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Deuterium NMR stereochemical analysis of *threo*–*erythro* isomers bearing remote stereogenic centres in racemic and non-racemic liquid crystalline solvents

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Abstract

We report the proton-decoupled deuterium NMR study of labelled diastereomers with remote stereogenic carbons dissolved in various mixtures of poly- γ -benzyl-L-glutamate (PBLG) and poly- γ -benzyl-D-glutamate (PBDG) liquid crystalline solutions. The evolution of quadrupolar splitting as well as the diastereomeric and the enantiomeric discrimination versus the proportion of PBLG and PBDG in the liquid crystalline phase is studied. It is shown that racemic liquid crystalline solutions of PBLG and PBDG may be used to measure diastereomeric excess (*de*). Thereafter the spectrum in PBLG solution allows for measuring the enantiomeric excess (*ee*) of each diastereomer. These first results suggest substantial prospects in the field of the analysis of diastereomers with remote stereogenic carbons. © 2000 Elsevier Science Ltd. All rights reserved.

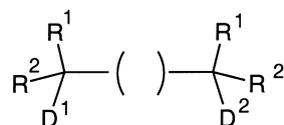
1. Introduction

NMR in chiral liquid crystalline solvents provides a powerful method for measuring enantiomeric purity in a large variety of molecules.¹ The chiral discrimination originates from the difference in the molecular ordering parameters of enantiomers when dissolved in such mesophases. This is easily observed via the orientation dependent NMR observables, namely the chemical shift anisotropy, $\Delta\sigma_i$, the dipolar coupling, D_{ij} , and the quadrupolar splitting, $\Delta\nu_{Qi}$, for spins larger than 1/2.² The best results have been observed using a lyotropic liquid crystal obtained by dissolving poly- γ -benzyl-L-glutamate (PBLG) in various organic solvents such as chloroform, dichloromethane or dimethylformamide.^{3–6}

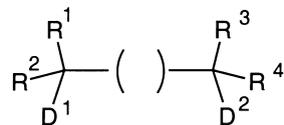
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More recently, we have reported that this technique allows *threo-meso* stereoisomers of compounds having remote stereogenic carbons that are not distinguishable through classical isotropic NMR to be distinguished and to be assigned unambiguously.⁷ Here again, the spectral discrimination originates from the order parameters that are different for these diastereomers. Furthermore, we have demonstrated that the spectral multiplicity was different for the *meso* and the *threo* compounds, leading to a non-ambiguous assignment of the relative configurations of the stereogenic centres.

Using a non-statistical mixture of *threo-meso* isomers of general formula, we have shown that proton-decoupled deuterium NMR ($^2\text{H}-\{^1\text{H}\}$) in PBLG allows all stereoisomers to be distinguished.⁷ For such compounds, where the deuterium atoms are located on the stereogenic carbons, we have demonstrated that the deuterons in the (*R,R*) isomer exhibit a single quadrupolar doublet, $\Delta\nu_Q(R,R)$, as they are equivalent (they are related through a C_2 axis). The same is true for the (*S,S*) isomer, but with a different quadrupolar splitting, $\Delta\nu_Q(S,S)$, because (*R,R*) and (*S,S*) molecules do not have the same orientation. In contrast, in the (*R,S*)-*meso* isomer, the deuterons are related through a plane of symmetry, so they are enantiotopic. It is known that, in this oriented chiral medium, enantiotopic nuclei are no longer equivalent.^{8–10} Consequently two quadrupolar doublets of equal intensities, $\Delta\nu_Q(R)$ and $\Delta\nu_Q(S)$ for the deuterons on the (*R*) and (*S*) centres, respectively, are observed. This fact allows a non-ambiguous assignment of such diastereomers in a non-racemic mixture of all three stereoisomers.



The purpose of this work concerns the more complex and general problem of the discrimination of *threo-erythro* diastereomers with remote stereogenic centres and the measurement of the *des* and the *ees* of these stereoisomers. The above symmetry arguments do not hold anymore when working on a mixture of such isomers because there is no more molecular symmetry. Let us imagine a molecule of general formula.



What can be expected in the deuterium NMR spectrum for a statistical mixture of all the possible diastereomers in PBLG solution? There are four stereoisomers (*R,R*), (*R,S*), (*S,R*), and (*S,S*), each of them bearing two non-equivalent deuterons. To the extent where all the isomers are oriented differently in the chiral liquid crystal, one should observe a quadrupolar doublet for each deuteron in each stereoisomer, namely four doublets centred on δ_{D1} and four doublets centred on δ_{D2} . Whenever the mixture is neither racemic nor statistic, i.e. a mixture of four stereoisomers with different concentrations, it will not be possible to know which is which, as is possible in the *threo-meso* case. A schematic $^2\text{H}-\{^1\text{H}\}$ NMR spectrum of a non-statistical *threo-erythro* mixture is shown in Fig. 1. As the signals of each diastereomer cannot be located on the spectrum, neither the *des* nor the *ees* can be evaluated.

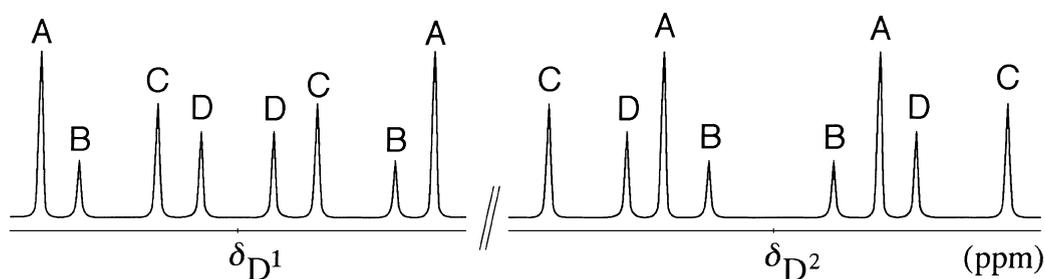
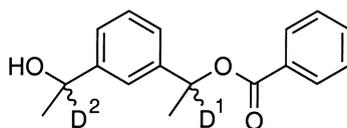


Figure 1. Schematic $^2\text{H}\{-^1\text{H}\}$ NMR spectrum of a non-statistical mixture of the stereoisomers of a dideuterated *threo-erythro* compound in PBLG liquid crystal. Four doublets denoted A, B, C and D are observed for each kind of deuterium. Doublets are to be regarded as arbitrarily located on the spectrum

Actually, this problem can be solved by acquiring less informative spectra. Indeed, when four doublets, for each deuterium, can be observed on the $^2\text{H}\{-^1\text{H}\}$ NMR spectrum, this is due to the solvent chiral discrimination of enantiomers and to the shape recognition of diastereomers through their molecular order parameters. So if we use a racemic liquid crystalline solvent, we should still be able to distinguish the spectra of diastereomers, since they have a different shape and consequently different order parameters, but the chiral discrimination of the enantiomers will vanish. This experiment can be performed using the liquid crystalline medium obtained with a solution of a racemic mixture of PBLG and its enantiomer the poly- γ -benzyl-D-glutamate (PBDG).⁸ In such a racemic oriented solvent, the dissolved molecules should be in the fast exchange limit by diffusing very rapidly, on the NMR time scale, from the vicinity of PBLG to PBDG fibres.⁸ Consequently we should observe only an average of these situations where diastereomers are still discriminated, allowing the *de* to be measured by NMR (Fig. 2). Then following the doubling of each NMR line from racemic PBLG/PBDG mixture to pure PBLG will allow the *ee* of each diastereomer to be measured as depicted in Fig. 2. It must be clear that this experiment will not permit the assignment of the signals to a particular diastereomer, nevertheless the *de* and the *ee* of each diastereomer can be measured with the usual NMR precision.

2. Results and discussion

To illustrate the method, the above principles have been applied on a non-statistical mixture of the four stereoisomers of α,α' -dideutero- α,α' -1,3-benzenedimethanol monobenzoate **2**, where the stereogenic carbons are four bonds remote. Compound **2** was prepared by enantioselective reduction of 1,3-diacetylbenzene with LiAlD_4 , according to Mosher's procedure,¹¹ followed by partial esterification of the mixture using dicyclohexylcarbodiimide (DCC).¹²



2

The $^2\text{H}\{-^1\text{H}\}$ NMR spectrum of a non-statistical mixture of the stereoisomers of **2** dissolved in PBLG liquid crystal is presented in Fig. 3a. The attribution of the different doublets to D^1 and D^2

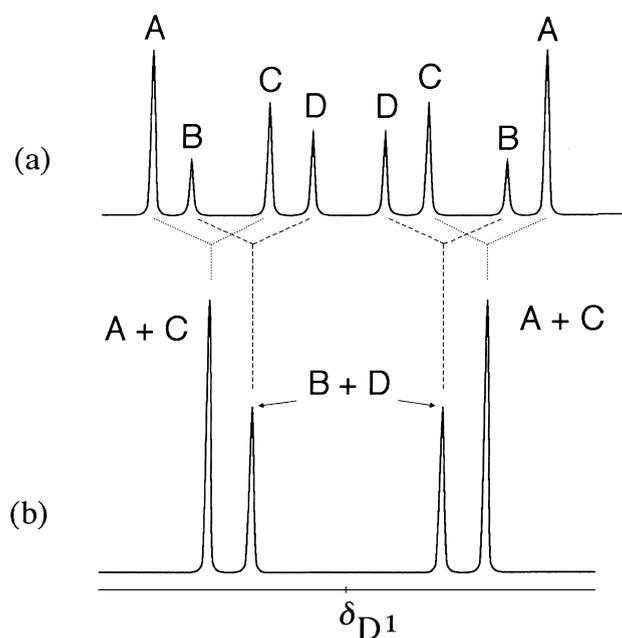


Figure 2. Evolution of the schematic $^2\text{H}\{-^1\text{H}\}$ NMR spectrum of a non-statistical mixture of the stereoisomers of a dideuterated *threo-erythro* compound for each deuterium from (a) PBLG to (b) a racemic mixture of PBLG/PBDG liquid crystals. Doublets which superimpose originate from the enantiomers of one diastereomer

is straightforward because the chemical shift of D^1 is larger than D^2 ($\delta_{\text{D}^1} - \delta_{\text{D}^2} = 1.22$ ppm, 46.8 Hz). As expected, we observe four quadrupolar doublets for D^1 and D^2 , associated with the four possible stereoisomers in the mixture. However, it is not possible to measure the *des* or the *ees* because there is no way to assign the signals of the enantiomers of each diastereomer. There are actually three possible solutions and only one is correct. To solve this problem, we need to compare this spectrum to that obtained with the same mixture of stereoisomers in the racemic PBLG/PBDG nematic solvent (Fig. 3b). It is clear that in this racemic medium the spectral multiplicity is reduced. The signal of D^2 shows two doublets, one for each of the *threo* [(*R,R*)+(*S,S*)] and *erythro* [(*R,S*)+(*S,R*)] isomers. The integration of these doublets allows the *de* to be measured at 42%. Subsequently, it becomes straightforward to deduce the *ee* of each diastereomer because the sum of the integrations of the signals from the two enantiomers must be consistent with the already known *de*. Here the largest and the smallest peaks belong to the enantiomers of the same diastereomer, whereas the signals which have the same intensities belong to the other diastereomer. This is the only combination of lines which leads to the same *de* than in Fig. 3b. We can therefore conclude, through integration, that one of the diastereomers is racemic, whereas the second is 89% *ee*.

The signal due to D^1 is reduced to a single doublet, which means that there is no diastereomeric discrimination on this site in the racemic PBLG/PBDG nematic solvent. Even in such a case, it is possible to determine the *de* by following the evolution of quadrupolar splittings as a function of PBDG/(PBLG+PBDG) weight fraction (see Table 1 and Fig. 4). In Fig. 4, the normalised values of $\Delta\nu_{\text{Q}}^{\text{D}^1}$'s are plotted against PBDG/(PBLG+PBDG) weight fraction. These normalised $\Delta\nu_{\text{Q}}^{\text{D}^1}$'s are corrected from the measured values to take into account the solvent order variations from one sample to another one.¹³ The correction factor applied was derived from the co-solvent (CH_2Cl_2)

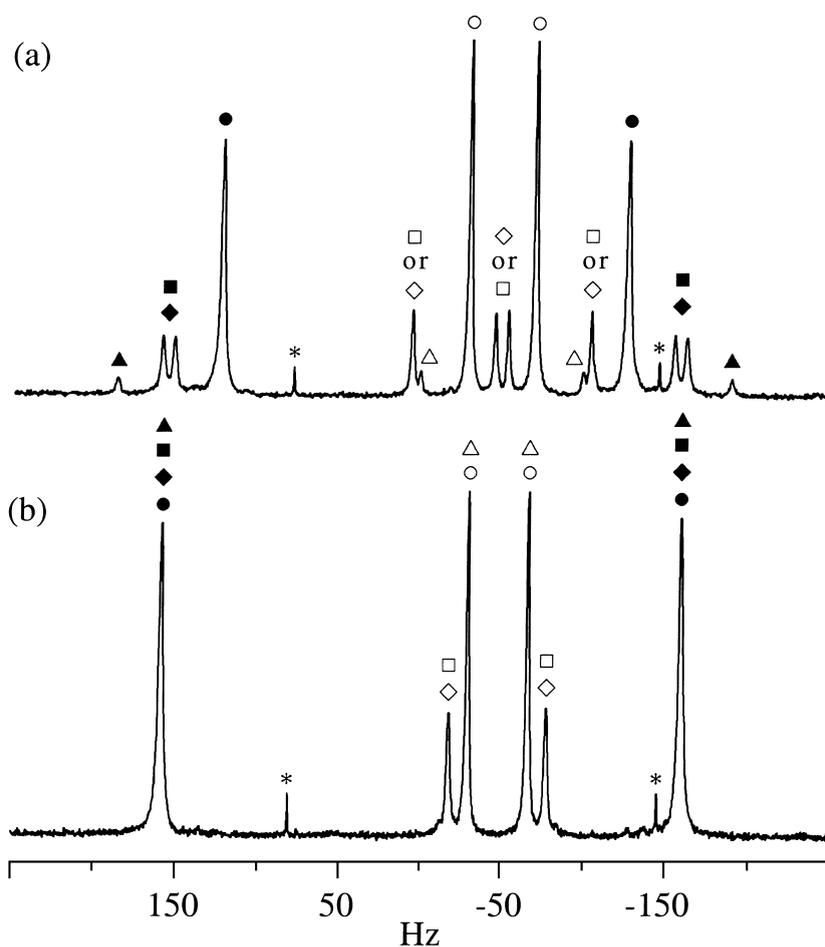


Figure 3. (a) Experimental $^2\text{H}\{-^1\text{H}\}$ NMR spectrum of a non-statistical mixture of the stereoisomers of **2** in PBLG/ CH_2Cl_2 (15.2 wt% PBLG) at $T=302$ K. (b) $^2\text{H}\{-^1\text{H}\}$ NMR spectrum of the same mixture in the racemic liquid crystal made of (0.5 PBDG/0.5 PBLG)/ CH_2Cl_2 (15.2 wt% (PBDG+PBLG)) at $T=302$ K. ▲, ●, ■, ◆ are the signals from D^1 and △, ○, □, ◇ are the signals from D^2 . ▲, ●, △, ○ are relative to the enantiomers of one diastereomer and ■, ◆, □, ◇ are relative to the enantiomers of the other diastereomer. * are the ^2H natural abundance signals of the co-solvent CH_2Cl_2

quadrupolar splitting, $\Delta\nu_{\text{Q}}^{\text{sol}}$ observed in natural abundance in the $^2\text{H}\{-^1\text{H}\}$ NMR spectrum. If experiments were perfect, this splitting would always be the same in all samples. As it is not the case, we measured a reference $\Delta\nu_{\text{Qref}}^{\text{sol}}$ on the sample where PBDG/(PBLG+PBDG) weight fraction equals 50%. Then the experimental $\Delta\nu_{\text{Q}}^{\text{D}^i}$'s were corrected through $\Delta\nu_{\text{Q}}^{\text{D}^i} \times (\Delta\nu_{\text{Qref}}^{\text{sol}} / \Delta\nu_{\text{Qsample}}^{\text{sol}})$, thus providing normalised $\Delta\nu_{\text{Q}}^{\text{D}^i}$'s. These normalised values are those we would have measured if the solvent order parameter was the same for all samples. Note that if the experiments are run with great care, the ratio $\Delta\nu_{\text{Qref}}^{\text{sol}} / \Delta\nu_{\text{Qsample}}^{\text{sol}}$ should be close to unity. We found that this ratio does not deviate from unity by more than 11%.

On Fig. 4, one can check that the evolution of the normalised quadrupolar splittings is essentially linear. At this point, it is important to emphasize that while a quadrupolar splitting can be positive or negative, only its absolute value can be measured in a spectrum. Assuming that all the $\Delta\nu_{\text{Q}}^{\text{D}^i}$'s have the same sign, the variation of the splittings is linear except for three transitions

Table 1

Experimental quadrupolar splittings (Hz) of **2** and of the solvent versus PBDG/(PBLG+PBDG) weight fraction. \blacktriangle , \bullet , \blacksquare , \blacklozenge are the signals from D¹ and \triangle , \circ , \square , \diamond are the signals from D². \blacktriangle , \bullet , \triangle , \circ are relative to the enantiomers of one diastereomer and \blacksquare , \blacklozenge , \square , \diamond are relative to the enantiomers of the other diastereomer

% PBDG	$\Delta v_Q^{D^1}$ (\blacktriangle)	$\Delta v_Q^{D^1}$ (\blacksquare)	$\Delta v_Q^{D^1}$ (\blacklozenge)	$\Delta v_Q^{D^1}$ (\bullet)	$\Delta v_Q^{D^2}$ (\square)	$\Delta v_Q^{D^2}$ (\triangle)	$\Delta v_Q^{D^2}$ (\circ)	$\Delta v_Q^{D^2}$ (\diamond)	Δv_Q^{solv}
0.0	376.2	321.5	306.9	249.3	110.0	100.0	-40.4	-7.9	224.5
16.5	344.7	312.0	302.8	265.2	90.0	76.8	-13.0	13.0	218.3
33.3	337.1	323.3	319.4	299.4	73.8	56.5	9.8	37.3	227.3
50.0	318.7	318.7	318.7	318.7	60.0	37.0	37.0	60.0	226.8
66.3	305.6	332.9	339.4	357.0	42.9	18.3	63.6	85.4	236.6
83.5	289.2	338.8	350.8	388.9	29.4	0.0	96.4	114.8	241.9
100.0	266.1	335.1	352.2	409.0	7.2	-25.7	113.2	126.2	238.3

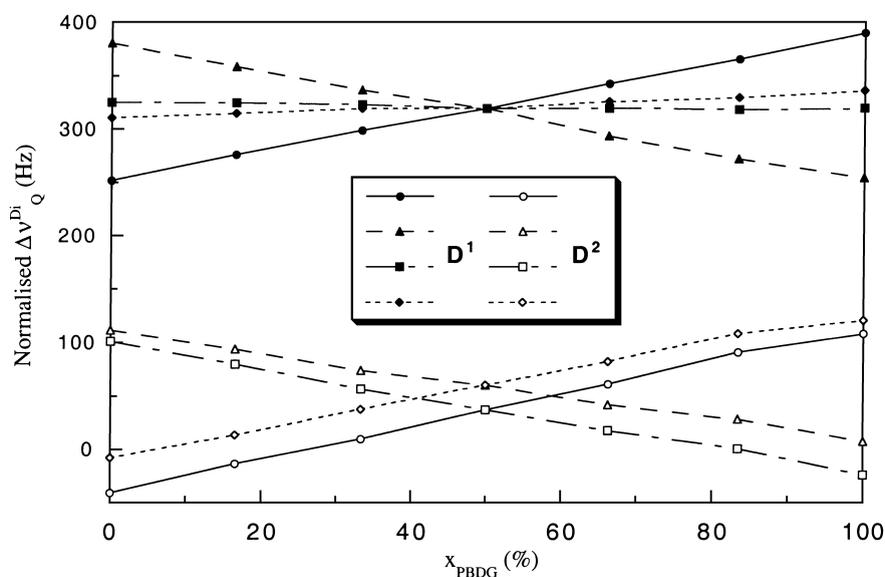


Figure 4. Evolution of the normalised $\Delta v_Q^{D^i}$'s versus the PBDG/(PBLG+PBDG) weight fraction. \blacktriangle , \bullet , \blacksquare , \blacklozenge are the signals from D¹ and \triangle , \circ , \square , \diamond are the signals from D². \blacktriangle , \bullet , \triangle , \circ are relative to the enantiomers of one diastereomer and \blacksquare , \blacklozenge , \square , \diamond are relative to the enantiomers of the other diastereomer

belonging to D² where the curves show a discontinuity at $\Delta v_Q^i = 0$ Hz. This discontinuity in the evolution is not conceivable and is actually associated to a change in the sign of the quadrupolar splitting. Such a sign change occurs when the average orientation on the C–D bond (and electric field gradient) goes across the magic angle. This is the reason why in Table 1 and Fig. 4 some splittings are reported with a relative sign opposite to the others. Consequently, measurements in PBLG/PBDG mixtures provide a novel method to obtain the relative signs of quadrupolar splittings. Furthermore, in situations like that observed for D¹ (where there is a fortuitous line superposition in the racemic medium), the ideal linear behaviour of normalised splitting versus the PBDG/(PBLG+PBDG) weight fraction allows the signals of each diastereomer to be determined.

3. Conclusion

In this study, we have demonstrated that racemic solutions of PBLG and PBDG can be used to measure the diastereomeric excess in a *threo-erythro* mixture. Then comparison of the spectra recorded in the racemic and non-racemic liquid crystalline phases allows the enantiomeric excess of each diastereomer to be determined. Still, the method does not allow for attributing the signals to a relative configuration like in the *threo-meso* case. This approach can be applied using deuterium NMR of isotopically labelled molecules and may be successfully extended to the natural abundance deuterium 2D NMR spectroscopy.^{14,15} These results suggest therefore substantial prospects in the field of diastereomeric analysis of compounds with remote stereogenic centres. In addition, the results show clearly that a rapid exchange of the chiral solute between PBLG and PBDG exists and that the enantiomeric discrimination averages to zero in the racemic mixture.

4. Experimental

4.1. General

¹H NMR spectra were recorded at 250.16 MHz and TMS was used as internal reference. ²H NMR spectrum was recorded at 38.39 MHz, and the CHDCl₂ residual signal, used as internal reference, was assigned to 5.32 ppm. Diastereomeric and enantiomeric excesses were measured by ²H-¹H NMR in PBLG liquid crystal and assignment was made as reported previously.⁷

4.2. Preparation of a non-statistical mixture of α,α' -dideutero- α,α' -1,3-benzenedimethanol **1**

An ether solution (32 ml) of (+)-(2*S*,3*R*)-4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol, ChiralD[®], (16.30 g, 57.5 mmol) was added in one minute at 0°C to a magnetically stirred solution of LiAlD₄ (1.05 g, 25.0 mmol) in ether (63 ml). Transfer was completed by rinsing the original flask with 16 ml of ether. Three minutes from the initial mixing, a solution 1,3-diacetylbenzene (1.622 g, 10.0 mmol) in ether (8 ml) was added dropwise to the precipitated reagent. The reaction was stirred for 22 hours at 0°C and then hydrolysed with an excess of hydrochloric acid. The undissolved precipitate was removed by filtration and the ether extracts were washed with water and dried with MgSO₄. Evaporation of ether gave a colourless oil (0.982 g, yield 58%), a mixture of stereoisomers of **1**. Stereochemical analysis gave: *threo-1* *ee* = 89%; *de* = 43% of *threo* isomer. ¹H NMR (250 MHz, CDCl₃) δ 7.40–7.25 (m, 5H), 2.00 (br s, 2H), 1.49 (s, 6H).

4.3. Preparation of a non-statistical mixture of α,α' -dideutero- α,α' -1,3-benzenedimethanol monobenzoate **2**

To a CH₂Cl₂ solution (19 ml) containing diol **1** (504 mg, 3.0 mmol), DCC (649.9 mg, 3.15 mmol) and 4-(dimethylamino)pyridine (40 mg, 0.33 mmol) was added benzoic acid (385 mg, 3.15 mmol). After 15 h the reaction mixture was separated by flash chromatography on silica gel, eluted with 4:6 ether:pentane. Monobenzoate was obtained as a colourless oil (353 mg, yield 43%). Stereochemical analysis gave: ((*R,R*)/(*S,S*))-**2** *ee* = 89%; ((*R,S*)/(*S,R*))-**2** *ee* = 0%; ((*R,R*)/(*S,S*))/((*R,S*)/(*S,R*)) *de* = 42%. ¹H NMR (250 MHz, CDCl₃) δ 8.11–8.06 (m, 2H), 7.60–7.53 (m, 1H), 7.48–7.29 (m, 6H), 1.85 (br s, 1H), 1.67 (s, 3H), 1.50 (s, 3H); ²H NMR (CH₂Cl₂) δ 6.09 (s, 1D), 4.87 (s, 1D).

4.4. Sample preparation and NMR measurements

Seven NMR samples were prepared as follows: 100 mg of (PBLG+PBDG) and 16.5 mg of **2** were weighed directly into a 5 mm o.d. NMR tube. Dichloromethane was added to obtain 15.2% w/w of (PBLG+PBDG) in the mixture. The weight fraction PBDG/(PBLG+PBDG) in the seven samples was 0.0, 16.5, 33.3, 50.0, 66.3, 83.5 and 100.0%. For this study, we have used polymers with relatively similar degrees of polymerisation (D.P.) (D.P._{PBLG} = 562 and D.P._{PBDG} = 914), both were purchased from Sigma and used without further purification. Samples were centrifuged back and forth until an optically homogeneous birefringent phase was obtained. Deuterium NMR measurements were performed on a Bruker AM 250 NMR spectrometer equipped with a selective 5 mm deuterium probe operating at 38.39 MHz. The temperature was controlled at 302 K using the Bruker BVT-1000 temperature regulation system and samples were spun at about 20 Hz. Broad-band proton decoupling was achieved by applying the WALTZ composite pulse sequence. 500 transients with 2 K of data points were acquired to obtain a good signal to noise ratio.

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