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The measurement of high enantiomeric excesses in chiral liquid crystals using ^{19}F NMR and exchangeable protons in ^{2}H NMR

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It is shown that the ^{19}F spectrum and the ^{2}H NMR spectrum of deuterated protons in a chiral liquid crystal medium can be used to measure high enantiomeric excesses.

Within the pharmaceutical industry measuring enantiomeric excess, or more particularly detecting small levels of the minor enantiomer is extremely important. This can be done in a number of ways by NMR using chiral derivatizing agents or solvating agents. All these methods rely on differences in chemical shift to resolve enantiomers, and in some cases no discrimination will be observed.[1] However one method uses a chiral liquid crystal solvent, such as a solution of poly-γ-benzyl-L-glutamate (PBLG) within an organic solvent. As the phase is liquid-crystalline, resonances of enantiomers are not resolved through the isotropic chemical shift but, because of the differential ordering of enantiomers, through chemical shift anistropies, $\Delta\sigma$, residual dipolar couplings, D_{ij}, and for spins > ½, the quadrupolar interaction, $\Delta\nu_Q$.[2] This increases the chances of observing some discrimination as a variety of different nuclei and parameters can be used to achieve separation.[3]

The largest discrimination, however, is observed with deuterium, as the quadrupolar interaction is much larger than the other interactions and so the separation between the resonances of enantiomers is large. The commonest approach therefore is to synthetically deuterate the molecule under question and acquire the ^{2}H-{^{1}H} spectrum.[4] Although this approach is amenable to observe small amounts of the minor enantiomer (deuterium is a sensitive nucleus)—the need for deuterium labelling is undesirable. In this case other nuclei must be considered as natural abundance deuterium methods are far too insensitive a technique for high e.e.'s.[5]

^{1}H NMR spectra are broad due to the many proton–proton dipolar couplings. This makes separating the resonances of enantiomers difficult, although it has been done using two-dimensional techniques.[6] ^{13}C-{^{1}H} NMR can provide good discrimination[7] but of course suffers from poor sensitivity. Therefore the best option, if available, is ^{19}F-{^{1}H} NMR as it is sensitive and sharp simple spectra are usually produced—we have measured enantiomeric excesses greater than 99%.

As an example compound we have used 2,2,2-trifluoro-(α-methylbenzyl)acetamide **1**.[8] The ^{19}F-{^{1}H} spectrum of the *R* enantiomer of **1** in PBLG/$CDCl_3$ is a triplet, due to the F–F dipolar coupling.[9,10] Addition of the other enantiomer creating a) a 12% e.e. of the *R*-form and b) 94% e.e. of the *R*-form (assuming enantiomers sourced from Aldrich are enantiomerically pure) showed additional triplets as shown in Fig. 1. Discrimination in Fig. 1a is achieved by a difference in chemical shift of 159 Hz and dipolar couplings of 24 Hz and 18 Hz.

Using linefitting techniques[11] for Fig. 1b an e.e. of 93% was calculated which is very close to the expected value of 94%.

To highlight the sensitivity of fluorine, a closer look at the 'pure' *R* enantiomer as shown in Fig. 2 reveals, at approximately 1/10th the size of the ^{13}C satellites, the S triplet—this corresponds to an approximate e.e. of 99.9%. At this level the accuracy of the measurement is not critical, but simply the fact that the minor enantiomer can be detected.

Whilst many pharmaceutical compounds contain fluorine there are equally as many that do not. We therefore propose returning to ^{2}H NMR and making use of slowly exchanging protons—such as amides which are present in many pharmaceutical compounds.

To do this **1** was selectively deuterated by dissolving it in MeOD. The solvent was then evaporated and the residue dissolved and evaporated in MeOD once more. A sample was then prepared in PBLG/$CHCl_3$ as before.[9]

The molecule now has one ND present so the ^{2}H-{^{1}H} spectrum contains one doublet due to the quadrupolar splitting of the deuterium. Since the ND is slowly exchanging the signals will appear relatively sharp. As shown in Fig. 3 enantiomeric excesses up to 98% can be measured relatively quickly. In Fig. 3b the *S*

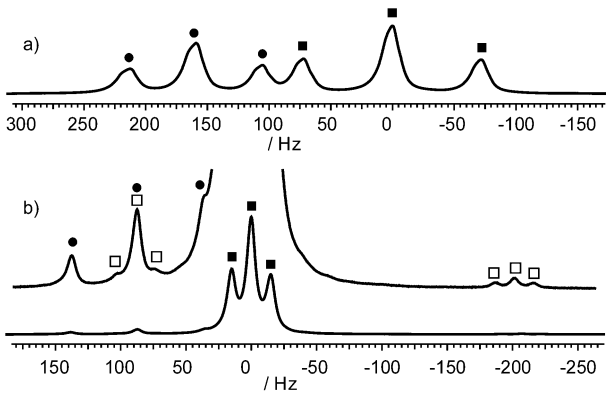


Fig. 1 ^{19}F-{^{1}H} NMR spectrum of **1** in a solution of PBLG/$CDCl_3$ (approx. 20 wt% PBLG) at 300 K a) 12% e.e *R*-form (experimental time 2 min) b) 94% e.e. *R*-form (experimental time 8 min). Peaks marked ■ belong to the *R*-form, those marked ● belong to the *S*-form and those marked □ are ^{13}C satellites.

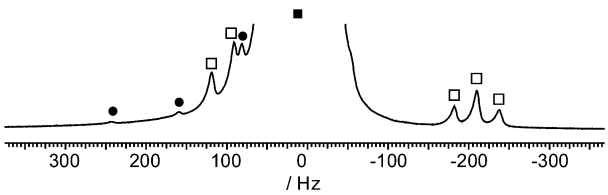
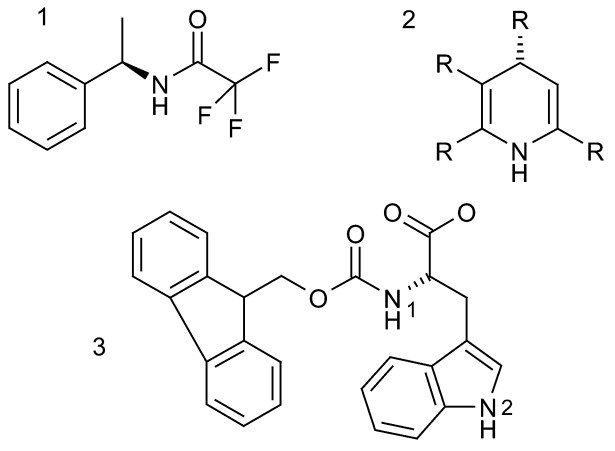


Fig. 2 ^{19}F-{^{1}H} NMR spectrum of 'pure' **1** in a solution of PBLG/$CDCl_3$ at 280 K (experimental time 2.3 hours). Peaks are labelled as per Fig. 1.



Scheme 1

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enantiomer had a quadrupolar splitting of 5013 Hz and the *R* enantiomer 4372 Hz, so the separation of the peaks, 641 Hz, is significantly greater than the linewidth of approximately 50 Hz.

Using linefitting techniques for Fig. 3a an e.e. of 97.6% was calculated which again agrees well with the expected 98.6%.

Firstly, to prove the method was not molecule specific, a current drug candidate **2** containing a slowly exchanging NH was considered. The sample was prepared in an identical fashion to **1** although **2** has very limited solubility in chloroform so the signal to noise ratio is significantly reduced. In addition since the NH is not an amide it might be expected that ND peaks will be slightly broader than for **1**.

Fig. 4 shows the expected discrimination but since the splitting of peaks 5165 Hz and 5366 Hz is comparable to the linewidth 80 Hz it was not possible to measure the enantiomeric excess accurately.

Secondly compound **3**, Fmoc-tryptophan, was examined. This is more challenging as it contains two possible sites for exchange and it is insoluble in $CDCl_3$. Fig. 5a shows a 24% e.e. sample of Fmoc-D-tryptophan prepared in PBLG/THF. At 300 K discrimination is seen in the ND(1) doublet with splittings of 1645 and 1207 Hz, but the doublet from ND(2) is not resolved. By increasing the temperature this ND(2) doublet is resolved (the ordering of the sample results in the doublet of ND(1) getting smaller with increased temperature, whilst the doublet of ND(2) gets larger)—Fig. 5b shows a 81% e.e. sample that discriminates enantiomers despite the splittings only being 155 and 68 Hz. Using linefitting the e.e. can be estimated as 83%.

In conclusion, we have shown that ^{19}F NMR can be used to measure extremely high enantiomeric excesses, and, if molecules contain slowly exchanging Hs, that by deuterating these exchangables very simple ^2H NMR spectra are produced which can be used to discriminate and quantify enantiomers. It should be noted that whilst the studies here have been limited to nitrogen bound protons the same principle could be applied to carbon bound exchangeable protons, such as methylenes next to carbonyl groups or other protons with relatively low pK_as.

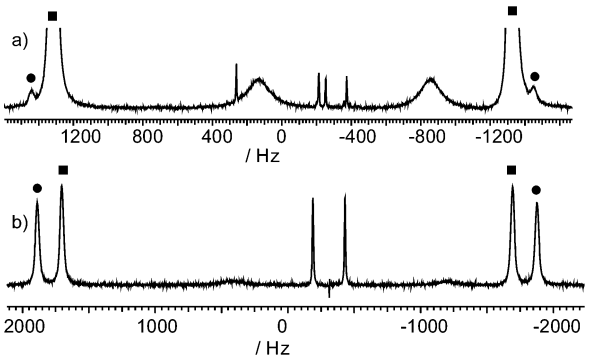

Fig. 3 ^2H-{^1H} NMR of deuterated **1** in a solution of PBLG/$CHCL_3$ at 300 K a) 98% e.e *R*-form (experimental time 17 hours) b) 8% e.e. *R*-form (experimental time 10 min). Peaks are labelled as per Fig. 1. The additional central peaks are from residual deuterated solvent.

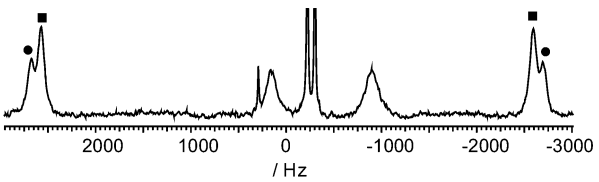

Fig. 4 ^2H-{^1H} NMR of deuterated **2** in a solution of PBLG/$CHCl_3$ at 329 K in a 24% e.e. *R*-form (experimental time 16.5 hours).

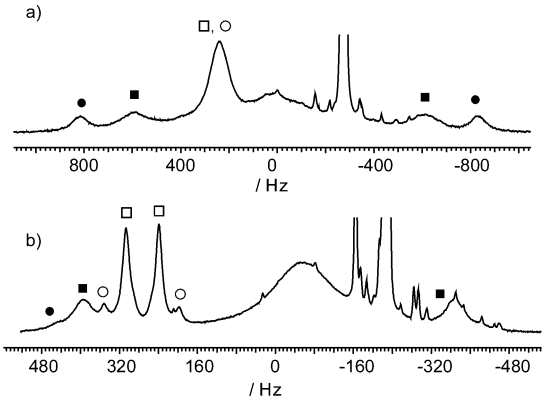

Fig. 5 ^2H-{^1H} NMR of deuterated **3** in a solution of PBLG/THF a) 300 K with a 24 % e.e. D-form (experimental time 2 hours) and b) 81% e.e. D-form (experimental time 3 hours). Peaks marked ■ arise from the D-form of ND(1), ● from the L-form of ND(1), □ from the D-form of ND(2) and ○ from the L-form of ND(2). Other peaks are from residual MeOD/d_8-THF and PBLG.

Notes and references

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9 *Sample preparation:* PBLG (180 mg, DP 502, MW 110 000, Sigma Chem. Co.) was placed in an NMR tube. For ^{19}F experiments up to 70 mg **1** was dissolved in approximately 750 ml $CDCl_3$ and then added to the NMR tube containing PBLG, which was then allowed to dissolve slowly. Finally the tube was sealed and mixed back-and-forth before being left to reach equilibrium for at least 30 minutes. For ^2H experiments the same procedure was used except that first the compound of interest was dissolved in 1 ml MeOD for up to 12 hours. The solvent was then evaporated off and the residue dissolved and evaporated in MeOD once more. This was then dissolved in $CHCl_3$ or THF as appropriate rather than $CDCl_3$.

10 Both ^{19}F and ^2H spectra were recorded on a Bruker DRX 500 using the lock coil to acquire deuterium. Adiabatic proton decoupling was used to prevent sample heating.

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