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Hydrogen bond formation may enhance RDC-based discrimination of enantiomers

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Abstract

The distinction of enantiomers based on residual anisotropic parameters obtained by alignment in chiral poly-γ-benzyl-L-glutamate (PBLG) is among the strongest in high-resolution NMR spectroscopy. However, large variations in enantiodifferentiation among different solutes are frequently observed. One hypothesis is that the formation of hydrogen bonds between solute and PBLG is important for the distinction of enantiomers. With a small set of three almost spherical enantiomeric pairs, for which $^1D_{CH}$ residual dipolar couplings are measured, we address this issue in a systematic way: borneol contains a single functional group that can act as a hydrogen bond donor, camphor has a single group that may act as a hydrogen bond acceptor, and quinuclidinol can act as both hydrogen bond donor and acceptor. The results are unambiguous: although camphor shows low enantiodifferentiation with PBLG and alignment that can be predicted well by the purely steric TRAMITE approach, the distinction of enantiomers for the other enantiomeric pairs is significantly higher with alignment properties that must involve a specific interaction in addition to steric alignment.

KEYWORDS

hydrogen bonding, partial alignment, PBLG, residual dipolar couplings, solute medium interaction

1 | INTRODUCTION

NMR spectroscopy, and in particular RDCs, is an indispensable tool for the configurational analysis of molecules.[1–3] Besides the determination of relative configuration, stereotopic assignment, and others,[4–9] the determination of absolute configuration is a long-standing but difficult goal of partially aligning NMR spectroscopy.[10,11] The distinction of enantiomers using chiral liquid crystalline[12–21] and gel-type alignment media[22–26] is well-known, but the determination of absolute configuration has been shown only in a case where well-characterized reference molecules have been available.[27]

The first and most widely used alignment medium for enantiomeric discrimination is poly-γ-benzyl-L-glutamate or simply PBLG. It has been shown that many difficult molecules,[28,29] even chiral alkanes,[30] can be distinguished using the chiral alignment medium. Many more

chiral alignment media have been shown to distinguish enantiomers, and different media provide varying capabilities of enantiodifferentiation, thereby strongly depending on the molecules studied. Apparently, a complex interaction between solute and alignment medium takes place that needs to be understood in more detail. A basic understanding of such interactions will enhance the choice of alignment media for enantiomeric differentiation but also may lead to techniques that allow a more widely applicable absolute configurational analysis. Next to theoretical calculations[31–33] on this subject, also targeted experimental studies will be necessary to learn more about the complex solute-alignment medium interactions.

The origin of enantiomeric distinction capability has been discussed since many years, with a nice summary of potential effects like H-bonding, carboxyl-dipoles, aromatic stacking, and overall dipoles given in Berdagué et al.[34] In this study, a PBLG/$CDCl_3$ chiral liquid crystalline phase was used as an alignment medium to distinguish enantiomers and to evaluate the role of potential H-bond formation in the alignment process and the corresponding enantiomeric distinction capabilities. Three enantiomeric pairs with close to spherical shapes have been used, where their active groups allow them to be H-bond donor, H-bond acceptor, or both. RDC measurements for all enantiomeric pairs are presented and discussed in detail with respect to their aligning and enantiomeric differentiation properties.

2 | THREE PAIRS OF ENANTIOMERS

Three enantiomeric pairs of molecules with very similar, spherical shape but different functional groups were selected to test the hypothesis that hydrogen bond formation may play a role in the alignment process and the capability to distinguish enantiomers using PBLG as the chirality distinguishing phase. The compounds are listed in Table 1 with their IUPAC names and their atom numbering throughout this study shown in Figure 1.

TABLE 1 Generic and systematic names of the compound used in this study.

Generic names	Systematic names
Borneol	Bicyclo[2.2.1]heptan-2-ol, 1,7, 7-trimethyl-, (1*S*, 2*S*, 4*S*) or (1*R*, 2*R*, 4*R*)
Camphor	Bicyclo[2.2.1]heptan-2-one, 1,7, 7-trimethyl-, (1*S*, 4*S*) or (1*R*, 4*R*)
Quinuclidinol	1-Azabicyclo[2.2.2]pctan-3-ol (3*R*) or (3*S*)

Borneol contains a hydroxyl group as the sole functional group of the molecule and may serve as a hydrogen bond donor. Camphor, instead, possesses a carbonyl at the very same position while otherwise having the same spherical structure as borneol, qualifying the molecule as a hydrogen bond acceptor. A third, bridged compound is quinuclidinol, which possesses a hydroxyl H-bond donor group and a tertiary amine that may act as an H-bond acceptor. All molecules are soluble in chloroform and therefore suitable for measurements in PBLG/$CDCl_3$.

To obtain experimental RDCs, isotropic and anisotropic samples were prepared. Next to an isotropic sample, two samples of enantiomerically pure compounds were prepared for each of the three constitutions. Within a concentration range of 12% to 25% (w/v), PBLG in chloroform acts as a weakly aligning chiral medium.[35] At this concentration, the solutions are very viscous, the mixing and homogenization steps take time, and they have to be done carefully. We therefore modified a vertical rotating

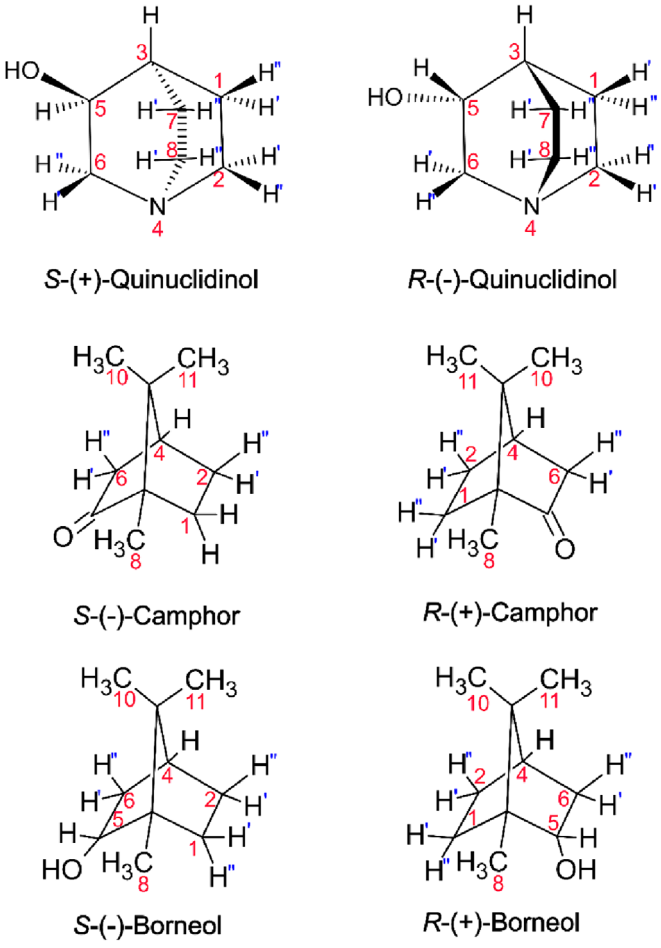

FIGURE 1 Chemical structures, generic names, and numbering of the three pairs of enantiomers used in this study. The absolute stereochemistry is indicated on the structure and with the generic name, and the optical rotation is given in brackets.

shaker 360° PTR-35 Grant-Bio so that 5 mm NMR tubes can be loaded, which heavily reduced manual labor and guaranteed well-mixed and homogenized samples after 24 h of rotation (see also a photo of the shaker in the SI). All relevant information regarding sample constitutions is summarized in Table 2.

3 | RDC MEASUREMENTS

After confirming assignments for the three compounds, ^{1}H-1D, 2D CLIP-HSQC[36] and P.E.HSQC spectra[37] using BEBOP[38,39] excitation, BIBOP[40,41] inversion pulses, and BURBOP universal rotation pulses[42,43] were recorded for all samples on a 500 MHz Bruker Avance III spectrometer in isotropic and aligned phases to obtain one-bond couplings. In addition, deuterium spectra and ^{2}H-imaging[44] experiments were recorded on the aligned samples to measure the quadrupolar splitting and to evaluate the homogeneity of alignment, respectively.

For the spectra, the typical proton spectral widths were 6 kHz for the isotropic and 12 kHz for the aligned samples, whereas the carbon spectral widths varied between 28 and 36 kHz, with 32 k time domain points recorded on the free induction decay (FID). Deuterium experiments were recorded with spectral widths of 28 kHz and 32 k time domain points. CLIP-HSQC experiments were typically acquired with a spectral width between 6 and 12 kHz and 16 k time domain points in the direct dimension, whereas in the indirect dimension, the spectral width was varied between 20 and 24 kHz with 256 increments. During data processing, zero filling to typically twice the number of points was applied in both dimensions.

For the measurement of residual dipolar couplings, the acquisition of spectra in isotropic and anisotropic states is needed, since the value obtained in the anisotropic state corresponds to the coupling in isotropic state plus the anisotropic coupling. With T corresponding to the *total* splitting observed in the anisotropic state, the scalar coupling J (as obtained in the isotropic state) is supposed to not change under weak alignment conditions and the residual dipolar splitting D (not to be confused with the residual dipolar coupling, which in most cases is half the dipolar splitting), and the differences of T and J values will provide the desired anisotropic parameters following the formula

$$T = J + D.$$

As the distance is known and approximately the same for all direct CH-bonds, $^1D_{CH}$ dipolar splittings obtained from CLIP-HSQC spectra directly contain valuable

TABLE 2 Preparation and concentrations of isotropic and anisotropic samples of the three pairs of enantiomers.

	Borneol			Camphor			Quinuclidinol		
		Anisotropic			Anisotropic			Anisotropic	
	Isotropic	*R*	*S*	Isotropic	*R*	*S*	Isotropic	*R*	*S*
Quantity [mg]	20	16.7	15.9	13	14	17	20	20.6	20.2
Solvent/PBLG [mg]	$CDCl_3$	$CDCl_3$/93.5	$CDCl_3$/92.9	$CDCl_3$	$CDCl_3$/90.1	$CDCl_3$/90.5	$CDCl_3$	$CDCl_3$/99	$CDCl_3$/98.76
Volume [mL]	0.75	0.6		0.75	0.6		0.75	0.6	
Monoisotopic mass [g/mol]	154.1			152.1			127.1		
Concentration [mM]	173	181	172	114	153	186	210	270	264
^{2}H splitting $\Delta\nu_Q$ of $CDCl_3$ [Hz]	-	299	263	-	262	285	-	305	261

angular information that can be used for structure determination or as applied here, for characterizing differences in alignment of the enantiomers. Couplings were extracted using the method described in Kummerlöwe et al.[45]: a row of the indirect carbon frequency is selected from the CLIP-HSQC, extracted and saved as a 1D spectrum. This 1D spectrum is overlaid with a copy of itself and then manually shifted to overlap the center of the α-component of the doublet with the center of the β-component. To estimate the error, a shift of one of the signals to the furthest right and the furthest left with still acceptable signal overlap are noted. The values obtained define then the individual maximum error estimate (MEE) of the coupling in Hz. For an experienced spectroscopist, this method gives reproducible and quite reliable results, marking roughly a 99% confidence level of the error.

Although samples were prepared thoroughly to ensure similar pairs of samples for the enantiomers, they can never be prepared fully identical. Therefore, after all dipolar splittings have been extracted with their experimental errors, the splittings were scaled according to the difference in the quadrupolar splitting of $CDCl_3$ to be able to fully compare the experimental dipolar splittings between the two enantiomers. If the set of splittings to be scaled is that of the (R)-enantiomer, it is simply multiplied by the ratio of the quadrupolar splittings $\Delta\nu_Q(S)/\Delta\nu_Q(R)$ of the deuterated solvent ($CDCl_3$) given in Table 2 (and vice versa for the other enantiomer). Corresponding scaled dipolar splitting values used for subsequent discussions are given in Table 3.

TABLE 3 Experimental $^1D_{CH}$ splittings of the three pairs of enantiomers.

Assignment	*S*-(−) Borneol [Hz]	*R*-(+) Borneol* [Hz]
CH-5	3.34 ± 1.5	29.16 ± 0.4
CH-4	4.91 ± 0.5	−20.53 ± 2
CH_2–6′	−30.94 ± 2	−11.15 ± 1
CH_2–6″	2.35 ± 1	−1.63 ± 1.3
CH_2–2′	−21.82 ± 3	−9.04 ± 2.4
CH_2–2″	24.25 ± 1.5	30.45 ± 0.6
CH_2–1′	19.13 ± 1	10.98 ± 0.8
CH_2–1″	−25.73 ± 5	−22.83 ± 1
CH_3–11	−1.65 ± 1.2	−0.25 ± 4
CH_3–10	5.92 ± 1	1.72 ± 0.4
CH_3–8	−1.12 ± 0.5	2.47 ± 0.5
Assignment	***S*-(−) Camphor [Hz]**	***R*-(+) Camphor* [Hz]**
CH_2–6′	7.8 ± 2	11.02 ± 0.4
CH_2–6″	29.4 ± 0.6	26.20 ± 0.7
CH-4	2.29 ± 3	4.11 ± 0.6
CH_2–1′		−26.38 ± 10
CH_2–1″		−5.47 ± 10
CH_2–2′	1.65 ± 3	5.97 ± 2
CH_2–2″	−41.51 ± 3	−52.93 ± 3
CH_3–10	−7.75 ± 1	7.19 ± 1.5
CH_3–11	15.84 ± 1	15.73 ± 0.3
CH_3–8	−6.14 ± 1	−6.87 ± 0.8
Assignment	***R*-(−) Quinu. [Hz]**	***S*-(+) Quinu.* [Hz]**
CH-5	22.71 ± 0.3	13.02 ± 0.3
CH_2–6′	−15.01 ± 1.5	−38.96 ± 0.4
CH_2–6″	−25.24 ± 0.5	−0.96 ± 0.8
CH_2–2′	41.34 ± 0.5	30.39 ± 5
CH_2–2″	−7.35 ± 0.5	−35.50 ± 5
CH_2–8′	−16.44 ± 1.4	−6.22 ± 0.4
CH_2–8″	32.86 ± 4	28.05 ± 0.5
CH-3	−4.93 ± 0.3	5.76 ± 0.5
CH_2–7′	23.05 ± 1	15.04 ± 1
CH_2–7″	10.52 ± 2	1.90 ± 1
CH_2–1′	9.1 ± 1	3.49 ± 1
CH_2–1″	−26.34 ± 0.5	−17.86 ± 1

Note: For camphor, two splittings were not extractable because of heavily broadened signals. Atoms highlighted in red represent stereogenic centers. In the column with *, dipolar splittings are scaled as described in the text.

4 | DISCRIMINATION OF ENANTIOMERS

The difference in measured dipolar splittings in some cases can be directly seen in the tables. However, as the different samples provide different alignment strengths, a comparison between the enantiomeric pairs has to be done by an adequate tool. As the agreement of the corresponding RDCs of the enantiomers is expected to be low in cases where the stereoisomers can be clearly distinguished, the correspondence of experimental data can best be visualized in correlation plots with correlation coefficients R^2 being a proper quality factor of overall distinction of enantiomers. For a proper comparison, the square of the so-called cosine similarity is used for R^2 instead of the conventional Pearson coefficient, as we can use the information that any straight line representing the correlation of sets of dipolar splittings must include the origin.[46] As shown in Figure 2, clear differences in the differentiation capacity of PBLG for the three pairs are found. For borneol and quinuclidinol, the obtained correlation coefficients are small, with values of 0.45 and 0.58, respectively. In both cases, a clear distinction of enantiomers is possible. In contrast, a high correlation

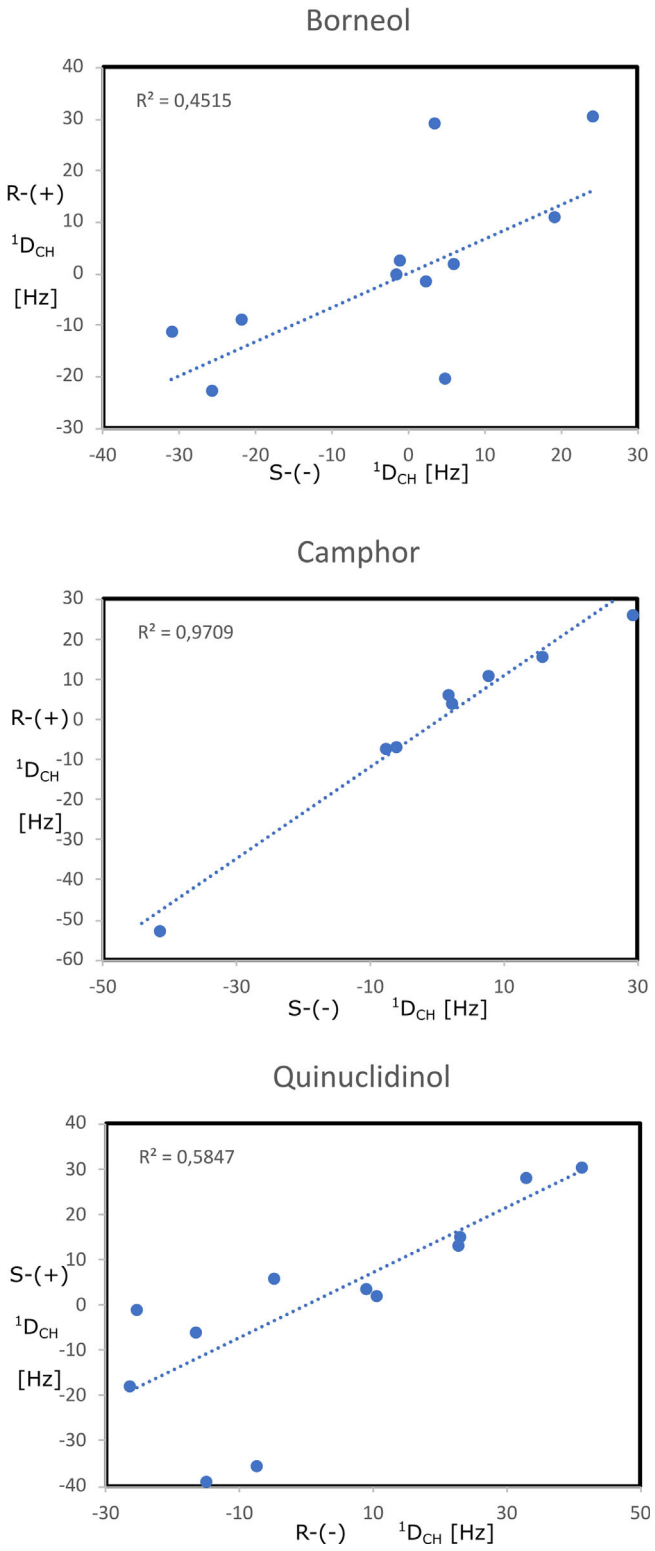

FIGURE 2 Comparison of the experimental RDCs extracted for the three pairs of enantiomers. For each pair, the experimental dipolar splittings measured for each enantiomer are plotted and corresponding cosine similarities (R^2) are determined.

with $R^2 = 0.97$ is found for camphor, which renders only a small chance to potentially distinguish the two enantiomers. The profoundly different alignment behavior of the enantiomeric pairs may be of interest for deciphering the underlying reasons and will be studied more closely in the following section.

5 | ALIGNMENT PROPERTIES

For a rigid molecule, the orientation of the molecule can be described by an alignment tensor, which can be derived by mathematical methods like the singular value decomposition (SVD)[48] if a minimum set of five independent experimental RDCs are available per rigid fragment and no more than three out of these five internuclear vectors lie in the same plane and no vectors are parallel. The alignment tensor can be also approximated, for example, by the TRAMITE prediction, for which the alignment tensor is assumed to coincide with the tensor of gyration.[49] Thus, calculated RDCs are assumed to depend on the molecular geometry only, with no other specific interaction interfering significantly. There are different programs that have the SVD approach being implemented, such as PALES,[50,51] ConArch+,[52] and MSpin[47] where the latter also contains the TRAMITE prediction and is used here. The MSpin program works with either single or few preselected fixed 3D conformations of the molecule under study. However, regarding the rigid nature of the bridged molecules studied, we decided to use the simplest fitting procedure with a single conformer single alignment tensor fitting approach. The experimental RDCs are given as input for the fitting and resulting fits give very good results ranging from a Cornilescu Q-factor[53] of 0.044 to 0.172 for the six individual compounds (see the Supporting Information for individual fitting parameters). Fits are worst for quinuclidinol, which may be expected regarding the potential flexibility of the bridged six-membered rings. TRAMITE as a simple predictive method was also used to obtain alignment tensors for the six individual compounds under study.

A quantitative analysis of the data is quite complex and leads to many numbers that can hardly be interpreted. We therefore attempted a visualization of data with respect to effective alignment in the lab frame: We know from elemental physics that negative RDCs (i.e., the directions with strongest negative values of the alignment tensor) correspond to an alignment where the couplings are mostly oriented along the external static magnetic field. We therefore oriented all molecules with negative resulting alignment tensor components from the SVD fits (indicated by reddish orbitals in Figure 3) along z, that is, the direction of the magnetic field. In addition, we rotated the molecules in the x,y-plane in such a way that the oxygens—indicative for

functional groups within the molecules—are visible as red bonds. We then left the molecules in the same orientations and visualized corresponding alignment tensors obtained by the TRAMITE approach (indicated by negative blue and positive yellow orbital components in Figure 3). The TRAMITE approach does not take into account the alignment medium, but only the shape and molecular weight distribution in the molecule. As all molecules possess an overall quite spherical appearance, oxygen as the heaviest atom will dominate the alignment, which can be clearly seen by the fact that all predicted alignment tensors point with their major axes in the direction of the oxygen. For camphor, the oxygen is predicted to point along the magnetic field, which correlates very well with the experimental alignment obtained from the SVD-fit. This correspondence can also be expressed in a quantitative measure, the so-called generalized cosine beta (GCB), which we calculated for the SVD-fitted alignment tensors versus their Tramite-predicted counterpart. S-(−)-camphor and R-(+)-camphor result in GCBs of 0.88 and 0.91, respectively. For borneol and quinuclidinol, instead, the hydroxyl groups are tilted with respect to the B_0-field and the TRAMITE predictions lead to alignment tensors that are far from the experimental results, which is also reflected in GCBs in the range of 0.2–0.3 (see the Supporting Information for actual numbers). Interestingly, all OH groups point in roughly the same direction relative to the *z*-axis. It is therefore likely

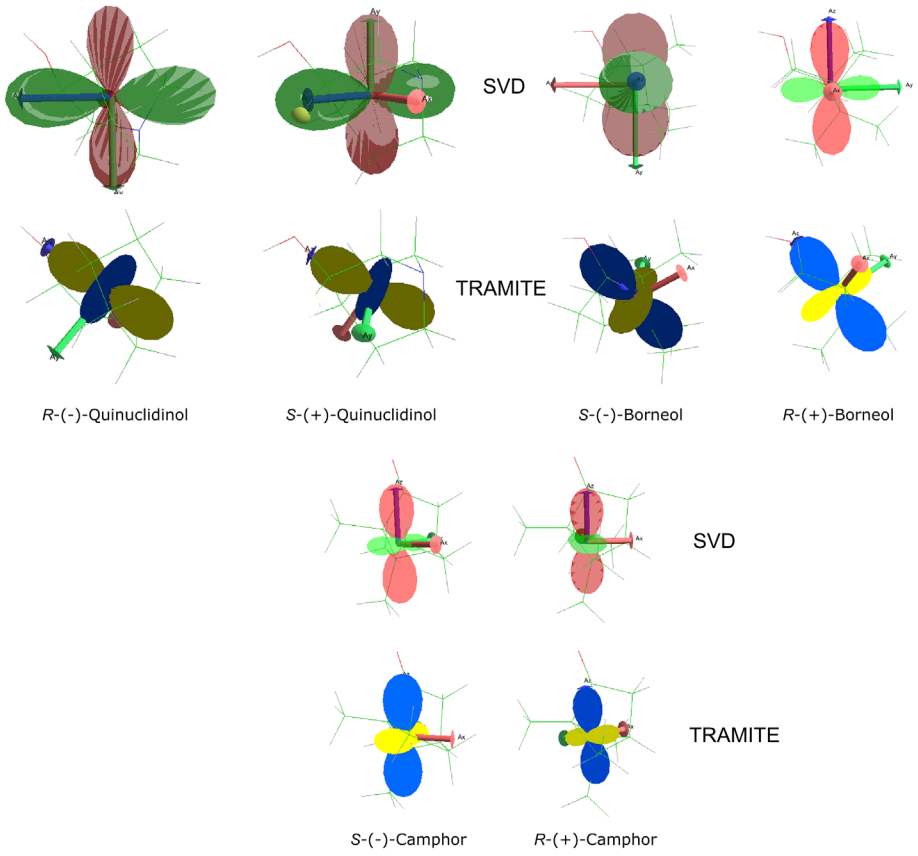

FIGURE 3 All molecules studied here with their corresponding alignment tensors as obtained from SVD fitting (red negative, green positive tensor components) and TRAMITE prediction (blue negative, yellow positive tensor components). All six molecules are oriented in a way that the negative component of the SVD-fitted alignment tensor points up/down, representing the predominant alignment of the molecule in PBLG with respect to the static magnetic field. Two identical molecules with the same orientation are shown on top of each other, the upper one with the SVD-fitted alignment tensor drawn at the center of mass, and the lower one with the TRAMITE-predicted alignment tensor. For the upper four molecules (quinuclidinol and borneol enantiomers), clearly, the H-bond donor groups (the white/red hydroxyl groups at the left upper side of each molecule) point at an angle to the magnetic field that is similar for all four molecules. The lower displayed camphor enantiomers, lacking an H-donor group, have the active carbonyl group pointing mostly along the magnetic field (negative alignment tensor components). Although the TRAMITE approach, which predicts alignment without considering charges, shows a high similarity to the SVD-fitted alignment tensors in the case of camphor, the alignment for all other molecules for the two types of calculations differs strongly. Clearly, the potential of H-bond formation might explain the difference. The differences in shading of the alignment tensors originate from the program MSpin used for tensor analysis.[47] Scaling and rotation of alignment tensors were performed manually and should be understood qualitatively.

that a specific interaction of the solutes with PBLG is present, causing this type of deviation of the alignment tensor from simple sterical prediction as with TRAMITE.

6 | DISCUSSION

We have studied the alignment behavior of three enantiomeric pairs, borneol, camphor, and quinuclidinol in the well-known liquid crystalline phase PBLG. All three compounds have a close to spherical overall shape but differ in their functional groups: The hydroxyl groups of borneol and quinuclidinol may serve as hydrogen bond donors, whereas the carboxyl group of camphor and the tertiary amine of quinuclidinol may serve as hydrogen bond acceptors.

Resulting dipolar splittings of the compounds partially aligned in PBLG/$CDCl_3$ give a very distinct picture of the alignment differences of each enantiomeric pair. Although the two compounds with hydrogen bond donors appear to have strongly differing dipolar splittings for the enantiomers, camphor, lacking a hydrogen bond donor, shows only very small differences in dipolar splittings for its enantiomers. A qualitative study of resulting alignment tensors shows in addition that the alignment of camphor can well be predicted by the TRAMITE approach based purely on the tensor of gyration of solute molecules. We can therefore conclude that the interaction of camphor molecules with PBLG is mainly steric, probably dominated by van der Waals-type potentials. As this interaction is weak, only small differences in RDCs can be measured.

The situation for borneol and quinuclidinol appears to be profoundly different. TRAMITE-predicted alignment tensors do not at all match the ones fitted using experimental data. The hydroxyl groups furthermore all show an angle of roughly 40–60° with respect to the most occupied orientation of the B_0-field in the molecular frame of reference—corresponding to the largest negative components of the SVD-fitted alignment tensors. This behavior can only be explained by an additional, specific interaction that is not present in camphor. Looking at the chemical structure of the side chain of PBLG (Figure 4), it is obvious that the ester of the protected glutamate may well serve as a hydrogen bond acceptor, explaining potentially the orientation of the hydroxyl groups of the solute molecules that are presumably imposed by the orientation of the resulting hydrogen bonds.

The explanation of differing alignment properties due to the formation of hydrogen bonds between PBLG and the solute molecules also directly explains the observed differences in enantiomeric differentiation capabilities of the different enantiomeric pairs. Hydrogen bonds in

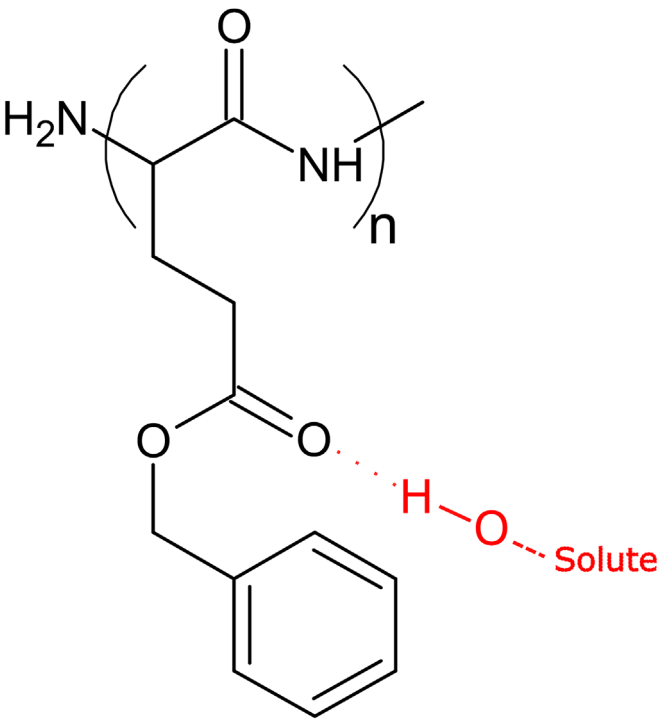

FIGURE 4 Chemical structure of poly-γ-benzyl-L-glutamate (PBLG) and the likewise formation of hydrogen bonds with solute molecules being able to act as hydrogen bond donors.

apolar organic solvents are relatively strong and provide a specific interaction site close to the chiral PBLG helix backbone. As such, the chiral distinctive power must be much larger than in the case of steric interactions only. We therefore predict that enantiomeric discrimination will be particularly high if specific hydrogen bonds between PBLG and a solute molecule can be established. As the amide groups of the PBLG backbone are already absorbed in helix-stabilizing hydrogen bonds, this implies that the solute molecule should be capable of acting as a hydrogen bond donor and that the molecular shape of the solute should be such that steric properties allow the hydrogen bond to be formed.

7 | CONCLUSION

With a small set of well-chosen spherical molecules, we were able to show profound differences in alignment behavior of chiral molecules in PBLG/$CDCl_3$ as a chiral liquid crystalline alignment medium. Although an enantiomeric pair with a functional group working as a hydrogen bond acceptor showed very little capability to distinguish its enantiomers by residual dipolar splittings, corresponding molecules with functional groups acting as hydrogen bond donors resulted in dramatic differences in their enantiomeric alignment. Apparently, the

possibility of solutes to form proper hydrogen bonds with the alignment medium PBLG in an apolar environment works as a strong enhancer in enantiodifferentiation. Even more, these data corroborate the findings of the Reggelin group,[54] which show similarly enhanced enantiodifferentiation capability for analytes with hydroxyl and amino groups for the use with poly (acetylene)-based alignment media and spectacular recent evidence for saturation transfer between poly-γ-benzyl glutamates and hydroxyl groups of solute molecules.[55] With all the evidence in hand, it can be safely concluded that the strongest known effect on the enantiomeric distinction power of chiral, helical alignment media in organic solvents are specific H-bonds with H-bond donor groups of a solute. Based on this result, we predict that a similar effect will be observed with H-bond acceptor molecules for chiral alignment media with H-bond donors in their backbone. We furthermore predict that the specific hydrogen bond should be as close to a chiral moiety as possible for best distinction of enantiomers. In an aqueous solution, on the other hand, the importance of hydrogen bonds will be less important and other effects like chiral hydrophobic stretches may be more decisive.

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