal was successfully used to efficiently analyze the enantiomeric composition of organic chiral building blocks involved in the syntheses of natural and synthetic bioactive products. The results reported here emphasize the high potential of this analytical strategy and prove its applicability for routinely determining enantiomeric excesses.

Organic chiral building blocks play an essential role in the development of rapid and efficient syntheses of natural and synthetic bioactive products. In connection with an ongoing total synthesis project, we have identified **1** as a potential chiral building block for the synthesis of several natural products possessing interesting biological activities, such as lasiodiplodin,[1] dolatrienoic acid,[2] dehydrocurcuvalin,[3] macrolactines,[4] leukotrienes,[5] diplodialide A,[6] brefeldin A,[7] and mutolide[8] (Scheme 1). Synthesis of enantiomerically pure compound **1** from smaller chiral building blocks has been previously described but requires lengthy routes (from lactate[2a] or glutamate[2b] esters) or expensive starting material (chiral nonracemic propylene oxide[9]). Alternatively, previously described stoichiometric[10] or catalytic[11] asymmetric

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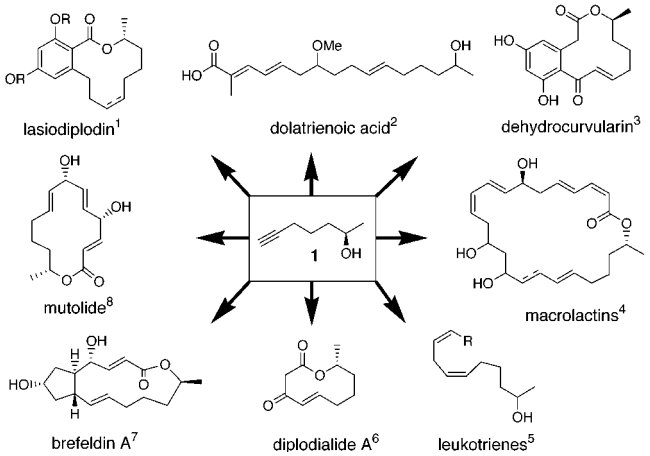
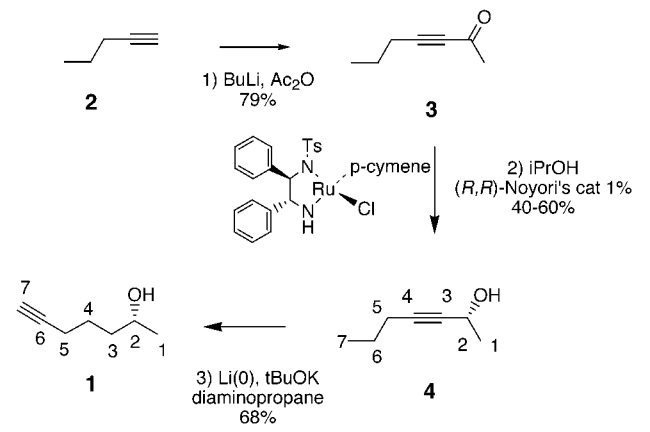
Scheme 1



reductions were moderately efficient in terms of enantioselectivity (78−83% enantiomeric excess). Consequently, we attempted to devise an efficient, rapid, inexpensive, and scalable route to enantiomerically pure **1** (Scheme 2).

Scheme 2



Following an asymmetric three-step route, access to **1** was achieved as follows. Ketone **3** was obtained in 79% yield by treating the lithium salt of commercial pentyne **2** with acetic anhydride. The ketone **3** was then asymmetrically reduced using 1% of Noyori's hydrogen transfer catalyst[12] to give alcohol **4** in 30−60% isolated yield.

At this stage of the synthesis, it was crucial to determine accurately the enantiomeric excess (ee) of **4**. For this purpose, we turned our attention to NMR in chiral liquid crystals (CLCs), which has been recently proposed in the field of enantiomeric analysis.[13] In particular we have explored the efficiency of natural abundance deuterium NMR (NAD NMR) in polymeric liquid-crystalline solvents made of poly-γ-benzyl-L-glutamate (PBLG) dissolved in organic solvents ($CDCl_3$, DMF, etc.) and its application for routine analyses.[14] This spectroscopic strategy provides an interesting and original alternative when conventional NMR methods used routinely in the laboratory (chiral solvating agents, chiral-lanthanide shift reagents, etc.)[15] fail or give rather poor analytical results. In this technique, the spectroscopic enantiodiscrimination principle is based on the fact that two enantiomers embedded in a chiral oriented solvent are not ordered in the same way, thus yielding the doubling of spectra (one for each enantiomer).[13] NAD NMR offers the major advantage that neither chemical modification nor isotopic labeling of solutes to be studied is required, while all deuterated sites are simultaneously probed, thus increasing the possibility to observe a chiral differentiation. Moreover, previous work has shown that despite the low natural abundance of deuterium nuclei (0.015%), routine spectrometers (400 MHz) were able to record overnight 1D or 2D NAD spectra with sufficient signal-to-noise (S/N) ratio to afford workable analytical information for (bio)chemists.[14]

The analysis of $^2H-\{^1H\}$ spectra acquired at natural abundance level in the PBLG phase is rather simple. Indeed, as a result of the absence of $^2H-^2H$ couplings, the spectra consist of the superposition of independent quadrupolar doublets (nuclei with spin $I = 1$) corresponding to all nonequivalent isotopomers in the mixture.[14,15] The separation between two components, centered on $\delta_{^2H}^{aniso}$ ($\approx \delta_{^1H}^{iso}$), is referred to as the quadrupolar splitting, denoted $\Delta\nu_Q$. When the discrimination between enantiomers occurs, we observe generally two doublets ($\Delta\nu_Q^S$ and $\Delta\nu_Q^R$) centered at the same frequency, one for each enantiomer.[13,14]

Figure 1a shows the 61.4-MHz natural abundance $^2H-\{^1H\}$ signal of the methyl group 1 of **4** (enantioselective route) dissolved in the PBLG/$CHCl_3$ system at 300 K (see numbering in Scheme 2).[16] This NAD spectrum (in fact a sum of columns) was extracted from a 2D autocorrelation experiment named *Q*-COSY, which has been specially designed to facilitate the analysis of overcrowded NAD spectra,[14] but in this example the analysis of the 1D NAD spectrum would be possible without the help of a 2D experiment. The NAD signals of the methyl group consist of one intense quadrupolar doublet ascribed to the majority isomer, *R*-(+)-**4**, while the minority isomer exhibits a

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(16) **Sample Preparation and NMR.** The CLC NMR samples of *R*-(+)-4 and (±)-4 were made from 100 mg of a chiral material, 100 mg of PBLG (MW ≈ 120 000), and 350 mg of dry $CHCl_3$. Hereafter we will use the notation: amount (in mg) of "solute/polymer (DP)/cosolvent". PBLG is available from Sigma. The sample preparation is described in ref 13a. NMR experiments were performed on a DRX-400 (using a 5 mm selective probe for ^2H and BBI probe for ^{13}C). Broad-band ^1H decoupling was applied using the WALTZ sequence. All 2D *Q*-COSY spectra were zerofilled to 1 k (t_1) × 2 k (t_2) data points prior to the double FT. Exponential filtering in both dimensions ($LB_{1,2} = 1$ Hz) was applied.

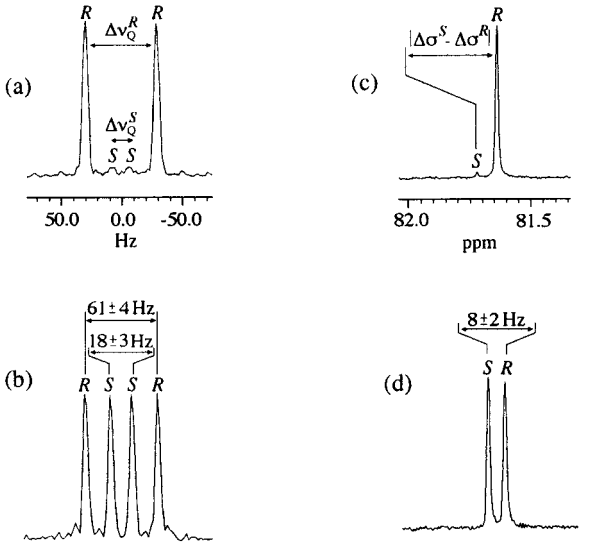

Figure 1. (a) Natural abundance $^2H-\{^1H\}$ signals associated with deuterons of the methyl group 1 and (c) $^{13}C-\{^1H\}$ signals of C-4 recorded for *R*-(+)-**4**. The 2D *Q*-COSY spectrum (magnitude mode) was acquired in 15 h, using 279 (t_1) × 1760 (t_2) data points with 400 scans per t_1 increments. The recycle delay (relaxation + acquisition) was ~0.6 s. The $^{13}C-\{^1H\}$ spectrum was recorded in 2 h by adding 2500 scans of 32 k data points and zerofilled to 64 k points to increase the digital resolution. The interferogram was acquired using a pulse angle of ~60° and a recycle delay of ~3 s. ^1H decoupling was applied during the totality of the experiment in order to benefit from the nuclear Overhauser effect. No digital filtering was applied. (b and d) Same experimental conditions as in a and c, but using (±)-**4**.

quadrupolar doublet with very low intensity. To validate this analysis, we have synthesized compound **4** using a racemic route and then recorded the 2D NAD *Q*-COSY spectrum of (±)-**4** with the same experimental conditions (NMR sample and spectroscopic parameters). In contrast with the NAD spectrum of *R*-(+)-**4**, we observe two intense doublets of same area, indicating that the ee is null as expected for a racemic mixture (see Figure 1b). Note that other chiral deuterated isotopomers show a chiral discrimination, but the methyl site is interesting for the ee measurement. Indeed it possesses three magnetically equivalent deuterons that contribute to the doublet intensity (optimal S/N ratio), and in this example it exhibits a large difference of quadrupolar splittings, allowing one to easily integrate the signal. The ee value for *R*-(+)-**4** calculated by simple peak integration of methyl group signals (averaged value on a series of integrations) or by a deconvolution process is 95 ± 5%.

One could argue that the accuracy of the ee is rather low as a result of the extremely weak deuterium sensitivity at natural abundance level (1.45×10^{-6} with respect to ^1H). To get clarification on this important point, proton-decoupled carbon-13 ($^{13}C-\{^1H\}$) spectra for *R*-(+)-**4** and (±)-**4** in the PBLG system were recorded.[15,20] In $^{13}C-\{^1H\}$ NMR, the dominant order-dependent interaction is the ^{13}C chemical shift anisotropy (CSA) denoted $\Delta\sigma$. ^{13}C CSA is particularly large for doubly and triply bonded and aromatic carbons. This is because the parameters governing the strength of the ^{13}C CSA mainly increase with the electronegativity of the substituents as well as the hybridization state of the carbons ($|\Delta\sigma(sp)| > |\Delta\sigma(sp^2)| > |\Delta\sigma(sp^3)|$).[13] In practice the spectroscopic enantiodiscrimination for a given carbon, *i*, occurs when two independent resonances separated by the quantity $|\Delta\sigma_i^S - \Delta\sigma_i^R|$ are observed.

Figure 1c and d shows the 100.6-MHz $^{13}C-\{^1H\}$ signals of the acetylenic carbon (4) for *R*-(+)-**4** and (±)-**4**, respectively. For this carbon, the difference of CSA causes a chemical shift difference between the two enantiomers of 0.08 ppm (~8 Hz). Note that the *R*-(+)-**4** isomer exhibits the most shielded signal, but this result is not general, because there is no straightforward correlation between the absolute configuration of enantiomers and their respective ^{13}C chemical shifts. Spectral enantioseparations of 4 and 8 Hz were also observed on C-1 and C-3 carbons, respectively (not shown). As previously, the large difference in peak areas for the enriched mixture of **4** indicates a strong ee, while no difference was measured for (±)-**4** within the experimental accuracy. This enables a simple and easy measurement of the ee. Here again the ee value of the enriched mixture obtained by simple peak integration of $^{13}C-\{^1H\}$ signals (averaged value on a series of integrations) or deconvolution is 95 ± 3%. The smaller error compared with the value calculated from the NAD spectrum is due to a better S/N ratio on the $^{13}C-\{^1H\}$ spectrum. This result confirms the value determined using NAD NMR and therefore validates the measurements performed by this technique.

Although already high, we have attempted to improve the enantioselectivity of the asymmetric reduction. Indeed, by carefully controlling the experimental conditions (ligand purity, degazing process, etc.), only the signal of the enriched enantiomer was spectroscopically detected using NAD NMR in PBLG/$CHCl_3$ (not shown). Results obtained by $^{13}C-\{^1H\}$ NMR show a very weak signal for the minority *S*-(−)-isomer that does not clearly emerge from noise. Both experimental results imply therefore an ee ≥ 97%. *R*-(+)-**4** was then submitted to an alkyne zipper reaction[17] to give *R*-(−)-**1** in a 68% yield (Scheme 2). Although the alkyne zipper reaction has been previously described as a racemization-free process,[17b] we were keen to check it out and test again the potential of NMR in CLC.

In contrast with compound **4**, the $^{13}C-\{^1H\}$ NMR for **1** gave poor spectral enantiodifferentiation ($|\Delta\sigma_i^S - \Delta\sigma_i^R| <$ 2 Hz) on all carbons of the molecule. This lack of separation indicates that the differential ordering effect (DOE) is too small to be clearly detected through a difference of ^{13}C CSA (at least at 100 MHz); this also includes the acetylenic carbons C-6 and C-7. This situation probably arises because the triple bond is now separated from the stererogenic center by a chain of four σ bonds exhibiting complex conformational dynamics. Even if $^{13}C-\{^1H\}$ NMR is certainly superior to the NAD NMR in terms of sensitivity and experimental time, this tool is not convenient to determinate

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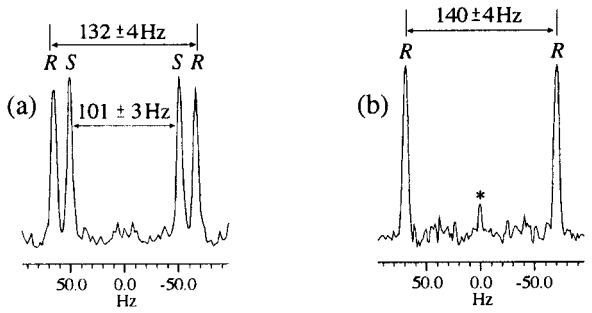

Figure 2. 61.4-MHz natural abundance $^2H-\{^1H\}$ signals associated with one of the diastereotopic deuterons of the methylene 3 for (±)-**1** (a) and *R*-(−)-**1**. (b) Dissolved in the PBLG/$CHCl_3$ system at 300 K. Both 2D *Q*-COSY spectra (magnitude mode) were recorded in 14 h using identical experimental conditions (211 (t_1) × 1600 (t_2) data points, 320 scans per t_1 increments, recycle delay = 0.6 s). The small central peak (labeled by an asterisk) is an artifact.

the ee of **1**, and consequently NAD NMR appears to be a very interesting alternative in this case.

Figure 2a and b reports the natural abundance $^2H-\{^1H\}$ signal associated with one of the deuterons of the methylene group 3 for (±)-**1** and *R*-(−)-**1**, respectively.[18] The assignment of this methylene was based on the analysis of ^1H COSY and HMQC 2D spectra, safely assuming that the $\delta_{^2H}^{aniso}$ is very close to $\delta_{^1H}^{iso}$. We first discuss the left trace (a) in the Figure 2 ((±)-**1**). Although various sites show a discrimination, we have focused our analysis on this methylene because it offers a large spectral enantiodifferentiation ($|\Delta\nu_Q^S - \Delta\nu_Q^R| \approx 30$ Hz) for one of its diastereotopic deuteron. Even if a single deuteron participates in the signal's intensity, this situation remains more attractive compared to that provided, for instance, by the methyl group's deuterons, in which case $|\Delta\nu_Q^S - \Delta\nu_Q^R| < 7$ Hz. The two deuterons in a methylene group are nonequivalent in chiral molecules. This causes in principle two pairs of two quadrupolar splittings (one for each enantiomer when the chiral discrimination occurs) centered on two distincts chemical shifts. In this example, the difference of $\delta_{^2H}^{aniso}$ between the two diastereotopic deuterons is around 0.1 ppm (~6 Hz). Although this difference is small, the *Q*-COSY spectrum allows the selection, using adequate slices of the 2D data set, of one of the diasterotopic deuterons. Figure 2a and b presents only slices for the deuteron of the methylene 3 exhibiting a differentiation, the other one showing no discrimination. This situation perfectly reflects the versatility of chiral discrimination in CLC when two distinct chiral isotopomers are considered. It also proves the undeniable advantage of the NAD NMR that probes simultaneously all possible deuterated sites of the chiral molecule.

In the NAD spectrum reported in Figure 2b, a single quadrupolar doublet is now visible, implying a large ee. As observed for compound **4**, the outer quadrupolar doublet corresponds to the *R*-(−)-**1** isomer. This fact is, however, fortuitous, because to date there is no simple correlation between the *R*/*S* (or ±) descriptors, the type of molecules studied, and the magnitude of quadrupolar splittings.[19] Considering the results obtained with the derivative **4**, the absence of signal for the *S*-(+)-isomer enables an estimate that the ee is > 97 %. This result is, therefore, fully consistent with that published by Midland et al., who described a racemization-free process for the alkyne zipper reaction.[17b]

This second example illustrates that the NAD NMR in PBLG can be considered as a highly valuable tool in the field of the enantiomeric analysis when other methods fail. The strong potential of this approach is mainly due to the large sensitivity of ^2H quadrupolar interaction to DOEs, compared to other anisotropic interactions (dipolar coupling or CSA). Thus even though the DOE between the *S* and *R* isomers is weak, their residual quadrupolar splittings may be sufficiently different to exhibit a spectroscopic enantioseparation. When an unsuccessful result is obtained using NAD NMR, the sample temperature, the composition of the mixture, the organic solvent, or the polypeptide can be changed.[13]

In summary, we have described an efficient asymmetric and multigram access to enantiomerically pure chiral building block **1**. Moreover, this study has clearly illustrated that NAD NMR in CLC can be routinely used, because it provides an efficient and practical solution for the enantiomeric analysis of chiral building blocks in which the control of the stereogenic carbons is crucial. Results reported here demonstrate also the outstanding capacity of the NMR in weakly oriented chiral medium in this respect, mainly due to the important flexibility of this tool compared with other isotropic NMR methods. The amount of solutes used in this work (100 mg) for NAD experiments can appear discouraging. That is why, using routine spectrometers (9.4 T), this tool is highly adapted for the analysis of chiral building blocks (MW ≈ 150), when a large amount of compound is still available. No doubt the use of higher field NMR spectrometers and/or cryogenic probe systems will overcome this disadvantage and greatly improve the efficiency of this technique for analyzing larger solutes. Finally, note that the enantiomers can always be extracted from the NMR sample.

Supporting Information Available: Experimental procedures for the synthesis of compounds **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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