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Helical Polyisocyanopeptides as Lyotropic Liquid Crystals for Measuring Residual Dipolar Couplings

Gao-Wei Li,[a] Jiang-Ming Cao,[a] Wen Zong,[c] Li Hu,[c] Mao-Lin Hu,[c] Xinxiang Lei,*[b] [c] Han Sun,*[d] and Ren Xiang Tan*[a]

Abstract: Residual dipolar coupling (RDC) emerged to be an important structural parameter for organic molecules, as well as biomolecules. Here, a new helical polyisocyanopeptide (L,L-PIAF-OBn) that forms lyotropic liquid crystals (LLC) in $CDCl_3$ is proposed as a novel weakly orienting medium for acquiring residual dipolar couplings (RDCs) of organic molecules. We demonstrate its application to the structural elucidation of strychnine and triptolide.

As an information-rich NMR structural parameter, residual dipolar coupling (RDC) has been widely employed in the elucidation of constitution, relative configuration, and conformation of organic molecules.[1] A combined approach using RDC analysis and chiroptical methods was proposed and demonstrated on the determination of the absolute configuration of a number of small molecules.[2] In general, RDCs can be only measured, if the molecule is partially aligned in an anisotropic environment, such as lyotropic liquid crystal (LLC) phases and stretched or compressed gels.[3] The method of reversible compression/relaxation of flexible cross-linked gels introduced by Gil *et. al* facilitates the measurement of residual dipolar coupling and residual chemical shift anisotropy with high efficiency and precision.[3f-h] As an alternative approach, LLCs are good options for RDC measurements, as they can be freely scaled by the variation of the concentrations and their ability to quickly align the sample. The most employed LLC-based alignment media for organic molecules are the α-helical homopolypeptides PBLG, PELG, PCBLL,[4] ACHC-rich β-peptides,[5] as well as recently introduced polymeric LLCs such as polyguanidines,[6] polyacetylenes[7] and polyisocyanides.[8] An effective and commonly used strategy in designing polymeric LLCs as new alignment media is to create a confined system which is composed of a stable polymer backbone. These self-assembled helical polymers usually display high persistence lengths that are capable of forming LLC media at relatively low concentrations, and therefore can be translated into a moderate degree of order for organic compounds.

To our knowledge, organic solution-based polymers that form stable LLCs and simultaneously exhibit low critical concentration are rather rare. Recently, the peptide-functionalized polyisocyanides have been extensively explored, showing their comprehensive applications to chirality sensing, drug-delivery and LC formation.[9] Polyisocyanopeptides with well-defined helical β-sheet architecture are thus promising candidates for polymeric LC design. Based on the study mentioned above and encouraged by our previous work on the development of graphene oxide (GO) based LC phases for aligning small molecules in polar solvents,[10] we designed and synthesized a new dipeptide-based polyisocyanide, which has been proven to be an effective and tuneable alignment medium for acquiring RDCs in apolar solvents such as $CDCl_3$.

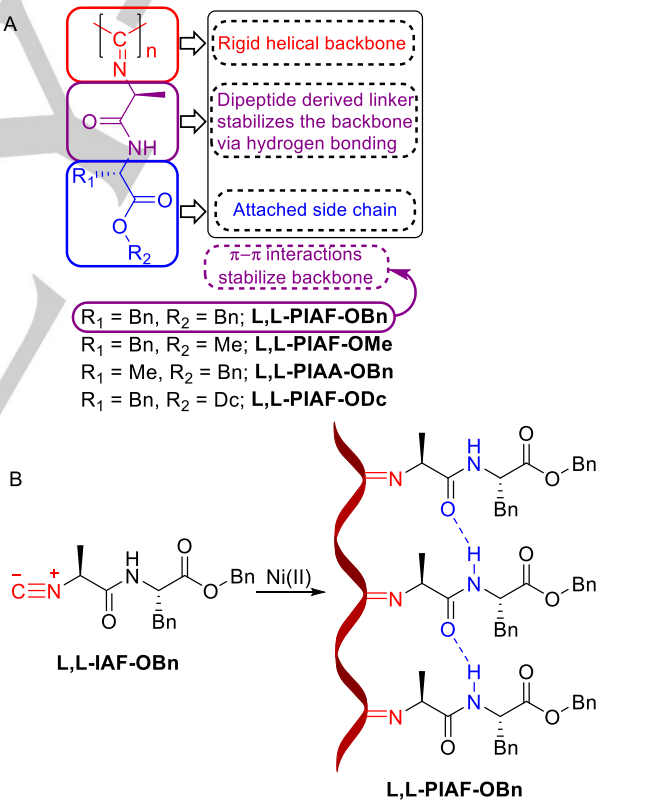

Figure 1. A) Basic principles employed in designing peptide-functionalized polyisocyanides, which form stable LLC phases in $CDCl_3$. B) Polymerization of the monomer L,L-IAF-OBn, with the proposed hydrogen bonding network between side chains for stabilizing the helical structure of the polymer.

In the first step, we designed four different polyisocyano-dipeptides (PIPs) sharing the same chemical backbone. Figure 1 shows the polymerization procedure of the representative monomer L-isocyanoalanyl-L-phenylalanine benzyl ester (L,L-IAF-OBn) using an achiral nickel catalyst. Other PIPs could be obtained using the same procedure. Polymerization details and

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the monomer synthesis are described in the Supporting Information. Interestingly, we found that although all the polymers presented in Figure 1A could be dispersed in $CDCl_3$, only poly(L-isocyanoalanyl-L-phenylalanine benzyl ester) (L,L-PIAF-OBn) was able to quickly form liquid crystals. When replacing one of the functional groups (R_1 and R_2, shown in Figure 1A) in L,L-PIAF-OBn by other alkyl groups (e.g., L,L-PIAF-OMe, L,L-PIAA-OBn and L,L-PIAF-ODc), the obtained polymers did not form the LC phases. In addition, the UV-visible absorption spectrum of L,L-PIAF-OBn revealed a distinct shoulder peak at 370 nm (Figure S1-1), which was attributed to the ordered packing of the helical main chain structure and aromatic π-stacking interactions. Therefore, we conclude that the π-π interactions formed by benzyl pendants in the side chains play a key role in the formation of the meso-phase in chloroform solution.

We next investigated the possibility of employing L,L-PIAF-OBn as a weak alignment medium for the RDC measurements. At a concentration of 11.5 wt %, we observed a maximal quadrupolar splitting of the deuterated $CDCl_3$ solvent signal of 108 Hz. The formation of homogenous LC phase could be additionally confirmed by the direct observation of strong birefringence with crossed polarizers using the naked eye (Figure 2B). Preparation of the anisotropic sample of L,L-PIAF-OBn LCs was fast and straightforward, only requiring addition of the polymers into the NMR tubes with the compound of interest dissolved in the solvent. Notably, the equilibrium could be reached in just a few minutes, and remained stable for several months (Figure 2A). The excellent solubility and intrinsic low viscosity enabled us to acquire high quality NMR spectra with narrow lines. Additionally, we verified the spatial homogeneity of the alignment in L,L-PIAF-OBn LCs by performing the deuterium NMR 1D imaging experiment,[11] showing that the anisotropic sample was well-equilibrated at different concentrations of the polymer (Figure S2.1-1).

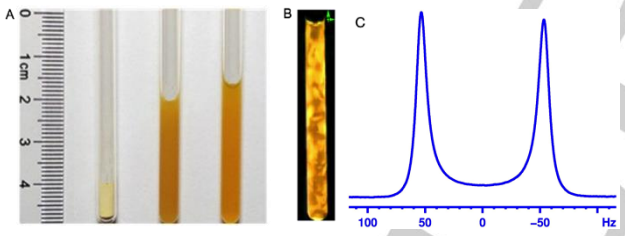


Figure 2. A) Preparation of the alignment media for the RDC measurements in 5 mm NMR tubes. From left to right: Dried L,L-PIAF-OBn polymer powder; Mixing of the LCs with solvent by shaking after 2 minutes; Addition of the compound of interest dissolved in a small amount of solvent. B) Optical micrograph of L,L-PIAF-OBn solution in a NMR tube observed through crossed polarizers, displaying the characteristic schlieren texture of LLC phase. C) 1D ^2H spectrum (25 °C, eight scans) of L,L-PIAF-OBn in $CDCl_3$ at 11.5 wt %, with a deuterium splitting of 108 Hz (line width: 10.2 Hz).

One of the remarkable property of L,L-PIAF-OBn is its ability to form LCs at very low concentrations. Comparable low critical concentration of LCs as alignment medium has only been found for a short water-based helical β-peptide.[5] However, until now reasonable size of the residual dipolar coupling could be only measured at relatively high concentration, as the ratio between the quadrupolar coupling and residual dipolar coupling measured in L,L-PIAF-OBn LCs is particularly high. For L,L-PIAF-OBn we found that the $\Delta\nu_Q$ observed in the ^2H NMR spectrum scaled up with the concentration of the dispersed polymer, as shown in Figure S2.1-2.

In order to test the compatibility of L,L-PIAF-OBn with organic molecules, we acquired RDCs of the highly rigid strychnine. One-bond proton-carbon *J*-couplings ($^1J_{CH}$) in the isotropic sample and the sum of the one-bond dipolar and *J*-couplings ($^1T_{CH}$) in the presence of the LLC phase were collected using the F2-coupled CLIP-HSQC experiment.[12] RDC values ($^1D_{CH}$) were extracted from the obtained spectra using the relation $^1D_{CH}=^1T_{CH}-^1J_{CH}$. The full 2D [$^1H$-$^{13}C$]-CLIP-HSQC spectra and representative aromatic region of strychnine (Figure S2.2-3) demonstrated the high quality of the spectrum with narrow lines, which were recorded on a sample with 6.3 wt % L,L-PIAF-OBn. The obtained RDC data range from -5.9 to +6.8 Hz (Table S2.2-1). As an alternative approach, we measured the $^1D_{CH}$ couplings of the analyte from the F1 dimension by using the *J*-scaled BIRD HSQC (JSB-HSQC) experiment.[13] The RDC values extracted from the JSB-HSQC experiment vary between -6.0 and +6.1 Hz, which are very close to the data obtained from the CLIP-HSQC experiment (Table S2.2-1 and S2.2-2). To verify the structure of solute using the acquired RDCs, we calculated the alignment tensor using the singular value decomposition (SVD) method [14] with the program package MSpin.[15] Theoretically predicted RDCs were calculated from the computed alignment tensor by using the DFT-optimized structure [16] as the input and further compared with the experimental determined ones. The quality factors (*Q* factors) between experimental and predicted RDCs were 0.15 (CLIP-HSQC data) and 0.16 (JSB-HSQC data), respectively (Figure S2.2-4 and S2.2-9). In a different batch of L,L-PIAF-OBn synthesis (L,L-PIAF-OBn*,Table S1-1; Figure S2.2-10), the corresponding medium shows significantly higher alignment strength with a quadrupolar coupling of $\Delta\nu_Q$ = 151 Hz. 22 $^1D_{CH}$ couplings of strychnine cover a favorable range of -15.2 to +21.2 Hz (Table S2.2-3). Furthermore, the full 2D spectra of strychnine demonstrated the high quality of the spectrum with narrow lines (Figure 3 and S2.2-11). The quality factors between experimental and predicted RDCs were 0.09 (CLIP-HSQC data) and 0.07 (JSB-HSQC data), respectively (Table 1). Currently, factors that could influence the alignment strength are still unknown and are under investigations in our lab.

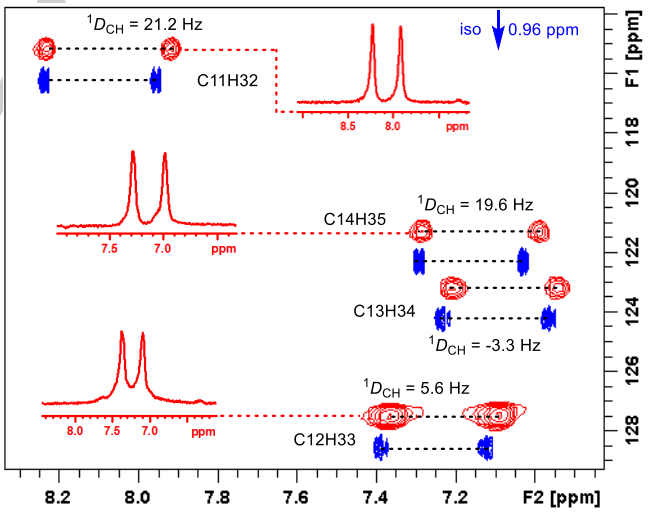
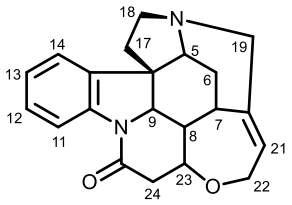


Figure 3. Expanded representative aromatic region of strychnine in the isotropic $CDCl_3$ phase (blue contours, down-shifted 0.96 ppm in the ^{13}C dimension) and in anisotropic 6.0 wt % PIP LCs (red contours) acquired on a 600 MHz spectrometer (^2H quadrupolar splitting of 151 Hz). The inserted trace from the anisotropic 2D spectra (C11/H32, C12/H33, C13/H34 and C14/H35) illustrates the favorable line widths achieved with L,L-PIAF-OBn LCs.

To further verify the scope of L,L-PIAF-OBn LC as a suitable alignment medium for other natural compounds, a biologically active triptolide was chosen as the analyte. RDCs for triptolide were in the range of -4.9 to +2.8 Hz. We employed the same

fitting procedure as described for strychnine, using the X-ray structures of triptolide and 14-*epi*-triptolide [17] as the input. A considerably lower quality factor (Q= 0.19 with CLIP-HSQC data, 0.18 with JSB-HSQC data) was obtained for triptolide (Figure S2.3-4 and S2.3-9), whereas the 14-*epi*-triptolide has a significantly higher Q factor (Q= 0.68 with CLIP-HSQC data, 0.55 with JSB-HSQC data, Figure S2.3-5 and S2.3-10). This result clearly demonstrates the value of the RDC data acquired in the L,L-PIAF-OBn for the stereochemical elucidation of triptolide.

Table 1. Experimental RDCs (Hz) of strychnine in anisotropic 6.0 wt % PIP LCs measured by using CLIP-HSQC and JSB-HSQC experiments, respectively [a]



Atom number	F_2-coupled RDCs	F_1-coupled RDCs
C12,H33	5.6	4.1
C21,H42	2.5	1.8
C13,H34	-3.3	-5.6
C14,H35	19.6	19.3
C11,H32	21.2	18.2
C23,H45	-10.0	-10.2
C22,H43	-15.2	-9.1
C22,H44	-5.7	-9.1
C5,H26	-1.9	-3.6
C9,H31	-11.0	-9.8
C19,H40	-4.6	-2.0
C19,H41	-1.4	-2.0
C18,H38	5.6	3.4
C18,H39	-0.9	3.4
C8,H30	-5.7	-3.8
C24,H46	11.6	0.8
C24,H47	-12.7	0.8
C17,H36	-0.5	-0.6
C17,H37	-0.5	-0.6
C7,H29	-11.5	-10.5
C6,H27	9.9	6.2
C6,H28	1.1	6.2
***Q* factor**	**0.08**	**0.07**

[a] CLIP-HSQC and JSB-HSQC experiments were recorded with the same anisotropic on a 600 MHz spectrometer.

In summary, we have presented L,L-PIAF-OBn LCs as a novel and effective alignment medium, which enables to introduce a low degree of alignment for RDC measurements of organic molecules. Comparing dipeptide-based polyisocyanides with different side chains suggested the high importance of the aromatic side chains in LLC formation. Additional stabilization of the helical structure was attained by the formation of hydrogen bonds between amide and carbonyl groups in the introduced peptide side chains. This work represents the first example of a polymeric LLC which is stable at extremely low critical concentration. Importantly, the excellent solubility and intrinsic low viscosity allowed us to acquire good quality NMR spectra with narrow lines. Taking different advantages of helical polyisocyanopeptide as a tuneable mesogen, our future plan is to increase the absolute RDC values for organic molecules by increasing its persistence length of L,L-PIAF-OBn via different catalysts and optimize the side chain of polyisocyanopeptides for other organic solvents.

Experimental Section

Experimental details, data for strychnine, triptolide, and preparation of the monomer are provided. (See Supporting Information)

Acknowledgements

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Keywords: polyisocyanodipeptides • liquid-crystalline medium • residual dipolar couplings • NMR spectroscopy • small organic molecules

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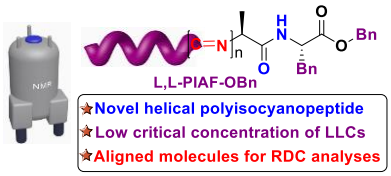
FULL PAPER

A helical polyisocyanopeptide (L,L-PIAF-OBn) can fleetly form lyotropic liquid crystal and adopt a striking low critical concentration in chloroform. The adjustable weakly alignment medium is easily handle and allows for acquiring residual dipolar couplings (RDCs) of organic molecules.



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