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A novel liquid crystalline system for partial alignment of polar organic molecules

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Abstract

A new system for partial alignment of polar organic molecules to measure residual dipolar couplings in NMR consists of a 1:1 or a 2:1 mixture of water and DMSO including 3–13% *n*-alkylpentaethylene glycol as the surfactant. Temperature and concentration dependence of the alignment system are investigated and, as examples, the $^{13}C,^{1}H$ residual dipolar couplings for the amino acid methionine **1** and for an α-methylene-γ-butyrolactone **2** have been obtained and are compared with those obtained from the alignment media consisting of *n*-alkylpentaethylene glycol, *n*-alkyl alcohol and water.

Keywords: NMR; Partial alignment; Liquid crystal; Residual dipolar couplings; Organic molecules

1. Introduction

Traditionally, the structure determination of relatively small organic compounds in solution is based on both one- and two-dimensional NMR spectroscopy such as COSY, HMQC, and HMBC [1]. Stereochemical questions are most often addressed with NOESY [2] which allow the determination of the distances between magnetic nuclei up to 5 Å. Increasing the number of atoms in a molecule and hence their distances results in NMR spectra, where *J* couplings and NOE values often are not more sufficient to adequately describe the conformation between separated parts of the molecules.

For this reason, a new approach for the structure determination of macromolecules has been worked out [3–5]. By partial alignment of the molecule in question in a lyotropic liquid crystalline medium, residual dipolar couplings (RDCs) between magnetic nuclei can be observed, where the actual values are taken from the difference between molecules dissolved in an isotropic and in the anisotropic medium.

The expression for the residual dipolar coupling $D_{IJ(\theta\varphi)}$ between two directly coupled nuclei can be simplified to the form [4]:

$$D_{IJ}(\theta,\varphi) = D_a^{IJ}\left\{A_a(3\cos^2\theta - 1) + \frac{3}{2}A_r\sin^2\theta\cos 2\varphi\right\}, \quad (1)$$

where

$$D_a^{IJ} = -\frac{\mu_0 h}{16\pi^3} S \frac{\gamma_I\gamma_J}{r_{IJ}^3}. \quad (2)$$

Here $A_a = \frac{1}{3}\left(A_{zz} - \frac{A_{xx}+A_{yy}}{2}\right)$ is the axial component of the molecular alignment tensor $\boldsymbol{A}$ characterizing the preferential orientation of the molecule relative to the static field direction; $A_r = 1/3(A_{xx} - A_{yy})$ is the rhombic component of $\boldsymbol{A}$; A_{xx}, A_{yy}, and A_{zz} are the principal axes of $\boldsymbol{A}$; θ and φ define the polar coordinates of the internuclear vector (between I and J) with respect to the static magnetic field direction; S is the generalized order parameter describing

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the internal motion of the internuclear vector; γ_I and γ_J are the gyromagnetic ratios of nuclei I and J; r_{IJ} is the distance between the nuclei I and J.

Different liquid crystalline media are used for the necessary partial alignment of the biomolecules, such as phospholipid bicelles [3], viruses [6], or membrane fragments [7]. Nematic phases may be made on the basis of cetyltrimethylammonium bromide [4], mixtures of cetylpyridinium bromide or cetylpyridinium chloride with *n*-hexanol [8,9] or mixtures of *n*-alkyl-poly(ethylene glycol) and *n*-alkyl alcohol in water (the so called Otting phases) [10]. These latter systems form a lyotropic liquid crystalline phase L_α under certain conditions, with the bilayer surface oriented parallel to the direction of the magnetic field [4]. As the nature of the solutes dictates, all varieties of alignment media for biological macromolecules have in common that they are based on water as solvent.

The application of this approach to determine the relative configuration of small organic compounds has been described in the literature only very recently [11–16] and the search for suitable alignment systems such as polymer gels for these molecules is currently under full swing [17–21]. The use of RDCs for structure determination becomes a significant improvement if the interpretation of the NOE enhancements for small molecules is hindered either by small correlation times τ_c of these molecules that lead to weak cross-peak intensities in NOESY or ROESY spectra with further complications by three spin effects or the circumstance that too few suitable protons are available. Sometimes the stereogenic centers that need to be related are too far away from each other or conformational equilibria impede a use of NOE data.

Small organic compounds are usually dissolved in organic solvents and hence, for using the alignment approach, it is necessary to find a liquid crystalline medium based on an organic solvent. As an extension of our previous work [11,16], we present here NMR investigations of a liquid crystalline medium based on a combination of the pentaethylene glycol monododecyl ether with a mixture of water (D_2O) and dimethyl sulfoxide (DMSO-d_6). Although the system does not consist solely of organic solvents, the use of a DMSO/water mixture provides sufficient solvating properties for a class of small polar organic compounds.

2. Experimental

Pentaethylene glycol monododecyl ether [$C_{12}E_5$, where 12 is the number of carbons in the *n*-alkyl group and 5 is the number of glycol units in the poly(ethylene glycol)] ($\geqslant$98% purity, Sigma), pentaethylene glycol monooctyl ether (C_8E_5, $\geqslant$98% purity, Sigma), *n*-octanol and *n*-hexanol were used without further purification. D_2O and DMSO-d_6 were of the highest purity commercially available. The lyotropic liquid crystalline phase was prepared by dissolving $C_{12}E_5$ in the mixture D_2O/DMSO-d_6 (1/1 or 2/1, wt/wt). The composition of the final solution is reported in weight percent for the ratio $C_{12}E_5$ to the mixture D_2O/DMSO-d_6. For comparison purposes the lyotropic liquid crystal was also prepared without DMSO by dissolving $C_{12}E_5$ and *n*-hexanol (molar ratio $r = 0.99$) in D_2O as described in the literature [10]. The tubes containing the stock solution were heated to 70 °C until clearance occurred and left to cool to room temperature.

For the amino acid containing sample, 8.5 mg of methionine (**1**) were mixed with a stock liquid crystal $C_{12}E_5$-D_2O/DMSO-d_6 (1/1). The final concentration was 1.5% (wt) for the amino acid and 5.5% (wt) of $C_{12}E_5$ in D_2O/DMSO-d_6 (1/1) for the liquid crystalline phase. The sample for comparison purposes was prepared by mixing 6 mg of **1** with a stock liquid crystal $C_{12}E_5$/*n*-hexanol/D_2O. The final concentration was 1% (wt) for **1** and 6.2% (wt) of $C_{12}E_5$ for the liquid crystalline phase.

For the lactone/$C_{12}E_5$ in D_2O/DMSO-d_6 sample, 7.6 mg of (**2**) was mixed with 741 mg of a stock liquid crystal $C_{12}E_5$ in D_2O/DMSO-d_6 (1/1) to a final concentration of 1.0% of lactone and 6.7% (wt) of $C_{12}E_5$. For comparison purposes also samples with $C_{12}E_5$/*n*-hexanol/D_2O and C_8E_5/*n*-octanol/D_2O with the lactone were prepared as follows: 8.4 mg of lactone **2** was dissolved in 666 mg of a stock solution of 5% (wt) $C_{12}E_5$ with *n*-hexanol (molar ratio $r = 0.85$) in D_2O for the $C_{12}E_5$/*n*-hexanol/D_2O sample. For the C_8E_5/*n*-octanol/D_2O sample 8.0 mg of lactone **2** were dissolved in 648 mg of a stock solution of 3% (wt) C_8E_5 with *n*-octanol (molar ratio $r = 0.87$) in D_2O leading to final concentration 1–1.2% for the lactone and 5% for $C_{12}E_5$ and 3% for C_8E_5, respectively.

The ^2H NMR spectra were recorded on a Bruker DRX-400 instrument equipped with a 5 mm BBO probe with the deuterium field frequency lock turned off. For monitoring the temperature dependence of the liquid crystalline system, a 15 min equilibration period was interleaved between successive temperatures. The ^1H,^{13}C couplings ($^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$) [22] for **1** were measured from HSQC spectra with no decoupling in the ^{13}C dimension using a new pulse scheme including a BIRD filter within the HSQC sequence [23] at the same instrument using a TBI probe. The measurements for **2** were performed on a Bruker DRX-600 instrument equipped with a 5 mm TBI probe. The corresponding ^1H,^{13}C couplings ($^1J_{CH} + {}^1D_{CH}$) were either measured from HSQC spectra with no decoupling in the ^{13}C dimension or from HSQC spectra without decoupling in the direct dimension [16]. The error in the measured C,H splittings values ranges from 0.3 to 0.5 Hz. For **2** the ^1H–^{13}C RDCs of the methyl group were converted into the corresponding ^{13}C–^{13}C RDCs using the formula in lit [13]. The alignment tensors for **2** were calculated using the bestfit module within PALES [24] and a structure obtained from geometry optimization of the compound in the program Spartan [25] using the Merck force field MMFF94 [26] (see Fig. 1).

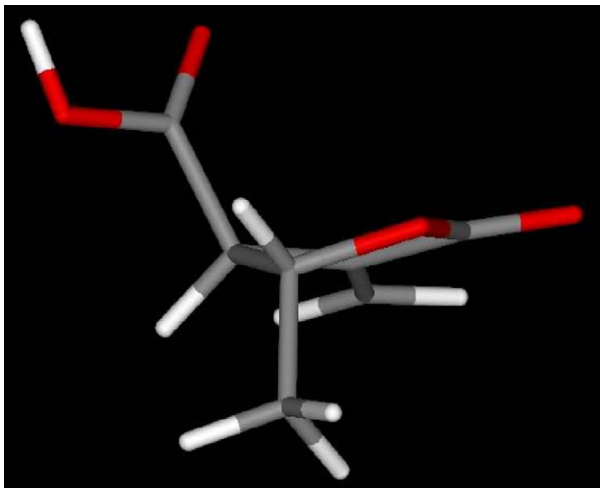


Fig. 1. Calculated structure of lactone **2** (MMFF94 within Spartan).

3. Results and discussion

3.1. Phase behavior of the water/DMSO/PEG systems

For the DMSO/water/$C_{12}E_5$ system used here, the presence of an ordered lamellar phase was monitored by the observation of quadrupolar splitting of the ^2H NMR signal of the solvent (D_2O). Two concentrations of DMSO have been investigated, one with a water/DMSO ratio of 1:1 (system I) and one with a ratio of 2:1 (system II), whereas the $C_{12}E_5$ concentration was varied between 3 and 13% (wt/wt). After placing the samples in the magnet, the quadrupolar splitting appeared within minutes and we were observing quadrupolar splitting in the ^2H NMR spectra of D_2O and of DMSO-d_6 (see Fig. 2). The splitting for D_2O is larger than for DMSO-d_6. This does, however, not necessarily mean that the degree of orientation for water is higher than for DMSO, but could also be due to a reduction of the quadrupolar splitting in the methyl groups of DMSO because of their mobility or due to a different orientation of alignment for DMSO itself.

Increasing the temperature from $T = 274$–293 K is not changing the shape of ^2H NMR signals of the solvents. Increasing the temperature of system I further to $T = 298$ K leads to singlets in the ^2H NMR signals of D_2O and of DMSO-d_6 thus indicating isotropic phase as can be seen in Fig. 2. System II shows isotropic behavior in the temperature region 303–328 K. Decreasing the temperature of the sample after heating allows the observation of the quadrupolar splitting in ^2H NMR pointing to the reversibility of the system (see also Figs. 5 and 6).

As expected, the quadrupolar splitting observed in the ^2H NMR spectrum increased with increasing surfactant to (water/DMSO) ratio. The ordering effect is determined mainly by the surfactant concentration ($C_{12}E_5$), with no significant difference between system I and system II. Fig. 3 demonstrates quadrupolar splitting of the ^2H NMR spectra of the D_2O signal in system I as a function of the concentration of $C_{12}E_5$ in water/DMSO at a constant temperature of $T = 283$ K. From our measurements we conclude that the dependence of quadrupolar splitting from concentration is linear for both systems.

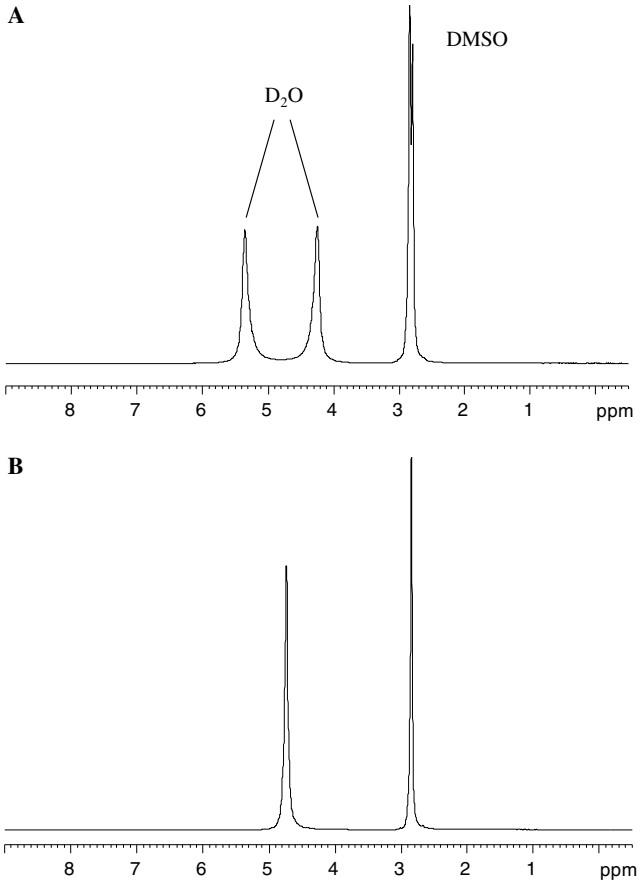


Fig. 2. The ^2H NMR spectra of the solvent (D_2O + DMSO-d_6) in dilute liquid crystalline system $C_{12}E_5$–D_2O + DMSO-d_6 (1/1); $C_{12}E_5$ to (D_2O + DMSO-d_6) ratio was 10.9 wt%. (A) $T = 296$ K; (B) $T = 303$ K.

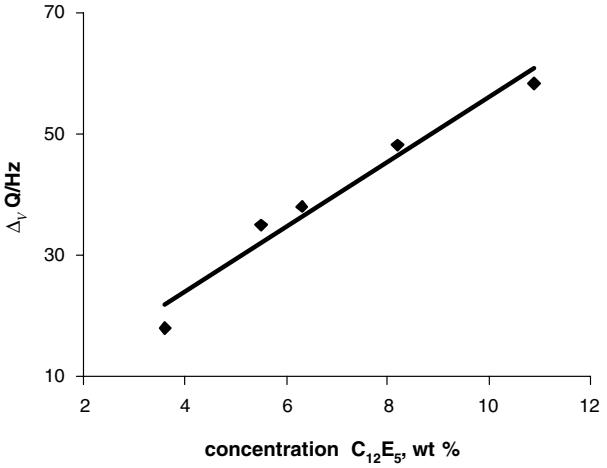


Fig. 3. Quadrupolar splitting of the ^2H NMR spectra of the D_2O signal in dilute liquid crystalline systems $C_{12}E_5$–D_2O + DMSO-d_6 (1/1) as function of concentration $C_{12}E_5$ in (D_2O + DMSO-d_6) for $T = 283$ K.

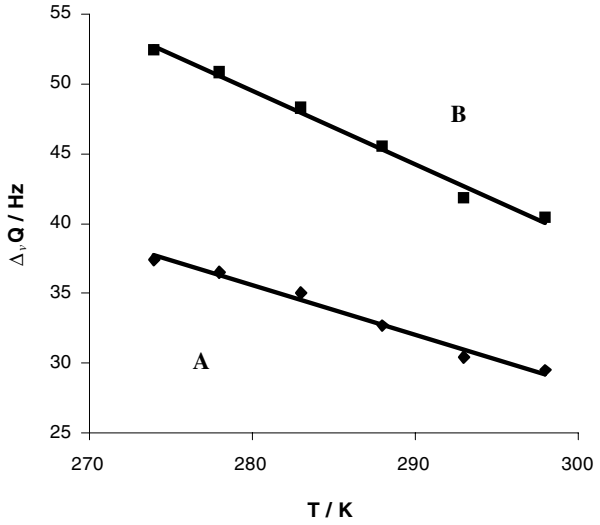


Fig. 4. Quadrupolar splitting of the ^2H NMR spectra of the D_2O signal in dilute liquid crystalline systems $C_{12}E_5$–D_2O + DMSO-d_6 (1/1) as function of temperature for two concentration $C_{12}E_5$ in (D_2O + DMSO-d_6): (A) 5.5%, (B) 8.2%.

We have investigated the dependence of the quadrupolar splitting on both system I and II as a function of the temperature. As shown in Fig. 4 these dependence is inversely linear, possessing a different slope and can be very well reproduced. It is in good agreement with a correlation between axial (A_a) and rhombic (A_r) components of the molecular alignment tensor and temperature given in Eq. (3) [5]

$$A_a = \Delta\chi[B^2/15\mu_0 kT]; \quad A_r = \delta\chi[B^2/15\mu_0 kT], \tag{3}$$

where $\Delta\chi$ and $\delta\chi$ are the terms of magnetic susceptibility anisotropies.

Figs. 5 and 6 show the temperature ranges, in which the lamellar phases (L_α) of the two systems were stable and gave well-resolved deuterium quadrupolar splitting with narrow line width. Areas, where quadrupolar splittings were only observed with broad lines and non-Lorentzian shapes, have not been considered. These graphs allow to estimate the range of stabilities. Apparently the systems are similar, but work best at different temperatures.

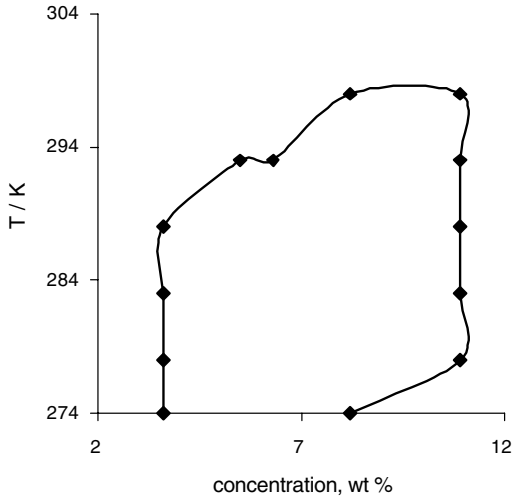


Fig. 5. Effects of concentration $C_{12}E_5$ in (D_2O + DMSO-d_6) and temperature on phase behaviour of the system $C_{12}E_5$–D_2O + DMSO-d_6 (1/1).

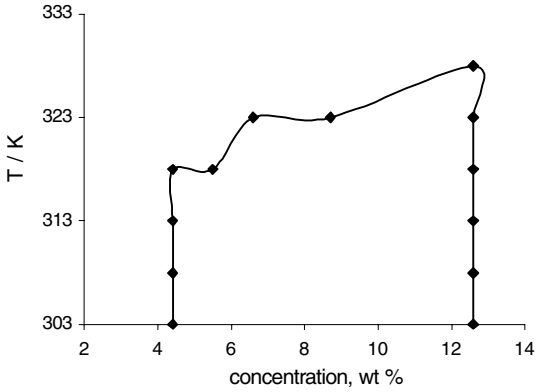
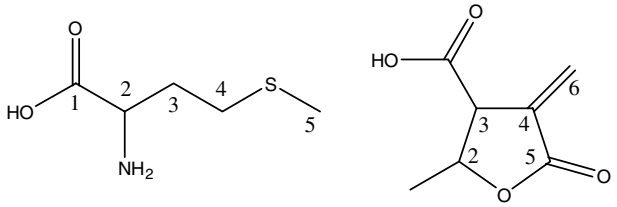


Fig. 6. Effects of concentration $C_{12}E_5$ in (D_2O + DMSO-d_6) and temperature on phase behaviour of the system $C_{12}E_5$–D_2O + DMSO-d_6 (2/1).

3.2. Alignment of solutes

To be of practical use, the liquid crystal systems must be stable after dissolution of organic compounds. This is clearly difficult for reagent mixtures as described here and depends very much on the nature of the solute. A general rule for the prediction of a working alignment system cannot be given yet. We had both experiences of disruption of the lamellar phase by dissolving organic compounds and of suitable alignment, however, with no clear structural insight, why some solutes destroy the lamellar phase and some do not. Furthermore, the concentration of the solutes seems to be an important factor. We demonstrate here the utility of system I for measuring $^1D_{CH}$ dipolar couplings in the amino acid methionine (**1**) and in the α-methylen-γ-butyrolactone (**2**).



1 **2**

The one-bond $^{13}C,^1H$ couplings of **1** dissolved in an isotropic solvent and in a lyotropic medium, were recorded. The scalar spin–spin couplings ($^1J_{CH}$) and line splittings (total coupling constants $^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$) for **1** obtained are shown in Table 1. The protons in one of the two methylene groups (S-CH_2) exhibit no diastereotopicity in neither isotropic case nor anisotropic case, thus also having the same scalar and dipolar couplings. In the other

Table 1
The scalar couplings ($^1J_{CH}$, Hz) and total spin–spin couplings ($^1J_{CH} + {}^1D_{CH}$, Hz) for methionine **1** dissolved in isotropic solvent and the lyotropic liquid crystalline media

	Isotropic	$C_{12}E_5$–D_2O + DMSO-d_6 (1/1)		$C_{12}E_5$/*n*-hexanol ($r = 0.99$)–D_2O	
	^1J/Hz	$^1(J + D)$/Hz	^1D/Hz	$^1(J + D)$/Hz	^1D/Hz
C2-H	147.5	143.6	−1.9	138.7	−4.4
-S-C4-H_a	139.1	124.5	−7.3	120.9	−9.1
-S-C4-H_a	139.1	124.5	−7.3	120.9	−9.1
-C3-H_a	131.5	116.5	−7.5	113.1	−9.2
-C3-H_b	130.7	116.5	−7.1	113.1	−8.8
-C5-H_3	139.4	141.5	1.0	146.1	3.4

CH_2-group scalar couplings of the two protons are slightly different and different dipolar couplings are observed for the two diastereotopic protons. For comparison purposes the total spin–spin couplings ($^1T_{CH} = {}^1J_{CH} + {}^1D_{CH}$) are also determined in the L_α phase consisting of $C_{12}E_5$/*n*-hexanol ($r = 0.99$)/D_2O (see Table 1).

To show the utility of system I the lactone **2**, which is soluble both in water and organic solvents, was aligned in system I and the two Otting phases ($C_{12}E_5$/*n*-hexanol/D_2O and C_8E_5/*n*-octanol/D_2O) [10]. The RDCs are shown in Table 2 together with some characteristics of the corresponding alignment tensors, calculated from these RDCs using the same structural model in all three cases (Table 3). Only 5 RDCs were accessible for **2** and therefore the alignment tensors cannot be calculated with high accuracy. It can, nevertheless, be stated that the alignment tensors obtained differ for the three systems. The collinearities and relative angles in five-dimensional space are given in Table 4. These are comparable in size with those obtained recently for norcamphor oriented in PS using different solvents [27].

These different alignment tensors for **2** in the three lamellar phases are indicative either for different orientational behavior of the three alignment systems for small organic molecules or for different conformational behaviour of the solute in the three alignment media. This latter aspect will be subject to further investigations.

4. Conclusions

The proposed pentaethylene glycol monododecyl ether in the mixture of D_2O and of DMSO-d_6 can be used as liquid crystalline system for the measurement of residual dipolar couplings of small polar organic molecules. The different systems investigated show stability in different

Table 2
The scalar couplings ($^1J_{CH}$, Hz) and total spin–spin couplings ($^1J_{CH} + {}^1D_{CH}$, Hz) for lactone **2** dissolved in isotropic solvent and the lyotropic liquid crystalline media

	Isotropic	$C_{12}E_5$/DMSO/D_2O		$C_{12}E_5$/*n*-hexanol/D_2O		C_8E_5/*n*-octanol/D_2O	
	^1J/Hz	$^1(J + D)$/Hz	^1D/Hz	$^1(J + D)$/Hz	^1D/Hz	$^1(J + D)$/Hz	^1D/Hz
H6a	163.73	184.28	10.27	179.83	8.05	170.61	3.44
H6b	164.23	212.03	23.90	212.54	24.16	185.86	10.81
H2	158.19	104.54	−26.83	80.61	−38.79	104.99	−26.60
H3	138.66	48.0	−45.33	25.00	−56.83	55.89	−41.39
Me	128.08	131.56	1.74	126.83	−0.63	125.05	−1.52
C1(Me)-C2[a]			−0.50		0.20		0.50

[a] As calculated from the $^1D_{C–H}$ using the formula given in [13].

Table 3
Alignment tensor properties for lactone **2** calculated from the data in Table 2 (after conversion of the Me group ^1H–^{13}C RDC into a ^{13}C–^{13}C RDC using the formula in Lit [13])

	$C_{12}E_5$/DMSO/D_2O	$C_{12}E_5$/*n*-hexanol/D_2O	C_8E_5/*n*-octanol/D_2O
$\Delta\nu_Q$/Hz[a]	22 (D_2O)	29	15
D_a/Hz[b]	6.26×10^{-4}	-7.37×10^{-4}	-5.61×10^{-4}
R[c]	0.61	0.55	0.54
A_{xx}[d]	5.4×10^{-5}	1.32×10^{-4}	9.51×10^{-5}
A_{yy}[d]	-1.2×10^{-3}	1.34×10^{-3}	9.37×10^{-4}
A_{zz}[d]	1.3×10^{-3}	-1.47×10^{-3}	-1.03×10^{-3}
EV A_{xx}[e]	0.93; 0.22; 0.29	0.99; −0.02; ×0.15	0.96; −0.28; 0.00
EV A_{yy}[e]	−0.08; −0.64; 0.76	0.09; −0.74; −0.67	0.19; 0.68; 0.70
EV A_{zz}[e]	−0.35; 0.74; 0.57	−0.12; −0.67; −0.73	−0.20; −0.67; 0.71
n(RDC)[f]	5	5	5

[a] $\Delta\nu_Q$ is the quadrupolar splitting of the solvent signal in the liquid crystal.
[b] D_a is a axial component of the alignment tensor.
[c] R is the rhombicity.
[d] A_{xx}, A_{yy}, A_{zz} are the principal components of the alignment tensor.
[e] EV A_{xx}, EV A_{yy}, EV A_{zz} are the eigenvectors for the corresponding main axes of the tensor.
[f] n(RDC) is the number of RDCs used for the calculation of the parameters with PALES.

Table 4
Relative angles (deg) and collinearities of the alignment tensores of lactone **2** in five-dimensional space for the three alignment media as obtained from as calculated with the program PALES

5D-angle/collinearity	$C_{12}E_5$/DMSO/D_2O	$C_{12}E_5$/*n*-hexanol/D_2O	C_8E_5/*n*-octanol/D_2O
$C_{12}E_5$/DMSO/D_2O	—	17.44/0.95	32.08/0.85
$C_{12}E_5$/*n*-hexanol/D_2O	17.44/0.95	—	15.09/0.96
C_8E_5/*n*-octanol/D_2O	32.08/0.85	15.09/0.96	—

temperature regions. This alignment medium was successfully used to measure the RDCs of the amino acid methionine **1** and a five-membered lactone **2**.

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