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Enhancing the Orienting Properties of Poly(γ-benzyl-L-glutamate) by means of Additives

Andreas Marx, Benjamin Böttcher, and Christina M. Thiele*[a]

Abstract: Residual dipolar couplings (RDCs) have recently become increasingly important in organic structure determination due to their unique information content. One main limitation for the use of RDCs in organic compounds is the orientation that needs to be induced to be able to measure RDCs. So far, there are very few possibilities to modulate the orientational properties of organic solutes and even less when chiral media are considered. Based on our recent findings that the critical concentration of the liquid-crystalline phase of homopolypeptides depends on their molecular weight, we sought for further ways to modulate the orienting properties. We were especially interested in seeing whether we could not only influence the induced degree of orientation, but whether we could also change the solute′s preferred orientation and even enhance enantiodifferentiation. We thus tried different aprotic and protic additives and were successful in all of the above-mentioned aspects by using CCl_4 as the additive. Furthermore, we consider DMSO to be a very useful additive. The LC phase of low MW poly(γ-benzyl-L-glutamate) (PBLG) is usually unstable when DMSO is added. The high MW PBLG used in this study, however, remained stable up to a DMSO/$CDCl_3$ ratio of 1:2. By using this combination of solvents, the alignment of the two enantiomers of a compound, which is insoluble in $CDCl_3$, namely, the HCl salt of a tryptophane ester, was possible leading to high-quality spectra. The two enantiomers of the tryptophane ester showed different couplings, thus indicating that enantiodifferentiation is taking place. Thus we were able to modulate the orienting properties (degree of orientation, preferred orientation and enantiodifferentiation) of PBLG by using additives and to increase the accessible solvent and solute range significantly.

Keywords: alignment media · enantiodifferentiation · homopolypeptides · liquid crystals · NMR spectroscopy

Introduction

The NMR spectroscopic determination of the three-dimensional structures of molecules usually relies on angular and distance restraints,[1] which are provided by ^3J couplings,[2,3] NOEs[4,5] and cross-correlated relaxation.[6–8] In contrast to these short-range NMR spectroscopic parameters, residual dipolar couplings (RDCs) provide global information and thus have had a massive impact on biomolecular structure determination[9] and more recently also on organic structure determination.[10–27]

As RDCs are anisotropic NMR parameters it is necessary to orient the compound in question with respect to the magnetic field. This is achieved by the use of orienting media. The two main classes of orienting media for the measurement of RDCs in organic compounds are liquid-crystalline phases (LC phases) based on homopolypeptides,[14,28,29] with poly-γ-benzyl-L-glutamate (PBLG) being the most prominent member, and stretched polymer gels.[18,30–34] The major advantage of the polypeptidic LC phases relative to available gels is their homochirality, which forms the basis for the excellent work on enantiodifferentiation done by Courtieu and Lesot et al.[15,16,28,35–40] One major drawback of these LC phases is the necessity of a certain minimum concentration (critical concentration) of the mesogen for the LC phase to be formed, which concomitantly results in a minimum degree of orientation that cannot be dropped down further.

[a] Dipl.-Chem. A. Marx, Dipl.-Chem. B. Böttcher, Dr. C. M. Thiele
Technische Universität Darmstadt
Clemens Schöpf Institut für Organische Chemie und Biochemie
Petersenstr. 22, 64287 Darmstadt (Germany)
Fax: (+49) 6151-16-5112
E-mail: cmt@punk.oc.chemie.tu-darmstadt.de

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In a first investigation towards the modulation of the orienting properties of PBLG, we were able to show that by application of very high molecular weight (MW) PBLG the critical concentration can be reduced to about 6%, thereby significantly increasing the spectral quality and reducing the degree of order.[41] We also showed that the degree of solute order can be varied by the concentration of the LC phase, while the preferred orientation of the solute remains unchanged. In accordance with results found in the literature,[35,37] we have also quantified the degree of enantiodifferentiation, showing that the difference in couplings and order tensors is only minute, but nevertheless can be determined reliably.[42]

If enantiodifferentiation in homopolypetidic liquid crystals shall be modulated, two homopolypeptides can be mixed as was recently reported by Lesot et al.[40]

Herein, we describe further investigations into modulating the orienting properties of PBLG LC phases revealing that, on the one hand, the induced degree of solute orientation can be reduced even further, and on the other hand, the degree of enantiodifferentiation in terms of different orientations can be significantly increased by the use of additives, such as CCl_4. Furthermore, a DMSO-compatible LC phase will be presented, which allows the measurement of RDCs of highly polar substances, such as hydrochlorides that are insoluble in $CDCl_3$.

Results and Discussion

For the applicability of LC-based orienting media, it is desirable to be capable of influencing the induced degree of solute orientation as far as possible. In a first step, we were looking for ways to diminish the inherent anisotropy of the LC phase. A promising report in this direction was made some time ago by Samulski et al.[43] who reported a significant decrease in the value of the anisotropy of the diamagnetic susceptibility of PBLG by the addition of small quantities of trifluoroacetic acid (TFA) to the LC phase of PBLG. Encouraged by that finding, we wanted to find out whether this can also be transferred to anisotropic observables, such as the quadrupolar splitting ($\Delta\nu_Q$) or RDCs.

The influence of protic additives: We started by preparing samples of PBLG with different amounts of added TFA (without solute). Figure 1 shows the course of $\Delta\nu_Q$ (●) as a function of the concentration of TFA. As can be seen, the value of $\Delta\nu_Q$ does indeed decrease significantly from approximately 210 Hz with no addition of TFA to about 40 Hz at a concentration of 20% TFA. When adding TFA, the concentration of

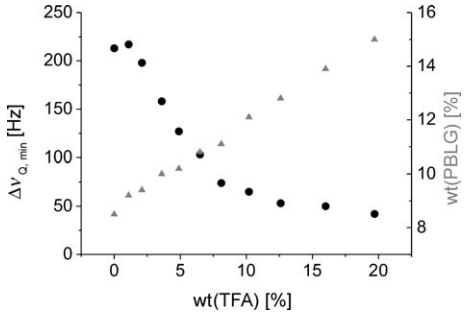
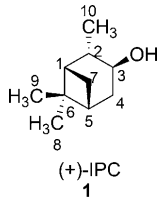


Figure 1. Course of the absolute value of the quadrupolar splitting ($\Delta\nu_{Q,min}$) (●) of $CDCl_3$ and the concentration of the LC phase (▲) of PBLG (for exact sample compositions see the Supporting Information) as a function of TFA concentration. All spectra were acquired at 300 K. The PBLG used had a number-averaged molar mass of 420 kDa and a polydispersity index (PDI) of 2.17.

PBLG (▲) has to be raised to retain a stable and homogeneous LC phase. Above 20% TFA, the LC phase is not stable because a helix-to-random-coil transition of PBLG takes place.[44] For other polypeptides with more fragile helix conformations relative to PBLG, it has been reported that this occurs at much lower concentrations of TFA.[45]

In the next step, we determined the RDCs of (+)-isopinocampheol ((+)-IPC, **1**) as a function of the TFA concentration. As before, we used IPC as the solute for the study of the orientational properties as it is a rigid compound that provides a sufficiently large number of RDCs to be able to determine the order tensor reliably.



(+)-IPC
1

In contrast to $\Delta\nu_Q$, some of the RDCs show a significant increase when TFA is added to the LC phase, whereas others slightly decrease (all values are given in the Supporting Information). The unequal change in the values of the RDCs indicates a change in the solute orientation. From the calculated orienting properties (axial component of the Saupe tensor (D_a) and generalised degree of order (GDO)), which are given in Table 1 (entries 1–5), it is clear that the degree of solute orientation is increased by the addition of

Table 1. Overview of the orientational properties of (+)-IPC in the LC phase of PBLG at different acid concentrations. All orienting properties were calculated by using the program hotFCHT.[48]

Entry	Additive	wt (additive) [%][a]	n (RDC)[b]	GDO [10^{-4}][c]	D_a [10^{-4}][d]	R[e]	RMSD[f]	Q[g]
1	TFA	0	9	12.1	5.36	0.383	0.239	0.029
2	TFA	2	9	18.1	8.66	0.331	0.677	0.053
3	TFA	4.5	7	17.7	8.20	0.486	0.267	0.019
4	TFA	7	7	18.0	7.79	0.475	0.253	0.018
5	TFA	10	7	17.5	8.07	0.481	0.231	0.016
6	PFBA[h]	2	9	15.2	7.31	0.314	0.836	0.078
7	PFBA[h]	4.5	6	19.5	9.56	0.236	0.104	0.008

[a] Weight concentration of the additive. [b] Number of $^1D_{CH}$ used for calculation of the parameters D_a, R, α, β and γ within hotFCHT. [c] Generalised degree of order. [d] Axial component of the order tensor. [e] Rhombicity. [f] Root-mean-square deviation of the calculated and measured RDCs. [g] Quality factor of the calculated fit versus measured RDCs as defined by Cornilescu.[49] [h] Perfluorobenzoic acid.

TFA. Furthermore, the spectral quality is reduced when TFA is added to the LC phase and TFA undergoes a slow esterification with the solute IPC.[46] As TFA is thus not the additive of choice, we have also tested other acids,[47] such as perfluorobenzoic acid (Table 1, entries 6 and 7; for more details see the Supporting Information), which does not form an ester with IPC on a timescale of several days. All tested acids showed a pronounced increase in solute orientation relative to the corresponding neat LC phase and a comparable decrease in spectral quality giving rise to significant increased uncertainties in the RDCs and in the orienting properties (see the Supporting Information). Hence, by adding protic additives, the solute orientation is changed, but the desired decrease in solute orientation is not possible, although one would have assumed that this is the case when looking at the $\Delta\nu_Q$ of the solvent.

It is known that PBLG forms aggregates in low polarity solvents, such as chloroform.[50,51] These aggregates are destroyed by the addition of acids or highly polar solvents, such as DMF.[52] Samulski et al.[43] were able to show that the effect of adding TFA to the LC phase of PBLG is also to increase the side-chain flexibility by hydrogen bonding of TFA to the ester side chain. At higher TFA concentrations (above 20%) the hydrogen-bonding network within the α-helix is destroyed, which leads to a helix-to-random-coil transition and, therefore, to a non-orienting medium. The origin of the increased solute orientation with TFA as the additive is most likely to be connected to effects such as disaggregation and hydrogen bonding to the ester side chain. We therefore continued to investigate further additives, thereby reducing stepwise the polarity of the additive. By lowering the polarity the effects of disaggregation and interaction with the ester side chains should be lowered. From this we hoped to find factors that would allow us to modulate the orienting properties in the desired way.

The influence of aprotic additives: As proposed by Gupta et al.[52] PBLG aggregates that are present in solvents such as chloroform are gradually destroyed by the addition of highly polar solvents, although relative to acids higher concentrations are necessary. Thus, we tested DMSO as an additive. In contrast to TFA, which is a strong hydrogen-bond donor, the interactions of DMSO with the ester side chains should be drastically reduced and hence should allow us to investigate whether the increase in solute orientation is presumably due to the disaggregation or the interaction with the ester side chain. The determined RDCs are summarised in Table 2 (entries 2–4; see also the Supporting Information) and the calculated orienting properties are summarised in Table 3 (entries 1–4). As can be seen, the D_a values are only slightly raised by the addition of DMSO. This indicates that the significant increase in solute orientation, which we observed for TFA as the additive, might be due to its hydrogen-bonding interactions. Again, there is an unequal change in the RDCs, which indicates a change in the solute orientation.

Although the spectral quality is reduced at high DMSO concentrations, the difference in RDCs and hence in the Euler angles between (+)- and (−)-IPC is still significant (see the Supporting Information). Nevertheless, relative to the neat LC phase, the degree of enantiodifferentiation is lowered.

When we applied THF as the additive we found a slightly different behaviour relative to DMSO. Firstly, the D_a values remain constant, which indicates that the degree of solute orientation is not raised. Secondly, the uncertainties of the RDCs are lower even at a higher concentration and a different behaviour of enantiodifferentiation is present (see Figure 2). In the neat and DMSO-containing LC phase the enantiodifferentiation of IPC is manifested by the difference in only one Euler angle, namely, the β angle. With THF as the additive a differentiation in the γ-Euler angle evolves at higher THF concentrations, whereas the difference in the β-

Table 2. Overview of the determined RDCs of (+)-IPC in the LC phase of PBLG with different aprotic additives. The couplings of C7–H7a and C7–H7s are omitted due to the possibility of strong coupling artefacts.

Entry	Additive	wt (additive)[a]	RDC [Hz]								
		[%]	C1–H1	C2–H2	C3–H3	C4–H4$_s$[b]	C4–H4$_a$[c]	C5–H5	C8–C6	C9–C6	C10–C2
1	DMSO	0	13.8±0.2	−6±0.2	12.45±0.2	13.3±0.2	−4.2±0.2	−4.8±0.2	−1.3±0.2	0.9±0.2	0.05±0.2
2	DMSO	4.5	15.3±0.5	−5.7±0.5	12.6±0.2	14.8±0.2	−3.8±0.5	−2.8±0.5	−1.5±0.5	1.0±0.2	0.2±0.2
3	DMSO	9	16.5±0.5	−5.0±0.5	12.4±0.2	16.0±0.2	−3.6±0.5	−0.7±0.5	−2.0±0.5	1.1±0.2	0.6±0.2
4	DMSO	13	–[d]	−4.45±0.5	14.0±0.5	17.4±0.5	−4.6±0.5	0.1±0.5	−2.2±0.5	1.2±0.5	0.7±0.5
5	THF	0	13.8±0.2	−6±0.2	12.45±0.2	13.3±0.2	−4.2±0.2	−4.8±0.2	−1.3±0.2	0.9±0.2	0.05±0.2
6	THF	5	14.7±0.2	−5.7±0.2	13.3±0.2	15.6±0.2	−4.0±0.2	−4.7±0.2	−1.6±0.2	1.1±0.2	0.1±0.2
7	THF	9	14±0.5	−4.9±0.2	12.7±0.2	15.9±0.2	−3.6±0.2	−4.3±0.5	−1.7±0.2	1.1±0.2	0.2±0.2
8	THF	13	14.0±0.5	−4.0±0.5	13.0±0.2	16.0±0.2	−3.1±0.2	−4.1±0.5	−1.9±0.2	1.1±0.2	0.3±0.2
9	CCl_4	0	14.9±0.2	−6.4±0.2	13.8±0.2	15.1±0.2	−4.8±0.2	−5.1±0.2	−1.4±0.2	1.1±0.2	0.1±0.2
10	CCl_4	9	15.4±0.2	−6.0±0.2	13.1±0.2	14.3±0.2	−4.3±0.2	−7.1±0.2	−1.3±0.2	1.0±0.2	−0.1±0.2
11	CCl_4	16	15.1±0.2	−6.0±0.2	11.9±0.2	13.5±0.2	−3.6±0.2	−8.4±0.2	−1.0±0.2	0.8±0.2	−0.1±0.2
12	CCl_4	21	15.5±0.2	−4.8±0.2	11.3±0.2	12.7±0.2	−3.2±0.2	−8.9±0.2	−0.9±0.2	0.8±0.2	−0.2±0.2
13	CCl_4	30	15.6±0.2	−4.0±0.2	10.6±0.2	12.2±0.2	−2.5±0.2	−11.5±0.2	−0.7±0.2	0.7±0.2	−0.4±0.2
14[e]	CCl_4	30	15.2±0.2	−4.6±0.2	11.5±0.2	13.2±0.2	−2.9±0.2	−10.5±0.2	−0.8±0.2	0.8±0.2	−0.4±0.2

[a] Weight concentration of the additive in the LC Phase of PBLG. [b] s stands for *syn* to the dimethylmethylene bridge in IPC. [c] a stands for *anti* to the dimethylmethylene bridge in IPC. [d] Coupling is omitted due to the possibility of strong coupling artefacts. [e] LC phase (including additive) with the same concentration as entry 9.

Table 3. Overview of the orientational properties of (+)-IPC in the LC phase of PBLG with different aprotic additives. All orienting properties were calculated by using the program hotFCHT.

Entry	Additive	wt (additive) [%][a]	n(RDC)[b]	GDO [10^{-4}][c]	D_a [10^{-4}][d]	R[e]	RMSD[f]	Q[g]
1	DMSO	0	9	12.1	5.73	0.371	0.287	0.033
2	DMSO	4.5	9	12.4	6.00	0.304	0.229	0.026
3	DMSO	9	9	13.4	6.51	0.278	0.308	0.034
4	DMSO	13	8	13.4	6.53	0.258	0.129	0.016
5	THF	0	9	12.1	5.91	0.385	0.223	0.025
6	THF	5	9	12.3	5.88	0.365	0.138	0.016
7	THF	9	9	12.0	5.72	0.367	0.188	0.022
8	THF	13	9	12.3	5.89	0.352	0.218	0.025
9	CCl_4	0	9	12.1	5.91	0.385	0.223	0.025
10	CCl_4	9	9	11.9	5.56	0.453	0.256	0.029
11	CCl_4	16	9	11.3	5.17	0.507	0.196	0.023
12	CCl_4	21	9	10.8	4.59	0.595	0.446	0.053
13	CCl_4	30	9	10.4	4.60	0.627	0.427	0.054
14[h]	CCl_4	30	9	10.9	4.85	0.580	0.469	0.054

[a] Weight concentration of the additive. [b] Number of $^1D_{CH}$ used for calculation of the parameters D_a and R within hotFCHT. [c] Generalised degree of order. [d] Axial component of the orienting tensor. [e] Rhombicity. [f] Root-mean-square deviation of the calculated and measured RDCs. [g] Quality factor of the calculated fit versus measured RDCs as defined by Cornilescu.[49] [h] LC phase (including additive) with the same concentration as entry 9.

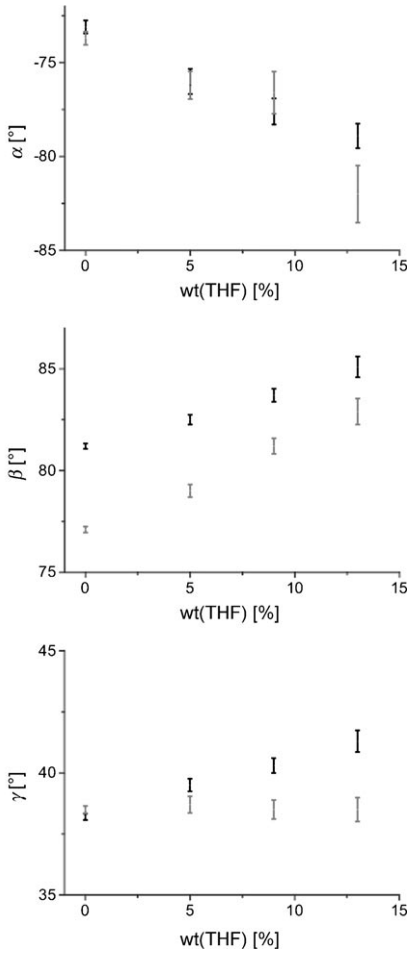


Figure 2. Evolution of the Euler angles of (+)- (black) and (−)-IPC (grey) as a function of the THF concentration. Interestingly, a differentiation in the γ-Euler angle evolves at higher THF concentrations while the difference in β decreases.

Euler angle is reduced. Thus, the solute orientation can be modified by using THF as the additive.

In the last step we tested highly non-polar additives. Most of these additives showed either phase separation (e.g., cyclohexane and other alkanes) or required a significant increase in the concentration of the LC phase. CCl_4, however, turned out to be a suitable non-polar additive.

The determined RDCs for (+)-IPC are listed in Table 2 (entries 9–13). Nearly all RDCs showed a pronounced decrease with higher CCl_4 concentration and hence the desired decrease of the degree of solute orientation, which is also confirmed by the drop of the D_a value (see Table 3, entries 9–13 and the Supporting Information). Interestingly, the critical concentration of the LC phase is reduced with the addition of CCl_4 from 7.9 (no CCl_4) to 6.5% (30% CCl_4), such that the decrease in RDCs and D_a could be due to dilution. Thus we compared the values of the RDCs of the neat LC phase with the LC phase containing 30% CCl_4 at an identical concentration (Table 2, entry 14; for the exact composition see the Supporting Information). It can be seen that the decrease in RDCs, and hence, in the degree of solute orientation is not solely caused by dilution.

In an earlier investigation, we found that with higher dilution of the neat LC phase the D_a value decreases, whereas the rhombicity R and the orientation remained constant.[41] For the LC phases containing CCl_4, the decrease in the D_a value is accompanied by an increase in the rhombicity R of the alignment tensor. In our eyes, this behaviour reflects a change in the interaction of the solute with PBLG caused by the additive CCl_4. We can only hypothesise on the reasons. By the addition of CCl_4, the overall polarity of the co-solvent of the LC phase is reduced. This is known to increase the intermolecular interactions of the PBLG strands and hence results in a pronounced formation of PBLG aggregates.[52] As a consequence, less PBLG surface is accessible to the solute IPC, which may be one reason for the observed decrease of solute orientation. Additionally, the reduction in the overall polarity of the co-solvent clearly also increases the intramolecular interaction of the ester side chain of PBLG, which results in an increase in the stiffness of the polymer, as indicated by the drop of the critical concentration.

When looking at the evolution of the Euler angles for (+)- and (−)-IPC (see Figure 3), one clearly sees that the degree of enantiodifferentiation is enhanced by the addition of CCl_4. The difference in the β-Euler angles does increase

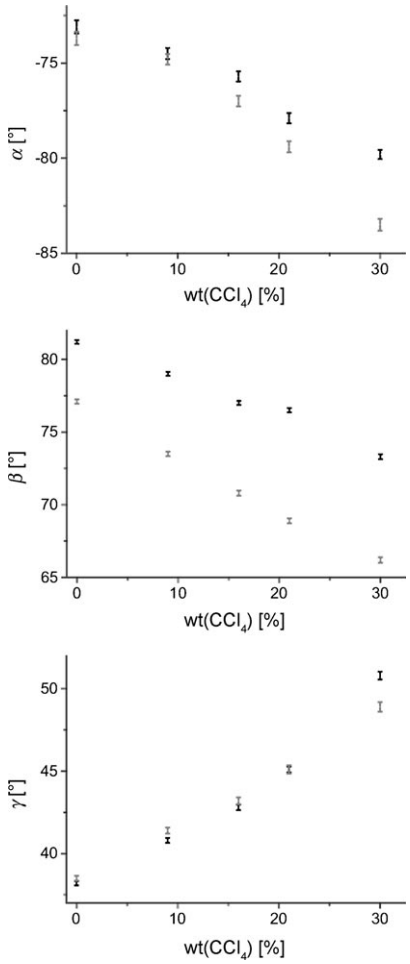


Figure 3. Evolution of the Euler angles of (+)- (black) and (−)-IPC (grey) as a function of the CCl_4 concentration.

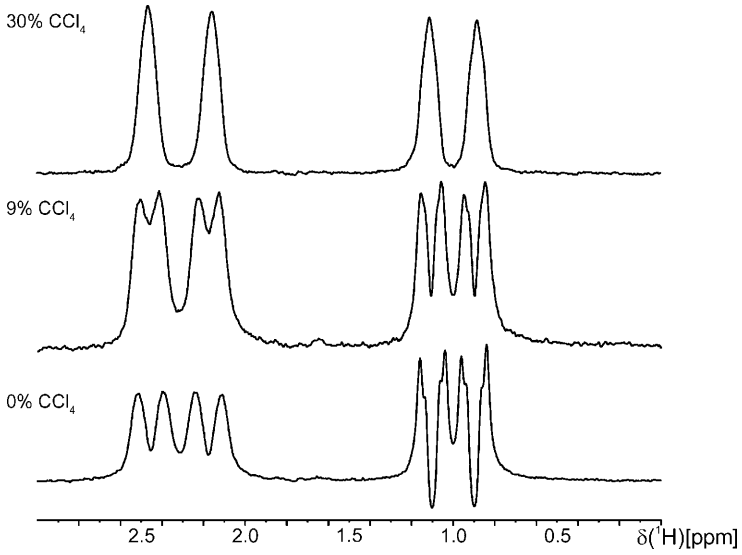
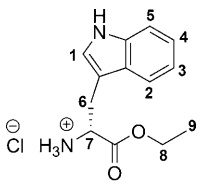


Figure 4. The ω_2 CLIP-HSQC[53] trace of the C7–H7 coupling shows the much improved line shape at higher CCl_4 concentration. Due to the decreased degree of solute orientation and/or the change in orientation, the homonuclear dipolar couplings are also significantly reduced, which means that the C7–H7 couplings can be evaluated.

significantly accompanied by an increasing differentiation in the α-Euler angle as a function of the CCl_4 concentration. Thus, CCl_4 leads to lower solute orientation and additionally to a more pronounced effect of enantiodifferentiation.

Another finding is that as a consequence of the lower solute orientation and/or altered order of the solute homonuclear couplings for some of the signals are significantly reduced. Figure 4 shows the ω_2 trace of the clean in-phase heteronuclear single-quantum correlation (CLIP-HSQC)[53] of the C7–H7 coupling of (+)-IPC. The homonuclear coupling, which causes a doublet-of-doublet-like pattern, is significantly reduced by the addition of CCl_4. At a concentration of 21% both RDCs show a clean doublet coupling pattern. In the neat and all other LC phases, the couplings of these diastereotopic protons are the only ones showing strong coupling artefacts due to large homonuclear couplings. As a consequence, these RDCs could not be evaluated thus far. By the addition of CCl_4, these artefacts are significantly reduced thus making these RDCs accessible.

Determination of the RDCs of a highly polar substance by the use of DMSO as the additive: Encouraged by the finding that the LC phase of the high MW PBLG is stable even at high DMSO concentrations, we additionally determined the RDCs of the hydrochloric salt of the tryptophane ester **2**, which is insoluble in pure chloroform.



D-Trp-OEt HCl

2

For natural products that are either isolated or, for reasons of stability, transformed to their salts, such as hydrochlorides or acetates, a highly polar solvent in which these compounds are soluble is required for the determination of the RDCs. In this context, DMSO is widely used. Consequently, a chiral alignment medium that is compatible with DMSO would be highly attractive. To the best of our knowledge, only a (very recently published) gelatine gel swollen in pure DMSO is available as a chiral DMSO alignment medium.[54]

For low MW PBLG ($M_n < 100$ kDa) the addition of DMSO leads either to partial precipitation of the polymer or to a substantial increase in the concentration of the LC phase to retain the stable homogenous LC phase. By the application of high MW PBLG ($M_n > 300$ kDa), stable LC phases were obtained up to a $CDCl_3$/DMSO ratio of approximately 2:1 at a weight concentration of the LC phase of 8.9%. All LC phases were long-term stable and no phase separation or precipitation occurred. Hence, the solvent mixture $CDCl_3$/DMSO is a good alternative to the [D_7]DMF-based LC phases of PBLG.

In the $CDCl_3$/DMSO solvent mixture the hydrochloride **2** is well soluble and hence can be oriented. Figure 5 shows the ω_2-coupled CLIP-HSQC of 7 mg of **2**. As can be seen,

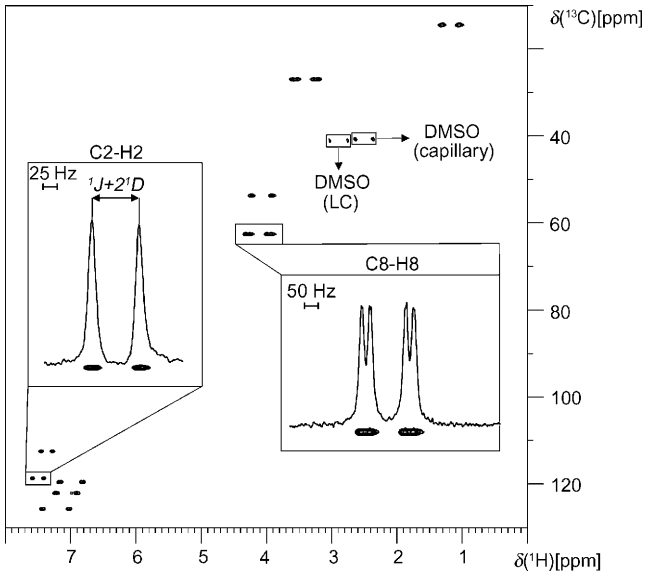


Figure 5. The ω_2-coupled CLIP-HSQC of **2** (7 mg) oriented in the LC phase of PBLG in a 2.5:1 mixture of $CDCl_3$ and DMSO. As can be seen, the overall spectral quality is very high and no residual polymer signals are present in the spectrum.

the spectral quality is excellent with no residual polymer signals or strong coupling artefacts being present.

The normalised RDCs for both enantiomers of the tryptophane ester are listed in Table 4. The observed RDCs are relatively low in value (except for the collinear vectors C2–H2 and C5–H5), which indicates that the degree of orientation is rather low. This is surprising as in our experience the degree of solute orientation is typically raised with increasing polarity of the solute in PBLG. The difference in the values of the normalised RDCs (see Table 4) indicates that enantiodifferentiation is taking place, although the difference in the values is rather small (as in the case of IPC).[42] The diastereotopic protons at C6 and C8 are isochronous in the solvent mixture in both the isotropic and the anisotropic sample. As we thus have only limited data, we did not determine the order tensors of the two enantiomers. The enantiodifferentiation, however, is clearly visible from the difference in normalised RDCs.

Conclusion

We have investigated the orientational properties of PBLG as a function of different additives. The main focus was to lower the induced degree of orientation, to modify the solute orientation and to enhance the degree of enantiodifferentiation. Therefore, we have started from the promising report by Samulski et al.[43] who described a strong decrease in the value of the anisotropy of the diamagnetic susceptibility when TFA was added to the LC phase of PBLG. We have observed that by adding TFA the absolute value of the quadrupolar splitting does indeed show a strong decrease from around 210 to as low as about 40 Hz. In contrast to this, some of the RDCs of (+)-IPC increased, while others decreased and the overall degree of solute orientation was increased by the addition of TFA.

When DMSO is applied as the additive, the degree of solute orientation is only slightly increased. Since DMSO lacks a hydrogen-bond donor site, we concluded that the hydrogen-bond interaction with the ester side chains is more likely to be the reason for the pronounced increase in the degree of solute orientation in the case of protic additives.

We have found CCl_4 to be a very suitable highly nonpolar additive. With increasing concentration of CCl_4, the RDCs are continuously lowered, which indicates the desired decrease in solute orientation relative to the neat LC phase. Furthermore, the critical concentration is lowered when CCl_4 is added. In addition to the lower degree of solute orientation, the additive CCl_4 also had an advantageous effect on enantiodifferentiation, which is monitored by the use of Euler angles. Both the α- and β-Euler angles showed an increasing difference for the two enantiomers as a function of the additive concentration, which indicates that the degree of enantiodifferentiation is clearly enhanced.

Furthermore, we have shown that highly polar substances, such as hydrochloric salts, can be dissolved and oriented in the LC phase of high MW PBLG by using the solvent mixture $CDCl_3$ and DMSO. The spectral quality of the oriented hydrochloride **2** was excellent with no residual polymer signals being present. Despite the high polarity, the hydrochloride **2** showed a rather low degree of orientation. Thus we could significantly enhance the accessible solvent range for PBLG.

Experimental Section

For the sake of simplicity only coupling data and orienting properties necessary to illustrate the observed results were given in the main text. The comprehensive data, particularly all measured RDCs and the resulting orienting properties, are fully listed in the Supporting Information.

All experiments for the determination of RDCs were carried out slightly above the critical concentration to ensure a stable LC phase. To confirm

Table 4. Overview of the normalised RDCs of **2** and *ent*-**2** in the LC phase of PBLG (60 mg) in $CDCl_3$ (390 mg)/DMSO (160 mg) solvent mixture. All RDCs for **2** were normalised by the ratio of the quadrupolar splittings of the corresponding samples.

Entry	$\Delta\nu_Q$[a] [Hz]	Compound	RDC_{norm} [Hz][b]								
			C1–H1	C2–H2	C3–H3	C4–H4	C5–H5	C6–H6	C7–H7	C8–H8	C9–H9
1	153	*ent*-**2**	14.8±0.2	−41.4±0.5	9.2±1.0	2.3±1.0	−41.5±0.5	15.6±0.2	6.7±0.2	9.5±0.5	1.8±0.2
2	141	**2**	12.8±0.2	−36.2±0.5	7.0±1.5	−0.2±1.0	−36.2±0.5	14.4±0.2	5.4±0.2	7.2±0.5	1.9±0.2

[a] Absolute value of the quadrupolar splitting of $CDCl_3$ in the LC phase. [b] Normalised RDCs.

the stability of the LC phase, a ^2H spectrum was recorded right before and after every experiment. For all LC phases PBLG was synthesised according to the literature[41] with an averaged molar mass of $M_n = 420$ kDa and a PDI of 2.17. All orienting properties were calculated by using the program hotFCHT originally developed by Berger et al.,[48] which was extended for the analysis of RDCs.[55]

Preparation of NMR samples: The LC phases were prepared directly in the NMR tube (5 mm o.d.). A total amount of 60 mg polymer and 30 mg of IPC were weighted directly into the NMR tube, which contained a [D_6]DMSO capillary to provide the lock signal. After adding the solvent $CDCl_3$ the polymer was allowed to dissolve overnight and the sample centrifuged back and forth until the ^2H signals were sharp and the line widths constant. All additives were added after the homogeneous phase was obtained (for exact compositions see the Supporting Information). For the LC phases containing high DMSO concentrations it is essential to first dissolve the polymer in $CDCl_3$ and then add DMSO in portions of approximately 80 mg. Otherwise a significantly longer time for the centrifugation procedure is necessary to obtain a stable phase. All concentrations reported are mass concentrations.

NMR experiments: All spectra of IPC in isotropic and oriented samples were recorded on a 500 MHz spectrometer (Bruker DRX-500) with a triple resonance inverse probe equipped with a z-gradient. All measurements were carried out without sample spinning at 300 K. The total coupling constants (^1T) and scalar coupling constants (^1J) were measured from ω_2 coupled CLIP- and CLAP-HSQC[53] experiments. An INEPT delay corresponding to 145 Hz was used. A total of 8 k data points were sampled in the direct dimension over a spectral width of 8 ppm to give a FID resolution of 0.48 Hz. In the indirect dimension, 256 data points were sufficient to prevent signal overlap. The spectra were processed with the use of an exponential window function with 1 Hz line broadening and zero filling by a factor of 2 to give a digital resolution of 0.24 Hz in the direct dimension. In the indirect dimension a $\pi/2$ shifted sine squared window function and zero filling by a factor of 4 was applied.

Calculation of orientational properties: The orientational properties were calculated by using the program hotFCHT.[48,55] Therefore, the scalar coupling (^1J) and the total coupling (^1T) constants as well as the coordinates of IPC were provided as an input.[41] Euler angles are reported as z, y', z' rotations with respect to the molecular axis frame. The dipolar couplings used for the determination of the orientational properties were calculated by using $T = J + 2D$. For the methyl groups, the measured $^1D_{CH}$ was converted to the corresponding $^1D_{CC}$ according to the literature,[56] which was used for the determination of the orienting tensor. All uncertainties were calculated by using a Monte Carlo simulation[57,58] with sufficient steps to achieve convergence of the Saupe values and Euler angles. For our systems these simulations required about 1500 steps until the change in the standard deviation was less than 1%. Contrary to Losonczi et al.,[57] we do not exclude steps from the averaging, for which the back calculated RDCs do not fall within the experimental errors.

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