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New amino acid-based anionic surfactants and their use as enantiodiscriminating lyotropic liquid crystalline NMR solvents

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Abstract—New amino acid-based anionic surfactants have been synthesized and their use as chiral oriented NMR solvents studied. A series of sulfonated amphiphilic L-Phe or L-Ala derivatives with pentyl to tetradecyl tails were prepared by reacting the corresponding amino acid esters with *o*-sulfobenzoic anhydride. Their critical micelle concentrations (CMCs) range from 1.6×10^{-5} to 1.4×10^{-2} mol/L and depend on the alkyl chain length as well as on the nature of the amino acid block. These values are comparatively lower than those of classical surfactants such as alkylbenzenesulfonates and *N*-acylamino-acid carboxylates. Their use as chiral liquid crystals (CLC) for analytical purposes is reported. In particular, it is shown that aqueous solutions of these chiral sulfonates in the presence of chlorinated solvents ($CHCl_3$, CH_2Cl_2, $C_2H_2Cl_4$, or CCl_4) provide homogeneous oriented NMR solvents able to differentiate between enantiomers of D,L-alanine-2-d_1 using proton and deuterium NMR spectroscopy.

1. Introduction

Amino acids and peptide-based chiral surfactants have attracted widespread attention over the last decade and have been exploited in various ways in chemistry, analysis, and biology. Molecular aggregates of amphiphilic amino acids and peptides have been designed as low molecular weight enzyme mimics and used in catalytic reactions.[1] The use of amino acid-based surfactants as chiral ligands or auxiliaries has been also reported.[2] Among the various analytical applications proposed in the literature, we should mention their use as chiral pseudostationary phases in micellar electrokinetic chromatography.[3] In these applications, it was shown that intermolecular hydrogen bonding between the amide moieties and the chiral solute as well as steric interactions play a significant role in chiral recognition.[3b–e] It was also found that even small changes in the polar headgroup could have a large impact on the enantioselectivity.[3f]

From an NMR point of view, amphiphilic chiral surfactants can provide interesting enantioselective oriented solvents.[4] Since the pioneering work of Sackmann et al.,[5] it is known that two enantiomers can be spectroscopically differentiated when they are dissolved in chiral liquid crystals. The choice of the oriented systems (organic or aqueous) depends obviously on the nature of enantiomers under study. For chiral organosoluble compounds, NMR in organic solutions of chiral polypeptide made of poly-γ-benzyl-L-glutamate (PBLG) or poly-ε-carbobenzyloxy-L-lysine (PCBLL) appears to be as one of the most promising and general methods for the evaluation of enantiomeric excess.[6,7] In contrast, for water-soluble chiral molecules, the choice of a suitable chiral oriented environment is rather limited and only a few reports of their enantioselective potential can be found in literature.[8–13] Consequently, the investigation of new chiral lyomesophases as a possible enantiodifferentiating oriented NMR solvents merits a continuous effort.

Various *N*-acylamino acid as carboxylate salts were designed as chiral surfactants and used to prepare oriented phases in H_2O or D_2O, most often in the presence of co-surfactants like fatty alcohols.[4,8–10] Thus cesium *N*-dodecanoyl-L-alaninate,[8] potassium *N*-dodecanoyl or *N*-tetradecanoyl-D,L-alaninate,[9] potassium *N*-hexadecanoyl-L-prolinate,[9a] sodium *N*-dodecanoyl-L-prolinate[11] and esters such as *O*-decanoyl-L-alaninate[10]

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or -prolinate[12] hydrochloride were used as chiral hosts. Most surfactants involved in lyomesophases self-aggregate to form a hydrophobic region, leaving the peptidic polar headgroups to interact with water. It was shown that intermolecular amide H-bonding near the headgroup plays a major role in the lyotropic liquid crystal assembly.[14]

One of the main disadvantages of carboxylate surfactants is that their solubility, and hence their surfactant properties are sensitive to protonation. On the other hand, it is well known that sulfonated surfactants such as alkali metal alkyl- or alkylaryl-sulfonates are much less sensitive to pH and consequently retain their properties over a wide range of pH.[15] Moreover, sulfonate anions can act as hydrogen-bond-accepting bases, and therefore play a role in chiral recognition.[3g]

Herein, we have designed new sulfonated amino acid-based surfactants and investigated their potential application as chiral lyomesophases. We first report a straightforward synthetic pathway to a series of new anionic chiral surfactants containing a lipophilic chain, a natural amino acid block and a benzenesulfonate polar headgroup. Their behavior in aqueous solution as well as their critical micelle concentrations are reported and discussed. Additionally, the use of these surfactants as oriented, chiral NMR solvents is studied. In particular we show their potential to provide high-resolution NMR spectra and to discriminate the NMR signals of hydrophilic enantiomers such as D,L-alanine-2-d_1.

2. Results and discussion

2.1. Synthesis and characterization

Most of the previously reported amphiphilic compounds derived from amino acids are formed by tethering an alkyl chain either through chemical[3h,i,16] or enzymatic[17] N-acylation or esterification. For all of them, the amino acid residue is the polar headgroup of the surfactant. To the best of our knowledge, the synthesis of amino acid-based surfactants containing an additional, more polar and less pH sensitive, sulfonate polar headgroup has never been described.

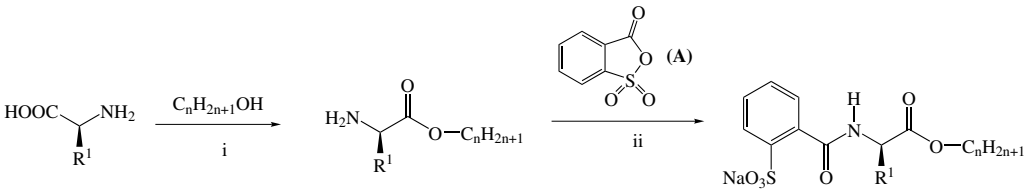
We choose to introduce an arylsulfonate residue via N-acylation with *o*-sulfobenzoic acid anhydride **A** that operates under mild conditions, and hence avoids racemization. This acylating reagent has been successfully used to prepare water-soluble ligands[18] and polymers[19] as well as sulfonated proteins.[20] Thus, L-phenylalanine derivatives **3a–e** with pentyl to tetradecyl chains and L-alanine derivative **4d** were readily synthesized in two steps from the corresponding natural amino acids (Scheme 1). Esters **1** and **2**, obtained by acid-catalyzed esterification in toluene, were acylated with almost quantitative yields. The sulfonated derivatives **3** and **4** were isolated after purification by chromatography with good overall yields (74–90%).

Arylsulfonates **3** and **4** were fully characterized by elemental analyses, optical rotation, IR and NMR spectroscopies. Complete assignments of the ^{1}H and ^{13}C NMR signals were achieved from COSY and HETCOR 2D spectra. The most characteristic ^{1}H NMR signals are summarized in Table 1. For all of these compounds, a single resonance was observed with a coupling constant $^3J_{NH-CH}$ of 6.2 Hz for the amide proton corresponding to the *anti* rotamer.[21] The presence of only one isomer in solution is further evidenced by the absence of any splitting of other ^{1}H and ^{13}C NMR signals. It is noteworthy that the most deshielded aromatic proton corresponds to the proton at the *ortho* position in regard to the sulfonate group.

2.2. Solution behavior and surfactant properties

Compounds **3** and **4** are fairly soluble in water for alkyls with chain lengths of up to 14 carbon atoms (Table 2). The solubility in water depends both on the nature of the amino acid residue and on the length of the aliphatic tail. Increasing the tail length by nine carbon atoms in Phe derivatives **3** lowers the solubility 1000-fold. The Ala derivative **4d** is much more soluble in water than its Phe analogue **3d**, thereby indicating a hydrophobic contribution of the benzyl group.

The critical micelle concentration (CMC) is the main characteristic of any surfactant. The CMC is the concentration above which surfactant molecules self-



Compounds	1a, 3a	1b, 3b	1c, 3c	1d, 3d	1e, 3e	2d, 4d
R^1	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph	CH_2Ph	CH_3
n	5	8	10	12	14	12

Scheme 1. Reagents and conditions: (i) APTS, Tol, reflux; (ii) a. Et_3N, THF, 60 °C, b. $NaHCO_3$.

Table 1. Main characteristic ^{1}H NMR resonances of **3d** and **4d**

	Chemical shift in DMSO-d_6[a]	
	3d (Phe)	**4d** (Ala)
Ph-$CH_2\beta^1$	2.95 (dd, $J_{H\beta1-H\beta2}$ 14, $J_{H\beta1-H\alpha}$ 8.8)	—
Ph-$CH_2\beta^2$	3.17 (dd, $J_{H\beta2-H\beta1}$ 14, $J_{H\beta2-H\alpha}$ 5.5)	—
CH_3–CH	—	1.36 (d, J 7.0)
OCH_2	3.90 (m)	4.04 (m)
CH α	4.62 (ddd, $J_{H\beta1-H\alpha}$ 8.8, $J_{H\beta2-H\alpha}$ 5.5, $J_{H\alpha-NH}$ 6.2)	4.42 (m)
$H_oC{=}O$[b]	7.59 (dd, J_o 2.0, J_m 7.0)	7.59 (dd, J_o 2.0, J_m 7.0)
$H_oSO_3^-$[c]	7.84 (dd, J_o 2.0, J_m 7.0)	7.85 (dd, J_o 2.0, J_m 7.0)
NH	9.70 (d, $J_{H\alpha-NH}$ 6.2)	9.54 (d, $J_{H\alpha-NH}$ 6.2)

[a] The chemical shifts are given in ppm and coupling constants in hertz.
[b] Aromatic proton adjacent to carbonyl group.
[c] Aromatic proton adjacent to sulfonate group.

Table 2. Physico-chemical data for sulfonates **3a**–**e** and **4d**

Entry	Compounds	N_C	Micellar parameters derived from CMC			Solubility in H_2O (mmol/L)[d]
			CMC (mmol/L)[a]	γ_{min} (mN/m)[b]	a_s per molecule (Å^2)[c]	
1	**3a**	5	14	30.5	127	1000
2	**3b**	8	1.4	29.0	95	50
3	**3c**	10	0.33	29.1	78	50
4	**3d**	12	0.063	30.4	85	10
5	**3e**	14	0.016	30.7	70	1
6	**4d**	12	0.41	33.0	77	1000
7	N–$C_{12}H_{25}$–Phe [22]	12	0.94			
8	N–$C_{12}H_{25}$–Ala[22]	12	12.7			
9	$C_{12}H_{25}$–C_6H_4–SO_3Na[23]	12	12.3	27.8	48	

[a] 25 °C.
[b] ±0.1.
[c] ±8 Å^2.
[d] Approximate values.

assemble into aggregates called micelles. Surface tension measurements demonstrate that sulfonated amino acid derivatives **3a**–**e** and **4d** are surfactants. The CMC values and other parameters derived from these measurements are given in Table 2. The CMCs of compounds **3** and **4**, that range from 14 to 0.016 mmol/L, are much lower than those of classical carboxylate amino acid-based surfactants and alkylbenzenesulfonates. Thus, the CMCs of **3d** and **4d** are more than 10-fold lower than those of *N*-dodecyl-alanine or phenylalanine[22] and 2-fold lower than sodium dodecylbenzenesulfonate[23] (entries 7–9 in Table 2). These results indicate that both the aromatic ring and the amino acid block favor the micellization probably via hydrophobic and/or dipole–dipole interactions.[24] The comparison of Phe and Ala derivatives, **3d** and **4d**, shows that the benzyl substituent has a significant hydrophobic contribution[25,26] and strengthens the interfacial packing: 10-fold reduction of the CMC as well as reduction of the minimum value of surface tensions γ_{min} above the CMC (about 30 mN/m for **3d** and 33 mN/m for **4d**).

The CMCs of Phe derivatives, **3a**–**e**, decrease with the lengthening of the alkyl chain. The graph log (CMC) displays a linear dependence on the number of carbon atoms N_C, according to Kleven's equation, like classical surfactants.[15] The comparison with alkylbenzenesulfonates, given in Figure 1, shows that surfactants **3** have CMC values similar to benzenesulfonates with longer aliphatic tails. In other words, the phenylalanine block

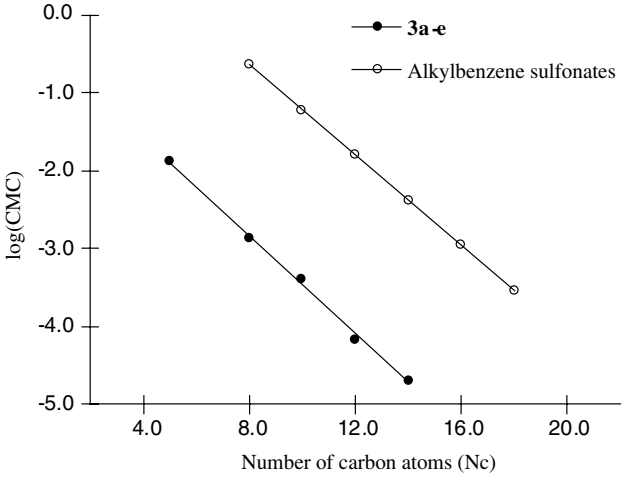


Figure 1. Log(CMC) versus number of carbon atoms in the aliphatic chain.

generates a hydrophobic contribution equivalent to an increase of the tail by seven to eight carbon atoms. This observation indicates that the benzyl substituent acts as a hydrophobic ramification. Furthermore the similar slopes observed for **3** and alkylbenzenesulfonates show that the contribution *per* aliphatic carbon atom to the free energy of micellization ΔG_{mic} are close together. The ease of micellization of our sulfonated surfactants arises from attractive interactions between the amino acid

blocks. The areas *per* head group at the air–water interface, a_s, for surfactants **3** and **4**, range from 70 to 130 Å^2 and decrease upon lengthening of the alkyl tail as previously described for classical surfactants.[27,14b] For a given tail, Ala and Phe derivatives **3d** and **4d** present similar areas, that are much larger than dodecylbenzene sulfonate, thereby indicating that the amino acid peptidic framework is located in the interfacial region.

For Phe derivatives, the location of the benzyl substituent on the hydrophobic micellar core is further evidenced by the ^{1}H NMR spectra of **3c** in D_2O below and above the CMC (respectively at 0.1 and 10 mmol/L). All the protons of the benzyl substituent (CH_2Ph) as well as methylene groups close to the ester function (OCH_2, OCH_2CH_2) are shifted to lower frequencies ($\Delta\delta = 0.1$–0.25 ppm) under aggregationing conditions. These results support the suggestion that the benzyl group is embedded within the micellar core. Similar occurrences were already reported for other surfactants like *N*-dodecyl-*N*,*N*′-dimethyl-*N*-benzyl ammonium chloride[25] or cationic phenylalanine-based surfactants.[26] Above the CMC, two signals were observed for the aromatic proton *ortho* to the sulfonate group in the ratio 97/3. These signals could be assigned, respectively, to aggregated surfactant molecules and free surfactant monomeric molecules, the concentration of the latter being equal to the CMC (0.33 mmol/L). The existence of the two separated domains in the aggregates may also account for these two signals as previously reported for surfactants like *N*-acylprolinate and *N*-acylmethyl glycinate.[11,28]

2.3. Some aspects of NMR in lyomesophases

The main advantage of NMR spectroscopy in chiral liquid crystals compared with classical NMR approaches dedicated to the enantiomeric analysis, is that no specific chemical functionalities or molecular topologies in the investigated compounds are a priori required.[29] Indeed only the selective orientational ordering of two enantiomers inside the phase induces the spectral enantiodiscrimination.[30] The second advantage is that NMR spectroscopy in anisotropic media provides basically numerous analytical tools able to differentiate between the signal of enantiomers.[6] Thus any magnetically active nuclei present (proton, carbon-13, deuterium for isotopically enriched compounds) in the molecule is a potential NMR probe for this aim.

In ^{1}H NMR, we mainly used ^{1}H–^{1}H residual dipolar couplings (RDC's) to probe the enantioselectivity of the phase.[6] In this case, each pair of interacting proton nuclei (*i* and *j*) may produce a direct dipolar coupling defined in hertz as

$$D_{HH} = -k_{HH} S_{HH} \quad \text{with } k_{HH} = \left(\frac{\mu_0}{4\pi}\right)\frac{h\gamma_H\gamma_H}{4\pi^2} \quad \text{and}$$

$$S_{HH} = \left\langle \frac{3\cos^2\theta_{HH}^{B_0} - 1}{2r_{HH}^3} \right\rangle, \qquad (1)$$

where the brackets denote an (ensemble or time) average over molecular tumbling and internal motions (vibrational motions, conformational changes…). In this equation, μ_0 is the magnetic susceptibility of free space, γ_H is the magnetogyric ratio of proton nuclei, r_{HH} is the internuclear distance between *i* and *j* protons, and S_{HH} is the order parameter for the internuclear vector r_{HH}. The magnitude of k_{HH} is equal to 120.07 kHz Å^3.

Deuterium NMR is also a powerful tool able to provide various information about the orientational behavior, the homogeneity and the enantioselectivity of a chiral lyomesophase in a static magnetic field B_0.[29,31] ^{2}H NMR spectra in oriented solvents are dominated by the quadrupolar interaction. Assuming axial symmetry of the electric field gradient along the C–D bond, this purely anisotropic interaction can be written as

$$\Delta\nu_Q = \frac{3}{2}K_D S_{C\text{–}D} \quad \text{with } K_D = \frac{e^2 Q_D q_{C\text{–}D}}{h} \quad \text{and}$$

$$S_{C\text{–}D} = \left\langle \frac{3\cos^2\theta_{C\text{–}D}^{B_0} - 1}{2} \right\rangle, \qquad (2)$$

where $K_{C\text{–}D}$ is the deuterium quadrupolar coupling constant and $S_{C\text{–}D}$ is the order parameter along the C–D relative to the magnetic field axis, B_0. $\theta_{C\text{–}D}$ is the angle between B_0 and the direction of the C–D bond. $K_{C\text{–}D}$ strongly depends on the hybridization state of the carbon atoms bonded to a given deuteron and varies between 170 and 210 KHz.[6]

2.4. Composition of the NMR samples

From an analytical point of view, an ideal amphiphilic oriented solvent used for NMR applications should satisfy several criteria. First, the system should be homogeneously and uniformly oriented in the static magnetic field, condition sine qua none to record high-resolution NMR for solutes. This condition is crucial for detecting small spectral enantiodiscrimination. Second, the amphiphilic system should form an oriented medium over the largest possible range of temperature, centered if possible on room temperature. This last point is important for investigating thermosensitive solutes. Third, the sample should orientate in the magnetic field as quickly as possible, ideally it should not exceed the time to reach the thermic equilibrium in order to ensure an important gain of experimental time. Fourth, it is necessary to reduce the chemical complexity of the lyomesophase by adding a minimum of components. This last occurrence facilitates the preparation of phases and improves their thermodynamical stability.

All of the surfactants synthesized in this work do not fulfill the various criteria mentioned above. In particular, it is known that lyomesophases are generally formed by surfactants having hydrocarbon chains beyond 10–12 carbon atoms.[8–12] As a consequence, we have disregarded derivatives **3a** and **b** as well as tetradecyl sulfonate **3e**, which featured too weak a solubility in water (Table 2). Although some preliminary tests using **3d** as surfactant showed some encouraging results, we have focused our study on **3c**.

Lyotropic structures can be ternary or quaternary systems. They are made up of a surfactant; water in which a co-surfactant (mainly a long chain alcohol) and/or an inorganic salt is added to destabilize hexagonal, cubic, or lamellar phases to obtain lyotropic nematic phases.[32]

Decanol or other fatty alcohols were widely used with classical surfactants,[4,8,10] but preliminary attempts performed with this type of co-surfactant (decanol) and **3c** did not provide a homogeneously oriented phase in the magnetic field (over a large range of concentrations and temperatures). This is the reason why we decided to explore the effect of weakly polar, chlorinated organic solvents such as $CHCl_3$, CH_2Cl_2, $C_2H_2Cl_4$, or CCl_4. Such additives are rarely used in amphiphilic organized molecular assemblies, and very few references can be found in literature. Thus Moulik and co-workers reported that the ternary system cetylpyridinium chloride/H_2O/$CHCl_3$ forms an anisotropic liquid crystalline phase in which $CHCl_3$ moiety plays the role of an oil or a very weak co-surfactant, as claimed by these authors.[33]

In amphiphilic systems, the choice of the composition of the mixture is essential to obtain oriented phases in the magnetic field. This choice should in theory be derived from the phase diagram of the system. This work (specially for a ternary mixture) is not really necessary for a preliminary study because we want to establish first the ability of the system to distinguish between enantiomers using NMR. Actually two conditions have imperatively driven our choice. First, the concentration of the surfactant should always be above the CMC. Second the various NMR criteria mentioned above should be fulfilled. As we will show later, these conditions were obtained using around 20–25% w/w of **3c**, 60–70% w/w of H_2O and 5–20% w/w of chlorinated organic solvent in a range of temperatures around 300–315 K.

As a molecular probe, we used a racemic and enantio-enriched mixture of D,L-alanine-2-d_1 (denoted (±)-**7d$_1$**) selectively deuterated at the stereogenic center. The main advantage of this chiral molecule is that we benefit from two abundant nuclei (proton and deuterium), thus permitting us to quickly record ^1H and ^2H NMR spectra with a small amount of chiral material. Furthermore, it is possible to explore very different internuclear directions (H···H or C–D) in the molecule, which should increase the possibility for us to observe chiral discrimination.[6]

2.5. Analysis of NMR results

The obtention of a purely anisotropic phase is rarely complete. Generally isotropic and oriented regions co-exist in the aligned sample, even if the magnetic field strength is sufficient enough to orientate the system. To illustrate this point, Figure 2a shows a typical example of ^1H spectra observed with (±)-**7d$_1$** embedded in **3c**/H_2O/$CHCl_3$ when the system is biphasic. In this case, the anisotropic ^1H signals of (±)-**7d$_1$** (labeled with open circles) is partially masked by the residual isotropic ^1H spectrum of **3c**, thus preventing their analysis. As the

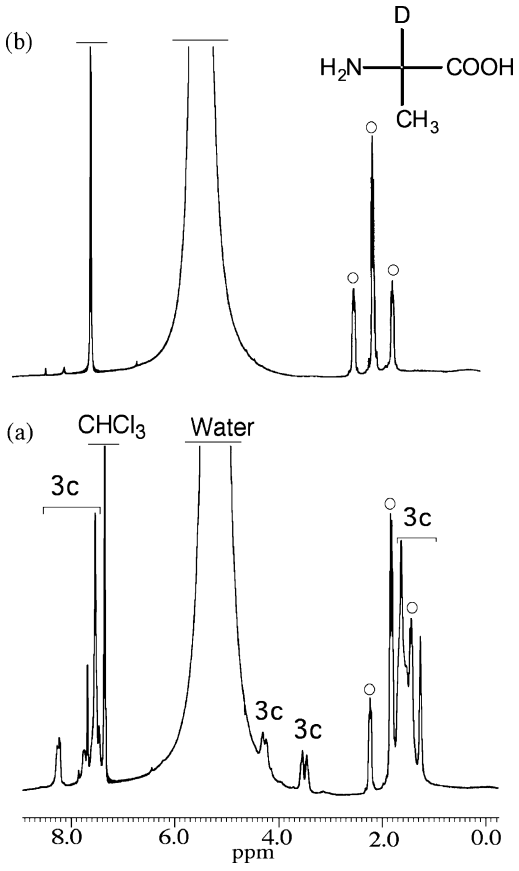


Figure 2. Example of 400.1 MHz 1H spectra of (±)-**7d$_1$** dissolved in **3c**/H_2O/$CHCl_3$ when the system is biphasic (a), and when the system is monophasic (b). Spectra (a) and (b) were recorded at 305 K, 45 and 120 min after introducing the sample in the magnetic field, respectively.

concentration in **3c** is largely above the CMC, the presence of isotropic spectral components indicates that the system has not reached its thermodynamical equilibrium yet, and hence the micellar self-organization is not ideal. We found that the proportion of isotropic signals in the spectra decreases as a function of time, and purely anisotropic spectra can be recorded after keeping the sample in the magnetic field for 1–3 h. In fact, it is more valuable to slightly adjust the sample temperature (increasing or decreasing) to fully eliminate any isotropic signals. Figure 2b shows the spectrum of (±)-**7d$_1$** in **3c**/H_2O/$CHCl_3$ when the system is monophasic and macroscopically homogeneous. In this case, the isotropic NMR resonances of the surfactant have totally disappeared, and hence we exclusively observe a broad and intense peak for water, a sharp and single peak for chloroform and an anisotropic spectral figure for the methyl group of (±)-**7d$_1$**. The elimination of signals of **3c** on proton spectra can be used as a pertinent test to assess if self-organization of the phase in the magnetic field is correctly achieved or not.

Figure 3a shows the proton signals of the methyl group of (±)-**7d$_1$**. This spectral pattern is featured by the superposition of two triplets of triplets resulting from RDC's within the three equivalent protons of the methyl group ($|T_{H\cdots H}| = |3D_{H\cdots H}|$) and the total H···D coupling

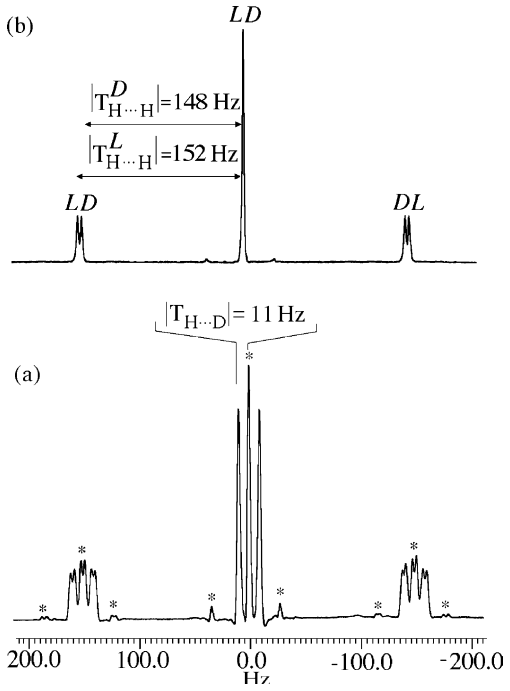


Figure 3. (a) 400 MHz ^{1}H signal (a) and ^{1}H-{^{2}H} signal (b) associated with the methyl group of (±)-**7d$_1$**. In both spectra, a Gaussian filtering (GB = 70%, LB = −5 Hz for (a), and GB = 55%, LB = −3 Hz for (b)) was applied to improve spectral enantiodiscrimination. The assignment of triplet in (b) is given according to the analysis of ^{1}H–{^{2}H} spectrum of (−)-**7d$_1$** (shown in Fig. 4).

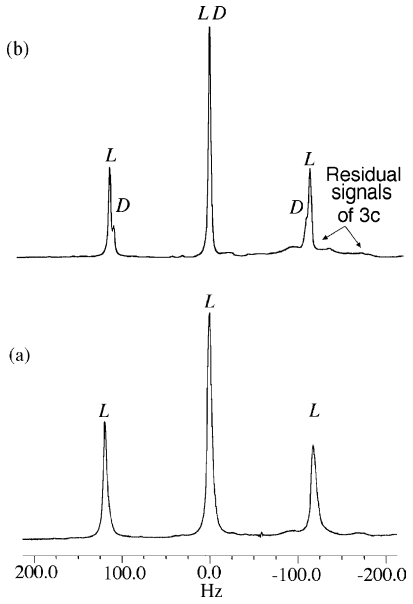


Figure 4. 400 MHz ^{1}H–{^{2}H} spectrum associated with the methyl group of **7d$_1$** using (a) pure L-isomer and (b) enantioenriched mixture in L-isomer (ee = 73%). A Gaussian filtering (GB = 45%, LB = −1 Hz) was applied in (b) to improve the spectral enantiodiscrimination.

($|T_{H\cdots D}| = |J_{HD} + 2D_{H\cdots D}| = 11$ Hz) between those protons and the deuterium nucleus (spin $I = 1$).[6] To check this spectral analysis, we recorded the deuterium-decoupled proton (^{1}H–{^{2}H}) spectrum of (±)-**7d$_1$**. As shown in Figure 3b, we clearly obtained two triplets with distinct dipolar splittings ($|T_{H\cdots H}| = 148$ and 152 Hz) centered on the same chemical shift. Considering the spin system of the molecule, such a spectral pattern implies that a chiral differentiation occurs on the basis of a difference of ^{1}H–^{1}H residual dipolar coupling at the methyl group.

Note here that the slight difference in peak intensity between the three (central) or six (outer) components of the triplet on the ^{1}H spectrum (Fig. 3a) is due to the superposition of a second (minority) spectral system (<5%) also made of two triplets of triplets (labeled by stars). Both coupling patterns (the minority and majority system) are centered on the same chemical shift. The minority system possesses similar values of $|T_{H\cdots H}|$ (149 and 151 Hz) but not the same $|T_{H\cdots D}|$ (18 Hz), when compared with the majority one. This system also exhibits a spectral enantiodiscrimination, which could originate from the acid form of the alanine (pH of the sample ~5). However the hypothesis of a biphasic lyotropic system exhibiting two types of homogeneous oriented domains cannot be excluded.

In order to ensure that the chiral discrimination observed on the ^{1}H spectra is not an artifact, we recorded the ^{1}H–{^{2}H} 1D spectrum using a scalemic mixture of **7d$_1$** enriched in an L-enantiomer (ee = 73%) as well as using the pure enantiomer (−)-**7d$_1$**. The sample compositions are the same as that for (±)-**7d$_1$** (see Table 4). Both spectra are shown in Figure 4a and b. As expected a single triplet is now observed for the enantiopure compound while two triplets of different intensities are obtained for the enantioenriched mixture. In this mixture, the smaller $T_{H\cdots H}$ corresponds to the D-isomer. This allows us to assign the signal for each optical isomer in the racemic mixture (see Fig. 3b). Conclusively, the analysis of these three spectra clearly demonstrates that the D- and L-amino acids are not ordered the same way in the phase.

The existence of two anisotropic spectra for (±)-**7d$_1$** clearly shows that a 9.4 T magnetic field is able to orient our lyomesophase. A lower magnetic field should also align the system. Such a study is currently underway. The range of proton RDC's indicates that the solute is more oriented than in case of organic solutions of polypeptide for instance.[6] However the ^{1}H linewidths measured without any digital filtering (6 and 8 Hz) indicates a relatively good macroscopic homogeneity. To support these points and provide better insight on the orientational behavior of this lyomesophase, we analyzed the ^{2}H–{^{1}H} spectra of (±)-**7d$_1$** dissolved in **3c**/H_2O/$CHCl_3$. Figure 5 shows an example of the ^{2}H–{^{1}H} spectra when the system is monophasic and homogeneously aligned. Peaks labeled with open circles correspond to the ^{2}H quadrupolar doublets ($|\Delta\nu_Q| = 3200$ Hz) of (±)-**7d$_1$**. The two components labeled with black circles are assigned to the natural abundance deuterium signal (a quadrupolar doublet) of water (HOD isotopomer)[34] in bulk solution. The quadrupolar splitting of $|\Delta\nu_Q| = 470$ Hz indicates that water molecules are also aligned in the bulk solution. The obser-

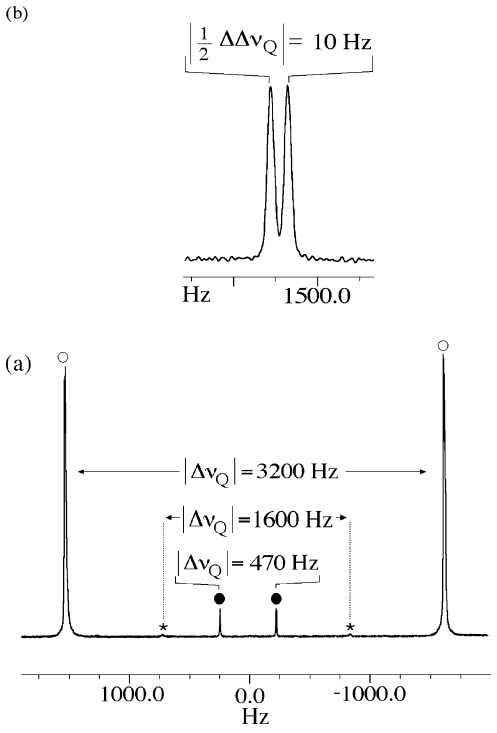


Figure 5. 61.3 MHz ^{2}H–{^{1}H} spectra of (±)-**7d$_1$** dissolved in **3c**/H_2O/$CHCl_3$ when the system is monophasic and highly homogenous. (b) ^{2}H–{^{1}H} spectrum showing a chiral discrimination. Only the deshielded component of the doublets is shown here. A Gaussian filtering (GB = 50%, LB = −9 Hz), was applied to improve the spectral enantiodiscrimination.

vation of very high-resolution ^{2}H NMR spectra for (±)-**7d$_1$** as well as HOD again proves a significant alignment of the phase in the external, static magnetic field, B_0. It can also be pointed out that the linewidths measured in this example are below 7 Hz while the magnitude of quadrupolar splitting is over 3000 Hz. This situation is particularly interesting because it indicates a very weak angular distribution of the C–D internuclear vector of **7d$_1$** relative to B_0 (see Eq. 1), and subsequently a very uniform and homogeneous alignment of the solute in the phase. It is noteworthy that linewidths decrease generally by keeping the sample in the magnetic field for several hours or by slightly adjusting the sample temperature. Comparatively, the linewidths measured here are in the same range of values obtained in polypeptide weakly ordering systems made of PBLG or PCBLL, but the quadrapolar splittings are larger.[29]

A deeper analysis of the deuterium spectrum also reveals the presence of a second doublet of very weak intensity (<1%) that does not clearly emerge from the spectral baseline (labeled with stars). These signals are more visible when the sample is biphasic. This doublet exhibits quadrupolar splitting equal to 1600 Hz, namely half of the previous splitting. This situation was observed when the director was perpendicular to B_0.[31] This secondary doublet is probably the residual signature of the inner wings of the deuterium powder pattern (Pake doublet) observed when the director of the phase is randomly distributed in respect to B_0.[34,35] As a consequence it appears that this lyotropic system behaves like a nematic of positive anisotropy of molecular diamagnetic susceptibility ($\Delta\chi_m > 0$), for which the director is homogeneously aligned parallel to B_0.[31] At this time, the low viscosity of the samples and the NMR results could indicate that this system forms a stable lyotropic (chiral) nematic phase in the temperature range of 305–315 K.[36] However, the determination of the mesophase structure (lamellar, nematic, hexagonal,...) is required and is currently under study.[36]

From the enantiomeric analysis, we also observed a chiral discrimination on the ^{2}H–{^{1}H} spectra. The measured separation between enantiomers is rather small ($|\Delta\nu_Q^L - \Delta\nu_Q^D| = 20$ Hz, and was only observed after keeping the sample in the magnetic field for several hours (Fig. 5b). Considering that the quadrupolar interaction is the most sensitive NMR interaction to an orientational order difference,[6] this result shows that the C–D internuclear direction relative to B_0 is very close in both enantiomers, and thereby a visible spectral discrimination only occurs when the phase inhomogeneities (due to temperature or concentration gradients) vanish. Here again this result confirms that the sample continues to slowly evolve for several hours to reach the most thermodynamically stable situation. Although the spectral separations in proton or deuterium are rather weak, enantiodifferential ordering forces exist in this phase, and these results prove the chiral recognition ability of the system.

Encouraged by these first successful results obtained using chloroform, we investigated other weakly polar chlorinated organic solvents such as CH_2Cl_2, $(CHCl_2)_2$, or CCl_4. The sample compositions are given in Table 4. Using similar concentrations for the three components of the mixture, high-resolution ^{1}H and ^{2}H–{^{1}H} spectra

Table 3. NMR results obtained for **7d$_1$** dissolved in the **3c**/water system using various chlorinated entities

Sample	Organic solvent (ratio chlorinated solvent/water w/w)[a]	Working sample temperature range (K)	^{2}H quadrupolar splitting range ($\Delta\nu_Q$/KHz)[a]	^{2}H half-height linewidth range ($\Delta\nu_{1/2}$/Hz)[a]	Chiral discrimination using ^{2}H NMR ($\Delta\Delta\nu_Q$/Hz)	Chiral discrimination using ^{1}H NMR (ΔT_{HH}/Hz)[b]
1	$CHCl_3$ (1/3.7)	305–320	3.2–3.3	5–10	Y (15–20)	Y (6–8)
2	CH_2Cl_2 (1/4.4)	305–315	3.2–3.3	7–10	Y (15–20)	Y (6–8)
3	$C_2H_2Cl_4$ (1/4.3)	320–330	3.0–3.2	5–7	N or <linewidth	Y (6–8)
4	CCl_4 (1/1.6)	330–340	2.8–3.0	8–10	N or <linewidth	Y (5–7)

[a] The composition of each sample is given in Table 4.
[b] Coupling between the three equivalent protons of the methyl group.

were obtained for each of these lyotropic mixtures in a range of temperatures varying in the 305–340 K. According to spectral data in Table 3, the range of deuterium quadrupolar splittings measured for the solute is very similar from a sample to another one, thus indicating that the molecular orientational behavior of the phase (and the solute) is almost unaffected by the type of chlorinated solvent used. This point suggests an identical role in the organization of the system for each of them. In contrast, the small variations in linewidths could indicate that the macroscopic homogeneity of the phase can be slightly affected by the nature of this mixture component. The comparison between the various experimental results shows that the system using tetrachloroethane as oil gives the optimal result since linewidths of 4–5 Hz were observed while the two components of the quadrupolar doublet are split by 3000 Hz.

3. Conclusion

The design and exploration of new surfactant types as chiral ordering agents using NMR spectroscopy is a noteworthy challenge, regarding the continuous efforts devoted to development of analytical methods for the determination of the enantiomeric purity. One of the advantages of using synthetic surfactants is that the structure can be readily varied.

Herein, we have reported the straightforward synthesis of the first series of sulfonated amino acid-based chiral surfactants with modulable properties depending on the nature of the amino acid residue (Phe or Ala) and the length of the aliphatic tail. These compounds self-aggregate in water at low concentration. Their low CMC as well as their large interfacial area, compared with classical surfactants, suggest that micellization is favored by attractive interactions between amino acid residues at the interface. Phenylalanine derivatives behave like branched surfactants with the benzyl substituent embedded within the micellar core.

From an analytical point of view, we have shown that these anionic surfactants aligned in a magnetic field can form homogeneous, lyotropic liquid crystalline phases in the presence of water and weakly polar chlorinated organic solvents around 320 K. They offer several practical advantages. The liquid crystal samples themselves are rather simple to prepare. They rapidly and homogenously align in the magnetic field and lead to sharp NMR resonances. The system behaves like a chiral nematic phase of positive anisotropy of the molecular diamagnetic susceptibility ($\Delta\chi_m > 0$), with the director uniformly and homogeneously aligned parallel to the static magnetic field. In addition we have demonstrated that enantioselective interactions between a chiral solute and the chiral polar head of the surfactant was sufficient to spectroscopically differentiate the NMR signal of enantiomers like D- and L-alanine-2-d_1.

The first NMR results using these new lyotropic systems as potential high-resolution oriented solvent are very promising and opens new prospects in the enantiomeric analysis of small, water soluble, organic compounds. Several questions arise, but are beyond the scope of this preliminary work. Among them it will be important to understand the exact role the chlorinated entities in the mesophase behavior. The second point will be to establish if the alanine molecules reside mainly in the interstitial water or is also able to penetrate into the micellar region, which is highly oriented. In particular, in which region of the aggregates the two enantiomers undergo enantiodifferential ordering forces induced by the asymmetry of the surfactant. Answering these questions should help us to improve the chiral selectivity of this system.

4. Experimental

4.1. General methods

All starting materials were purchased from currently available commercial sources. Solvents were distilled by conventional methods. Reactions were monitored by TLC on plates coated with 0.25 mm silica gel 60 F_{254}, which were visualized under UV at 254 nm, 5% sulfuric acid in ethanol or ninhydrin solution (0.2%) in ethanol as developing agents. Silica gel Merck Gerduran SI60 (40–63 μm) was used for column chromatography. Routine ^1H and ^{13}C NMR spectra in isotropic solvents were recorded on a Bruker AC 300 at 300 and 75 MHz, respectively. The chemical shifts δ were referenced to residual chloroform (7.27 and 77.0 ppm), water (4.63 ppm), or DMSO (2.50 and 39.50 ppm). ^1H–^1H homonuclear (COSY) and ^1H–^{13}C heteronuclear (HETCOR) correlations allowed to elucidate several structures. IR spectra were obtained on a Magna-IR Spectrometer 550 (KBr pellets) and spectral bands are reported in cm^{-1}. Mass spectra were obtained using an electrospray ion-source in negative mode (ES−) on an MS-Engine HP5989B in methanol/water with 1% triethylamine and peaks are reported as m/z. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter at 25 °C. Melting points are uncorrected. Elemental analyses were obtained from the Service Central d'Analyses (CNRS, Vernaison, France).

4.1.1. Synthesis of phenylalanine esters 1

4.1.1.1. (2*S*)-Amino-3-phenyl-propionic acid pentyl ester 1a. A mixture of L-phenylalanine (1.00 g, 6.1 mmol), pentanol (0.64 g, 7.3 mmol), and PTSA (1.15 g, 7.3 mmol) in toluene (50 mL) was heated at reflux overnight, using Dean–Stark apparatus. After removal of the solvent, the reaction mixture was diluted with Et_2O, washed successively with NaOH (1 M) and brine and dried over Na_2SO_4. The crude product isolated after removal of the solvent was purified by column chromatography on silica gel with EtOAc/cyclohexane 1/2, then EtOAc to provide the ester **1a** as oil (1.23 g, 86%): R_f 0.38 (EtOAc/cyclohexane 1/2); ^1H NMR (DMSO-d_6),

δ (ppm): 0.84 (t, 3H, *J* 7.0 Hz, CH_3), 1.19 (m, 4H, CH_2), 1.46 (m, 2H, CH_2CH_2O), 1.89 (br s, 2H, NH_2), 2.79 (dd, H, *J* 13.5Hz, *J* 7.0Hz, CHHPh), 2.85 (dd, H, *J* 13.5 Hz, *J* 6.6 Hz, CHHPh), 3.54 (dd, H, *J* 7.0 Hz, *J* 6.6 Hz, CH), 3.93 (m, 2H, CH_2O), 7.20 (m, 5H, H_{arom}).

4.1.1.2. (2*S*)-Amino-3-phenyl-propionic acid octyl ester 1b. Prepared as described above for **1a**, starting from L-phenylalanine (1.00 g, 6.1 mmol), octanol (0.94 g, 7.3 mmol), and PTSA (1.15 g, 6.1 mmol) in toluene (50 mL) to provide ester **1b** as oil (1.40 g, 83%); R_f 0.30 (EtOAc/cyclohexane 1/2); ^1H NMR ($CDCl_3$), δ (ppm): 0.89 (t, 3H, *J* 7.0 Hz, CH_3), 1.24 (m, 10H, CH_2), 1.47 (m, 2H, CH_2CH_2O), 3.32 (dd, H, *J* 14 Hz, *J* 7.4 Hz, CHHPh), 3.47 (dd, H, *J* 14 Hz, *J* 5.4 Hz, CHHPh), 4.03 (t, 2H, *J* 6.6 Hz, CH_2O), 4.44 (dd, H, *J* 7.4 Hz, *J* 5.4 Hz, CH), 7.30 (m, 5H, H_{arom}).

4.1.1.3. (2*S*)-Amino-3-phenyl-propionic acid decyl ester 1c. Prepared as described above for **1a**, starting from L-phenylalanine (2.00 g, 12.1 mmol), decanol (2.30 g, 14.5 mmol), and PTSA (2.30 g, 12.1 mmol) in toluene (100 mL) to provide the ester **1c** as oil (3.50 g, 95%): R_f 0.35 (EtOAc/cyclohexane 1/2); $[\alpha]_D = +14.8$, $[\alpha]_{436} = +28.8$, $[\alpha]_{365} = +48.8$ (*c* 1, DMF); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 7.0 Hz, CH_3), 1.23 (m, 14H, CH_2), 1.46 (m, 2H, CH_2CH_2O), 1.73 (br s, 2H, NH_2), 2.77 (dd, H, *J* 13.5 Hz, *J* 7.0 Hz, CHHPh), 2.84 (dd, H, *J* 13.5 Hz, *J* 6.6 Hz, CHHPh), 3.54 (dd, H, *J* 7.0 Hz, *J* 6.6 Hz, CH), 3.95 (m, 2H, CH_2O), 7.20 (m, 5H, H_{arom}); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.93 (CH_3), 22.09, 25.27, 28.03, 28.61, 28.89, 28.91, 31.28 (CH_2), 40.95 (CH_2Ph), 55.78 (CH), 63.87 (CH_2O), 126.19 (C_p), 128.04 (C_o), 129.14 (C_m), 137.90 (C_{ipso}), 174.99 (C=O).

4.1.1.4. (2*S*)-Amino-3-phenyl-propionic acid dodecyl ester 1d. Prepared as described above for **1a**, starting from L-phenylalanine (3 g, 18.2 mmol), dodecanol (4.05 g, 21.8 mmol), and PTSA (3.45 g, 18.2 mmol) in toluene (150 mL) to provide the ester **1d** as oil (4.82 g, 80%): R_f 0.29 (EtOAc/cyclohexane 1/2); $[\alpha]_D = +13.1$, $[\alpha]_{436} = +26.4$, $[\alpha]_{365} = +47.2$ (*c* 1, DMF); ^1H NMR ($CDCl_3$), δ (ppm): 0.84 (t, 3H, *J* 6.5 Hz, CH_3), 1.23–1.60 (m, 22H, CH_2 and NH_2), 2.86 (dd, H, *J* 13.4 Hz, *J* 7.9 Hz, CHHPh), 2.84 (dd, H, *J* 13.4 Hz, *J* 5.5 Hz, CHHPh), 3.72 (dd, H, *J* 7.9 Hz, *J* 5.5 Hz, CH), 4.10 (t, 2H, *J* 6.9 Hz, CH_2O), 7.25 (m, 5H, H_{arom}); ^{13}C NMR ($CDCl_3$), δ (ppm): 14.23 (CH_3), 22.80, 25.98, 28.03, 28.67, 29.34, 29.46, 29.61, 29.68, 29.76, 32.03 (CH_2), 41.31 (CH_2Ph), 56.00 (CH), 65.25 (CH_2O), 126.89 (C_p), 128.64 (C_o), 129.39 (C_m), 137.40 (C_{ipso}), 175.25 (C=O).

4.1.1.5. (2*S*)-Amino-3-phenyl-propionic acid tetradecyl ester 1e. Prepared as described above for **1a**, starting from L-phenylalanine (2.00 g, 12.1 mmol), tetradecanol (3.11 g, 14.5 mmol), and PTSA (2.30 g, 12.1 mmol) in toluene (100 mL) to provide the ester **1e** as oil (3.60 g, 85%): R_f 0.22 (EtOAc/cyclohexane 1/2); ^1H NMR ($CDCl_3$), δ (ppm): 0.89 (t, 3H, *J* 7.0 Hz, CH_3), 1.27 (m, 24H, CH_2), 1.58 (m, 4H, CH_2CH_2O and NH_2), 2.88 (dd, H, *J* 13.6 Hz, *J* 7.7 Hz, CHHPh), 3.09 (dd, H, *J* 13.6 Hz, *J* 5.5 Hz, CHHPh), 3.73 (dd, H, *J* 7.7 Hz, *J* 5.5 Hz, CH), 4.10 (t, 2H, *J* 7.0 Hz, CH_2O), 7.23 (m, 5H, H_{arom}); ^{13}C NMR ($CDCl_3$), δ (ppm): 14.11 (CH_3), 22.68, 25.86, 28.53, 28.61, 29.22, 29.34, 29.49, 29.56, 29.64, 29.66, 29.67, 31.90 (CH_2), 41.19 (CH_2Ph), 55.88 (CH), 65.14 (CH_2O), 126.78 (C_p), 128.51 (C_o), 129.27 (C_m), 137.29 (C_{ipso}), 175.15 (C=O).

4.1.2. Synthesis of alanine ester 2. 4.1.2.1. (2*S*)-Amino-propionic acid dodecyl ester 2d. Prepared as described above for **1a**, starting from L-alanine (1.00 g, 11.2 mmol), dodecanol (2.50 g, 13.4 mmol), and PTSA (2.13 g, 11.2 mmol) in toluene (25 mL) to provide ester **2d** as oil (2.71 g, 94%); R_f 0.27 (EtOAc/cyclohexane1/2); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 6.5 Hz, CH_3CH_2), 1.15 (d, 3H, *J* 7.0 Hz, CH_3CH), 1.24 (m, 20H, CH_2 and NH_2), 1.56 (m, 2H, CH_2CH_2O), 3.38 (q, H, *J* 7.0 Hz, CH), 4.03 (m, 2H, CH_2O); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.94 (CH_3CH_2), 15.72 (CH_3CH), 22.08, 25.14, 27.93, 28.59, 28.69, 28.90, 28.95, 29.02, 31.28 (CH_2), 47.80 (CH), 65.52 (CH_2O), 174.03 (C=O).

4.1.3. Synthesis of arylsulfonated Phe esters 3

4.1.3.1. Sodium, 2-(1-pentyloxycarbonyl-2*S*-phenyl-ethyl carbamoyl)-benzene sulfonate 3a. To a solution of ester **1a** (1.13 g, 4.8 mmol), triethylamine (6.60 mL, 48.0 mmol) in dry THF (60 mL) under nitrogen was added *o*-sulfobenzoic acid cyclic anhydride (1.30 g, 7.1 mmol). After stirring for 6 h at 60 °C, THF was evaporated and the resulting mixture solubilized in EtOAc, washed with HCl (1M), $NaHCO_3$ saturated, brine, dried over Na_2SO_4. The crude product was isolated after removal of the solvent and purified by column chromatography on silica gel with EtOAc, then EtOAc/MeOH (9/1) to give sulfonate **3a** (1.82 g, 86%) as white solid: mp 73 °C; R_f 0.28 (EtOAc/MeOH 9/1); $[\alpha]_D = +27.6$, $[\alpha]_{436} = +62.9$, $[\alpha]_{365} = +115.9$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3421, 3266 (NH), 1736 (OC=O), 1652 (NHC=O), 1541 (NH), 1240–1081 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.81 (t, 3H, *J* 7.0 Hz, CH_3), 1.14 (m, 4H, CH_2), 1.37 (m, 2H, CH_2CH_2O), 2.97 (dd, H, *J* 13.6 Hz, *J* 8.4 Hz, CHHPh), 3.18 (dd, H, *J* 13.6 Hz, *J* 5.5 Hz, CHHPh), 3.89 (m, 2H, CH_2O), 4.63 (ddd, H, *J* 5.5 Hz, *J* 8.4 Hz, *J* 6.3 Hz, CH), 7.26 (m, 5H, $H_{arom\text{-}Phe}$), 7.44 (dt, 2H, J_o 7.3 Hz, J_m 1.8 Hz, H_{arom}), 7.59 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 7.84 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 9.70 (d, H, *J* 6.3 Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.92 (CH_3), 21.82, 27.47, 27.68 (CH_2), 37.36 (CH_2Ph), 55.11 (CH), 64.34 (CH_2O), 126.71, 126.82, 128.39, 129.26, 129.36, 129.86, 130.37 (C_{arom}), 133.18 (C_{ipso}–CO), 137.00 ($C_{ipso\text{-}Phe}$), 144.36 (C_{ipso}–SO_3), 167.72 (CONH), 171.13 (COO); Anal. Calcd for $C_{21}H_{24}NNaO_6S{\cdot}1/2H_2O$: C, 55.99; H, 5.77; N, 3.11. Found: C, 55.74; H, 5.64; N, 3.15.

4.1.3.2. Sodium, 2-(1-octyloxycarbonyl-2*S*-phenyl-ethyl carbamoyl)-benzene sulfonate 3b. Prepared as described above for **3a**, starting from ester **1b** (0.62 g, 2.0 mmol), triethylamine (2.20 mL, 15.8 mmol), and *o*-sulfobenzoic anhydride (0.54 g, 3.0 mmol) in dry THF (35 mL) to

provide the sulfonate **3b** (0.88 g, 93%) as a white solid: mp 104 °C; R_f 0.30 (EtOAc/MeOH 9/1); $[\alpha]_D = +29.6$, $[\alpha]_{436} = +64.9$, $[\alpha]_{365} = +118.7$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3421, 3266 (NH), 1741 (OC=O), 1650 (NHC=O), 1544 (NH), 1240–1082 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 7.0 Hz, CH_3), 1.20 (m, 10H, CH_2), 1.37 (m, 2H, CH_2CH_2O), 2.95 (dd, H, *J* 14.0 Hz, *J* 8.5 Hz, C*H*HPh), 3.15 (dd, H, *J* 14.0 Hz, *J* 5.5 Hz, CH*H*Ph), 3.90 (m, 2H, CH_2O), 4.62 (ddd, H, *J* 5.5 Hz, *J* 8.5 Hz, *J* 6.2 Hz, CH), 7.25 (m, 5H, $H_{arom\text{-}Phe}$), 7.45 (dt, 2H, J_o 7.3 Hz, J_m 1.8 Hz, H_{arom}), 7.59 (dd, 1H, J_o 6.6 Hz, J_m 2.0 Hz, H_{arom}), 7.86 (dd, 1H, J_o 7.4 Hz, J_m 1.8 Hz, H_{arom}), 9.70 (d, H, *J* 6.2Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.94 (CH_3), 22.07, 25.18, 27.87, 28.53, 28.55, 31.17 (CH_2), 37.24 (*C*H_2Ph), 54.94 (CH), 64.17 (CH_2O), 126.51, 126.70, 128.22, 129.08, 129.12, 129.65, 130.22 (C_{arom}), 133.09 (C_{ipso}–CO), 136.95 ($C_{ipso\text{-}Phe}$), 144.43 (C_{ipso}–SO_3), 167.56 (CONH), 170.95 (COO); Anal. Calcd for $C_{24}H_{30}NNaO_6S{\cdot}3/4H_2O$: C, 57.99; H, 6.31; N, 2.82. Found: C, 58.06; H, 6.40; N, 2.89.

4.1.3.3. Sodium, 2-(1-decyloxycarbonyl-2*S*-phenyl-ethyl carbamoyl)-benzene sulfonate 3c. Prepared as described above for **3a**, starting from ester **1c** (1.50 g, 4.9 mmol), triethylamine (5.40 mL, 39.3 mmol), and *o*-sulfobenzoic anhydride (1.60 g, 8.8 mmol) in dry THF (75 mL) to provide the sulfonate **3c** (2.38 g, 95%) as white solid: mp 102 °C; R_f 0.21 (EtOAc/MeOH 9/1); $[\alpha]_D = +26.4$, $[\alpha]_{436} = +59.4$, $[\alpha]_{365} = +108.0$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3421, 3290 (NH), 1745 (OC=O), 1643 (NHC=O), 1544 (NH), 1245–1086 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.86 (t, 3H, *J* 6.6 Hz, CH_3), 1.23 (m, 14H, CH_2), 1.37 (m, 2H, CH_2CH_2O), 2.97 (dd, H, *J* 13.6 Hz, *J* 8.6 Hz, C*H*HPh), 3.17 (dd, H, *J* 13.6 Hz, *J* 5.3 Hz, CH*H*Ph), 3.89 (m, 2H, CH_2O), 4.61 (ddd, H, *J* 5.3 Hz, *J* 8.6 Hz, *J* 6.2 Hz, CH), 7.25 (m, 5H, $H_{arom\text{-}Phe}$), 7.44 (dt, 2H, J_o 7.3 Hz, J_m 1.8 Hz, H_{arom}), 7.59 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 7.84 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 9.71 (d, H, *J* 6.2 Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.97 (CH_3), 22.10, 25.18, 27.87, 28.60, 28.69, 28.89, 31.27 (CH_2), 37.25 (*C*H_2Ph), 54.97 (CH), 64.16 (CH_2O), 126.55, 126.76, 128.25, 129.16, 129.70, 130.21 (C_{arom}), 133.16 (C_{ipso}–CO), 136.99 ($C_{ipso\text{-}Phe}$), 144.40 (C_{ipso}–SO_3), 167.69 (CONH), 171.01 (COO); Anal. Calcd for $C_{26}H_{34}NNaO_6S{\cdot}1/4H_2O$: C, 60.56; H, 6.84; N, 2.72. Found: C, 60.39; H, 6.54; N, 2.55.

4.1.3.4. Sodium, 2-(1-dodecyloxycarbonyl-2*S*-phenyl-ethyl carbamoyl)-benzene sulfonate 3d. Prepared as described above for **3a**, starting from ester **1d** (2.50 g, 7.5 mmol), triethylamine (8.30 mL, 60.0 mmol) and *o*-sulfobenzoic anhydride (2.10 g, 11.4 mmol) in dry THF (125 mL) to provide the sulfonate **3d** (3.72 g, 92%) as white solid: mp 85 °C; R_f 0.41 (EtOAc/MeOH 9/1); $[\alpha]_D = +24.7$, $[\alpha]_{436} = +55.6$, $[\alpha]_{365} = +101.5$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3418, 3255 (NH), 1738 (OC=O), 1650 (NHC=O), 1544 (NH), 1245–1081 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 6.6 Hz, CH_3), 1.22 (m, 18H, CH_2), 1.36 (m, 2H, CH_2CH_2O), 2.95 (dd, H, *J* 14 Hz, *J* 8.8 Hz, C*H*HPh), 3.17 (dd, H, *J* 14 Hz, *J* 5.5 Hz, CH*H*Ph), 3.90 (m, 2H, CH_2O), 4.62 (ddd, H, *J* 5.5 Hz, *J* 8.8 Hz, *J* 6.2 Hz, CH), 7.25 (m, 5H, $H_{arom\text{-}Phe}$), 7.44 (dt, 2H, J_o 7.3 Hz, J_m 1.8 Hz, H_{arom}), 7.59 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 7.84 (dd, 1H, J_o 6.9 Hz, J_m 1.8 Hz, H_{arom}), 9.70 (d, H, *J* 6.2 Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.99 (CH_3), 22.14, 25.23, 27.93, 28.65, 28.75, 28.92, 28.99, 29.04, 29.08, 31.34 (CH_2), 37.28 (*C*H_2Ph), 54.98 (CH), 64.25 (CH_2O), 126.56, 126.77, 128.26, 129.16, 129.70, 130.22 (C_{arom}), 133.16 (C_{ipso}–CO), 137.00 ($C_{ipso\text{-}Phe}$), 144.40 (C_{ipso}–SO_3), 167.69 (CONH), 171.01 (COO); SM (-ES, m/z): 516 (M−Na, 100%); Anal. Calcd for $C_{28}H_{38}NNaO_6S{\cdot}H_2O$: C, 60.30; H, 7.23; N, 2.51. Found: C, 60.61; H, 7.31; N, 2.63.

4.1.3.5. Sodium, 2-(1-tetradecyloxycarbonyl-2*S*-phenyl-ethyl carbamoyl)-benzene sulfonate 3e. Prepared as described above for **3a**, starting from ester **1e** (1.60 g, 6.2 mmol), triethylamine (6.70 mL, 48.3 mmol), *o*-sulfobenzoic anhydride (1.68 g, 9.1 mmol) in dry THF (100 mL) to provide the sulfonate **3e** (2.64 g, 92%) as white solid: mp 71 °C; R_f 0.37 (EtOAc/MeOH 9/1); $[\alpha]_D = +25.8$, $[\alpha]_{436} = +57.5$, $[\alpha]_{365} = +104.2$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3421, 3266 (NH), 1740 (OC=O), 1647 (NHC=O), 1541 (NH), 1240–1085 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 6.6 Hz, CH_3), 1.24 (m, 22H, CH_2), 1.37 (m, 2H, CH_2CH_2O), 2.98 (dd, H, *J* 13.6 Hz, *J* 8.5 Hz, C*H*HPh), 3.15 (dd, H, *J* 13.6 Hz, *J* 5.5 Hz, CH*H*Ph), 3.89 (m, 2H, CH_2O), 4.63 (ddd, H, *J* 5.5 Hz, *J* 8.5 Hz, *J* 6.2 Hz, CH), 7.25 (m, 5H, $H_{arom\text{-}Phe}$), 7.44 (dt, 2H, J_o 7.3 Hz, J_m 1.8 Hz, H_{arom}), 7.58 (dd, 1H, J_o 7.0 Hz, J_m 1.8 Hz, H_{arom}), 7.86 (dd, 1H, J_o 7.0 Hz, J_m 1.8 Hz, H_{arom}), 9.69 (d, H, *J* 6.2 Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.95 (CH_3), 22.10, 25.19, 27.89, 28.61, 28.71, 28.88, 28.95, 29.03, 29.05, 31.29 (CH_2), 37.24 (*C*H_2Ph), 54.93 (CH), 64.18 (CH_2O), 126.50, 126.71, 128.21, 129.08, 129.65, 130.19 (C_{arom}), 133.11 (C_{ipso}–CO), 136.96 ($C_{ipso\text{-}Phe}$), 144.40 (C_{ipso}–SO_3), 167.62 (CONH), 170.96 (COO); Anal. Calcd for $C_{30}H_{42}NNaO_6S$: C, 63.47; H, 7.46; N, 2.47. Found: C, 63.02; H, 7.52; N, 2.41.

4.1.4. Synthesis of arylsulfonated Ala ester 4d

4.1.4.1. Sodium, 2-(1-dodecyloxycarbonyl-2S-ethyl carbamoyl)-benzene sulfonate 4d. Prepared as described above for **3a**, starting from ester **2d** (1.60 g, 6.2 mmol), triethylamine (6.70 mL, 48.3 mmol), and *o*-sulfobenzoic anhydride (1.68 g, 9.1 mmol) in dry THF (100 mL) to provide the sulfonate **4d** (2.59 g, 90%) as white solid: mp 71 °C; R_f 0.33 (EtOAc/MeOH 9/1); $[\alpha]_D = +10.4$, $[\alpha]_{436} = +36.0$, $[\alpha]_{365} = +49.3$ (*c* 1.5, EtOH 96%); IR (KBr), ν_{max} (cm^{-1}): 3412, 3279 (NH), 1740 (OC=O), 1647 (NHC=O), 1554 (NH), 1085 (S=O); ^1H NMR (DMSO-d_6), δ (ppm): 0.85 (t, 3H, *J* 6.6 Hz, CH_3CH$_2$), 1.24 (m, 18H, CH_2), 1.36 (d, 3H, *J* 7.0 Hz, CH_3CH), 1.56 (m, 2H, CH_2CH_2O), 4.04 (m, 2H, CH_2O), 4.42 (dq, H, *J* 7.0 Hz, *J* 6.2 Hz, CH), 7.44 (dt, 2H, J_o 7.4 Hz, J_m 1.8 Hz, H_{arom}), 7.59 (dd, 1H, J_o 6.6 Hz, J_m 2.2 Hz, H_{arom}), 7.85 (dd, 1H, J_o 6.6 Hz, J_m 2.2 Hz, H_{arom}), 9.54 (d, H, *J* 6.2 Hz, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 13.95 (*C*H$_3$CH$_2$), 16.71 (*C*H$_3$CH), 22.09, 25.26, 28.07, 28.62, 28.71, 28.94, 28.99, 29.03, 31.29 (CH_2), 48.62 (CH),

Table 4. Compositions of liquid-crystalline NMR samples made of (±)-**7d$_1$** in surfactant **3c–d**

Sample	Surfactants			H_2O		Organic solvent			Solute	
		/mg[a]	/% w/w	/mg[a]	/% w/w		/mg[a]	/% w/w	/mg[a]	/% w/w
1	**3c**	110	24	260	58	$CHCl_3$	70	16	10	2
2	**3c**	111	25	262	59	CH_2Cl_2	60	14	10	2
3	**3c**	110	25	260	59	$C_2H_2Cl_4$	60	14	12	2
4	**3c**	111	21	262	48	CCl_4	160	29	11	2
5	**3c**	110	24	270	60	Toluene	60	14	10	2
6	**3c**	113	25	261	58	Decanol	70	15	10	2
7	**3d**	110	27	261	63	$CHCl_3$	33	8	7	2

[a] The accuracy on the weighting is ±1 mg.

64.29 (CH_2O), 126.74, 129.04, 129.58, 130.12 (C_{arom}), 133.19 (C_{ipso}–CO), 144.46 (C_{ipso}–SO_3), 167.40 (CONH), 172.49 (COO); SM (-ES, m/z): 440.5 (M−Na, 100%); Anal. Calcd for $C_{22}H_{34}NNaO_6S{\cdot}1/2H_2O$: C, 55.92; H, 7.47; N, 2.96. Found: C, 55.58; H, 7.36; N, 3.06.

4.2. Surface tension measurements

Surface tension measurements were obtained by the Du Noüy method with a platinum ring using a Krüss K 10T tensiometer, thermostated at 25 °C. The aqueous solutions of compounds **3** and **4** were prepared using ultrapure water (MilliQ) and the surface tension γ was measured at the equilibrium (15 smin) for various concentrations.

Below CMC, the superficial tension, γ decreases when the surfactant concentration C increases, according to Gibbs' equation (Eq. 3):

$$d\gamma = -2 \times 2.3\Gamma RTd(\log(C)) \quad (3)$$

assuming a complete dissociation of the counter ion.

Above the CMC γ reaches a minimum value, γ_{min}, and remains constant. The CMC value is determined from the inflection point in the γ versus $\log(C)$ curve. The superficial excess Γ in mol/m^2 is deduced from the slope of γ versus $\log(C)$ below the CMC. The area per head group at the air–water interface a_s (Å^2/molecule) is given by Eq. 4:

$$a_s = \frac{1}{N\Gamma} \quad (4)$$

with N being Avogadro's number.

4.3. NMR spectroscopy in amphiphilic chiral oriented solvents

4.3.1. Sample preparation. The composition of oriented NMR samples is listed in Table 4. The amount of the components was optimized in order for the total volume of the sample to be optimal when compared to the length of the coil of a 5 mm diameter NMR probe-head. In the first step, around 110 mg of surfactant (21–27% w/w), 7–12 mg chiral material **7d$_1$** (2% w/w), and around 260 mg of water (63–48% w/w) were directly weighted into a 5 mm od NMR tube. The NMR sample was then centrifuged during 30 s and inverted from head to tail and recentrifuged again (several cycles) to optimize the mixing of various components of the mixture. Using a colorless solute, a nonoriented, fluid, and transparent sample was obtained. In a second step, chlorinated solvent ($CHCl_3$, CH_2Cl_2, $C_2H_2Cl_4$, CCl_4) was added to the mixture by portion of 10–20 mg and the sample mixed (by centrifugation) until a rather clear optically homogeneous birefringent phase was obtained. Note that a very viscous, rather white, mixture was observed if the w/w concentration in chlorinated entity was too low. When this concentration was optimal (8–29% w/w), a rather fluid, transparent phase was obtained. The changes in the macroscopic characteristics of the sample allowed us to easily control the optimal amount of organic solvent to be added. Note that the reproducibility of NMR results strongly depend on sample preparation, and in particular the homogeneity of the mixture.

4.3.2. NMR spectroscopy. The ^1H, ^1H–{^2H} and ^2H–{^1H} 1D NMR experiments were performed at 9.4 T on a Bruker DRX 400 high-resolution spectrometer equipped with a BBO probe and a standard variable temperature unit (BVT 3200). The samples were not spun in the magnetic field. Note however that the spinning of the mixture does not affect the micellar organization of the system. In order to remove the proton–deuterium scalar and dipolar couplings, the protons were broadband decoupled using a WALTZ-16 composite pulse sequence. Other experimental NMR parameters or details are given in the figure captions.

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