Natural Abundance Deuterium NMR Spectroscopy in Polypeptide Liquid Crystals as a New and Incisive Means for the Enantiodifferentiation of Chiral Hydrocarbons

Philippe Lesot,* Muriel Sarfati, and Jacques Courtieu[a]

Abstract: Polymeric chiral liquid-crystalline solvents based on homopolypeptides are of interest with the view to discriminate between enantiomeric pairs of chiral hydrocarbons using proton-decoupled deuterium one- and two-dimensional NMR spectroscopy at natural abundance level. This method offers the major advantage that neither chemical modification nor isotopic labelling of the solutes to be studied is required. Chiral differentiation between optical isomers is observed through a difference in residual deuterium quadrupolar splittings. The spectroscopic separations and the S/N ratio from the spectra are usually large enough to measure the enantiomeric excess with an accuracy varying between 5 to 10%. This analytical approach is successfully applied to a large collection of chiral, rigid or flexible unsaturated as well as saturated hydrocarbons, including cases of axial chirality, atropoisomerism, and moieties existing as a mixture of enantiomers interconverting by ring inversion. Using the results reported in literature, a systematic comparison with other analytical strategies (NMR, GC, HPLC, VCD) is made and discussed. Also, a tentative proposal to rationalise the various results in terms of chiral differentiation and enantioselective shape recognition is presented. We show that this original tool provides an attractive and incisive alternative to the existing analytical techniques for studying nonfunctionalised chiral materials.

Keywords: deuterium · enantioselectivity · hydrocarbons · liquid crystals · NMR spectroscopy

Introduction

In the field of chiral analysis, NMR analytical strategies were found to have some practical advantages over HPLC or GC methods. Shorter experimental times in particular are an advantage, as NMR does not need any long conditioning or calibration procedures. In addition, chiral columns can prove to be rather expensive as well as very compound specific. However, in spite of the widespread development of isotropic NMR techniques for stereochemical characterisations and enantiomeric-purity determinations, there is so far no universal, attractive NMR methodology which can be successfully applied to all classes of organic molecules, namely polar and nonfunctionalised compounds, as well as rigid or highly flexible structures with large or small molecular shape anisotropy.[1, 2] Consequently, the development of direct, accurate and rather inexpensive NMR alternatives, accessible to almost all research groups, is a substantial task, and numerous efforts are continuously devoted to this aim.[3]

[a] Dr. P. Lesot, M. Sarfati, Prof. Dr. J. Courtieu
Laboratoire de Chimie Structurale Organique
Université de Paris-Sud, Bât. 410
ICMMO, UMR 8074, 91405 Orsay cedex (France)
Fax: (+33) 1 69 15 47 70
E-mail: philesot@icmo.u-psud.fr

Classical NMR methods used to differentiate enantiomers necessitate chiral auxiliaries, either a chiral derivatising agent (CDA), a lanthanide chiral shift reagent (LCSR) or a chiral solvating agent (CSA).[1, 2] The lack of generality of these techniques arises from the fact that only functionalised chiral molecules can interact with these chiral auxiliaries to convert enantiomers either to stable diastereomers or to distinguishable diastereomeric adducts. As a direct consequence, the unsaturated and saturated chiral hydrocarbons, which do not possess any polar groups, are not good candidates for the common means used in laboratories. This situation is a major disadvantage for simple and rapid investigations in asymmetric synthesis involving chiral hydrocarbons such as asymmetric hydrogenation of olefinic compounds, allenic compounds, or asymmetric enzymatic chemical transformations such as kinetic resolution of alkanes for instance.

Some advanced NMR analytical tools in isotropic phases have been proposed for analysing chiral hydrocarbons, and sporadic reports can be found in the literature.[4–28] However, these different approaches were often considered, sometimes unfairly, as rather exotic or too sophisticated to be used routinely by organic (bio)chemists. Among them are NMR techniques using enantiopure fluorinated compounds (2,2,2-trifluoro-1-(9-anthryl)ethanol) as CSA,[4] NMR methods using either binuclear complexes obtained by the combination

  0947-6539/03/0908-1724 $ 20.00+.50/0

of achiral silver salt and optically active lanthanide (L = Yb, Eu, Pr) complexes ([Ag(fod)]/[(+)-L(hfbc)$_3$]) as CSR,[5–8] organometallic CDAs involving *trans*-chlorinated-[chiral amine] platinium(II) complexes (Am*-Pt^{II}(Cl$_n$)),[9–11] or C_2-symmetric palladium(0) or platinum(0) complexes ((diop)-M^0).[12, 13] More recently, NMR approaches using dirhodium complexes [Rh$_2$(mtpa)$_4$][14] as well as C_2-symmetric rhodium complexes [Rh(nbd)[-bdpp]ClO$_4$][15] as CSRs have been described. Although these spectroscopic strategies have been successfully realised for specifically discriminating some chiral allenic, olefinic or aromatic hydrocarbons, they do not always guarantee resonance separations large enough for a convenient enantiomeric purity determination. Besides, none of them provides a general tool for efficiently analysing any chiral hydrocarbons.

Other NMR methodologies, based on the use of chiral torus-shaped macromolecules such as α,β,γ-cyclodextrins (abbreviated α,β,γ-CDs) or chemically modified homologues in solutions as hosts, have received much attention over recent years for studying the discrimination of chiral organic guests. These methods, which involve cyclic oligosaccharides as chiral solvating agent, have provided some successful practical applications for chiral hydrocarbons.[16–18] Due to their relatively hydrophobic interiors, CDs have the ability to form inclusion complexes with substrates of different chemical structures, including apolar molecules. The key point for enantioselective recognition within CDs is their ability to accommodate enantiomers of varying polarity into the apolar cavities by virtue of attractive hydrophobic interactions, and then to produce short-lived diastereomeric solvates having anisochronous NMR resonances through differential shielding effects. Among the reported results, the successful spectroscopic differentiation of some chiral bicycloalkenes,[19, 20] chiral trisubstituted allenes[21–24] or chiral aromatic hydrocarbons[23–25] have been described.

Although interesting and original, the NMR approaches mentioned above yielded very limited results for chiral alkanes, even if the NMR enantioseparation at low temperature of one example of chiral bicycloalkane, *cis*-decaline, complexed with β-CD, is noteworthy.[26] To the best of our knowledge, all these strategies have failed to solve the problem of the discrimination between enantiomeric pairs of branched acyclic alkanes. Two reasons may explain this situation. Firstly, since enantiomeric alkanes have no functional groups, it is not possible to derivatise them or to form distinguishable diastereomeric complexes as in the case of chiral olefins. Secondly, it seems that the lack of polar interactions as well as a strong molecular shape anisotropy results in small energetic and structural differences in alkane-cyclodextrin complexes, so that anisochronous NMR resonances are poorly resolved. It is clear that this class of compound lacks molecular features able to undergo "intensive" diastereoisomeric interaction with CDs to be discernible by NMR, even though the enantioresolution of some linear chiral alkanes by chiral GC using CD as chiral stationary phase (CSP) has been proved.[27, 28] Actually this failure also emphasises that the observable NMR effect used in isotropic solvents for differentiating between diastereoisomeric complexes, namely a variation of chemical shifts induced by differential shielding effects, is certainly not sensitive enough to clearly separate the NMR signals of chiral acyclic alkanes in CDs. If this lack of sensitivity cannot be overcome using the highest accessible magnetic fields, other NMR analytical strategies have to be designed, keeping in mind that the differentiation between two enantiomers requires the need of another chiral species.

The alternative strategy we propose consists of using a chiral ordering agent (COA) as a chiral selector. COAs are mainly chiral liquid crystals (CLCs), such as the organic solutions of chiral polypeptidic polymers as discussed below.[29, 30] Approaches involving COA are based on three major features. Firstly, NMR spectra in (weakly) oriented media are affected not only by the usual chemical shifts and scalar couplings, but also by anisotropic interactions such as the chemical shift anisotropy, ($\Delta\sigma_i$), the dipolar coupling (D_{ij}) and for spin $I > \frac{1}{2}$ nuclei, and the quadrupolar splitting ($\Delta\nu_{Qi}$).[29, 31] These order-dependent terms, which are averaged to zero in the liquid state, provide new and powerful observable NMR parameters as far as the spectral enantiodiscrimination is concerned.[29] Secondly, the intermolecular enantioselective interactions between the chiral solute and the chiral phase sufficiently affect the ordering of the enantiomers to produce spectroscopic separation. In other words, NMR enantiodiscrimination in CLCs reflects a selective ordering of the enantiomers, and it does not necessitate specific chiral short-range strong interactions that are able to affect the electronic shielding of enantiomers differently, as in isotropic NMR.[32] Thirdly, the method is quantitative and enantiomeric composition of a chiral mixture can be assessed by peak integration.[33]

The first work describing how chiral liquid-crystalline solvents can be used in this way was reported by Sackmann et al.[34] A long period followed when this approach was rejected by (bio)chemists, primarily because the liquid-crystalline phases employed were not good solvents for a wide range of compounds.[35] Unexpectedly, organic solutions of synthetic homopolypeptides, such as poly-γ-benzyl-L-glutamate (PBLG) or poly-ε-carbobenzyloxy-L-lysine (PCBLL) have shown an amazing chiral discrimination power when used as the chiral selector, as well as a large ability to dissolve organic molecules.[29, 30] Thus it was found that differences in enantioselective interactions between the *S* and *R* isomers and the polypeptide helices were sufficiently large to provide chiral recognition for a large variety of functionalised molecules using either deuterium, carbon-13, or fluorine-19 NMR spectroscopy.[29]

It is obvious, however, that the choice of the nuclei to observe in a chiral molecule, and the associated anisotropic observable parameters, are crucial since all anisotropic interactions do not exhibit the same sensitivity towards the differential ordering effect (DOE) of enantiomers. This should be of special importance when investigating non-functionalised enantiomers, as we assume a priori that the enantioselective interactions between the polypeptide helices and such solutes should be rather weak, thereby leading to small DOE values. This is probably the reason why we reported in an earlier study that the proton-decoupled carbon-13 (^{13}C-{^{1}H}) NMR in PBLG failed in differentiating

  0947-6539/03/0908-1725 $ 20.00+.50/0

chiral hydrocarbons such as (±)-α-pinene, (±)-limonene and (±)-3-methylpentene on the basis of chemical shift anisotropy differences.[36] A rough estimation of the sensitivity of the various ^{1}H, ^{2}H, ^{13}C or ^{19}F anisotropic interactions towards the DOE indicates clearly that $|\Delta\nu_{Q_i}| > |D_{ij}| > |\Delta\sigma_i|$.[29] Consequently the initial investigations focused on chiral discrimination using proton-decoupled deuterium NMR in PBLG using monodeuterated solutes.[37–39] The large sensitivity of the deuterium quadrupolar interaction allowed for the visualization of various families of chiral functionalised molecules. Among them, enantiomers of polar molecules that are chiral by virtue of isotopic substitution were successfully differentiated in PBLG.[40] More interestingly was the discrimination, by $^{2}H\{^{1}H\}$ NMR in PBLG, between the two enantiomers of (±)-4-deuterobicyclo[3.2.1]oct-2-ene, for which the deuterium probe was stereoselectively introduced into the *endo*-position.[39] Indeed this first example of spectral enantiodistinction of a bicycloalkene suggested fruitful prospects for examining other chiral hydrocarbons.

Although many synthetic strategies can be found in the literature, the selective introduction of deuterium nuclei into chiral molecules, without racemisation, is neither always possible nor easy to do. In particular the site-specific isotopic labelling of chiral nonfunctionalised hydrocarbons, and the purification procedures, can be very tedious. As this step may present a serious limitation, we recently turned our attention towards the possibility of recording high-resolution natural abundance deuterium (NAD) NMR spectra of solutes in the polypeptidic oriented phases. We have demonstrated that NAD NMR spectra of chiral functionalised compounds can be recorded with satisfying quality, using routine NMR equipment (on a 400 MHz and even a 250 MHz spectrometer!), within a reasonable experimental time (overnight) and 80–100 mg of a racemic mixture.[41, 42] Even though the level of natural abundance (0.015%) and the NMR sensitivity of deuterium are extremely low compared with ^{1}H, the main advantage of NAD NMR is its ability to provide rather simple spectra (no $^{2}H-^{2}H$ couplings). Moreover, the method simultaneously probes the signals of all possible isotopomers of a molecule. This latter occurrence strongly increases the probability of distinguishing between enantiomers compared to selectively labelled chiral material. In this context, it became possible to investigate the enantioselectivity of PBLG or PCBLL towards a large variety of chiral apolar solutes without any chemical transformation of the molecules to be studied.

In this article, we present an extended study of the visualisation of chiral compounds devoid of polar functional groups in the PBLG system using $^{2}H-\{^{1}H\}$ NMR spectroscopy at natural abundance level. In Sections 2 and 3 NAD NMR spectroscopy in liquid crystals is introduced. In Section 4 we investigate a collection of twenty apolar hydrocarbons such as bicyclic and acyclic alkenes, alkanes, alkynes, aromatic compounds, including the cases of chiral molecules with no stereogenic carbon. The experimental results are discussed and compared with those obtained by other analytical methods when data were found in the literature. The goal of these discussions is to convince chemists that this NMR method in CLCs provides substantial prospects in the field of chirality. Besides this, in Section 5, the significance of the results will be briefly discussed. We will attempt to rationalize some results in terms of chiral differentiation and shape recognition in order to propose some qualitative insights of the dominant factors responsible for enantiodifferentiation of hydrocarbons in these chiral mesophases.

Background—Enantiodiscrimination of Hydrocarbons Using the NAD NMR in PBLG Systems

Oriented organic solutions of a polypeptide in a magnetic field: Organic solutions of an homopolypeptide are prepared by dissolving the appropriate amount of polymer (PBLG or PCBLL) in various neat organic solvents or mixtures of solvents. Both synthetic polymers are commercially available from Sigma. The most useful organic solvents are $CHCl_3$, CH_2Cl_2, THF, dioxane and DMF, the latter being a good aprotic polar solvent.[29] Due to the large number of possible solvents, almost all polar and apolar chiral compounds may be dissolved in such mixtures.

When dissolved in these helicogenic solvents, the polypeptidic chain adopts a rigid α-helical conformation,[43] while the side chains, branched from the main helix, are appreciably extended in the transverse direction to the helix, thus forming a rather mobile, secondary helix.[44–47] Within certain concentration ranges these organic solutions of α-helical rods form stable cholesteric phases. When placed in the probe of the NMR instrument, the strong static magnetic field unwinds the cholesteric supramolecular helix, thus yielding a chiral nematic phase with director, n, homogeneous orientated parallel to B_0.

Optimisation of this ternary mixture, chiral material/polymer/organic solvent, indicated that the best NMR results are usually obtained for samples prepared with a concentration of polymer varying between 12 to 30% by weight depending on the organic solvent, with a degree of polymerisation (DP) in the range of 350–600. Polypeptidic polymers with higher DPs may also be used but NMR linewidths begin to increase due to the sample viscosity. When the polymer concentration is below 10%, the samples are generally polyphasic. Apart from some molecules that are able to precipitate the polymer fibres, the addition of as much as 80 to 200 mg of a compound to be studied to a 100 mg of polymer dissolved in 350–500 mg of organic solvent mixture does not disrupt the liquid-crystalline properties of the sample. The possibility to use large amounts of solute is an important advantage for NAD NMR applications because we can investigate compounds with a molecular weight up to 200 $g\,mol^{-1}$, and hence partially overcome the low natural abundance of deuterium. The PBLG or PCBLL systems usually provide a very homogeneous anisotropic mesophase, with a low to moderate viscosity at room temperature. This depends not only on the organic solvent, but also on the size, the chemical type and the amount of solute added as well. The lower the viscosity of the sample, the better the resolution and S/N ratio. It should be noted that the viscosity of PBLG phases in the presence of hydrocarbons is generally higher for

0947-6539/03/0908-1726 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

a given organic co-solvent at room temperature than that found with functionalised analogues. This situation may arise from a restricted mobility of PBLG side chains due to the tendency of PBLG fibres to aggregate in some solvents.[48] The increase of the sample temperature or the use of trifluoroacetic acid (TFA) can reduce this effect.[48] Last but not least in NAD, the solid-like NMR spectrum of PBLG does not interfere with that of the solutes. Consequently it is not necessary to use "cosmetics" to suppress the lines due to PBLG molecules.

Proton-decoupled deuterium NMR in oriented media: In deuterium NMR in liquid-crystalline media, the partially averaged magnetic interactions are dominated by the quadrupolar couplings. Consequently we obtain simple spectra that make deuterium NMR very useful in this respect.[31] The ^{2}H{^{1}H} NMR spectrum of two monodeuterated enantiomers oriented differently in a chiral ordered environment consists of two independent quadrupolar doublets, one for each stereoisomer. Since ^{2}H chemical shift anisotropy is negligible in all chemical situations, the doublets are usually centered on the same chemical shift. The separation between the two lines is referred to as the quadrupolar splitting. Expressed in Hertz it is equal to:[29, 31]

$$\Delta\nu_{Q_i}^{S\,\text{or}\,R} = \tfrac{3}{2}\left(\frac{e^2 Q_{D_i} q_{C-D_i}}{h}\right) S_{C-D_i}^{S\,\text{or}\,R} \qquad (1)$$

The ratio $e^2Q_{D_i}q_{C-D_i}/h$, noted hereafter K_{C-D_i}, is the deuterium quadrupolar coupling constant (QCC). K_{C-D_i} is a priori the same for two enantiomers, but varies from one deuterium atom to another depending on the hybridization state of the bonded carbon atom. It is approximately equal to 170 ± 5 kHz, 185 ± 5 kHz and 210 ± 5 kHz, for sp^3, sp^2 and sp carbon atoms, respectively.[31] $S_{C-D_i}^{S\,\text{or}\,R}$ is the order parameter of the C–D$_i$ bond for the *S* or *R* enantiomers relative to the magnetic field axis. Here it is assumed that the electric field gradient (EFG) tensor is axially symmetric along the C–D bond.[49] Equation (1) indicates clearly that chiral discrimination is only detected when $|\Delta\nu_{Q_i}^S - \Delta\nu_{Q_i}^R| \neq 0$. The relatively large magnitude of K_{C-D} for deuterium nuclei implies that even though the difference of orientation between the *R* and *S* isomers is small, their residual quadrupolar splittings may be sufficiently different to provide spectral separation of enantiomer's signals. A chiral discrimination is observed when $|\Delta\nu_{Q_i}^S| \neq |\Delta\nu_{Q_i}^R|$ even if one of the splittings is averaged to zero (Figure 1e). This latter situation, sometime encountered experimentally, is obtained when the average orientation of a C–D internuclear axis lies fortuitously along the magic angle direction, for which the order parameter $S_{C-D_i} = 0$.[29, 31]

To quantitatively analyse the "differential ordering effect" associated with given C–D directions in both enantiomers, and get better insight on the phenomenon, we define the DOE factor as:

$$|\,\text{DOE}_i\,| = 2 \times \frac{|\Delta\nu_{Q_i}^{\text{larger}}| - |\Delta\nu_{Q_i}^{\text{smaller}}|}{|\Delta\nu_{Q_i}^{\text{larger}}| + |\Delta\nu_{Q_i}^{\text{smaller}}|} \qquad (2)$$

The stereochemical descriptors *R* and *S* are replaced by the superscripts "larger" and "smaller" because, to date, there is no correlation between the amplitude of the splittings, $\Delta\nu_{Q_i}$, and the absolute configuration of the enantiomers. The use of absolute values in Equation (2) is also due to the lack of information about the sign of $\Delta\nu_{Q_i}$. The reason for Equation (2) is that there is no (known) correlation between molecular orientation and chiral discrimination. As a matter of fact, the magnitude of chiral differentiations, $\Delta(\Delta\nu_{Q_i})$, seems uncorrelated to the magnitude of quadrupolar splittings $\Delta\nu_{Q_i}$. In other words, a large chiral differentiation does not necessarily imply large quadrupolar splittings, and reciprocally. This above DOE factor must be seen as the degree of differential orientation of a C–D bond in enantiomers with respect to the average orientation of the same C–D bond in the achiral oriented phase made of a racemic mixture of PBLG and its enantiomer, PBDG (denoted PBG in the following). This interpretation supposes, however, that the quadrupolar splittings have the same sign. Sign changes are necessary in Equation (2) when the signs of $\Delta\nu_{Q_i}^{\text{larger}}$ and $\Delta\nu_{Q_i}^{\text{smaller}}$ are different. This rather rare latter situation can easily be controlled when comparing spectra obtained of PBLG and PBLG/PBDG in organic solutions or through variable temperature experiments. Note that in PBG mixtures, two enantiomers are diffusing very rapidly, on the NMR time scale, from the vicinity of PBLG and PBDG. Consequently we observe only an average of these situations, thus eliminating the chiral discrimination.[50] Disregarding the simultaneous cancellation of quadrupolar doublets for both enantiomers, Equation (2) indicates that the DOE factor varies from zero (no chiral discrimination) (Figure 1c), to two, corresponding to the spectral situation for which the residual quadrupolar splitting for one of the enantiomers is averaged to zero as shown (Figure 1e). Although insufficient for an

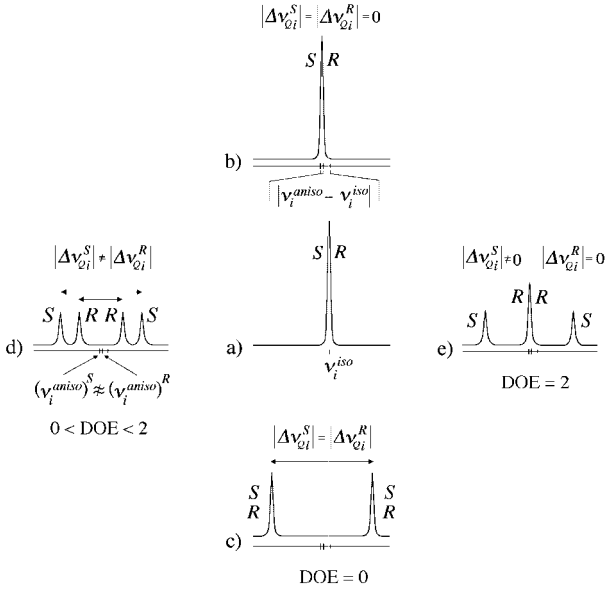


Figure 1. Schematic representation of possible ^{2}H{^{1}H} spectral patterns associated with two monodeuterated enantiomers embedded in a CLC compared to the isotropic spectrum. a) Isotropic spectrum. b)–e) see figure. Only patterns d) and e) show a spectral enantiodiscrimination. The various spectra are plotted to scale, and the assignments *S* and *R* given in all spectral patterns are arbitrary. Due to the weakness of ^{2}H chemical shift anisotropy in all chemical situations, the difference of the chemical shift anisotropies between two enantiomers, $\Delta\Delta\sigma_{^2H} = |\Delta\sigma_{^2H}^S - \Delta\sigma_{^2H}^R|$, is assumed to be too small to produce significant effects on the spectra.

absolute comparison, the DOE factor already provides a quantitative measurement of the differential ordering effect between two given enantomeric C–D directions in the CLC, and hence may be used to rationalize numerous experimental results. Furthermore, it is often necessary to take into account small order variations due to any change in working temperature or concentration when DOE values are to be compared from one sample to an other. For this purpose the $\Delta\nu_Q$ values measured in a sample may be normalised, for instance to the ratio ($\Delta\nu_Q$ of $CDCl_3$ in one sample divided by $\Delta\nu_Q$ of $CDCl_3$ for the sample).

Natural abundance proton-decoupling deuterium 1D and 2D NMR: Due to the very low probability to observe two interacting deuterium atoms in the same isotopomer, the $^2H/^2H$ spin–spin couplings are not detected at natural abundance levels. Consequently, as all the couplings to protons are eliminated through broadband decoupling, the NAD spectra in the PBLG phase are rather simple as they consist of the superposition of independent quadrupolar doublets corresponding to all nonequivalent deuterium atoms in each of the enantiomers.[41, 42] Disregarding any quadrupolar doublet originating from organic solvent, we can expect $2n$ doublets to be visualized in the NAD spectrum for a racemic mixture of chiral hydrocarbons possessing n nonequivalent deuterium atoms, assuming that neither line overlaps nor null quadrupolar splittings occur and that all deuterated chiral isotopomers are discriminated.

We found it convenient to characterise the efficiency of the spectroscopic chiral discrimination in terms of deuterated site numbers differentiated in the molecule. For this purpose we define the ratio of deuterated sites showing a chiral separation of signals over the total number of nonequivalent sites in the molecule. This ratio, hereafter denoted R_c, allows for instance for a simple comparison between homologous derivatives. Between two solutes, the variations in R_c can result from a lack of an enantiorecognition ability of the polypeptide, a lack of spectral resolution or the nonobservation of deuterium signals when quadupolar doublets do not emerge clearly from noise (as we will see in the Discussion Section).

For large chiral molecules, the identification of the two components for each quadrupolar doublet in NAD spectra is, however, not straightforward due to numerous peak overlaps. This situation arises because the largest quadrupolar splittings in PBLG solutions have approximately the same amplitude as the deuterium chemical shift dispersion at 9.4 T. Consequently, the NAD 1D-NMR spectra in PBLG do not exhibit an approximately symmetrical aspect as in strongly ordered liquid crystals (for which the correlation between two components is usually trivial). Nevertheless, this can be seen as a practical advantage because it enables the assignment of almost all quadrupolar splittings on the basis of chemical shifts. To facilitate the analysis of overcrowded NAD spectra by establishing the correlation between components of each deuterium doublet, we have developed several proton-decoupled deuterium 2D NMR experiments referred to as QUOSY (for QUadrupole Ordered SpectroscopY). Among them, the 2D autocorrelation experiment named Q-COSY was found to be the most suitable and useful 2D sequence for NAD NMR in terms of signal sensitivity.[42] The role of Q-COSY 2D experiments is to simplify congested NAD 1D spectra (Figure 2).

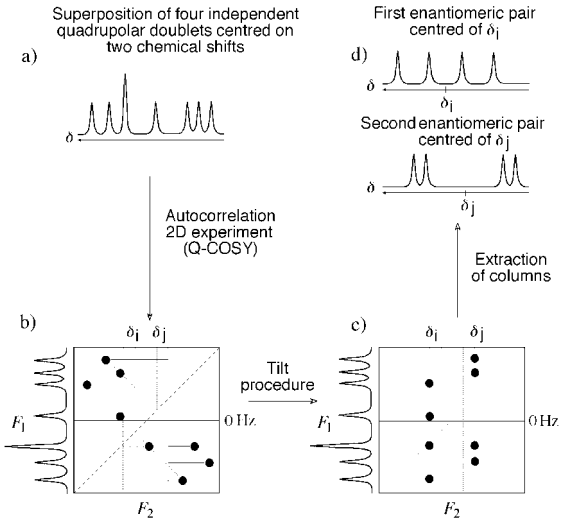


Figure 2. Principle of the spectroscopic separation of independent quadrupolar splittings associated with enantiomeric isotopomers using a NAD 2D Q-COSY experiment. a) NAD 1D spectrum exhibiting the overlap of two quadrupolar components. b) NAD Q-COSY spectrum showing the separation of two pairs of quadrupolar splittings centred on chemical shifts d_i and d_j. c) Tilted Q-COSY spectrum. Note the elimination of quadrupolar splittings in the F_2 dimension and the scale factor (equal to 2) on the chemical shifts. d) Columns extracted from the 2D tilted contour plot and showing separately the two pairs of quadrupolar splittings.

The Q-COSY experiment consists basically of a simple two-pulse sequence ($90^\circ_x - t_1 - 180^\circ_x - t_2$) which presents a formal analogy with some sequences applied for spin $I = ½$, but it cannot be simply compared.[42, 51] In this sequence, the first pulse creates one-quantum coherences that evolve during the t_1 period. The 180° pulse produces a total coherence transfer between the one-quantum coherences that evolve again during the detection period t_2. The effect of nonideal pulses, which generates a degradation of the S/N ratio and undesirable residual on-diagonal peaks in the Q-COSY, have been corrected by replacing each single pulse in the sequence by composite pulses which have a greater tolerance to imperfections.[42, 51] One limitation of the Q-COSY sequence is that the 2D contour plot should be displayed in the magnitude mode to remove the phase-twist line shapes, thus reducing the spectral resolution. It could be argued that 2D NMR experiments giving pure absorption peaks in both dimensions are more valuable to analyse overcrowded spectra. For NAD NMR applications we have however shown that the phase sensitive variant of the Q-COSY sequence, referred to as Q-COSY-ph, was less sensitive by a factor $\sqrt{2}$ than the Q-COSY itself.[42, 51] Consequently, the Q-COSY-*ph* 2D experiments are well adapted for the analysis of *per*deuterated chiral molecules dissolved in polypeptide liquid crystals, but not for NAD applications. As on-diagonal peaks are absent in the Q-COSY 2D contour plot, it is possible and useful to tilt the data as in a *J*-resolved 2D NMR experiment.[52] In this case, all quadrupolar doublets line up parallel to the F_1 axis

0947-6539/03/0908-1728 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

with a scaling factor of 2 on the chemical shift in the F_2 dimension.[42, 51]

This data manipulation produces a deuterium chemical shift spectrum in the F_2 dimension, and allows the separation of the sub-spectra of each isotopomer in the mixture, extracting a column at their chemical shift (Figure 2c). From the various sums of columns extracted from the tilted 2D data set, it is trivial to observe the two components of each quadrupolar doublet and to measure their residual splittings.

Results and Discussion

The twenty compounds investigated in this work were specifically chosen for their structural features and are quoted as compounds **1**–**20**. To organise the discussion, chiral solutes have been divided into five distinct classes of structurally related compounds. For each of these molecular classes, one or two selected examples of NAD spectra are shown in the corresponding figure and are discussed in detail in the text. The first class of structure (Table 1) gathers rigid bicyclic hydrocarbons such as pinene and various isomers. Here the word "rigid" implies that the carbon skeleton of molecules does not possess any rotating C–C bond other than C–CH_3. Besides, we assume that the effects due to molecular vibrations are negligible, in particular their correlation between vibrations and reorientations, are neglected.[53] The second class (Tables 2 and 3) contains functionalized molecules such as aromatic, ethylenic and acetylenic derivatives which possess both an unsaturated functional group and an asymmetric carbon atom in a flexible chain. The third class (Table 4) contains flexible chiral cyclic hydrocarbons such as *trans*-1,2-dimethylcyclohexane. The fourth class (Table 5) contains nonfunctionalized flexible, acyclic molecules such as 3-methylhexane which is the simplest chiral alkane. Lastly, the fifth class of compounds (Table 6) gathers some chiral hydrocarbons without a stereogenic carbon atom, but that exist in enantiomeric forms. The structure of the molecules, the sample composition and temperature, the quadrupolar splitting of the solvent (column 1), the deuterated site evaluated (column 2), the associated chemical shift and quadrupolar splittings for both enantiomers when the assignment was clearly possible (columns 3 and 4), $\Delta\Delta\nu_Q$ (column 5), the DOE factor (column 6); the different values and the various methods (reported in literature) which have already provided successful chiral discrimination (columns 7 and 8) are given in all tables.

For almost all compounds, the assignment of quadrupolar doublets is mainly based on chemical shifts of corresponding protons found in the literature. This is possible because the ^{2}H chemical shifts are very close to those of in proton NMR (in ppm). Although the ^{2}H chemical shift anisotropy is negligible, the δ values reported in the tables can exhibit small discrepancies with respect to literature values obtained in isotropic solvents. These upfield or downfield shift solvent effects seem a direct consequence of the strong polarity of the polymer (ca. 3.5 debye per peptidic residue).[54] When no data were available, the assignment of each doublet was not always trivial to achieve, noticeably in apolar alkanes where ^{2}H chemical shift dispersion is rather small.[41, 42] In these situation, assignments were performed through classical methods in the isotropic state, COSY, HMQC and INADEQUATE, until a clear assignment of ^{13}C and ^{1}H NMR signals could be made. The assignments were confirmed, when necessary, by measuring the proton–carbon dipolar couplings, $^1D_{C-H}$, in the ^{13}C spectra of compounds in PBLG.[29] Indeed, because $S_{C-D} = S_{C-H}$, the $|\Delta\nu_{Q_{C-D}}/D_{C-H}|$ ratio is approximately fixed by the quadrupolar and dipolar constants at ≈ 12. Thus, from the D_{C-H} value measured the magnitude of $|\Delta\nu_{Q_{C-D}}|$ for a given site can be reasonably approximated, and therefore compared with experimental values.

Rigid chiral bicyclic hydrocarbons: Historically, the first successful enantiomeric discrimination of a chiral hydrocarbon using deuterium NMR in PBLG was observed with the (±)-*endo*-4-deuterobicyclo[3.2.1]oct-2-ene, namely a chiral bicycloalkene.[39] As the first examples of using NAD NMR in PBLG, it was therefore pertinent to test various other chiral bicycloalkenes such as α-pinene [(±)-**1**] and regioisomers such as β-pinene and camphene. Chiral monoterpene hydrocarbons are common natural products and are prevalent constituents of essential oils.[55] Some of these are also constituents in certain insect pheromones, and are used as a starting material for a number of enantioselective chemical transformations.[55, 56] In addition, these chiral compounds have been extensively employed as model molecules to investigate the efficiency of numerous analytical methods to differentiate enantiomeric pairs of chiral apolar alkenes. Among the numerous successful methods already used for (±)-**1**, we can cite the chromatographic approaches (GC and HPLC) using cyclodextrins as CSP,[27, 56, 62] NMR using chiral lanthanide/silver binuclear shift reagents,[6] dirhodium complexes,[14] as well as CD derivatives in aqueous solutions as chiral solvating agent.[19, 20, 63] More recently, it was shown that by using vibrational circular dichroism (VCD) it was possible to differentiate between the two enantiomers of (±)-**1**.[64, 65] Figure 3a presents the NAD Q-COSY map of (±)-α-pinene in the PBLG phase recorded at 298 K. Projections show a strongly congested NAD 1D NMR spectrum, which is rather difficult to decipher unambiguously. Such a spectrum perfectly illustrates the usefulness of QUOSY 2D experiments. As described schematically in Figure 2b, only the cross-peaks autocorrelating the two components of each quadrupolar doublet appear on the 2D spectrum, improving the legibility of the 2D spectrum. The tilted spectrum shows that only ^{2}H chemical shifts are observed along the F_2 axis (Figure 3b). Under these conditions the ^{2}H spectrum in this spectral dimension is formally identical with the NAD 1D-NMR spectrum recorded in an isotropic solvent. This situation usually allows the assignment of numerous quadrupolar doublets on the basis of their chemical shifts δ_{2H}. The (±)-α-pinene assignments were based on that published by Martin et al in the context of the well-known SNIF-NMR method.[66] Among the various deuterated sites showing a distinct chiral differentiation, we report in Figure 3c and d the traces of the methyl deuterium atom 8 and the deuterium atom 7′ in the *endo*-position, respectively.

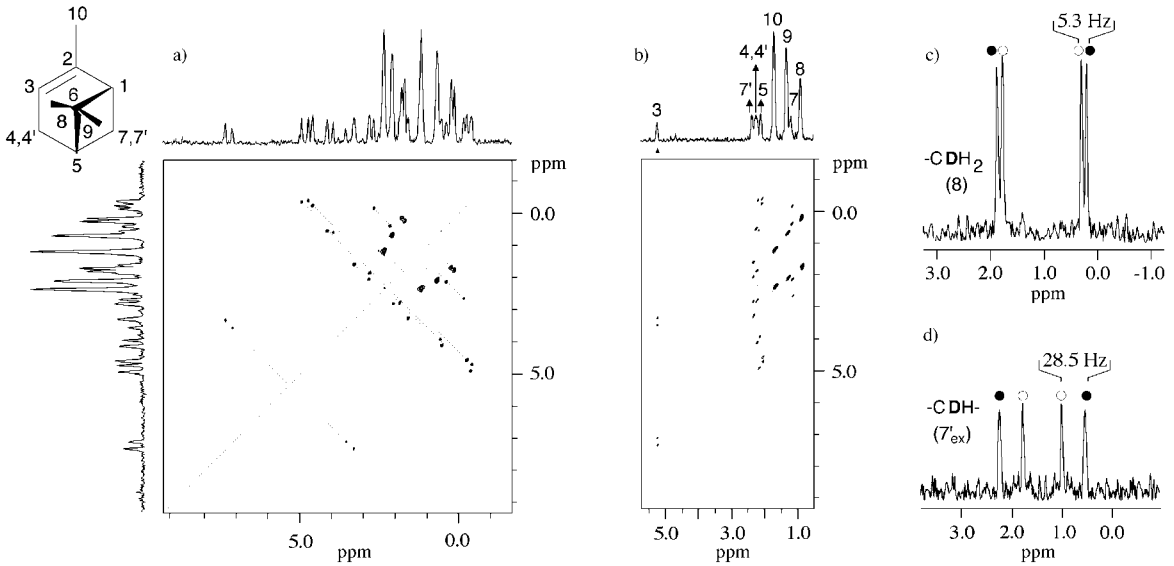


Figure 3. a) NAD Q-COSY spectrum of (±)-*trans*-α-pinene in the PBLG/$CHCl_3$ phase recorded at 298 K. The 2D matrix is 313 (t_1) × 1400 (t_2) data points. No filtering was used. b) 2D contour plot after tilting. Note the cancelling of quadrupolar doublet in the F_2 dimension. (c and d) Columns extracted from the 2D tilted spectrum and showing the spectral enantiodistinction on the methyl group 8 and the *exo* deuterons 7′.

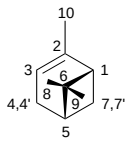
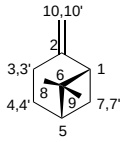
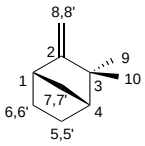
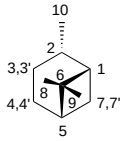
The values of quadrupolar splittings for both enantiomers as well as the corresponding DOE are summarized in Table 1 (entry 1). For this first example, we can visualize that seven out of ten possible couples of chiral isotopomers in the mixture are discriminated, leading to a total of sixteen quadrupolar splittings visible on the 2D contour plot. This spectral situation corresponds to $R_c = 0.70$. The DOE factors at 298 K vary between 0 and 1.16. Thus, the deuterated site 4′ exhibits the largest DOE factor while no chiral discrimination is detected on the methyl deuterium atoms 9 and 10. The range of values perfectly reflects the versatility of chiral discrimination between two chiral isotopomers, and shows the undeniable interest of NAD 2D NMR to simultaneously probe all possible deuterated sites of the molecule. It should be noted that the quadrupolar doublets associated to deuterium atom 1 do not clearly emerge from the noise. Disregarding the fact that a single isotopomer deuterated in position 1 contributes to the NMR signal, and not three as for methyl groups, two reasons can be proffered for explaining this lack of signal. First, the T_1 relaxation time may be unusually large compared with values usually measured in PBLG (typically $T_1 \approx 200$–400 ms). In this case our relatively high recycling rate could produce a significant loss of signal. Relaxation effects could be problematic since the recycling rate used in the 2D experiment may affect the accuracy on quantitative measurements. This argument is actually not valid because, within the experimental errors, all our attempts to give evidence of any difference in the deuterium longitudinal relaxation time between two enantiomers failed.[68] Secondly, the site-specific isotopic ratio (H/D) for the corresponding site can be low because of depletion effects, thus reducing the S/N ratio for this site in the NAD spectrum considerably. In the case of site 1, the isotropic NAD NMR spectrum clearly shows a significant depletion effect relative to the corresponding deuterated isotopomers.[66, 69]

As the enantiomers were commercially available, we prepared an enriched sample in the *R* enantiomer (*ee* 50 %), keeping the concentration (*w*/*w*) of solute, polymer and co-solvent constant (compared to the racemic sample). The difference in peak intensities allowed each pair of quadrupolar splittings to be assigned to a particular enantiomer. The analysis of these data (Table 1) emphasises that there is no direct correlation between the measured quadrupolar splittings and the absolute configuration. In other words, the set of smaller (larger) quadrupolar splittings is not associated with a single enantiomer. This is a consequence of the fact that molecular ordering is a second-rank tensorial property and not a single scalar number.[31]

Further chiral rigid bicycloalkenes we have investigated were (±)-β-pinene [(±)-**2**] and (±)-camphene [(±)-**3**] as regioisomers of (±)-**1**. These molecules have also been extensively employed in assessing the enantioselectivity of CDs grafted as CSP in GC or HPLC.[56–62] The results collected using NAD NMR in PBLG are listed in entries 2 and 3 of Table 1, but their respective NAD spectra are not shown. In case of (±)-**3** and (±)-**4**, the solute–solvent mixture was rather viscous. In order to enhance the fluidity of such samples, we increased the working temperature slightly compared with (±)-**1**. The results obtained for (±)-**1**, (±)-**2** and (±)-**3** (also including the (±)-*endo*-4-deuterobicyclo[3.2.1]oct-2-ene),[39] show good enantioselectivity of the oriented polypeptide helices toward this kind of rigid bicyclic compound. As in the case of solute (±)-**1**, the range of DOE factors is large (from 0 to 1.5) and the R_c factor is high (from 0.66 to 0.75). Such an analysis enables an easy choice of the best site to measure an eventual *ee*. Disregarding the numerical differences in DOE values from one site to another, it can be claimed that the enantiomeric recognition ability of PBLG is not really sensitive to either the position of the double bond, or to that of the *gem*-dimethyl group.

0947-6539/03/0908-1730 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

Table 1. Data for chiral rigid bicyclic hydrocarbon compounds (**1**–**4**).

Entry Structure Sample composition[a] T/ $\lvert \Delta\nu_Q^{solvent} \rvert$ [b]	Site	δ[c] [ppm]	$\lvert \Delta\nu_{Q_i}^{smaller} \rvert / \lvert \Delta\nu_{Q_i}^{larger} \rvert$ [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
1	D1	NO[i]	NO			GC	
	D3	5.26	218.7 (*R*)/247.3	28.6	0.123	CD[j]	[56,57,59,61,63]
	D4,4′	2.19	205.8/325.1 (*R*)	119.3	0.449	MCD[j]	[27,58,60]
		2.24	58.2 (*R*)/220.5	162.3	1.164	HPLC	
	D5	2.09	298.2 (*R*)/315.5	17.3	0.056	CD	[62]
	D7en	1.23	108.3/173.5 (*R*)	65.2	0.462	NMR	
	D7′ex	2.38	46.9 (*R*)/103.9	57.0	0.756	MCD[j]	[19,20]
	D8	0.92	90.1/100.7 (*R*)	10.6	0.111	BNC[j]	[6-8]
51+50/100/350	D9	1.31	87.3	0	0	DC[j]	[14]
298/827	D10	1.70	71.5	0	0	VCD	[64,65]
2	D1	NO				GC	
	D3,3′	1.92	47.3	0	0	CD	[56,57,59,61,63]
		1.39	47.6/67.3	19.7	0.343	MCD	[27,58,60]
	D4,4′	1.73	20.5/175.8	155.3	1.583		
		1.22	230.2/357.3	127.1	0.432	HPLC	
	D5	1.64	222.1/235.4	13.3	0.058	CD	[62]
	$D7_{en}$	1.25	121.8/283.4	161.6	0.798		
	$D7'_{ex}$	1.73	39.6/131.3	91.7	1.073	NMR	
	D8	1.06	80.5/95.9	15.4	0.174	BNC	[7]
	D9	1.09	97.1	0	0		
51+51/100/350	D10,10′	4.52	153.1	0	0		
298/803		4.75	244.1/283.9	39.8	0.151		
3	D1	NO				GC	
	D4	1.91	54.7/73.5	18.8	0.293	CD	[59,61]
	D5,5′	1.40	91.1/142.3	51.2	0.439		
		1.72	153.9/199.5	45.6	0.258	HPLC	
	D6,6′	1.27	182.2	0		CD	[62]
		1.64	211.2/231.3	20.1	0.090		
	D7,7′	1.22	166.1/193.9	27.8	0.154	NMR	
		1.73	35.9/59.8	23.9	0.499	BNC	[6-8]
	D8,8′	4.52	198.1	0	0		
		4.76	11.6/34.9	23.3	1.002		
97/101/353	D9	1.05	16.8/54.2	37.4	1.054		
303/858	D10	1.09	42.1/59.8	17.7	0.347		
4	D1	NO				GC	
	D2	2.11	410.3	0	0	CD	[57]
	D3,3′	1.27	129.9/143.3	13.4	0.098	MCD	[70,71]
		1.71	217.2	0	0		
	D4,4′	1.77	247.2/305.1	60.1	0.209		
		1.82	106.5/164.2	57.7	0.426		
	D5	1.91	126.1/191.0	66.1	0.409		
	$D7_{en}$	1.40	424.4/441.8	17.4	0.040		
	$D7'_{ex}$	2.08	5.5/38.6	33.1	1.501		
	D8	0.90	111.4	0	0		
41+42/100/371	D9	1.28	110.2/119.6	9.4	0.082		
303/858	D10	0.92	128.2	0	0		

[a] Sample composition (x mg of solute/x' mg of polymer/x'' mg of organic solvent). [b] Sample temperature [K] and quadrupolar splitting [Hz] of the C–D bond of the solvent. [c] Deuterium chemical shift measured in the chiral liquid crystal. The solvent signal is used as internal reference. For chloroform, it was calibrated at 7.24 ppm. [d] adrupolar splittings for each enantiomer. [e] Difference of splittings: $\Delta\Delta\nu_{Q_i} = \lvert \Delta\nu_{Q_i}^{larger} \rvert - \lvert \Delta\nu_{Q_i}^{smaller} \rvert$. [f] DOE factor for a given deuterium atom i (see text). [g] Analytical methods (NMR, VCD, HPLC, GC, optical) able to differentiate between enantiomers of the compound and already reported in literature. [h] Numbering of references given in the text. [i] NO: No observation of deuterium signals. The quadrupolar doublets do not clearly emerge from noise. [j] BNC: binuclear complex, CD: (α,β,γ)-native cyclodextrin, DC: dirhodium complex, MCD: chemically modified cyclodextrin, NR: no report found in literature, PC: platinium complex, RC: rhodium complex. [number] Suggested assignment, but may be reversed.

We also recorded the NAD spectrum of (±)-*trans*-α-pinane [(±)-**4**] in the system PBLG at 303 K. The result was very successful since unexpectedly, 50% of deuterated sites (R_c = 0.5) exhibited an enantiomeric spectral differentiation with a maximum DOE of 1.5 for the deuterium atom 7′ in the *exo*-position. This is the first example of the enantiomers of a "rigid" chiral bicyclic alkane being discriminated by using NMR spectroscopy. This result is important because it shows that the intermolecular potential experienced by this bicyclic hydrocarbon when in the chiral environment of PBLG is sufficiently large to produce an efficient DOE, even if the molecule lacks π-electrons. From a more fundamental point

of view, this could indicate that the presence of chemical functions on the solute is not necessary in the global chiral differentiation mechanism occurring in this homopolymer, and that selective topological recognition plays a significant role in the case of chiral rigid solutes. Moreover, we will see below that shape-selective aspects of molecular recognition in a polypeptide are sufficiently large for differentiating between enantiomeric pairs of flexible, chiral saturated hydrocarbons. This result emphasizes that NMR in chiral oriented media is able to overcome the inefficiency of analytical NMR techniques in isotropic media for this type of compounds.[9–12] To our knowledge no previous reports of the chiral differentiation of (±)-**4** using NMR was found in literature. However, a GC enantiomeric separation of (±)-**4**, on permethylated β-CD and on perpenthylated γ-CD was reported in 1989.[57, 70, 71]

Semi-rigid chiral aromatic, acetylenic and ethylenic derivatives: Chiral compounds belonging to this second class can be seen as semi-rigid molecular structures. They are featured by an electron-rich rigid group (aromatic, acetylenic, ethylenic group) and a flexible part containing the chiral center (Tables 2 and 3). Contrary to the previous series of molecules, only a small number of strategies to discriminate the enantiomers of these compounds have been published. NMR using rhodium or platinum complexes gave positive results in case of (±)-2-phenylbutane [(±)-**5**] and (±)-3-methylpentene [(±)-**8**],[9] while NMR methods involving β-CDs as solvating agents exhibited separate resonances for the enantiomers of (±)-**5** and (±)-2-α-naphthylbutane [(±)-**6**].[24, 25] Note that for this latter compound, optical activity measurements were described.[72] No reports were found in the literature for other compounds, even if it was shown that peralkylated β-CDs were suitable for the enantiomeric separations of some chiral dienic derivatives.[73] Note that the lack of experimental results for these specific compounds does not necessarily mean that they cannot be differentiated using the various approaches already referenced, but only that they have not been retained as model molecules to explore their respective analytical efficiency. The NAD NMR results obtained in the PBLG phase are summarized in Tables 2 and 3 (entries 5–10). In all examples investigated, the enantioselectivity of the polypeptide yields separated NAD signals for each enantiomer in numerous deuterated sites. In particular, all chiral isotopomers of compound **5** are differentiated with DOEs varying between 0.01 to 1.32 (Figure 4).

In this example, the ratio R_c is equal to 1, and indicates an optimal situation in terms of enantiomeric discrimination. Results obtained with (±)-**6** and (±)-3-phenyl-but-1-ene [(±)-**7**] also show high discrimination efficiency of PBLG toward these chiral moieties, since the R_c values are equal to 0.66 and 0.75, respectively. Note that the assignment of benzenic signals in **5** and **7** is based on the group-contributing method for protons, while for **6** it is based on reports in literature.[74]

Of particular interest is the discrimination of flexible olefinic enantiomers such as (±)-3-methylpentene [(±)-**8**] and (±)-3-methylhexene [(±)-**9**]. No previous NMR report

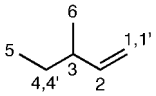
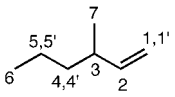
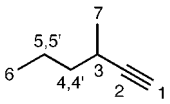
Table 2. Data for chiral semiflexible aromatic compounds (**5**–**7**).

Entry Structure Sample composition[a] T/ | $\Delta\nu_Q^{solvent}$ | [b]	Site	δ[c] [ppm]	| $\Delta\nu_{Q_i}^{smaller}$ | / | $\Delta\nu_{Q_i}^{larger}$ | [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
5	D1	1.46	4.5/21.9	17.4	1.318	NMR	
	D2	2.81	616.9/625.2	8.3	0.013	RC[j]	[15]
	D3,3′	1.81	343.1/362.4	19.3	0.055		
		1.81	55.7/64.9	9.2	0.152	NMR	
	D4	1.05	97.2/108.4	11.2	0.102	MCD	[24,25]
	D6	7.43	349.4/361.9	12.5	0.035		
102/102/350	D7	7.53	347.8/359.2	11.4	0.032		
299/680	D8	7.43	544.1/555.8	11.7	0.021		
6	D1	1.59	8.1/23.6	15.5	0.978	NMR	
	D2	3.74	465.1/514.5	49.4	0.100	MCD	[24,25]
	D3,3′	1.93	158.5	0	0		
		2.06	166.3/231.5	65.2	0.327		[72]
	D4	1.16	57.1/71.5	14.4	0.224		
	D6	7.65[1]	464.5/483.7	19.2	0.041	Optical	
	D7	7.66[1]	434.2/512.2	78.0	0.164	activity	
	D8	7.92	328.1/363.7	35.6	0.103		
	D10	8.12	276.9/312.4	35.5	0.122		
	D11	7.67[2]	477.3	0	0		
99/100/370	D12	7.67[2]	434.2	0	0		
299/619	D13	8.43	270.1/305.1	35.0	0.122		
7	D1,1′	NO				NR	
	D2	6.27	331.1/376.2	45.1	0.127		
	D3	3.68	581.7/596.7	15.0	0.025		
	D4	1.58	6.4/19.6	13.2	1.015		
	D6	7.44	242.7/255.7	13.0	0.052		
	D7	7.53	247.1/260.2	13.1	0.052		
102/100/350 299/648	D8	7.43	586.7/602.9	16.2	0.027		

[a–j] See the footnotes of Table 1.

0947-6539/03/0908-1732 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

Table 3. Data for chiral semiflexible ethylenic and acetylenic compounds (**8**–**11**).

Entry Structure Sample composition[a] $T/\lvert\Delta\nu_Q^{solvent}\rvert$ [b]	Site	δ[c] [ppm]	$\lvert\Delta\nu_{Q_i}^{smaller}\rvert/\lvert\Delta\nu_{Q_i}^{larger}\rvert$ [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
8	D1,1′	4.90	102.4/112.2	9.8	0.091	HPLC	
		4.99	367.3/371.3	4.0	0.011	PC	[9]
	D2	5.71	356.5	0	0		
	D3	2.07	417.2	0	0	NMR	
	D4,4′	1.36	57.7/61.8	4.1	0.068	PC	
		1.37	152.7/165.8	13.1	0.082		[9]
100/101/350	D5	0.83	86.2	0	0		
298/832	D6	0.90	25.8/33.8	8.0	0.268		
9	D1,1′	4.98	364.5/366.3	1.80	0.017	NR	
	D2	5.70	648.5/655.1	6.6	0.010		
	D3	2.16	771.5/783.7	12.2	0.016		
	D4,4′	1.31[1]	563.2	0	0		
		1.35[1]	412.7/422.1	9.4	0.023		
	D5,5′	1.28[1]	391.9/408.5	16.6	0.041		
		1.30[1]	496.3/508.9	12.6	0.025		
99/101/402	D6	0.92	158.2	0	0		
298/1121	D7	1.01	14.9/23.1	8.2	0.431		
10	D1	1.98	255.3	0	0	NR	
	D3	2.36	349.2/397.6	48.4	0.130		
	D4,4′	1.36	257.3/289.7	32.4	0.118		
		1.69	260.6/274.7	14.0	0.052		
	D5,5′	1.29	296.7	0	0		
		1.32	313.3	0	0		
81/82/428	D6	0.87	91.1/96.9	5.8	0.061		
300/804	D7	1.11	79.3/85.1	5.8	0.071		

[a–j] See the footnotes of Table 1.

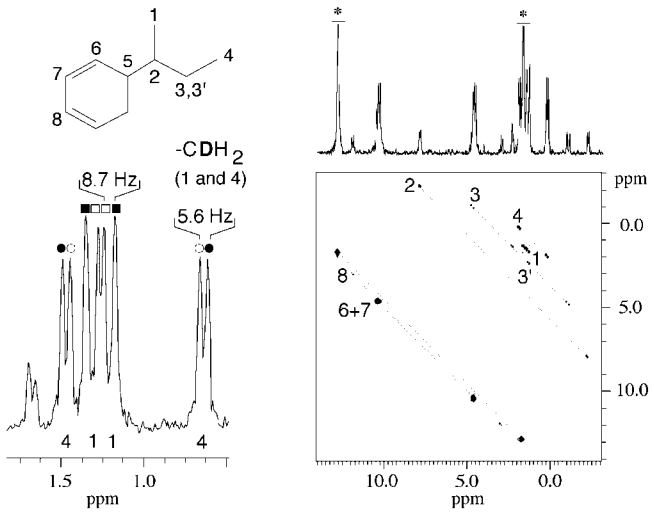


Figure 4. On the right: NAD Q-COSY spectrum of (±)-2-phenylbutane in the PBLG/$CHCl_3$ phase recorded at 299 K and using 331 (t_1) × 1600 (t_2) data points. No filtering was applied. The chloroform signals are marked by an asterix on the F_2 projection. Left: Sum of columns showing enantiomeric discrimination on the methyl groups 1 and 4.

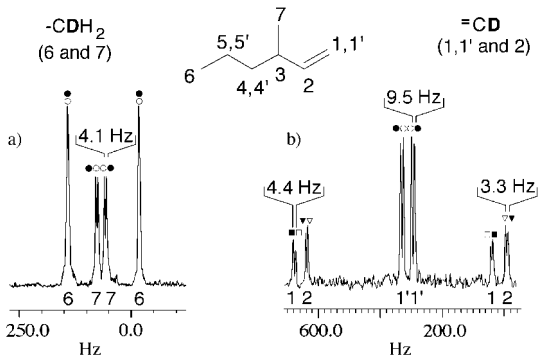


Figure 5. NAD NMR signals associated to the methyl groups 6 and 7 (a) and ethylenic deuterium atoms 1, 1′, 2 (b) extracted from the tilted Q-COSY spectrum of (±)-3-methylhexene in the PBLG/$CHCl_3$ phase at 298 K. The spectrum was recorded as a 2D matrix of 300 (t_1) × 1750 (t_2) data points and 400 scans were added per t_1 increment. An exponential window ($LB_{1,2}$ = 1 Hz) was applied in both dimensions. The doublets due to each enantiomer is arbitrarily labelled.

concerning these two compounds was found, but Schurig and Nowotny have described the enantioseparation of (±)-2,4-dimethyl-1-heptene using GC on derivatised γ-CD.[60] Numerous spectral enantiodifferentiations in PBLG are observed for (±)-**8** (R_c = 0.62) and (±)-**9** (R_c = 0.83) (Figure 5). Thus, the spectral analysis of (±)-**9** shows a chiral differentiation on deuterium atoms in the methyl group 7, on deuterium atom D_3 located on the asymmetric carbon, as well as deuterium atoms on the *gem*-(D_2), *trans*-($D_{1'}$) and *cis*-(D_1) positions in the vinyl group, but not on the methyl group 6. Note that the quadrupolar splittings for the *cis*- and *gem*-deuterium atoms in the vinyl group are very close.

This is because the associated C–D bonds EFG are more or less parallel, and hence they must have a very similar orientation relative to the magnetic field. This fact enables us to simply assign the ethylenic deuterium atoms. Similar behaviour can be observed for the vinyl group of (±)-**8**. The

large difference in peak intensities exhibited by *gem*-(D_2) and *cis*-(D_1) compared with *trans*-($D_{1'}$) in (±)-**9** originates from an important depletion effect,[66] already observed in the isotropic NAD 1D NMR spectrum. The same depletion mechanism explains why quadrupolar doublets for *cis*-(D_1) and *trans*-($D_{1'}$) in (±)-**7** do not clearly emerge from the noise.

Comparison of the data found for solutes (±)-**8** and (±)-**9** are informative. It shows that the magnitude of quadrupolar splittings, and hence order parameters, increases with the length of the main alkyl chain. This is due to the classical increase of order parameters with the shape anisotropy (length/diameter) from (±)-**8** to (±)-**9**. In contrast, the DOE values do not seem strongly affected by this increase in the molecular ordering. This remark suggests that molecular order and chiral discrimination may not be correlated to the same molecular properties. Finally, we have studied the case of chiral acetylenic compounds using a racemic mixture of 3-methylhexyne [(±)-**10**]. Here again the NAD spectrum exhibits numerous separate resonances for the two enantiomers with a relatively good R_c ($R_c = 0.62$).

In this class of derivatives (Tables 2 and 3), it should be noted that compound (±)-**7** differs slightly from the set of other derivatives because two groups with π-electrons are present in the molecular structure. The comparison of the quadrupolar splittings and the DOE factors with (±)-**5** shows that the orientational behaviour of both derivatives toward the oriented polypeptide helices does not differ strongly. This could indicate that the vinyl group plays a minor role in terms of molecular recognition ability, probably because the difference in the size and shape anisotropy of both molecules is rather negligible.

Several conclusions can be drawn from the examination of all the results collected using this class of molecules:

a) A chiral discrimination can be observed in the flexible as well as in the rigid part of these molecules.

b) The quadrupolar splittings measured on the methyl groups are generally among the smallest in a molecule. Actually this is true whatever the class of molecule concerned. This is due to the rotation of the methyl group around the C–CH_3 axis.[75] Consequently, calculating the average orientation, the order parameter associated to a methyl C–D bond appears equal to the order parameter of the C–C bond times $[3\cos^2(\pi - 2\theta_m) - 1]/2 = -⅓$, where $2\theta_m$ is the tetrahedral angle. This $-⅓$ factor justifies the above remark in that sense that the average order parameter of a methyl group will be always three times smaller that the order parameter of the C–C direction it turns around.

c) All C–D bonds that are roughly oriented along the molecular longest axis give rise to the largest quadrupolar splittings. This is particularly visible for the deuterium atom in the *para*-position in the monosubstituted benzene derivatives (±)-**5** and (±)-**7**. Probably the same reason explains why in (±)-**6** the deuterium atoms 11 and 12, as well as 6 and 7 which are colinear to 11 and 12, respectively, exhibit the largest quadrupolar splittings. Note that this situation is not observed for the acetylenic deuterium atom of **10**, and it means that in this case the most oriented axis does not coincide with the acetylenic C–D direction.

d) Deuterium atoms on the chiral centers exhibit generally large quadrupolar splittings but small DOEs. So far, we have no clear explanation for this particular behaviour.

Flexible chiral alkanes: We next turned our attention to the flexible chiral saturated aliphatic hydrocarbons with the general formula $CHR^1R^2R^3$ (R = alkyl), such as 3-methylhexane (**10**) and higher homologues.[76] Indeed, these chiral species feature a high conformational flexibility which may severely reduce or eliminate the polypeptide helices' ability to be enantioselective. Although the enantiomeric separation of these trialkylmethanes have been achieved by GC using undiluted cyclodextrins as CSP,[27, 28] to the best of our knowledge, all isotropic NMR techniques failed in discriminating such chiral alkanes.

All results obtained in CLC are summarised in Table 4. Figure 6 reports the NAD Q-COSY map of (±)-**10** as well as two different traces associated with deuterium atoms referred to as 4′ and 5. On this 2D spectrum, we can observe twelve different quadrupolar doublets. This result clearly indicates that two deuterium atoms in the molecule are discriminated, as only ten quadrupolar doublets would be detected if no enantiomeric separation occurred.

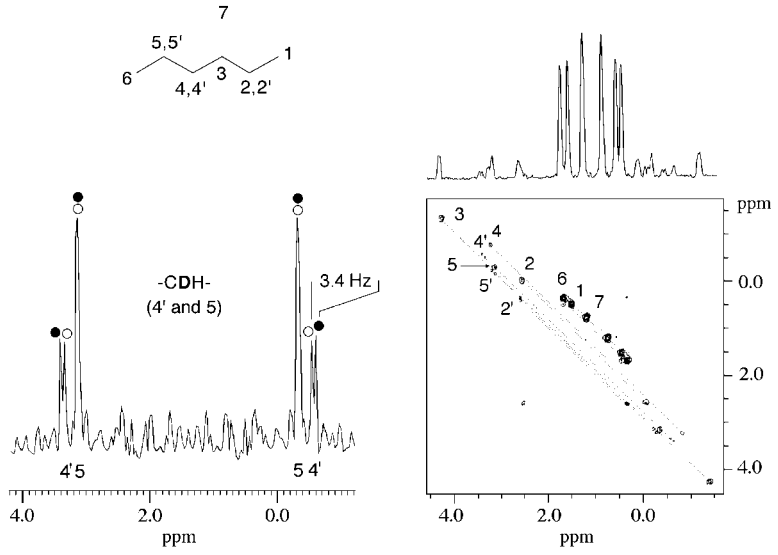
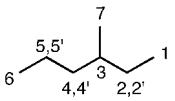
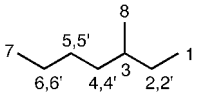
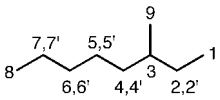


Figure 6. Right: NAD Q-COSY spectrum of (±)-3-methylhexane recorded at 298 K using 300 (t_1) × 1700 (t_2) data points. 400 Transients for each t_1 increment were added. A Gaussian filtering was used in both dimensions. The chloroform doublet is not shown in the spectrum. Left: sum of columns showing a spectral enantiomeric discrimination on the deuterium atom 4′.

  0947-6539/03/0908-1734 $ 20.00+.50/0

Table 4. Data for chiral flexible acyclic alkane compounds (**11**–**13**).

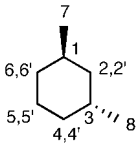
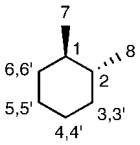
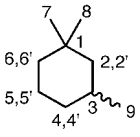
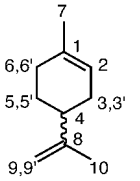
Entry Structure Sample composition[a] $T/\lvert\Delta\nu_Q^{solvent}\rvert$ [b]	Site	δ[c] [ppm]	$\lvert\Delta\nu_{Q_i}^{smaller}\rvert/\lvert\Delta\nu_{Q_i}^{larger}\rvert$ [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
11	D1	0.86	64.5	0	0	GC	
	D2,2′	1.12	160.0	0	0	MCD	[27,28]
		1.33	135.7	0	0		
	D3	1.30	346.1	0	0		
	D4,4′	1.08	247.4	0	0		
		1.27	239.1/245.9	6.8	0.028		
	D5,5′	1.28	213.0	0	0		
		1.33	211.6/203.4	8.2	0.039		
80/100[k]/445	D6	0.88	81,8	0	0		
298/585	D7	0.85	27.2	0	0		
12	D1	0.86	112.7	0	0	GC	
	D2,2′	1.12	296.9/307.5	10.6	0.035	MCD	[27,28]
		1.33	258.9/258.9	0	0		
	D3	1.30	696.7/703.5	6.8	0.01		
	D4,4′	1.09	580.2	0	0		
		1.29	[508–529]*	–	–		
	D5,5′	1.23	[508–529]*	–	–		
		1.27	[508–529]*	–	–		
	D6,6′	1.28	470.8/479.6	8.8	0.018		
		1.29	[508–529]*	–	–		
50/100/400	D7	0.89	143.5	0	0		
298/882	D8	0.85	69.9	0	0		
13	D1	0.84	123.7	0	0	GC	
	D2,2′	1.11	301.6/313.2	11.6	0.038	MCD	[27,28]
		1.32	279.6/289.8	10.2	0.036		
	D3	1.27	794.2	0	0		
	D4,4′	1.08	[671–696]*	–	–		
		1.27	[671–696]*	–	–		
	D5,5′	1.23	746.2/751.4	5.2	0.007		
		1.27	[671–696]*	–	–		
	D6,6′	1.22	639.0/653.8	14.8	0.023		
		1.24	[671–696]*	–	–		
	D7,7′	1.28	546.7/562.7	16.0	0.029		
		1.29	529.5/546.7	17.2	0.032		
60/100/400	D8	0.87	168.7	0	0		
298/896	D9	0.84	98.9	0	0		

[a–j] See the footnotes of Table 1. [k] 50 mg (DP = 562)+50 mg (DP = 1078). * The exact measurement of the quadrupolar splitting was not possible, consequently only an interval of values is given for information.

The assignment of each doublet (Figure 6a) was achieved using the procedure described at the beginning of this section (Table 4, entry 11). Thus, we were able to deduce that the 4′ and 5′ deuterium atoms are discriminated while deuterium atoms 2 and 3 show only broad peaks indicating a spectroscopically unresolved chiral discrimination. In contrast, no chiral discrimination was detected on the methyl signals referred to as 1, 6 and 7. The analysis of NAD NMR spectra of 3-methylheptane [(±)-**11**] and 3-methyloctane [(±)-**12**] recorded at 298 K in a PBLG/$CHCl_3$ phase have unambiguously shown the doubling of numerous peaks; this indicates that enantiomers of these solutes are significantly discriminated in this oriented phase. Here again, the identification procedure previously described shows that the largest chiral separations are measured for the diastereotopic deuterium atoms noted 2, 6 for (±)-**12** and 2, 2′, 6, 7, 7′ for (±)-**13**. As for (±)-**12**, none of the methyl groups is discriminated, probably because the factor $-1/3$ introduced above makes the DOE too small to be observed here. A small chiral separation is visible on the deuterium atoms attached to the asymmetric carbon atom of these compounds. Even if the spectral differentiation obtained are small for these chiral alkanes, it must be stressed that no alternative NMR methods are available for this series of compounds.

Flexible chiral monocyclic hydrocarbons: We have investigated the chiral monocyclic six-membered-ring hydrocarbons, exhibiting ring conformational changes during the flipping process, with the purpose of exploring the influence of conformational dynamics in the enantioselective recognition in PBLG. The case of chiral *trans*-disubstituted cyclohexanic derivatives is especially interesting (Table 5, entries 14–17). Except for compound **17**, no report in literature was found involving NMR differentiation of the enantiomers of these molecules.[7, 8, 14] Schurig et al. have, however, described the enantioseparations of these three analytes on CDs using GC, as well as for a series of homologues such as *cis*/*trans*-1-ethyl-2-methylcyclohexane and *cis*/*trans*-1-methyl-2-*n*-propylcyclohexane.[60, 70]

Table 5. Data for chiral flexible cyclic hydrocarbon compounds (**14**–**17**).

Entry Structure Sample composition[a] $T/\lvert\Delta\nu_Q^{solvent}\rvert$ [b]	Site	δ[c] [ppm]	$\lvert\Delta\nu_{Q_i}^{smaller}\rvert/\lvert\Delta\nu_{Q_i}^{larger}\rvert$ [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
14	D1/D3	1.81	309.5/320.5	11.0	0.035	GC	
	D2/D2′	1.31	166.4/185.4	19.0	0.108	MCD	[70]
	D4,4′/D6,6′	1.15	120.7/141.1	20.4	0.156		
		1.55	250.1/258.5	8.4	0.033		
	D5/D5′	1.49	180.2/201.1				
	D7/D8	0.94	57.5/65.9	8.4	0.136		
99/101/349 298/891							
15	D1/D2	0.94	553.6/567.5	13.9	0.025	GC	
	D3a/D6a	0.95	543.5	0	0	MDC	[70]
	D3′e/D6′e	1.62	24.2/54.2	30.0	0.765		
	D4a/D5a	1.24	571.6/528.5	43.1	0.078		
	D4′e/D5′e	1.68	294.5/308.1	13.6	0.045		
	D7/D8	0.89	84.2	0	0		
101/100/351 298/884							
16	D2a	0.81	423.3	0	0	GC	
	D2′e	1.38[1]	178.0/198.7	20.7	0.110	MCD	[70]
	D3	1.56[2]	189.9	0	0		
	D4a	0.77	423.3	0	0		
	D4′e	1.69	213.3/239.9	26.6	0.117		
	D5a	1.46	440.2	0	0		
	D5′e	1.55[2]	453.4	0	0		
	D6a	1.09[1]	453.1	0	0		
	D6′e	1.39	408.8/408.8	0	0		
	D7	0.94	33.6/43.3	9.7	0.253		
102/100/351	D8	0.94	110.3	0	0		
298/865	D9	0.89	123.5	0	0		
17	D2	5.38	29.3	0	0	GC	
	D3,3′	1.88	496.6/574.3	77.7	0.145	CD	[59,61]
		1.91	107.3/317.4	210.1	0.989	MCD	[58,67]
	D4	2.06	631.9/662.2	30.3	0.047		
	D5,5′	1.45	679.1/726.7	47.6	0.068	HPLC	
		1.81	182.7	0	0	CD	[62]
	D6,6′	2.03[1]	329.4	0	0		
		2.05[1]	429.3	0	0	NMR	
	D7	1.61	351.1	0	0	BNC	[7,8]
	D9,9′	4.68	167.0	0	0	DC	[14]
52+52/100/350		4.68	1184.5	0	0		
298/877	D10	1.71	76.5	0	0		

[a–j] See the footnotes of Table 1.

As a first illustration, we focused on the (±)-*trans*-1,3-dimethylcyclohexane [(±)-**14**]. Such a molecule can be seen as chiral with an average C_2 symmetry on the NMR time scale. This is a typical example of molecules whose averaged symmetry is higher than the symmetry of chiral contributing conformers (C_1).[64] The inversion of the chair conformation (a,e and e,a) leads to the formation of a single isomer that is nonsuperimposable with its mirror image (Figure 7a). Consequently, like two chiral "rigid" enantiomers, the PBLG fibres should interact differently with the two enantiomeric conformers of *trans*-1,3-dimethylcyclohexane, thereby permitting their spectroscopic differentiation using NAD NMR. Since we detect only monodeuterated isotopomers which are different from the totally protonated molecule in NAD NMR, an important question arises at this stage of the discussion. One could argue that a monodeuterated isotopomer in the (a,e) position is no longer identical with the isotopomer in the (e,a) position as the axial deuterium atom becomes equatorial and vice versa. Actually, in PBLG as solvent, the order parameters are very small (in the 10^{-3} to 10^{-4} range), and consequently no isotopic effect on the ordering of solutes has ever been detected to date. Consequently, molecular orientational ordering of a protonated molecule is essentially identical to that of its monodeuterated isotopomers.[40]

The NAD Q-COSY map of (±)-**14** is displayed in Figure 7b. The assignment given in the 2D contour plot is based on that proposed by Grant and co-workers.[77] Note the assignment of quadrupolar doublets is much simpler than in acyclic alkanes because the average C_2 symmetry axis reduces the number of nonequivalent deuterium atoms by a factor of

  0947-6539/03/0908-1736 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

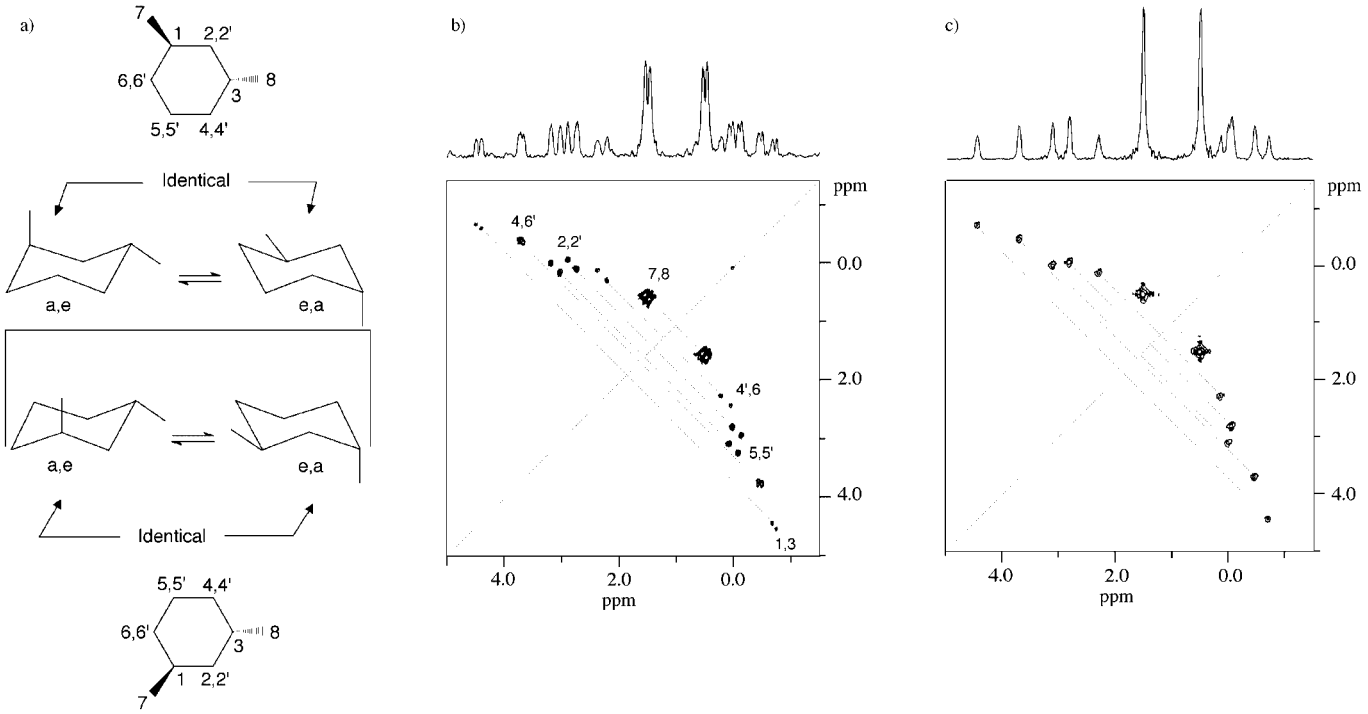


Figure 7. a) Representation of the conformers of (±)-*trans*-1,3-dimethylcycohexane and their stereochemical relationships. b) and c) NAD Q-COSY spectrum of (±)-*trans*-1,3-dimethylcyclohexane in the PBLG/$CHCl_3$ phase and the PBG/$CHCl_3$ phase, respectively. Both 2D spectra recorded at 298 K and using 300 (t_1) × 1400 (t_2) data points. An exponential window ($LB_{1,2} = 2.0$ Hz) was applied in both dimensions. The chloroform signals are not shown. Note the collapsing of pairs of quadrupolar doublet into a single doublet in the achiral phase.

two. The analysis of the NAD 2D NMR spectrum showed a spectral differentiation on all chiral isotopomers of the molecule because six pairs of quadrupolar doublets centred on six distinct chemical shifts were observed ($R_c = 1.0$).

In regard to the above discussion relating to isotopic effects on orientation, we have confirmed our assertion by recording the spectrum of (±)-**14** in an organic solution of PBG. Experimentally only one quadrupolar doublet (instead of two) should be observed in the NAD Q-COSY spectrum for each nonequivalent deuterium atom in this achiral nematic phase. The NAD Q-COSY spectrum recorded in the achiral solvent PBG is shown in Figure 7c. As expected, the various quadrupolar doublets associated with two chiral isomers (which were differentiated in PBLG) collapse now into six doublets in the nonchiral phase. The result obtained is, therefore, consistent with our previous analysis, and subsequently it can be concluded in the light of this result that PBLG helices differentiate between the two enantiomers of (±)-**14** at room temperature.

The stereochemical behaviour of (±)-*trans*-1,2-dimethylcyclohexane [(±)-**15**] differs from that of (±)-**14** because the isomerisation process involves the interconversion of two diastereomeric chair conformations (a,a and e,e) of different energy. At room temperature the difference in steric energy (11.5 kJ mol^{-1}) between the (e,e) and (a,a) *trans*-conformers leads to a Boltzmann distribution of 99% (e,e) and 1% (a,a).[64] Consequently, only the (e,e) isomer and its enantiomer are detected on NAD NMR spectra. Results extracted from the NAD spectrum (Table 5, entry 15) indicate unambiguously the spectral separation of enantiomers of (±)-**15**, but only four of six possible chiral isotopomers exhibit a chiral discrimination ($R_c = 0.66$). Also NAD NMR in PBLG is a suitable method to visualise the two enantiomers of (±)-1,1,3-trimethylcyclohexane [(±)-**16**]. Note that our assignment of quadrupolar doublets agrees with that made in the earlier literature.[78] The R_c ratio is quite weak ($R_c = 0.25$) and notably reduced compared with that of solutes **14** and **15**. We have no clear explanation for such a result, however, only a single site is sufficient if the corresponding S/N and DOE are sufficient to be used for quantitative measurements.

Monocyclohexenes exist in half-chair form as depicted in Figure 8a.[64] In case of limonene (±)-**17**, the ring inversion produces diastereomers with two distinct conformations. However, the rather low inversion barrier of this type of cycloalkenes implies a fast conformational equilibrium between the two conformers at room temperature. Consequently, on the ^{2}H NMR time scale in PBLG the two conformational isomers cannot be observed separately, and hence the six-membered ring can be considered as planar. However, due to the propylenic ligand, the molecule still possesses an overall C_1 symmetry and exists in two enantiomeric forms. NMR, using the binuclear shift reagents Yb(Hfc)$_3$/Af(fod) and dirhodium complex Rh_2(MTPA)$_4$, has been applied successfully for this chiral cyclohexene.[7, 8, 14] Besides, the enantioresolution of (±)-**17** by GC using CDs as CSP was extensively investigated.[58, 59, 61, 67] Figure 8b and c display the tilted NAD Q-COSY spectrum of (±)-**17** and one column showing a chiral differentiation.

The identification of quadrupolar doublets of (±)-**17** (as well as in (±)-**16**) is based on the identification proposed in

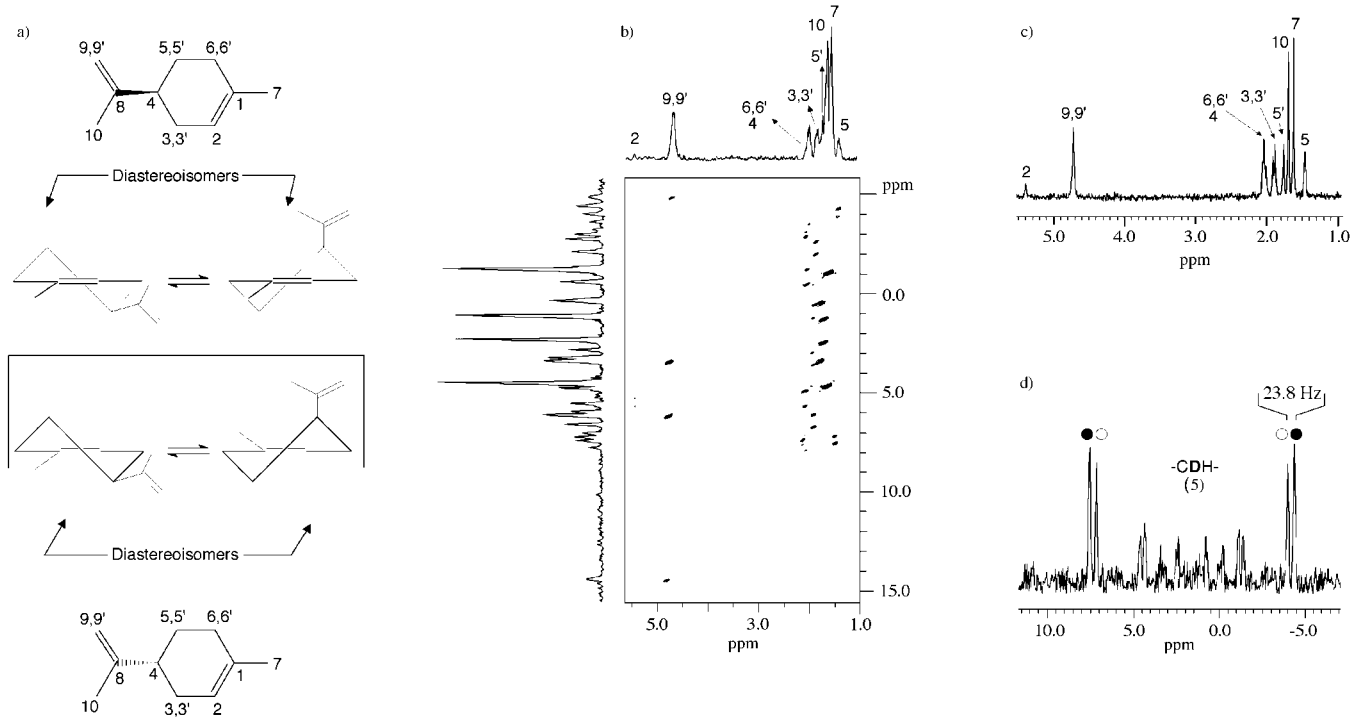


Figure 8. a) Representation of the conformers of (±)-limonene and their stereochemical relationships. b) Tilted NAD Q-COSY spectrum of (±)-limonene in the PBLG/$CHCl_3$ phase recorded at 298 K. The data matrix is 256 (t_1) × 1400 (t_2) data points. An exponential window ($LB_{1,2} = 1.0$ Hz) was applied in both dimensions. c) NAD spectrum recorded at 298 K in an isotropic solution of $CHCl_3$. d) A column showing enantiomeric discrimination on the methyl deuterium atom 5.

ref. [77]. Unambiguously, the analysis of this spectrum shows a chiral discrimination on several signals ($R_c = 0.25$), in particular those belonging to the cyclohexylene part. To confirm this result we have recorded the NAD Q-COSY spectrum of (±)-**16** in the racemic PBG/$CHCl_3$ liquid crystal (not presented), and clearly observed the elimination of pairs of quadrupolar doublets.

Chiral hydrocarbons without a stereogenic carbon atom: To broaden the application of this method, we finally examined derivatives exhibiting enantiomeric isomers but lacking stereogenic tetrahedral carbon atoms (Table 6, entries 18–20). Thus, we have investigated allenic molecules with axial chirality,[64] an example of atropoisomerism typically provided by the chiral 1,1′-binaphthyl derivatives,[64] and a chiral molecule known to exist as a mixture of enantiomers interconverting by ring inversion as the *cis*-decahydronaphthalene.[64]

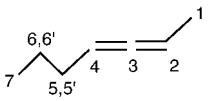
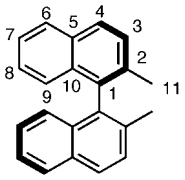
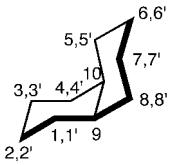
In the literature, several papers dealing with the enantiodiscrimination of chiral allenic compounds can be found. In 1986, Mannschreck et al. obtained fair spectral discriminations induced by a mixture of achiral salt Ag(fod) and the optically active complex (+)-Yb(hfbc)$_3$ for some chiral 1,3-disubstituted allenic hydrocarbons through ^{1}H NMR spectroscopy.[79] More recently, Salvadori and co-workers have investigated another approach and shown the analytical potential of CDs as CSA for the determination of the enantiomeric composition of a small collection of chiral trisubstituted allenic hydrocarbons using ^{1}H NMR.[21–24] Here we have investigated a chiral disubstituted allene, the hepta-2,3-diene in racemic mixture [(±)-**18**]. The NAD Q-COSY spectrum of (±)-**18** in a PBLG system and related traces are shown in Figure 9. Here again, the ability of PBLG to interact differently with the two enantiomers yields spectral enantiodiscrimination on numerous sites of the molecule ($R_c = 0.63$).

The most significant spectral discrimination is obtained on the diastereotopic sites 5 and 5′ of the molecule, but diastereotopic sites 6 and 6′ also exhibit spectral discrimination. Note that the doubling of the deuterium signals for the methyl group 7 in the flexible chain does show a small chiral separation while no separation is obtained for methyl group 1 directly bonded to the rigid part of the molecule. Again this situation perfectly illustrates the complexity of determining a priori the deuterated site providing the best chiral differentiation.

We next studied a binaphthyl compound in the cisoid conformation (Figure 10). In this conformation, the dihedral angle between the two naphthalene rings is less than 90°,[80] and hence the molecule exhibits two atropoisomeric enantiomers.[64] This molecule is featured by an overall C_2 symmetry, reducing the number of chiral isotopomers by a factor of two. In Figure 10a and b, we present the F_2 projection of the NAD Q-COSY spectrum of (±)-2,2′-

  0947-6539/03/0908-1738 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

Table 6. Data for chiral hydrocarbon compounds without a stereogenic carbon atoms (**18**–**20**).

Entry Structure Sample composition[a] $T/\lvert\Delta\nu_Q^{solvent}\rvert$ [b]	Site	δ[c] [ppm]	$\lvert\Delta\nu_{Q_i}^{smaller}\rvert/\lvert\Delta\nu_{Q_i}^{larger}\rvert$ [Hz][d]	$\Delta\Delta\nu_{Q_i}$[e] [Hz]	DOE_i[f]	Other methods used	Ref.[h]
18	D1	1.64	72.4	0	0	NR	
	D2	NO	–	–	–		
	D4	NO	–	–	–		
	D5,5′	1.96	268.2/278.2	10.0	0.037		
		1.98	235.1/244.4	9.2	0.038		
	D6,6′	1.43	185.1/192.9	7.8	0.041		
102/100/399		1.43	205.6/213.9	8.3	0.039		
300/661	D7	0.93	80.5/88.5	8.0	0.095		
19	D11[l]	2.12	103.7/129.7	26.0	0.222	NR	
		1.98	78.5/105.2	27.2	0.289		
	D3–9[l]	NO NO					
50/100/400 ($CHCl_3$)							
300/652							
58/134/258 (DMF)							
313							
20	D1/D5a	1.52	126.2/196.9	70.7	0.437	NMR	[26]
	D1′D5′e	1.10	244.8/266.6	21.8	0.085	CD	
	D2/D6a	1.14	214.7/229.8	15.1	0.067		
	D2′/D6′e	1.64[n]	133.3/144.3	11.1	0.080		
	D3/D7a	1.25	283.6/303.1	19.5	0.066		
	D3′/D7′e	1.29	537.9/576.4	38.5	0.069		
	D4/D8a	1.38[p]	128.1/198.3	70.2	0.430		
	D4′/D8′e	1.38[p]	223.2/244.6	21.4	0.091		
11/60[n]/603 (CH_2Cl_2)	D9/D10	1.56	222.7/232.3	9.6	0.042		
230/no data							

[a–j] See the footnotes of Table 1. [l] Values given in the first and the second line correspond to the splittings measured in the PBLG/$CHCl_3$ phase and the PBLG/DMF phase, respectively. [m] The DP of polymer used is 1352. [n] The most deshielded quadrupolar doublet is used as internal reference with $\delta =$ 1.64 ppm.

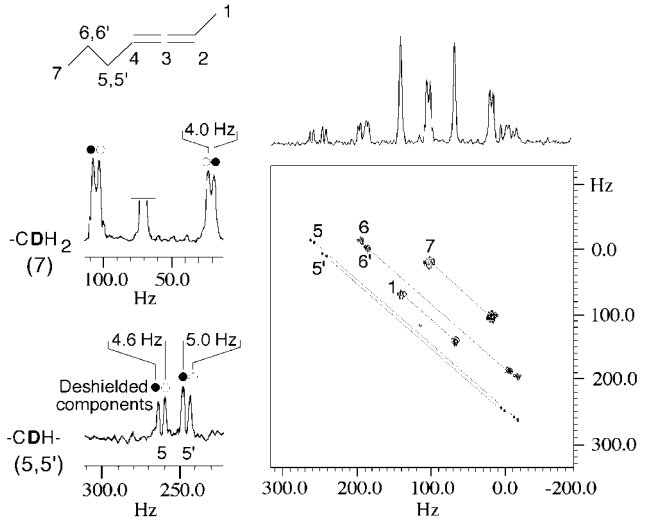


Figure 9. Right: NAD Q-COSY spectrum of (±)-hepta-2,3-diene in the PBLG/$CHCl_3$ phase recorded at 300 K and using 340 (t_1) × 1750 (t_2) data points. 496 scans were added per t_1 increment. An exponential filtering ($LB_1 = 1.0$ Hz, $LB_2 = 0.5$ Hz) was applied in both dimensions. The chloroform signals is not shown. Left: signals of the methyl deuterons 7 and the diastereotopic deuterium atoms 5,5′, both showing a spectral enantiodiscrimination. Only the deshielded components of quadropolar doublets are displayed. The peaks due to each enantiomer are arbitrarily labelled by (●) and (○).

dimethyl-1,1′-binaphthyl [(±)-**19**] dissolved in the PBLG/$CHCl_3$ phase and in the PBLG/[D_7]DMF phase, respectively. Both experiments were recorded under similar NMR conditions (Table 6, entry 19). As mentioned in the Experimental Section, the comparison of both spectra emphasises the strong advantage of using chloroform instead of other organic solvents having numerous distinct deuterated isotopomers. For DMF, three quadrupolar doublets centered on three chemical shifts are expected to be found in the NAD spectrum of the co-solvent at room temperature. Because of the conformational steric hindrance, the two methyl groups in the N-*gem* position are nonequivalent, and so they exhibit distinct ^2H chemical shifts and quadrupolar splittings in NAD spectra.

In both experiments, the quadrupolar doublets associated with biaryl groups of solutes are not clearly discernable from the noise, and only the signals of methyl deuterium atoms provides a useful signal. This is, however, sufficient to measure an eventual enantiomeric excess. The reason is that the deuterium signal of the methyl groups is the sum of six equivalent isotopomers for each enantiomer. The presence of two doublets located on the chemical shift of the methyl group shows the enantiodifferentiation of this molecule in both oriented phases.

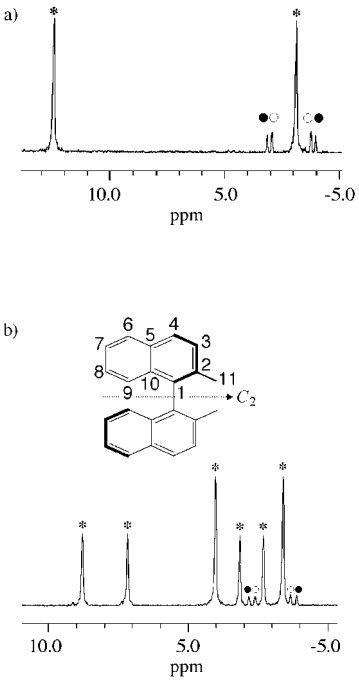


Figure 10. F_2 projection of the NAD Q-COSY spectrum of (±)-2,2′-dimethyl-1,1′-binaphthyl in the PBLG/$CHCl_3$ phase (a) and in the PBLG/DMF phase (b). Both 2D spectra were recorded at 300 K and 313 K respectively using 245 (t_1) × 1760 (t_2) data points and 496 scans were added per t_1 increment. A gaussian window (GB = 40 %, LB = − 3.0 Hz) was applied in both dimensions. Note the difference of the spectral fingerprint associated with the deuterium signal of chloroform and DMF (∗). The doublets of the methyl groups are arbitrarily labelled (●) and (○).

Some years ago we presented an experimental study of the visualisation of enantiomers through proton-decoupled carbon-13 NMR ($^{13}C\{^1H\}$) in natural abundance.[36, 81] By this technique spectral discrimination between enantiomers is observed through a difference of carbon-13 chemical-shift anisotropies, visible on the $^{13}C\{^1H\}$ spectra through a simple difference of resonance frequencies. In particular, we have described the chiral discrimination of 2,2′-dihydroxy-1,1′-binaphthyl mixtures enriched in the *R* enantiomer (*ee* 31 %), dissolved in the PBLG/[D_7]DMF phase at 313 K.[29, 36] The results were remarkable because, at 100 MHz, 8 out of 10 of the aromatic carbon atoms exhibited two different resonances with differences varying from 10 to 17 Hz. Such separations allowed for easy measurment of the *ee* by peak integration. It was therefore of interest to examine the $^{13}C\{^1H\}$ spectrum of (±)-**19** in order to compare these two solutes. For this purpose the $^{13}C\{^1H\}$ 1D spectra of (±)-**19** in PBLG/$CHCl_3$ and in PBLG/DMF were recorded at 300 and 313 K, respectively. Only the spectrum in the $CHCl_3$/PBLG phase is reported in Figure 11. The assignment of carbon atoms of aromatic group proposed in the spectrum is tentatively based on the group-contribution method.[82] In both oriented solvents, the results are quite amazing because almost all aromatic carbons (90 % in $CHCl_3$ and 80 % in DMF) show a chiral discrimination on the basis of ^{13}C frequency differences. The spectral separations measured in the $CHCl_3$/PBLG phase vary between 2 and 6 Hz, while those found in the PBLG/DMF solvent vary between 3 to 9 Hz (spectrum not shown). The chemical-shift differences measured in spectra are globally smaller than values measured with the (±)-2,2′-dihydroxy-1,1′-binaphthyl in the PBLG/DMF system (10 – 17 Hz), but still allow for an easy determination of the enantiomeric composition by peak integration. The results obtained with (±)-**19** using $^{13}C\{^1H\}$ NMR are important. Until now almost all others chiral molecules investigated in this work were not differentiated (alkane) or gave too small

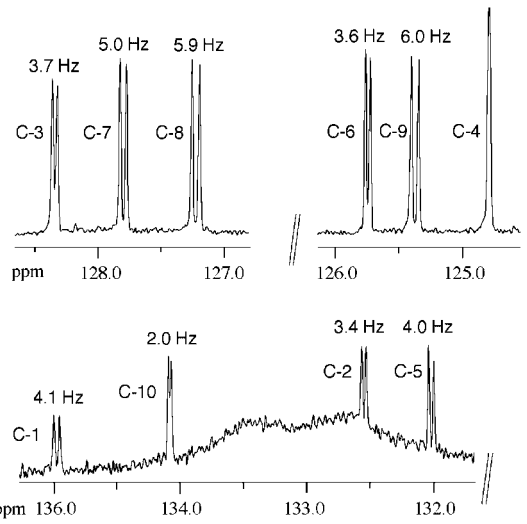


Figure 11. 100.4 MHz $^{13}C\{^1H\}$ NMR signals associated with the aromatic carbon atoms of (±)-2,2′-dimethyl-1,1′-binaphthyl embedded in the PBLG/$CHCl_3$ phase. The spectrum was recorded at 300 K using 2800 scans and 28 k data points, respectively. A gaussian filtering (GB = 36 %, LB = − 2.0 Hz) was applied to enhance the spectral appearance, and the FID was zerofilled to 64 k data points to increase the digital resolution.

spectral separations to be useful (alkenes, alkynes). As the sample composition of (±)-**19** and the hydroxy derivative in the PBLG/DMF system are the same, a comparison between these molecules is possible in terms of enantioselectivity.

Thus it appears that the replacement of a polar functional group (such as OH) by a nonpolar one (CH_3) does not avoid the chiral discrimination, but only affects its magnitude. The fact that almost all aromatic carbon atoms shows a chiral discrimination through a difference of ^{13}C chemical-shift anisotropy suggests that orientational parameters are strongly different for the two mirror-image isomers. Actually the ^{13}C chemical-shift anisotropy of sp^2 carbon nuclei is rather small and only large DOEs may result in such visible NMR discriminations.[29, 36] Consequently ^{13}C anisochronous resonances of (±)-**19** imply a large difference in the orientational behaviour of these atropoisomeric enantiomers. This result gives new and important evidence of the role of shape recognition in the mechanisms governing the differential ordering effect in the PBLG oriented phase.

NMR differentiation of chiral compounds existing as a mixture of enantiomers interconverting by ring inversion is of practical importance in numerous applications, and is an interesting challenge in stereochemical analysis. Especially intriguing is the example of *cis*-decahydronaphthalene (Table 6, entry 20). For this chiral flexible bicyclic alkane, also called *cis*-decalin, the interconversion between two predominant, chair-chair conformers of equal energy with an overall C_2-symmetry, noted **20a** and **20b**, is possible at room temperature (Figure 12a). By contrast, the freezing of the ring inversion at low temperature leads to a racemic mixture of chiral "rigid" invertomers.[83] Based on the chromatographic separation of *cis*- and *trans*-decalin using β-CD,[84], Dodziuk et al. have recently found that $^{13}C\{^1H\}$ NMR involving β-CD as chiral solvating agent was able to discriminate between chiral conformers **20a** and **20b** at 223 K.[26] This elegant

0947-6539/03/0908-1740 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

approach shows modest chemical-shift differences between diastereomeric complexes, since the largest peak separation measured using a 11.7 T magnetic field does not exceed 7 Hz. To explore the chiral recognition ability of PBLG toward chiral nonfunctionalised invertomers as well as to compare the results with those obtained using CDs, we have prepared a sample of *cis*-decalin dissolved in the PBLG/CH_2Cl_2 phase.[85] To avoid too long an experimental time associated with NAD NMR experiments at very low temperature, we have used the perdeuterated *cis*-decalin (**20**) which is commercially available. Figure 12b reports the ^2H Q-COSY phased spectrum of **20** recorded at 230 K. The analysis of the autocorrelated peaks visible in the 2D contour plot unambiguously shows seventeen distinct quadrupolar doublets (the doublets b′ and d are overlaping). To understand the spectrum multiplicity, it must be recalled that, due to the overall C_2 axis, only nine nonequivalent pairs of C–D directions exists in **20a** (or **20b**) when the ring inversion is frozen. Thus, in an achiral oriented solvent, nine quadrupolar doublets are expected to be observed if there is no overlapping of signals.

Assuming now that the molecular ordering for the enantiomers **20a** and **20b** are different inside the chiral phase, we may expect to observe up to eighteen doublets, if all lines are well resolved. Experimentally, we detect eight further doublets compared with the theoretical maximum expected in an achiral liquid crystal. This result must be interpreted as the evidence of the chiral recognition of *d*,*l*-conformers of *cis*-decalin by the polypeptide. To confirm this analysis, the ^2H Q-COSY phased spectrum of **20** was recorded in the achiral solvent PBG/CH_2Cl_2 at 230 K (Figure 12c). The various quadrupolar doublets associated with equivalent deuterium atoms in **20a** and **20b**, which were differentiated in PBLG, now collapse into nine doublets in the achiral phase. This result proves unambiguously that PBLG helices differentiate between the two chiral invertomers of *cis*-decalin at low temperature and leads to a surprising high R_c factor ($R_c = 1.0$), with two sites exhibiting a DOE factor larger than 0.4. Note that the assignment of quadrupolar doublets is based on the work published recently by Abraham and co-workers.[86] We have explored the enantioselective ability of two other chiral liquid-crystalline phases: the PBLG/$CHCl_3$ and the PCBLL/$CHCl_3$ systems. In both cases, these chiral oriented systems interact enantioselectively with the two invertomers of *cis*-decalin.[85]

Prospects

The various experimental results described here emphasize the feasibility, the versatility and the large analytical potential of natural abundance deuterium (NAD) NMR spectroscopy in chiral liquid-crystalline solvents. Thus this approach provides a suitable solution to chiral hydrocarbon DOE measurements in PBLG without site-specific isotopic labelling. It may be argued that the sensitivity of this tool is inherently limited by the low sensitivity of deuterium nuclei and its very low natural abundance level. Indeed, in the conditions we explored, we generally estimate that the accuracy of the enantiomeric excess measurements in NAD NMR is somewhere between 5 and 15%. Furthermore, it depends on the available amount of material under investigation in respect to its molecular weight. In practice, we have experimentally shown that it was not possible to safely quantify *ee*'s over ca 95% with our current experimental means and a reasonable amount of NMR time.[87] Below this limit the signal of minor enantiomers is not observed, even on a methyl group for which the amplitude of signal is three times larger than for a monodeuterated isotopomer.

Also, it is clear that the amount of racemate used in this work (80–100 mg) can appear discouraging for organic chemists, even if the enantiomers can always be extracted from the liquid-crystalline sample after the NMR measure-

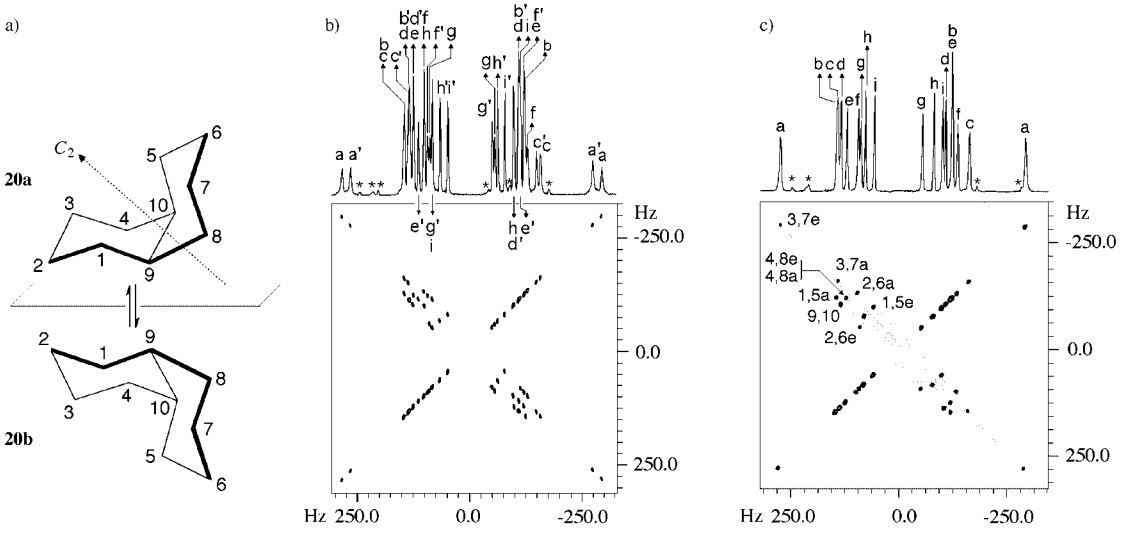


Figure 12. a) Representation of the two interconverting *d*,*l*-conformational forms of *cis*-decalin. b) and c) Deuterium phased Q-COSY spectra for *cis*-[D_{18}]decalin at 230 K in the PBLG/CH_2Cl_2 phase and in the PBG/CH_2Cl_2 phase, respectively. Both spectra were acquired as a matrix of 512 (t_1) × 1024 (t_2) data points with 64 scans for each t_1, and then filtered using a sine window prior to the complex Fourier transformed according to the echo–anti-echo mode. Peaks marked by an asterisk arise from the deuterium nuclei of perdeuterated *trans*-decalin impurities.

  0947-6539/03/0908-1741 $ 20.00+.50/0

ments. This situation is not an insurmountable obstacle. There is no doubt that, by taking advantage of higher magnetic field NMR spectrometers now available in many laboratories, it should be possible to acquire the NAD spectra with less chiral compounds, to reduce the experimental time (EXPT) or to determine the *ee* with higher accuracy.[29, 88] Thus, when recording the NAD spectra of a chiral molecule at 122.8 MHz (18.79 T or 800 MHz for ^{1}H), the S/N ratios would be increased by a factor of 2.8 for the same EXPT. Conversely, the EXPT would be reduced by a factor of 6.6 to obtain the same S/N ratio that is obtained at 61.4 MHz with a 9.39 T field. The second advantage of using very high-field magnets is obviously a better readability of the NAD NMR spectra due to a larger dispersion of deuterium chemical shifts.

Another, and much less expensive, solution to improve the S/N ratio of NAD spectra in PBLG would consist of using selective cryogenic probes which have shown increasing success. The most recent developments of those cryoprobes have shown a very significant gain of the signal sensitivity (a factor around 4) compared to standard probes.[89] To give a simple comparison, calculations indicate that the sensitivity of a 600 MHz spectrometer equipped with a selective deuterium cryoprobe would be equivalent to a 1500 MHz spectrometer equipped with a standard probe. Consequently, the use of higher magnetic fields combined with deuterium cryoprobes will yield such an enhancement of sensitivity that the above results should be acquired with optimal conditions for a very accurate determination of enantiomeric purity.

The cheapest way to improve the sensitivity of NAD experiments could consist of using proton-to-deuterium polarisation transfer.[90] To date the various experimental tests performed in our group have shown a dramatic loss of deuterium signal in ^{2}H – ^{1}H correlation 2D experiments compared with direct measurements. The main reason is that the low magnitude of ^{2}H – ^{1}H scalar and dipolar couplings in very weakly oriented solvents, such as those provided by organic solutions of PBLG, requires unacceptably large delays for the transfer periods in the NMR sequences with respect to the deuterium ^{2}H relaxation times.

Some considerations on the mechanisms of enantioselective recognition in a polypeptide oriented phase: Although the twenty compounds investigated here have different structural topologies, all of them have shown spectral enantiodiscriminations in PBLG. It was possible to obtain these results because the oriented polypeptide helices are always able to interact enantioselectively with rigid or flexible, nonfunctionalized enantiomers. Furthermore, the sensitivity of the quadrupolar interaction towards the DOE is sufficiently large to reveal small to very small orientational differences. The results obtained with flexible molecules imply that the intermolecular shape recognition mechanisms in the polypeptide system are rather insensitive to fast conformational changes, as in the case of linear chiral alkanes or (±)-*trans*-disubstituted cyclohexane derivatives at room temperature. If this was not the case, then the results for nonrigid molecules would be poor.

Beyond the practical applications, the screening of apolar enantiomers is a necessary task in the context of a systematic exploration of the various parameters governing the differential ordering in polypeptidic liquid-crystalline solvents. Indeed from an electrostatic point of view, the various molecules chosen are rather free of appreciable permanent dipole moments, in particular alkanes. This enables us to neglect strong site-specific interactions such as hydrogen bonding or charge-transfer interactions, as in case of polar functionalised solutes. In such compounds the leading electrostatic interactions involve the polarisability which, as shown in ref. [91], seems to have a relatively negligible contribution to solute ordering in liquid crystals. Consequently, it should also have a negligible effect on the differential ordering of two enantiomers in CLC. This remark leads to the idea that geometrical aspects could be dominant. At least, the results reported here clearly indicate that interactions involving strong host – guest electrostatic interactions are not the only mechanism involved in chiral recognition of solutes in polypeptidic oriented solvents. Clearly, if strong interactions were required, no chiral discrimination could be observed for alkanes and cycloalkanes. On the other hand, the enantioselective contributions derived from the short-range repulsive intermolecular forces, correlated with the molecular-shape anisotropy, seems to play an important role.[53, 91, 92]

At present some reasonable assumptions on enantiodiscriminating forces responsible for chiral recognition in a polypeptide oriented phase can be made. Regarding the above results and those already obtained with a wide variety of polar functionalised molecules, it appears that the polarity of the solutes and the related site-specific interactions is important but not the unique factor in the mechanism of chiral differentiation. For molecules devoid of polar functions other short-range interactions are sufficiently large in the chiral discrimination mechanism to produce a visible spectral effect. Among them, we may imagine that π-stacking effects between the aromatic rings of the lateral chains of PBLG and aryl moieties of solute molecules contribute to the chiral differentiation by reinforcing the short-range solute – polypeptide chiral interactions. This could explain why relatively large DOE values were measured with aryl derivatives (entries 5 – 7, 19). Nevertheless this hypothesis has to be balanced against the bulky van der Waals volume of an aryl group that contributes strongly to the molecular shape anisotropy of the solute. Hitherto there is no absolute or direct experimental evidence to substantiate the role of any π – π interactions, but such an effect is clearly plausible.

Before this investigation we were almost totally convinced, in a crude molecular representation, that electrostatic host – guest interactions were the predominant factors to account for the mechanism of differential ordering of enantiomers, while the shape selectivity might be considered as a secondary contribution.[36] In the light of these new results, it now appears that we have underestimated the contribution of intermolecular shape recognition, and now reverse can be suggested for these compounds. The quantification of relative contributions of electrostatic terms and geometrical shape recognition related to the steric character in the chiral discrimination mechanism is a major task for obtaining a deeper understanding of the phenomenon. It will, however,

0947-6539/03/0908-1742 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 8

stimulate our future studies aimed at elucidating the mechanisms of enantioselectivity in polypeptidic oriented solvents.

Conclusion

The differentiation of hydrocarbon enantiomers and their stereochemical characterisation is the ultimate challenge in asymmetric synthesis. It requires methodologies which can be successfully applied to the widest possible range of chemical types. This is an important development in the field of enantioselective synthesis, where there is an immense need for routine, accurate, reliable and universal methods for the determination of optical purities. So far no analytical approach has completely answered the expectation of organic chemists involved in asymmetric synthesis.

As an alternative to the isotropic NMR methods, we believe that NAD NMR in chiral oriented solvents can play a major role as illustrated in this paper. The experimental results presented here for twenty chiral hydrocarbons broaden the scope of the method, and therefore amplify the undeniable potential of NMR using polypeptidic COAs, which have been illustrated earlier for a wide range of polar chiral molecules. From a practical point of view, the major advantage of this methodology is based on the remarkable enantiorecognition capacity of homopolypeptide helices, compared to other chiral selectors, to interact enantioselectively with rigid as well as flexible chiral apolar derivatives. Consequently, none of the alternative methods (chiroptical, chromatographic or other NMR techniques) currently available for hydrocarbons seems to be as general as that proposed in this work. Finally the price-to-performance ratio should push chemists involved in the analysis of chiral material to adopt this original analytical strategy.

So far the main criticism for routine use of this approach could eventually be the low sensitivity of deuterium NMR at natural abundance level. There is no doubt, however, that the advent of very high-field NMR spectrometers and/or the widespread use of cryogenic probe systems will enhance the present potential and the range of applications of this tool in the near future.

The ultimate challenge of our upcoming research is obviously the determination of the absolute configuration of chiral molecules in these chiral oriented media. This goal necessitates the evaluation a priori of the order parameters of any *ij* internuclear vectors, S_{ij}, for each of the enantiomers. In terms of chiral differentiation, we should be able to predict (at least qualitatively) whether $S_{ij}^{R} > S_{ij}^{S}$ or $S_{ij}^{R} < S_{ij}^{S}$. Hence, it should be possible to assign the larger (or smaller) deuterium quadrupolar doublets for one of the enantiomers for a given site. It is highly probable that a direct correlation between the CIP symbols, which ascribes priorities of ligands on the stereogenic tetrahedral centers on the basis of atomic number, and the magnitude of quadrupolar splitting is not possible.[64, 93] Priorities based on the ligand volumes, their electronic profile and their chemical nature will probably provide more reliable correlations.[94–96] Whatever the system priority proposed, it will be necessary to develop a realistic model in order to take into account all contributions involved in the chiral discrimination and their subtle balance. We believe the experimental results presented in this paper will contribute to this task, because they demonstrate the role of geometrical factors in the chiral recognition mechanisms. The comparison between theoretical results and experimental data collected using NAD NMR should enable numerous parameters of the model to be adjusted. Here again, the advent of powerful computer-aided molecular-dynamics (MD) simulations based on reliable molecular force-field parameters for the polypeptide will facilitate this work.[45, 46, 97]

Besides, if two enantiomers can be discriminated by NMR using organic solutions of PBLG, it would be of interest to investigate its use as mesogenic chiral stationary phase in HPLC.[98] In a preliminary publication, it has recently been shown that a large set of nonchiral olefinic compounds were resolved by HPLC using PBLG as stationary phase.[99] The investigation of the enantioseparation of chiral hydrocarbons by this kind of CSPs is now in progress.

Experimental Section

Materials and general comments: In NAD NMR applications, the preparation of oriented samples is crucial because a lack of macroscopic homogeneity may strongly affect the quality of spectra, in particular the linewidths, and hence the signal-to-noise (S/N) ratio for solute signals. The typical procedure to prepare suitable samples for NAD NMR consists of directly weighing 80–100 mg of the investigated material, 80–100 mg of polymer and adding about 350–500 mg of co-solvent into a 5 mm NMR tube.[29] Under these conditions, the total volume of the sample is optimal compared to the length of the coil of a 5 mm diameter probe head. The exact composition of each NMR sample is given in column 1 of Tables 1–6. The accuracy of the weighing procedure is ± 0.5 mg. Unless other specified, we have utilised PBLG with a degree of polymerisation (DP) of 562 ($M_w \approx 120\,000$) and dry chloroform as organic solvent in this study. Chloroform dissolves a large variety of organic material, and the temperature of PBLG/$CHCl_3$ samples can be varied over a wide range (from 230 to 360 K).[85] The rapid solute motions in this oriented phase generally leads to narrow lines (3–10 Hz) in the $^2H\{^1H\}$ spectra. Furthermore, chloroform contains a single deuterated isotopomer that gives rise to a single additional quadrupolar doublet in the NAD spectrum which is easily recognised in the spectra. Note that the shape and the linewidth of chloroform signals provided a strict control of the magnet stability as well as possible time evolution of the sample homogeneity during the NAD experiments. In addition, the number of deuterium atoms per unit volume is not exceedingly large relative to those of the chiral solutes, thus minimising the digitisation problems associated with the dynamic range of the analogue-to-digital converter (ADC).[100] The last advantage of chloroform as co-solvent is to provide a strong proton signal which can be efficiently used to shim the magnet of the spectrometer from the proton FID. To avoid the evaporation of deuterochloroform during long NMR experimental time, it is convenient to seal the samples. Note that it can also be useful to perform several freeze-pump-thaw cycles to remove paramagnetic oxygen dissolved in the sample that may contribute to NMR linewidths of solutes, but this step is not usually required. Before recording the NAD NMR spectra, it is recommended to keep the sample in the magnetic field for about 15–30 min in order to achieve good thermal equilibration. When spectral resolution is rather hard to achieve, centrifugation and rehomogenization of the sample to remove concentration gradients in the sample is needed for a successful observation of good quality NAD spectra.

NMR measurements: NAD 1D and 2D NMR experiments in polypeptidic oriented solvents can be performed on routine spectrometers equipped with a direct or inverse BB probe, and hence no additional hardware is required.[41, 42] Nevertheless, the use of high magnetic field spectrometers and a selective deuterium probe is advantageous to enhance the detection of deuterium nuclei at natural abundance level.[100] In this work, all NAD NMR spectra were recorded on a Bruker DRX-400 high-resolution NMR

spectrometer (9.4 T) equipped with a 5 mm selective deuterium probe (61.4 MHz) with fluorine lock (376.5 MHz) developed by Bruker France, and a standard variable temperature control unit (BVT 3000). The fluorine field-frequency-lock device was required for an overnight acquisition only if the field drift gave rise to a significant line broadening. All NAD 1D and 2D NMR experiments presented here were recorded with digital filtering and over-sampling to enhance the dynamic range of the ADC and applying the WALTZ-16 composite pulse sequence to decouple protons. Note that the proton decoupling in NAD applications did not necessitate more power than for isotropic samples because of the small magnitude of the residual $^1H-^2H$ dipolar couplings (few hertz). The NMR tube was not spun in the magnet and its temperature was regulated carefully (temperature for each sample is given in Tables 1–6). Obviously, for each sample, the tuning and the matching of the deuterium coil were optimized to induce maximum NMR signal. Unless otherwise specified, the NAD Q-COSY experiments were recorded using 320 FIDs for each t_1 increment, using a recycling delay around 0.5 s. All 2D spectra were zero-filled to 1024 (t_1) × 2048 (t_2) data points prior to 2D FT and then symmetrized.

Acknowledgements

The authors thank Prof. H. Kagan for the kind gift of a racemic mixture of 2,2′-dimethyl-1,1′-binaphthyl. We are indebted to Dr. J. M. Péchiné for the gift of (±)-**5**, (±)-**7** and (±)-**18**. We thank Prof. J. W. Emsley and Prof. M. Longeri for fruitful discussions.

[1] D. Parker, *Chem. Rev.* **1991**, *91*, 1441.
[2] R. Rothchild, *Enantiomer* **2000**, *5*, 457.
[3] P. Schreier, A. Bernreuther, M. Huffer, *Analysis of Chiral Organic Molecules*, de Gruyter, Berlin, **1995**.
[4] W. H. Pirkle, C. W. Boeder, *J. Org. Chem.* **1977**, *42*, 3697.
[5] T. J. Wenzel, R. E. Sievers, *Anal. Chem.* **1981**, *53*, 393.
[6] T. J. Wenzel, R. E. Sievers, *J. Am. Chem. Soc.* **1982**, *104*, 382.
[7] W. Offermann, A. Mannschreck, *Tetrahedron Lett.* **1981**, *22*, 3227.
[8] W. Offermann, A. Mannschreck, *Org. Magn. Reson.* **1984**, *22*, 355
[9] M. Goldman, Z. Kustanovitch, S. Weinstein, A. Tishbee, E. Gil-Av, *J. Am. Chem. Soc.* **1982**, *104*, 1093.
[10] P. Salvadori, G. Uccello-Barretta, R. Lazzaroni, A. Caporusso, *Chem. Commun.* **1990**, 121.
[11] For an overview of the use of chiral platinium(II) complexes, see: G. Uccello-Barretta, R. Bernardini, F. Balzano, P. Salvadori, *Chirality* **2002**, *14*, 484.
[12] D. Parker, R. J. Taylor, *Chem. Commun.* **1987**, 1781.
[13] D. Parker, R. J. Taylor, *Tetrahedron* **1988**, *44*, 2241
[14] K. Wypchlo, H. Duddeck, *Tetrahedron: Asymmetry* **1994**, *5*, 27
[15] J. M. Buriak, J. A. Osborn, *Chem. Commun.* **1995**, 689
[16] To find more information, an entire issue of Chemical Review was devoted to cyclodextrins: *Chem. Rev.* **1998**, *98*, 1751–2076.
[17] W. A. König in *Gas Chromatography: Enantiomer Separations with Modified Cyclodextrins*, Hüthig, Heidelberg, **1992**.
[18] C. J. Easton, S. F. Lincoln, *Chem. Soc. Rev.* **1996**, *25*, 163.
[19] A. Botsi, K. Yannakopoulou, E. Hadjoudis, B. Perly, *Chem. Commun.* **1993**, 1085.
[20] A. Botsi, B. Perly, E. Hadjoudis, *J. Chem. Soc. Perkin Trans. 2* **1997**, 89.
[21] G. Uccello-Barretta, F. Balzano, A. M. Caporusso, P. Salvadori, *J. Org. Chem.* **1994**, *59*, 836.
[22] G. Uccello–Barretta, F. Balzano, A. M. Caporusso, A. Iodice, P. Salvadori, *J. Org. Chem.* **1995**, *60*, 2227.
[23] G. Uccello–Barretta, F. Balzano, P. Salvadori, R. Larazoni, A. M. Capurosso, R. Menicagli, *Enantiomer* **1996**, *1*, 365.
[24] G. Uccello-Barretta, F. Balzano, A. Cuzzola, R. Menicagli, A. M. Capurosso, P. Salvadori, *Gaz. Chim. Ital.* **1997**, *127*, 383.
[25] G. Uccello-Barretta, F. Balzano, R. Menicagli, P. Salvadori, *J. Org. Chem.* **1996**, *61*, 363.
[26] H. Dodziuk, J. Sitkowski, L. Stefaniak, J. Jurerczak, D. Sybilska, *Chem. Commun.* **1992**, 207.
[27] W. A. König, D. Icheln, T. Runge, I. Pforr, A. Krebs, *J. High Resolut. Chromatogr.* **1990**, *13*, 702.
[28] U. J. Meierhenrich, W. H. P. Thiemann, F. Goesmann, R. Roll, H. Rosenbauer, *Chirality* **2001**, *13*, 454.
[29] M. Sarfati, P. Lesot, D. Merlet, J. Courtieu, *Chem. Commun.* **2000**, 2069, and references therein.
[30] C. Aroulanda, M. Sarfati, J. Courtieu, P. Lesot, *Enantiomer* **2001**, *6*, 281.
[31] J. W. Emsley, J. C. Lindon, *NMR Spectroscopy Using Liquid Crystal Solvents*, Pergamon Press, Oxford, **1975**.
[32] P. Lesot, D. Merlet, T. T. Rantala, J. Jokisaari, J. W. Emsley, J. Courtieu, *J. Phys. Chem.* **1997**, *101*, 5719
[33] a) I. Canet, A.Meddour, J. Courtieu, J. L. Canet, J. Salaün, *J. Am. Chem. Soc.* **1994**, *116*, 2155; b) V. Madiot, P. Lesot, D. Grée, J. Courtieu, R. Grée, *Chem. Commun.* **2000**, 169.
[34] E. Sackmann, S. Meiboom, L. C. Snyder, *J. Am. Chem. Soc.* **1968**, *90*, 2183.
[35] a) A. S. Tracey, P. Diehl, *FEBS Lett.* **1975**, *59*, 131; A. S. Tracey, K. Radley, *J. Phys. Chem.* **1984**, *88*, 6044; b) J. Courtieu, J. P. Bayle, E. Lafontaine, *J. Am. Chem. Soc.* **1989**, *111*, 8294.
[36] A. Meddour, P. Berdagué, A. Hedli, J. Courtieu, P. Lesot, *J. Am. Chem. Soc.* **1997**, *119*, 4502.
[37] J. Courtieu, E. Lafontaine, J. M. Péchiné, C. L. Mayne, *Liq. Cryst.* **1990**, *7*, 293.
[38] J. P. Bayle, J. Courtieu, E. Gabetty, A. Loewenstein, J. M. Péchiné, *New J. Chem.* **1992**, *16*, 837.
[39] I. Canet, J. Courtieu, A. Loewenstein, A. Meddour, J. M. Péchiné, *J. Am. Chem. Soc.* **1995**, *117*, 6520.
[40] A. Meddour, I. Canet, A. Loewenstein, J. M. Péchiné, J. Courtieu, *J. Am. Chem. Soc.* **1994**, *116*, 9652.
[41] P. Lesot, D. Merlet, A. Loewenstein, J. Courtieu, *Tetrahedron: Asymmetry* **1998**, *9*,1871.
[42] D. Merlet, B. Ancian, J. Courtieu, P. Lesot, *J. Am. Chem. Soc.* **1999**, *121*, 5249.
[43] A. L. Lehninger, *Biochemistry*, Worth Publishers, New York, **1978**.
[44] K. Czarniecka, E. T. Samulski, *Mol. Cryst. Liq. Cryst.* **1981**, *63*, 205.
[45] A. Abe, N. Kimura, *Macromolecules* **1989**, *22*, 2138 and A. Abe, N. Kimura, *Macromolecules* **1989**, *22*, 2145.
[46] N. Kimura, A. Abe, *Rep. Prog. Polymer Phys. Jpn.* **1992**, *35*, 547 and references therein.
[47] A. Abe, N. Kimura, *Computer Aided Innovation New Mater.* **1993**, 1409.
[48] R. W. Duke, D. B. Du Pré. W. A. Hines, E. T. Samulski, *J. Am. Chem. Soc.* **1976**, *98*, 3094.
[49] C. P. Slitcher, *Principles of Magnetic Resonance*, Springer, Berlin, **1990**.
[50] C. Canlet, D. Merlet, P. Lesot, A. Meddour, A. Loewenstein, J. Courtieu, *Tetrahedron: Asymmetry* **2000**, *11*, 1911.
[51] D. Merlet, M. Sarfati, B. Ancian, J. Courtieu, P. Lesot, *Phys. Chem. Chem. Phys.* **2000**, *2*, 2283.
[52] The tilting process is achieved by using the computational algorithm developed for the *J*-resolved 2D experiments and usually implemented in the NMR software.
[53] a) J. Lounila, P. Diehl, *J. Magn. Reson.* **1984**, *56*, 254; b) J. Lounila, P. Diehl, *Mol. Phys.* **1984**, *52*, 827; c) J. Lounila, *Mol. Phys.* **1986**, *58*, 897; d) E. E. Burnell, C. A. de Lange, *Chem. Rev.* **1998**, *98*, 2359.
[54] Y. Uematsu, I. Uematsu, *Adv. Polymer Sci.* **1984**, *59*, 37.
[55] a) S. Dev, *CRC Handbook of Terpenoids*, *Vol. 1*, CRC press, Boca Raton (USA), **1982**; b) J. B. Harbone, *Introduction to Ecological Biochemistry*, Academic Press, London, **1977**; c) A. Botsi, K. Yannakopoulou, E. Hadjoudis, *Carbohydr. Res.* **1993**, *241*, 37.
[56] T. Koscielski, D. Sybilska, J. Jurczak, *J. Chromatogr.* **1983**, *280*, 131.
[57] T. Koscielski, D. Sybilska, J. Jurczak, *J. Chromatogr.* **1986**, *364*, 299.
[58] V. Schurig, H. P. Nowotny, *J. Chromatogr.* **1988**, *441*, 155.
[59] M. Lindström, T. Norin, J. Roeraade, *J. Chromatogr.* **1990**, *513*, 315.
[60] V. Schurig, H. P. Nowotny, *Angew. Chem.* **1990**, *102*, 969; *Angew. Chem. Int. Ed.* **1990**, *29*, 939, and references therein.
[61] a) R. J. Ochocka, D. Sybilska, M. Asztemborska, J. Kowalczyk, J. Goronowicz, *J.* of *Chromatogr.* **1991**, *543*, 171; b) D. Sybilska, J. Kowalczyk, M. Asztemborska, R. J. Ochocka, H. Lamparczyk, *J. Chromatogr. A* **1994**, *665*, 67.
[62] D. W. Armstrong, J. Zukowski, *J. Chromatogr.* **1994**, *666*, 445.

  0947-6539/03/0908-1744 $ 20.00+.50/0

[63] A. Botsi, K. Yannakopoulou, E. Hadjoudis, B. Perly, *Chem. Commun*. **1993**, 1085.
[64] A. L. Eliel, A. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**.
[65] T. J. Johnson, S. Burke, C. P. Schultz, B. Jun, D. L. Weaver, H. H. Drews, M. Boese, *Bruker Report* **2000**, *148*, 44.
[66] G. J. Martin, M. L. Martin, B. L. Zhang, *Plant Cell Environ.* **1992**, *15*, 1037.
[67] D. W. Armstrong, W. Li, C. D. Chang, J. Pitha, *Anal. Chem*. **1990**, *62*, 914.
[68] D. Merlet, P. Lesot, J. Courtieu, Unpublished results.
[69] S. Hanneguelle, J. N. Thibault, N. Naulet, G. J. Martin, *J. Agric. Food Chem*. **1992**, *40*, 81.
[70] V. Schurig, H. P. Nowotny, D. Schmalzing, *Angew. Chem*. **1989**, *101*, 785; *Angew. Chem. Int. Ed*. **1989**, *28*, 736.
[71] W. A. König, R. Krebber, G. Wenz, *J. High Resolut. Chromatogr*. **1989**, *12*, 790.
[72] R. Menicagli, O. Piccolo, L. Lardicci, M. L. Wiss, *Tetrahedron* **1979**, *35*, 1301
[73] J. Ehlers, W. A. König, S. Luts, G. Wenz, H. Dieck, *Angew. Chem*. **1988**, *100*, 1614; *Angew. Chem. Int. Ed*. **1988**, *27*, 1556.
[74] L. Ernst, P. Schulz, *Mag. Reson. Chem*. **1992**, *30*, 73.
[75] J. W. Emsley, *Encyclopedia of NMR* (Eds.: D. M. Grant, R. K. Harris), Wiley, Chichester, **1996**, pp. 2781.
[76] M. Sarfati, J. Courtieu, P. Lesot, *Chem. Commun*. **2000**, 1113.
[77] J. Curtis, D. K. Dalling, D. M. Grant, *J. Org. Chem*. **1986**, *51*, 136.
[78] D. M. Grant, J. Curtis, W. R. Croasmun, D. K. Dalling, *J. Am. Chem. Soc*. **1982**, *104*, 4492.
[79] A. Mannschreck, W. Munninger, T. Burgemeister, J. Gore, B. Cazes, *Tetrahedron* **1986**, *42*, 399.
[80] L. Pu, *Chem. Rev*. **1998**, *98*, 2405.
[81] P. Lesot, D. Merlet, A. Meddour, A. Loewenstein, J. Courtieu, *J. Chem. Soc. Faraday Trans.* **1995**, *91*, 1371.
[82] H. A. Kalinowski, S. Berger, S. Braun, *Carbon-13 NMR Spectroscopy*, Wiley, New York, **1988**.
[83] a) D. M. Grant, D. K. Dalling, L. F. Johnson, *J. Am. Chem. Soc*. **1971**, *93*, 3678; b) D. K. Dalling, D. M. Grant, E. G. Paul, *J. Am. Chem. Soc*. **1973**, *95*, 3718.
[84] T. Koscielski, D. Sybilska, J. Lipkowski, A. Miediokritskaja, *J. Chromatogr*. **1986**, *351*, 512.
[85] M. Sarfati, C. Aroulanda, J. Courtieu, P. Lesot, *Tetrahedron: Asymmetry* **2001**, *12*, 737.
[86] R. J. Abraham, M. A. Warne, L. Griffiths, *J. Chem. Soc. Perkin Trans. 2* **1997**, 31.
[87] A. Parenty, J.-M. Campagne, C. Aroulanda, P. Lesot, *Org. Lett*. **2002**, *4*, 1663.
[88] a) K. Tabayashi, K. Akasaka, *J. Chem. Phys.* **1997**, *101*, 5108; b) K. Tabayashi, K. Akasaka, *Liq. Cryst.* **1999**, *26*, 127.
[89] Y. W. Kim, W. L. Earl, R. E. Norberg, *J. Magn. Reson*. **1995**, *116*, 139.
[90] R. R. Ernst, G. Bodenhausen, A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford, **1987**.
[91] A. F. Terzis, D. J. Photinos, *Mol. Phys.* **1994**, *83*, 847.
[92] A. F. Terzis, C. D. Poon, E. T. Samulski, Z. Luz, R. Poupko, H. Zimmermann, K. Müller, H. Toriumi, D. J. Photinos, *J. Am. Chem. Soc*. **1996**, *118*, 2226.
[93] R. S. Cahn, C. K. Ingold, V. Prelog, *Angew. Chem*. **1966**, *78*, 413; *Angew. Chem. Int. Ed.* **1966**, *5*, 385.
[94] H. J. Krabbe, H. Heggemeier, B. Schrader, E. H. Korte, *J. Chem. Res.* **1978**, (S) 238, (M) 3023.
[95] A. Meddour, A. Loewenstein, J. M. Pechiné, J. Courtieu, *Tetrahedron: Asymmetry* **1997**, *8*, 485.
[96] P. Chalard, M. Bertrand, I. Canet, V. Théry, R. R. Remuson, G. Jeminet, *Org. Lett*. **2000**, *2*, 2431.
[97] a) E. Alvira, J. Breton, J. Plata, *Chem. Phys.* **1991**, *155*, 7; J. Helfrich, R. Hentschke, U. M. Apel, *Macromolecules* **1994**, *27*, 472; b) G. Celebre, *J. Chem. Phys.* **2001**, *115*, 9552.
[98] L. Sojak, I. Ostrovsky, G. Kraus, T. G. Andronikashvili, *J. Chromatogr*. **1988**, *436*, 47; b) P. E. Sonnet, R. R. Heath, *J. Chromatogr.* **1985**, *321*, 127.
[99] O. Ferroukhi, S. Guermouche, M. H. Guermouche, P. Berdagué, J. Courtieu, J. P. Bayle, *Chromatographia* **2000**, *51*, 701.
[100] R. Freeman, *Nuclear Magnetic Resonance*, 2nd ed., Cambridge University, Addison, Wesley Longman, **1997**.

Received: July 15, 2002
Revised: October 21, 2002 [F4251]