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NMR in chiral polypeptide liquid crystals: the problem of amines

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Abstract—It is shown that lyotropic liquid crystal mixtures made of poly-γ-benzyl-L-glutamate (PBLG) dissolved in *N*,*N*-dimethylformamide (DMF) are efficient anisotropic NMR solvents to distinguish the enantiomers of chiral amines through the effects of the differential ordering of enantiomers. This type of solvent overcomes problems often encountered when dissolving amines into the more conventional PBLG/$CHCl_3$ or PBLG/CH_2Cl_2 liquid crystals. Furthermore, it is shown that perdeuterobenzyl chloride is an excellent achiral deuterated derivatizing agent for enantiomeric excess measurements of chiral amines in conjunction with the PBLG/DMF solvent.

1. Introduction

Over the last few years, we have been developing an original NMR method to distinguish and quantify enantiomers. This method is based on the use of chiral ordering media as NMR solvents.[1,2] The basic phenomenon is that two enantiomers dissolved in chiral liquid crystals are not ordered the same and consequently all the order sensitive NMR interactions are different, namely, the chemical shift anisotropies, the dipolar couplings and the quadrupolar splittings for spins larger than 1/2 such as deuterium. The advantages of this technique are numerous.

First, the method is easy to implement on any spectrometer and most of the time only requires a simple 1D NMR experiment, although it may require some skill in the sample preparation (≈1 h).[1]

Second, the method is very efficient as it works with all types of compounds or chemical functions including hydrocarbons.[3]

Third, the chiral discrimination is not only seen through a chemical shift process like conventional methods.[4] It can be observed through the measurement of any of the above-mentioned anisotropic interactions. This means that if, for one reason or another, enantiomers cannot be observed through the observation of a dipole–dipole coupling, one can then try to observe a chemical shift anisotropic effect or a deuterium quadrupolar splitting effect, even at a natural abundance level.[1] Indeed, there are so many opportunities to observe the effects of the differential ordering of enantiomers that, statistically over hundreds of examples treated to date, the method failed to give the expected answer in less than 1% of the cases.

Fourth, the price of the analysis is quite inexpensive as quoted in Ref. 5.

Currently, the best results have been obtained using lyotropic chiral liquid crystals made up of organic solutions of synthetic homo-polypeptides such as poly-(γ-benzyl)-L-glutamate (PBLG), poly-(ε-carbobenzoxy)-L-lysin (PCBLL) and poly-(γ-ethyl)-L-glutamate (PELG). A large variety of organic co-solvents can be used such as chloroform, dichloromethane, 1,1,2,2-tetrachloroethane, tetrahydrofuran, 1,4-dioxane or *N*,*N*-dimethylformamide (DMF). The only condition is to use a helicogenic solvent in order to preserve the α-helical conformation of the polypeptide backbone, an essential condition in order to obtain a homogeneous liquid crystalline solution.

Amongst the collaborations with various chemical laboratories working on asymmetric synthesis, we have recently been involved with a group working on chiral amines.[6,7]

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On this occasion we encountered an uncommon problem that made our technique inefficient. Owing to the importance of amines, noticeably in pharmaceutical industries, herein we report a description of the problem encountered and the solutions investigated to solve it.

2. Results and discussion

2.1. Problem

Lasne et al. are involved in the asymmetric synthesis of *N*-arylaminopyrrolidines in the research of new ligands of serotoninergic receptors[6] and of new asymmetric reactions using chiral palladium organometallic-amine complexes.[7] Over the course of arylation of *N*-phenyl-3-aminopyrrolidine, diamine **1** was obtained (Fig. 1). The absence of any reaction of this amine with MTPA prevented the use of the Mosher's method to separate the enantiomers. Chiral capillary electrophoresis or chiral HPLC were unsuccessful neither. Consequently we decided to try the chiral liquid crystal technique.

Owing to the high sensitivity of quadrupolar splitting to the differential ordering of enantiomers, we decided to work first with a deuterated isotopomer of **1**, $\mathbf{1d}_5$, which can be easily synthesized (Fig. 1). The proton decoupled deuterium (^{2}H–{^{1}H}) NMR spectrum of $\mathbf{1d}_5$ dissolved in PBLG/$CHCl_3$ is reported in Figure 2a.

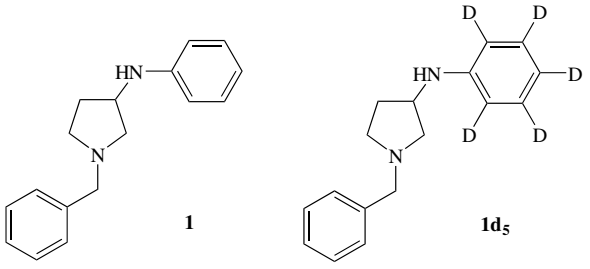


Figure 1.

This spectrum exhibits anomalous wide lines, with linewidth $\Delta\nu_{1/2} = 150$–200 Hz, and no chiral discrimination could be observed. In several other occasions working with amines with the same solvent we came across this type of problem of wide lines whose origin were still unclear. At first we thought this problem was the result of some type of exchange phenomenon. However, when heating or cooling the sample, no coalescence process was observed. To date we believe that the amines interact strongly with the polypeptide fibres, maybe through hydrogen bonds. Consequently, the molecular motion of the amine may be strongly affected and whenever the motion becomes too slow, T'_2s are shortened to values where high resolution is lost due to the efficiency of the quadrupolar relaxation process.

Believing that the problem could originate from the polypeptidic fibres we used a PCBLL/$CHCl_3$ liquid crystal solvent. The linewidth was narrower than in Figure 2a, but

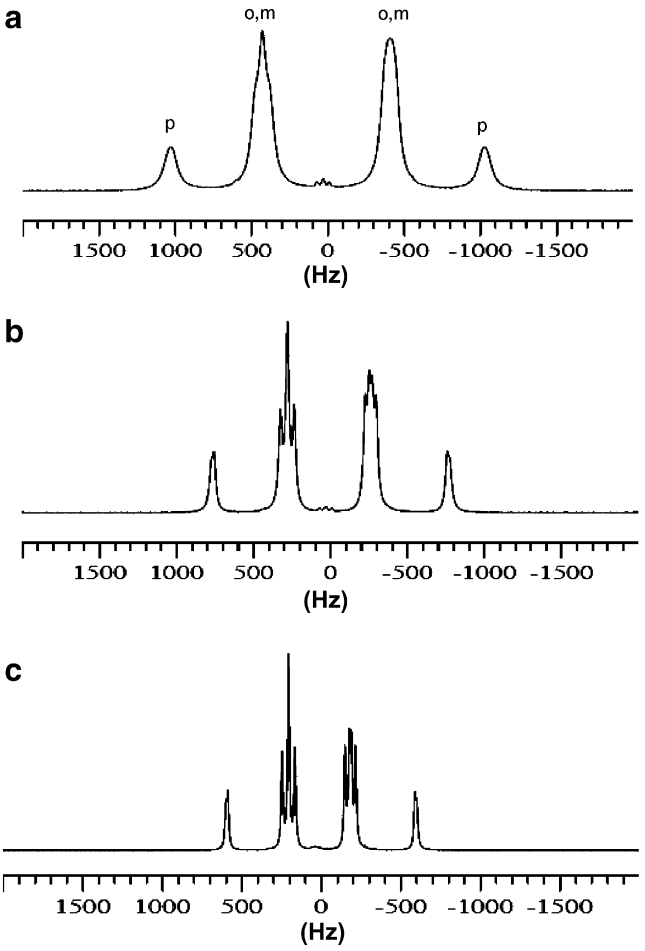


Figure 2. ^{2}H–{^{1}H} NMR spectra of racemic $\mathbf{1d}_5$ in PBLG liquid crystals at $T = 310$ K. a: PBLG/$CHCl_3$ (PBLG weight fraction $X_M = 19.5\%$); b: PBLG/$CHCl_3$/DMF (100.0/407.3/30.5; $X_M = 18.6\%$); c: PBLG/$CHCl_3$/DMF (100.0/404.6/61.5; $X_M = 17.7\%$).

not enough to observe a clear chiral discrimination. We then decided to add a small amount of DMF into the PBLG/$CHCl_3$ liquid crystal as this aprotic polar solvent is known to have a strong dissociative influence on electrostatic intermolecular associations. Furthermore, it is a good solvent for PBLG. Spectra b and c in Figure 2 were obtained by adding 7% and 13%, respectively, of DMF in the chloroform co-solvent of PBLG. Clearly the lines appear thinner as the DMF proportion in the co-solvent increases. At the same time, the molecular order parameters decrease with the amount of DMF as can be seen through the general lowering of the quadrupolar splittings. This last phenomenon supports the idea of an association to the PBLG fibres that is lowered by the addition of DMF to the $CHCl_3$ co-solvent.

The decrease in the NMR linewidth induced by the DMF led us to the conclusion that the latter solvent could be the ideal PBLG co-solvent to study chiral amines with this technique, despite the fact that PBLG/DMF is not the easiest liquid crystal to work with, since PBLG/DMF forms a gel at room temperature. Consequently, both the important steps of PBLG/DMF/chiral solute sample preparation

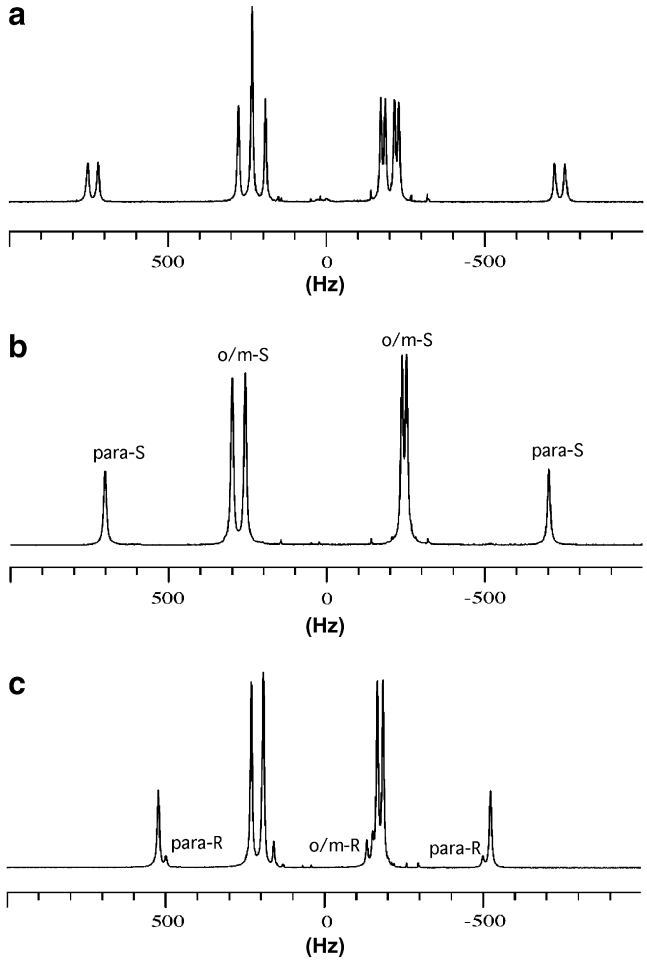


Figure 3. $^{2}H–\{^{1}H\}$ NMR spectra of a: Racemic $\mathbf{1d}_5$ in PBLG/DMF (PBLG weight fraction $X_M = 36.4\%$) at $T = 330$ K; b: *S*-enriched $\mathbf{1d}_5$ (22.0 mg), in PBLG/DMF ($X_M = 33.9\%$) at $T = 320$ K; c: *S*-enriched $\mathbf{1d}_5$ (22.0 mg) plus racemic $\mathbf{1d}_5$ (5.7 mg) in PBLG/DMF ($X_M = 29.0\%$) at $T = 320$ K.

and NMR experiments must be carried out at a temperature above 310 K.

In Figure 3a the $^{2}H–\{^{1}H\}$ NMR spectrum of 22 mg of racemic $\mathbf{1d}_5$ dissolved in PBLG/DMF chiral liquid crystal at 330 K is reported. This time, the spectrum is of a good quality and the line separations between enantiomeric signals are large enough to allow the measurement of an eventual enantiomeric excess. In Figure 3b, the spectrum of the (*S*)-enriched enantiomeric mixture obtained through asymmetric synthesis is presented. In this spectrum, no signal from the (*R*)-enantiomer could be detected as only three quadrupolar doublets are observed, one for each kind of deuterium (*ortho*, *meta* and *para*). Nevertheless, the presence of a very small amount of (*R*) cannot be excluded, the signal of which could be hidden in the foot of the (*S*)-enantiomer lines. In order to determine an accurate experimental value for the enantiomeric excess we added a known quantity of racemic $\mathbf{1d}_5$ (5.7 mg) to sample B. Spectrum 3-C corresponds to this mixture, where the signals from the (*R*) enantiomer are now observable. Careful integration and deconvolution of the *para* proton resonances allows evaluating the ee as 77%. Straightforward calculations lead to a minimum value ee >97% in B sample. In all these spectra, very small peaks are observed, which are due to the natural abundance deuterium signals of DMF. These may be used as an internal reference if needed.

2.2. Generalization of the method

The above result was first obtained owing to the convenient properties of the PBLG/DMF liquid crystal towards the amine orientation, but also to the simple introduction of the deuterated moiety over the course of the synthesis of $\mathbf{1d}_5$. The latter point cannot be made general for every amine synthesis and consequently, other techniques must be tested in order to demonstrate the efficiency and the generality of the PBLG/DMF anisotropic solvent in the enantiomeric quantification of chiral amines. Amongst the various possibilities derived from the above result we explored the idea of using perdeuterobenzyl chloride **2** as an achiral deuterated derivatizing agent to introduce deuterium nuclei into the molecule to be studied.

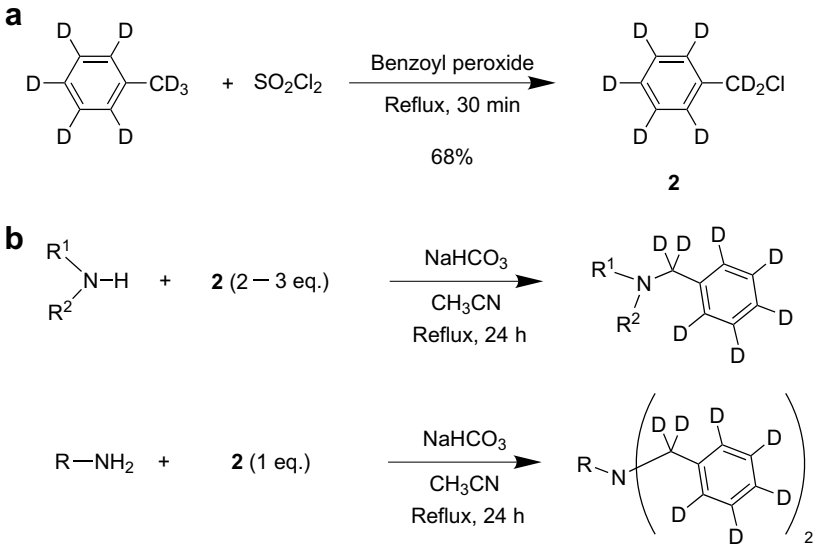
The idea of achiral deuterated derivatizing agents (ADDA) for alcohols acids, amines or amino acids is not new. The advantages of this strategy are:

1. There is no need to be concerned about the enantiomeric purity of the derivatizing agent since this one is not chiral.
2. There is no need to be concerned about the completeness of the reaction between the achiral derivatizing agent and the enantiomers to be studied since no kinetic resolution can take place.
3. The derivatizing agent is deuterated only to bring the deuterium spy nuclei into the adduct in order to take advantage of the deuterium quadrupolar splitting to observe enantiomers using a chiral liquid crystalline solvent such as PBLG/DMF.

We previously proposed various possible ADDA's for amines or amino acids such as acetyl chloride-d_3,[8] trifluoroacetic anhydride (using it as a fluorinated achiral derivatizing agent for fluorine NMR)[9] or pentadeuterated benzoyl chloride.[10] The main inconvenience of these derivatizing agents is that they yield to amides for which a possible chemical equilibrium between the *s-cis* and *s-trans* conformations of the amide group often causes some complexity in the spectral analysis along with a tremendous linewidth broadening. The use of perdeuterobenzyl chloride **2** as an ADDA can lead to amines and not amides, which therefore will not cause such problems. The latter, although commercially available, can be easily prepared from the much cheaper perdeuterotoluene with a standard procedure (Scheme 1a).

Monobenzylation of secondary amines and dibenzylation of primary amines were achieved in refluxing acetonitrile using one to three equivalent of benzyl chloride **2** (Scheme 1b).

The $^{2}H–\{^{1}H\}$ NMR spectrum of a single enantiomer of such perdeuterobenzylamines in PBLG-DMF liquid crys-



Scheme 1. a: Preparation of benzyl chloride **2** from perdeuterotoluene. b: Benzylation of amines.

tal was expected to contain ten peaks; that is, five quadrupolar doublets: three doublets with intensity one corresponding to the *para*, and the α and α′ diastereotopic deuterium of the methylene group and two others with intensity two corresponding to the *ortho* and *meta* deuterium. For a mixture of enantiomers, the spectrum should be doubled, meaning that five doublets for each enantiomer should be observed.

The first perdeuterobenzylamine to be tested was derived from a non-racemic mixture of 2-(hydroxymethyl)-pyrrolidine enantiomers (ee = 34.3% ± 0.1 (*S*)). The $^{2}H–\{^{1}H\}$ NMR spectrum of this compound in PBLG/DMF at 350 K is presented in Figure 4. It exhibits the spectral features depicted above.

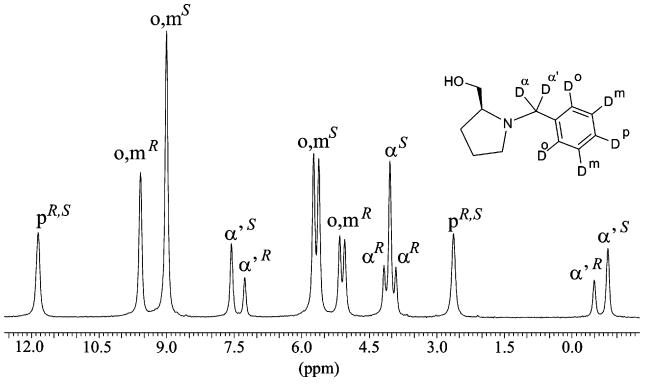


Figure 4. 61.4 MHz $^{2}H–\{^{1}H\}$ NMR spectrum the perdeuterobenzylamine derived from (*S*)-2-(hydroxymethyl)-pyrrolidine 34.3% ee in PBLG/DMF at $T = 350$ K. (PBLG weight fraction $X_M = 26.2\%$).

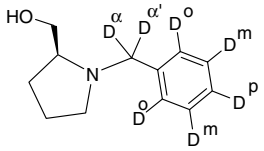
The assignment of the quadrupolar doublets to the corresponding deuterium and enantiomer was achieved using chemical shifts and integrations. Chemical shift anisotropy is negligible for deuterium NMR. Consequently, the chemical shifts of the deuterium nuclei in PBLG/DMF mesophase are essentially the same as those measured from the isotropic spectrum (7.35 and 7.32 ppm for *ortho* and *meta*; 7.24 ppm for *para*; 4.05 and 3.29 ppm for α and α′ deuterium). Furthermore, as the mixture was not racemic, it was possible to assign all the doublets of a given enantiomer.

The signals of all the deuterium atoms are doubled, that is, a proper signal to each enantiomer is observed, except for the *para*. This is a good illustration of having several different deuterium nuclei in an ADDA. Depending on the difference in orientation between the enantiomers, it can happen that no discrimination is observable along a given carbon–deuterium (C–D) direction, although this is almost never the case for all the other C–D directions. It should be noted in Figure 4 that the splitting of the α^S deuterium is null at this temperature. This means that the average angle between this C–D bond and the magnetic field is equal to the magic angle (54.7°) for the (*S*)-enantiomer.

Table 1 presents the absolute values of the quadrupolar splittings measured for each type of deuterium in each enantiomer ($\Delta\nu_Q^R$ and $\Delta\nu_Q^S$) at several temperatures. It also includes the value $|\Delta\nu_Q^R - \Delta\nu_Q^S|/2$, which is the spectral line separation of enantiomers for a given deuterium. Thus, for this example, the best separations are obtained for the *ortho* and *meta* deuterons. Increasing the temperature resulted in a decrease of the quadrupolar splittings as well as the NMR linewidth.

A simple integration of the different well separated signals gave an NMR estimated enantiomeric excess of 33% ± 2, which is in good agreement with the value of 34.4% ± 0.1 of the weighed preparation. A better precision on the NMR measured ee may be obtained by carrying out several independent acquisitions on the same sample, then taking an average of the measured integrations. With a signal to noise ratio larger than 100 and ten independent measurements, the mean standard deviation (MSD) on the average

Table 1. Absolute values of deuterium quadrupolar splittings ($\Delta\nu_Q^R$, $\Delta\nu_Q^S$) and spectral separation, $|\Delta\nu_Q^R - \Delta\nu_Q^S|/2$, for a perdeuterobenzylamine derived from (*S*)-2-(hydroxymethyl)-pyrrolidine 34.3% ee in PBLG/DMF (PBLG weight fraction $X_M = 26.2\%$) at different temperatures

Amine	*T* (K)	Site	$\lvert\Delta\nu_Q^R\rvert$ (Hz)	$\lvert\Delta\nu_Q^S\rvert$ (Hz)	$\lvert\Delta\nu_Q^R - \Delta\nu_Q^S\rvert/2$ (Hz)
	310	α	726	746	10
		α′	96	138	21
		o,m	468	355	56.5
		o,m	460	347	56.5
		p	686	654	16
	320	α	647	673	13
		α′	67	100	16.5
2-(Hydroxymethyl)-		*o,m*	410	308	51
pyrrolidine		*o,m*	402	301	50.5
ee = 34.3% (*S*)		*p*	647	625	11
PBLG/DMF $X_M = 26.2\%$	330	α	582	612	15
		α′	40	64	12
		o,m	360	269	45.5
		o,m	352	261	45.5
		p	616	602	7
	350	α	477	513	18
		α′	16[a]	0	8
		o,m	278	208	35
		o,m	272	201	35.5
		p	566	566	0

[a] Temperature evolution indicates a change in the sign of this quadrupolar splitting around 345 K.

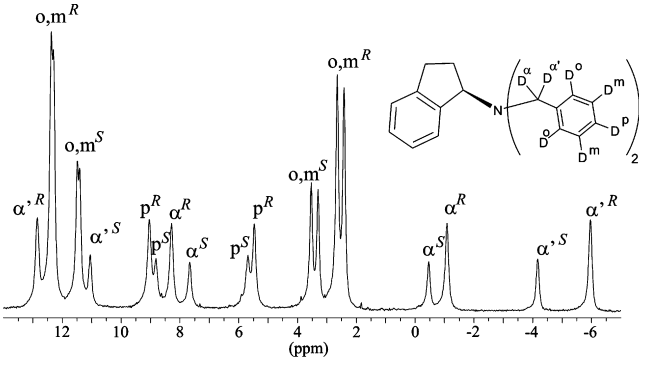
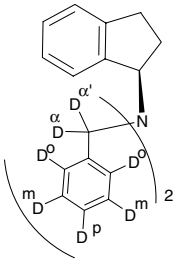


Figure 5. 61.4 MHz ^2H–{^1H} NMR spectrum the bis-(perdeuterobenzyl)amine derived from (*R*)-1-aminoindane 32.2% ee in PBLG/DMF (PBLG weight fraction $X_M = 26.2\%$) at $T = 320$ K.

ee should reach values below 1%. Benzyl chloride **2** thus appears to be an excellent ADDA for secondary amines associated with the PBLG/DMF liquid crystalline solvent.

For primary amines, the problem is more complex. The substitution conditions required to obtain mono-benzylated amines with a reasonable yield would necessitate the reaction of an excess of amine against benzyl chloride **2**. This is not suitable because in the majority of cases, the chemists will furnish a small amount of the amine to be studied. As a result, we recommend an excess of ADDA be used in order to obtain the di-benzylated amine with a good yield. With such a di-benzylated amine derivative, the expected spectrum is similar to that of a mono-benzylated one. Actually, due to rapid nitrogen atom inversion the two benzyl groups are equivalent.

Table 2. Absolute values of deuterium quadrupolar splittings ($\Delta\nu_Q^R$, $\Delta\nu_Q^S$) and spectral separation, $|\Delta\nu_Q^R - \Delta\nu_Q^S|/2$, for a perdeuterobenzylated amine derived from (*R*)-1-aminoindane 32.2% ee in PBLG/DMF (PBLG weight fraction $X_M = 26.2\%$) at different temperatures

Amine	*T* (K)	Site	$\lvert\Delta\nu_Q^R\rvert$ (Hz)	$\lvert\Delta\nu_Q^S\rvert$ (Hz)	$\lvert\Delta\nu_Q^R - \Delta\nu_Q^S\rvert/2$ (Hz)
1-Aminoindane ee = 32.2% (*R*)	310	α	1270	1050	110
		α′	649	573	38
		o,m	675	564	55.5
		o,m	660	549	55.5
		p	206	186	10
	320	α	1156	935	110.5
		α′	576	499	38.5
		o,m	612	503	54.5
		o,m	593	484	54.5
		p	219	192	13.5
	330	α	1053	838	107.5
		α′	508	434	37
PBLG/DMF $X_M = 26.2\%$		*o,m*	554	451	51.5
		o,m	535	432	51.5
		p	234	200	17

An example of using a non-racemic mixture of 1-aminoindane enantiomers (ee = 32.2% ± 0.1 (*R*)) as the chiral amine is presented in Figure 5. Again, a total assignment of the quadrupolar doublets was possible with the help of both the known isotropic chemical shifts and enantiomeric excess. The signal assignments are noted in Figure 5. The quadrupolar splittings of the various deuterium nuclei are reported in Table 2 for each enantiomer, together with their evolution with temperature. Here, the largest spectral separation is observed on one of the benzylic deuterium (α′).

Integration led to an NMR estimate of the enantiomeric excess ee = 33.3% ± 2, which correlate well with the 32.2% ± 0.1 ee of the weighed preparation and indicates that no racemization occurred during the reaction with **2**.

3. Conclusions

We have shown that a lyotropic liquid crystal made up of a dimethylformamide solution of poly-γ-benzyl-L-glutamate with a PBLG weight fraction around 26% is efficient for the NMR study of chiral amines. In this chiral anisotropic solvent, the NMR linewidths are small enough to discriminate enantiomers of amines in contrast with the more common PBLG/chloroform liquid crystal. It seems that DMF is able to break some strong intermolecular interactions between the polypeptide fibres and amines, which causes broad NMR lines in the chloroform liquid crystal solution.

Furthermore, perdeuterobenzyl chloride appears to be a good achiral deuterated derivatizing agent for primary and secondary amines. The reaction is mild and does not seem to induce any racemization. We have shown two demonstrative examples but the technique was also successful with (±)-*sec*-butylamine, (±)-2-aminopentane, (±)-3-aminoheptane, (±)-2-amino 1-butanol, (±)-α-methyl-benzylamine and (±)-2 methylpiperidine.[11]

We think these results should be of interest to chemists involved with the asymmetric synthesis of amines, noticeably in pharmaceutical industries.

References

1. Sarfati, M.; Lesot, P.; Merlet, D.; Courtieu, J. *Chem. Commun.* **2000**, 2069–2081.
2. Canet, I.; Courtieu, J.; Loewenstein, A.; Meddour, A.; Pechiné, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 6520–6526.
3. Lesot, P.; Sarfati, M.; Courtieu, J. *Chem. Eur. J.* **2003**, *9*, 1724–1745.
4. Parker, D. *Chem. Rev.* **1991**, *91*, 1441–1457.
5. Science/Technology concentrate, *Chemical Engineering News* **1997**, *6*, 24.
6. Rouden, J.; Bernard, A.; Lasne, M.-C. *Tetrahedron Lett.* **1999**, *40*, 8109; Jean, L.; Rouden, J.; Maddaluno, J.; Lasne, M.-C. *J. Org. Chem.* **2004**, *69*, 8893–8902.
7. Corruble, A.; Davoust, D.; Desjardins, S.; Fressigné, C.; Giessner-Prettre, C.; Harrison-Marchand, A.; Houte, H.; Lasne, M.-C.; Maddaluno, J.; Oulyadi, H.; Valnot, J.-Y. *J. Am. Chem. Soc.* **2002**, *124*, 15267–15279.
8. Canet, J. L.; Canet, I.; Courtieu, J.; Da Silva, S.; Gelas, J.; Troin, Y. *J. Org. Chem.* **1996**, *61*, 9035–9037.
9. Jakubova, M.; Meddour, A.; Pechine, J. M.; Baklouti, A.; Courtieu, J. *J. Fluorine Chem.* **1997**, *86*, 149–153.
10. Meddour, A.; Loewenstein, A.; Pechine, J. M.; Courtieu, J. *Tetrahedron: Asymmetry* **1997**, *8*, 485–494.
11. Solgadi, A. PhD Thesis, Université Paris-Sud 11, No. 7804, 2004.