Synthesis and aggregation of two-headed surfactants bearing amino acid moieties†

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The synthesis of bolaamphiphiles with two amino acid heads having the general structure AA—X—AA, where AA denotes an amino acid (D- or L-alanine or L-histidine) and X an alkyl chain of varying length, is described. Micellization was observed for the bolaamphiphile with a twenty-carbon atom alkyl chain. For compounds with shorter chain lengths, light scattering and electron microscopy suggest the formation of vesicles or fibrous aggregates. These bolaamphiphiles can also form gels at higher concentrations in water or ethanol. Fibrous structures were observed in these gels by electron microscopy.

Synthèse et agrégation d'amphiphiles à deux têtes portant un motif acide aminé. Des bolaamphiphiles de structure AA—X—AA, où AA représente un motif acide aminé (D- ou L-alanine ou L-histidine) et X est un segment lipophile de longueur variable, ont été synthétisés. Les phénomènes d'agrégation en solution aqueuse de ces bolaformes ont été étudiés. Seuls les composés présentant une longueur de chaîne de vingt atomes de carbone forment des micelles. Pour les bolaamphiphiles ayant des chaînes hydrocarbonées plus courtes la formation de vésicules, de fibres ou d'hélices est observée diffusion de la lumière et microscopie électronique. Ces bolaamphiphiles à motif acide aminé forment aussi des gels dans l'éthanol ou dans l'eau. Dans ce cas, des structures fibreuses ont pu être observées par microscopie électronique.

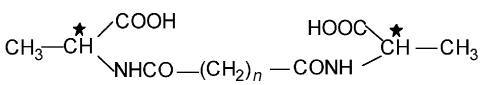
Biocompatible surfactants have become increasingly important considering their biological and biochemical applications. New compounds, analogues of natural products, have been synthesized and their physical, chemical and biological properties determined.[1–8]

The easiest way to obtain chiral amphiphilic molecules is to introduce a sugar or an amino acid head group. So, many unipolar amino acid surfactants with one or two hydrocarbon chains have been described in the literature,[9–17] particularly salts of long-chain *N*-acyl amino acids that are currently used as detergents, foaming agents and shampoos because they are mild, non-irritating to human skin and highly biodegradable. In water these *N*-acyl amino acid amphiphiles form aggregates, the morphology of which has been widely studied. Because of their chirality, they can form gels containing helical fibers that are stabilized by amide hydrogen bonds.[18]

Only two bolaamphiphiles with one amino acid head group (D- or L-lysine or ornithine) and one ammonium head group have been described in the literature. Using electron microscopy the authors showed that these molecules form molecular monolayers, rods and tubules in water.[19]

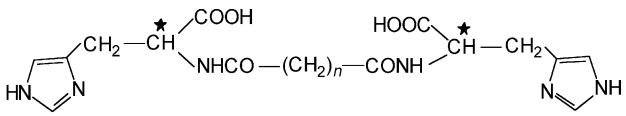
In this paper, we describe, for the first time, the synthesis of symmetric bolaamphiphiles having two amino acid head groups (D- or L-alanine or L-histidine) and various hydrophobic spacers (Fig. 1, 2). Then we will discuss the behaviour of these bolaamphiphiles at the water–air interface as studied by tensiometry, and their properties in water explored by conductometry, light scattering and electron microscopy. Their aggregation in organic solvents was also studied and the influence of their chirality on their organization mode examined.

Depending on the pH of the aqueous medium, the above compounds are ionic or non-ionic. Therefore, the aggregate formed will be influenced by both the pH and the alkyl chain length. Usually bolaamphiphiles are known to organize in an aqueous environment to give aggregates of various morphologies: spheres, small spherocylinders, large cylinders, discs, lamellae and vesicles. It has been shown that a chiral centre can induce the formation of fibers (twisted ribbon or helical rope) with a single twist direction.[20]



D or L : **1** (n = 10) ; **2** (n = 12) ; **3** (n = 20)

Fig. 1 Structure of alanine bolaamphiphiles.



L : **4** (n = 10) ; **5** (n = 12) ; **6** (n = 20)

Fig. 2 Structure of L-histidine bolaamphiphiles.

† *Supplementary material available:* Synthesis and characterization of the dimethyl ester bolaamphiphiles. For direct electronic access see http://www.rsc.org/suppdata/nj/1999/447/, otherwise available from BLDSC (No. SUP 57486, 8 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/njc).

Results and discussion

Synthesis

D- or L-alanine methyl esters (obtained by esterification of D- or L-alanine) were reacted with a long chain diacyl chloride in the presence of triethylamine to give the bolaamphiphile methyl esters. We checked that after this step the optical rotations of the two corresponding stereoisomers were still opposite. The D- and L-bolaamphiphiles **1**, **2**, **3** were then obtained after hydrolysis with 50, 50 and 30% yields, respectively. In this synthesis the eventuality of racemization at the hydrogen-bearing carbon centres had to be considered. In the ^{1}H NMR spectra signals corresponding to diastereoisomers were not observed. Moreover, when the basic hydrolysis was achieved in MeOD–D_2O, deuteration was not detected by NMR (^{1}H and ^{13}C). Since under these conditions the rate of racemization would be equal to that of hydrogen–deuterium exchange,[21,22] we can assume that racemization of the amino acid moiety does not occur during the reaction.

The L-histidine bolaamphiphiles (**4**, **5** and **6**) were synthesized by the reaction of L-histidine methyl esters (obtained from L-histidine) with decan-1,10-dioic acid or dodecan-1,12-dioic acid or docosan-1,20-dioic acid, respectively, in the presence of *N*-ethyl-*N*′-(γ-dimethylaminopropyl)carbodiimide and l-hydroxybenzotriazole.[23] Then sodium hydroxide hydrolysis of the ester functions gave bolaamphiphiles **4**, **5** and **6** with 25, 30 and 23% yields, respectively.

Study of self-organization

Micelle formation. Previous results with bolaamphiphilic compounds show that it is possible to observe vesicles or micelles, depending on the length of the spacer.[24] When the spacer is long enough, folding of the chain becomes possible and micelles occur, whereas when the spacer is too short, folding is not possible and vesicles are observed. For each bolaamphiphile we tried to identify the type of aggregate formed spontaneously in water.

First, the solubility of these compounds was studied in distilled water (Table 1). The solubility of bolaamphiphiles with ten and twelve methylene units (**1**, **2**, **4**, **5**) is high. Bolaamphiphiles with a spacer of twenty methylene units (**D-3**, **L-3**, **6**) form gels at their concentration solubility limit. A Krafft point was observed for **D-3** and **L-3** at 15 °C.

The self-organization studies were undertaken in water at 30 °C using two methods: tensiometry (Fig. 3–6) and conductometry. In our concentration range (10^{-5}–10^{-2} mol l^{-1}), the pH of the alanine bolaamphiphile solutions varied from 6.15 to 9.5. In this pH range, these compounds are always in the anionic form. So, we preferred not to have a fixed pH and thus to avoid salt effects brought about by buffer. For histidine bolaamphiphiles however, it was necessary to fix the pH because of the protonation equilibra involving the imidazole group. In view of further biological applications, the pH was

Table 1 Solubility in water at 30 °C

Compound	Solubility/mol l^{-1}
D-1	$>5 \times 10^{-1}$
L-1	
D-2	$>5 \times 10^{-1}$
L-2	
D-3	Give gels for
L-3	$c \geq 4 \times 10^{-2}$
4	9×10^{-2}
5	5×10^{-2}
6	$<10^{-4}$

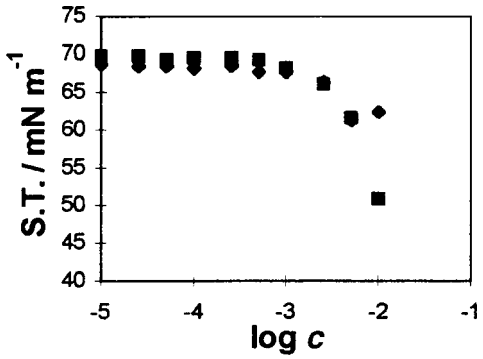


Fig. 3 Surface tension (S.T.) *versus* concentration of aqueous solutions of bolaamphiphiles **D-1** (◆) and **L-1** (■) at 30 °C.

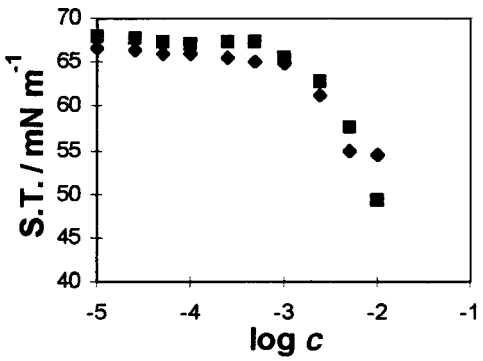


Fig. 4 Surface tension *versus* concentration of aqueous solutions of bolaamphiphiles **D-2** (◆) and **L-2** (■) at 30 °C.

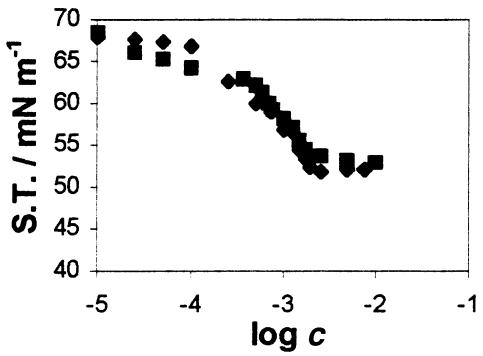


Fig. 5 Surface tension *versus* concentration of aqueous solutions of bolaamphiphiles **D-3** (◆) and **L-3** (■) at 30 °C.

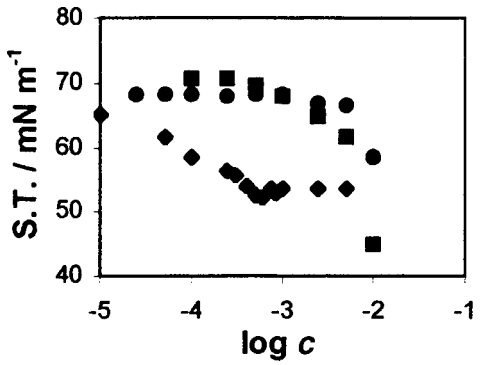


Fig. 6 Surface tension *versus* concentration of aqueous solutions of bolaamphiphiles **4** (●), **5** (■) and **6** (◆) at 30 °C and at pH = 7, 7 and 11.5, respectively.

fixed at 7. So the solutions were buffered by the imidazole ring of the molecule. At pH values of around 7, compounds **4** and **5** are present in two forms, anionic and zwitterionic. Due to the insolubility of compound **6** at pH 7, we had to study its organization at pH 11.5 (in phosphate buffer).

The shape of the surface tension curves of bolaamphiphiles **1**, **2**, **4** and **5**, between 10^{-5} and 10^{-2} mol l^{-1}, are not characteristic of micelle formation. The conductometry study (for concentrations of 10^{-5} to 5×10^{-2} mol l^{-1}) did not show aggregation phenomena either. Moreover, these amphiphiles have a weak tensioactive character, perhaps due to their high solubility in water. For bolaamphiphiles **D-3**, **L-3** and **6**, the shape of the tensiometry curves is similar to that obtained for a classic surfactant (Fig. 5, 6). For bolaamphiphiles **3**, no difference between the two isomers, D and L, was observed: these compounds seem to form micelles at a concentration of 2×10^{-3} mol l^{-1}. Bolaamphiphile **6** seems to form micelles at a concentration of 6×10^{-4} mol l^{-1}. The same value is obtained from the conductometry study.

So, among all the studied compounds, only bolaamphiphiles **D-3**, **L-3** and **6** presented a micellar behaviour and it was possible to determine their aggregation number (average number of detergent molecules per micelle unit). Information concerning the micelle aggregation number (N) can be obtained from the fluorescence quenching of a probe, $Ru(bipy)_3^{2+}$ by a hydrophobic quencher: 9-methylanthracene.[25] The mean aggregation number is related to the measured ratio of luminescence intensities in the absence (I°) and in the presence (I) of the quencher Q by the following expressions:

$$\ln(I^\circ/I) = \frac{[Q]N}{[C] - [CMC]} \quad (1)$$

The concentrations of the amphiphiles [C] were five fold the critical micellar concentration (CMC) and the ratio [amphiphile] : [luminescent probe] was 20 : 1. At low concentrations of quencher (1) was obeyed and from the slopes, the mean aggregation numbers N were calculated with a precision of ± 1 to give N equals 15, 14 and 5 for bolaamphiphiles **L-3**, **D-3** and **6**, respectively.

The range of micellar aggregation numbers found in the literature is very large. For monopolar amphiphiles, this number, determined in aqueous media, varies from 15 to 400[26] and for bipolar amphiphiles, from 5[27] to 250.[28] The aggregation number increases with the hydrophobicity, with the binding of counter ions to the micelle and with decreases in the size and charge of the hydrophilic head group. Generally, with bolaamphiphiles, the presence of a second head group increases the solubility of the amphiphile in water and leads to the formation of small aggregates (aggregation numbers ≈ 15) when compared to one-headed amphiphiles (≈ 70).[29,30] A folded conformation has been suggested to explain this. As amphiphiles having a 12 carbon alkyl chain and one amino acid head have aggregation numbers between 60 and 70,[31] our results are consistent with the above hypothesis. The small aggregation number of bolaamphiphile **6** can be compared to similar aggregation numbers (5 to 11) observed for surfactants having aromatic head groups like naphthalene disulfonic acid or urocanic acid.[5,32]

No differences were observed between enantiomers: they have the same CMC and a similar aggregation number. These results show that optically active bolaamphiphiles present the same lack of enantiomeric effect on micellization as the chiral one-headed surfactants described in the literature.[33]

The results concerning the micellization of amino acid bolaamphiphiles are in accordance with the hypothesis that when the spacer is long enough, folding of the chain permits the formation of micelles. Similarly, one can expect the formation of vesicles for shorter spacer lengths.

Formation of other types of aggregate. After sonication at 0 °C for 15 min (power output 110 watts, 80% duty cycle), an aqueous solution (5×10^{-3} mol l^{-1}) of each bolaamphiphile was observed first by quasi-elastic light scattering and then by electron microscopy. These solutions had a pH of 9.1 for bolaamphiphiles **1**, **2**, **3**, a pH of 7 for bolaamphiphiles **4**, **5** and a pH of 11.5 for **6**. Using light scattering, very small objects (1–10 nm) and larger aggregates were observed for bolaamphiphiles **1**, **4** and **5**. The larger ones are in the range of known vesicular bolaamphiphile diameters.[34] Table 2 shows the diameters and the size distribution of these aggregates. The results are the average of 10 to 20 tests for each size determination, the standard deviation is about 20%. No aggregates were detected in other alanine bolaamphiphile solutions (**2** and **3**) or histidine bolaamphiphile **6**.

The sonicated solutions were also studied by electron microscopy. A staining technique and a platinum shadowing method were used to visualize the aggregates directly.[35–37] Vesicles and fibers can be observed by these methods, but not micelles because they are dynamic and too small. For bolaamphiphiles **1**, **4** and **5** the formation of vesicles was observed. The vesicles formed by compounds **1** had a diameter of around 50 nm, vesicles of bolaamphiphiles **4** and **5** were more polydisperse with diameters between 30 and 100 nm. In all cases, the vesicles tended to aggregate into larger objects. This observation could explain the difference in sizes measured by light scattering and electron microscopy.

Twisted fibrous aggregates were observed by electron microscopy for enantiomeric amphiphiles **2** but not for the **L-2** + **D-2** racemate. It seems that the twisted fibers are left-handed for **L-2** [Fig. 7(*a*)] and right-handed for **D-2** (Fig. 8). This observation agrees with literature results since enantiomeric amphiphiles are known to produce mirror-image helices.[20,38–41] Amide bonds in surfactants play an important part in the formation of these highly-ordered structures.[42]

A mechanism proposed for fiber formation is the aggregation of globular particles.[43] It is possible that vesicles were not observed for compound **2** because they were already aggregated at the concentration used. In order to verify this hypothesis, solutions of two-fold diluted **L-2** were studied by electron microscopy and monodisperse spherical vesicles of 60 nm diameter were detected [Fig. 7(*b*)]. This demonstrates that there is a transition dependent on the concentration between the two types of aggregates (vesicles and fibers).

For bolaamphiphiles **3** and **6** neither vesicles nor fibers were observed by electron microscopy, confirming the light scattering results.

Gel formation in water or organic solvents was also studied. The gels were obtained from saturated solutions of bolaamphiphiles in ethanol or water. The solutions were heated, then cooled slowly to ambient temperature.[44] Because of the difficulties inherent in the study of gels a more powerful transmission electron microscope and a scanning electron microscope were used.

In water, only bolaamphiphiles **3** and **6** formed translucid gels at 4×10^{-2} and 10^{-4} mol l^{-1}, respectively. The structures obtained for bolaamphiphiles **3** are thermosensitive and

Table 2 Diameters (ϕ) and size distribution (%) of aggregates observed by light scattering in 5×10^{-3} mol l^{-1} aqueous solutions of bolaamphiphiles **1** (pH = 9.1), **4** (pH = 7) and **5** (pH = 7)

L-1		**D-1**		**4**		**5**	
ϕ/nm	%	ϕ/nm	%	ϕ/nm	%	ϕ/nm	%
1	30	1	50	1	67	10	15
82	30	67	10				
196	40	134	40	172	33	191	85

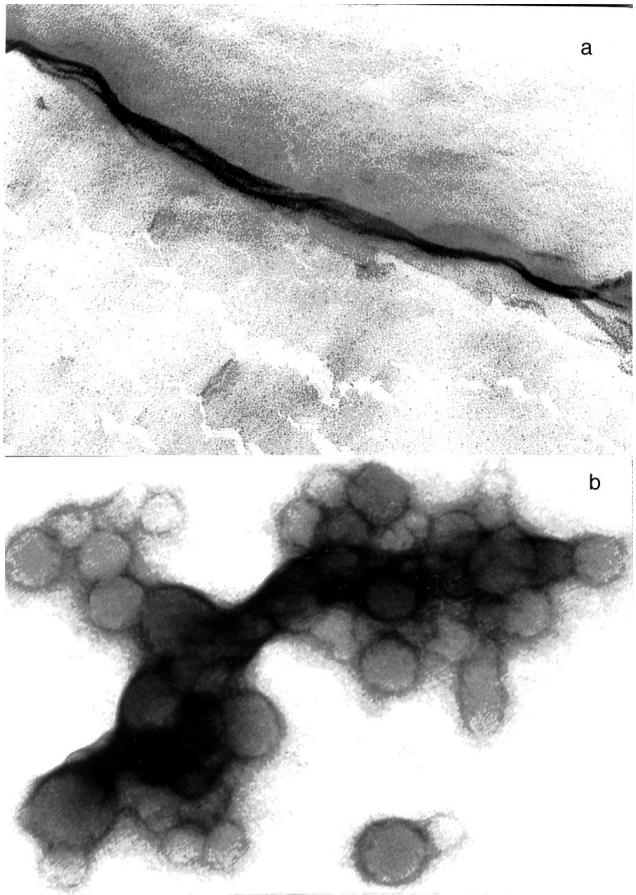


Fig. 7 Transmission electron micrograph of aggregates formed from **L-2** (1 cm = 70 nm). (a) Twisted fibers, Pt shadowing and (b) vesicles obtained after dilution of the twisted fibers, negative stained with uranyl acetate.

lose their organization at temperatures above 20 °C. They are stable at 7 °C for several months. Fibrous structures had been detected by optical microscopy, but it was not possible to obtain electron micrographs. The thermal instability of the structure may explain this fact. The other bolaamphiphiles did not give gels in water; this can be related to their high solubility.

When 0.1 mol l^{-1} hot ethanol solutions of compounds **1** and **2** were allowed to cool, they afforded gels. The gels given by **1** were observed by the two electron microscopy techniques (scanning and transmission electron microscopy). Twisted fibers were observed but the twist direction was not obvious (Fig. 9).

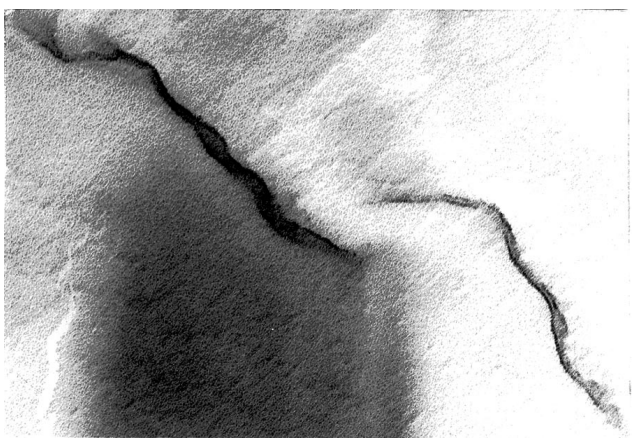


Fig. 8 Transmission electron micrograph of formed twisted fibers (Pt shadowing) from **D-2** (1 cm = 70 nm).

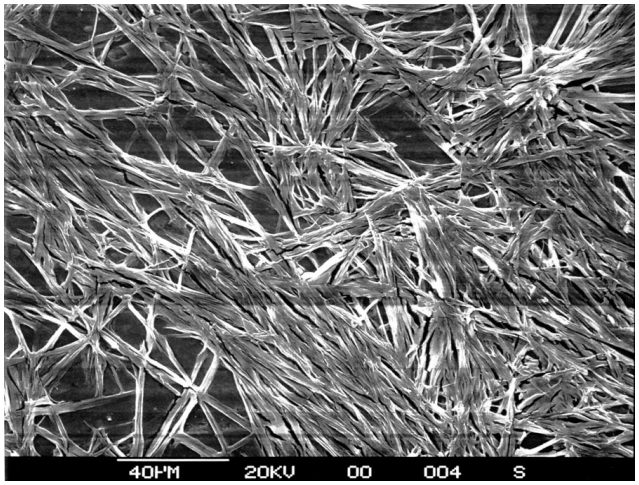


Fig. 9 Fibrous structures (scanning electron micrograph) formed by compound **L-1**.

A similar type of organization has been described in the literature.[43] Aqueous or organic solutions of optically active amphiphilic compounds form gels in which fibrous structures are observed.

Conclusion

Various bolaamphiphiles with two amino acid head groups were synthesized and found to have the same behaviour as the bolaamphiphiles already described: (*i*) when the spacer is long enough to allow folding of the chain, micelles can be formed; (*ii*) with a shorter chain, there is aggregation into vesicles or fibrous structures; (*iii*) the shape and size of various aggregates are analogous to those reported in the literature.

In summary, this new family of amino acid bolaamphiphiles presents interesting behaviour in aqueous solution. This study could lead to much wider use of these molecules owing to the properties of the amino acids they contain. Novel research perspectives involving this class of surfactants could include. (*i*) formation of mixed molecules with a sugar head and an amino acid head; (*ii*) interaction with cholesterol to mimic membrane stabilization; (*iii*) interaction with nucleic acid bases; and (*iv*) synthesis of immunooadjuvants.

Experimental

All the solvents (Prolabo or Carlo Erba) were used without further purification. The reagents were purchased from Aldrich, Acros or Lancaster (>98% purity). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 and AC 400 spectrometers. The DCI, NH_3 or CH_4 mass spectra were recorded on a Nermag R10-10 apparatus and the FAB mass spectra on a ZAB-MS apparatus (WG-Analytical, Manchester, UK). Infrared spectra were recorded on a Perkin Elmer 683b spectrophotometer and UV spectra on a Hewlett Packard 8452 A spectrophotometer. Melting points were determined on an Electrothermal apparatus (capillary tubes). Microanalysis was carried out at the ENSCT (Toulouse, France) on a Carlo Erba 1106 instrument.

Syntheses

The experimental data of bolaamphiphile methyl esters is given in the electronical supplementary information.

L-1. Yield = 49%; $[\alpha]_D^{25} = -15°$ (water, $c = 10^{-3}$ g ml^{-1}). ^{1}H NMR (DMSO-d_6, δ): 1.12 (d, $J_1 = 6.9$ Hz, 6H, CH_3); 1.22 (m, 12H, CH_2); 1.44 (m, 4H, $CH_2\beta CO$); 2.05 (t, $J = 7.3$ Hz,

4H, $CH_2\alpha CO$); 3.68 (qd, $J_1 = 6.9$ Hz, $J_2 = 6.7$ Hz, 2H, CH); 7.24 (d, $J = 6.7$ Hz, 2H, NHCO). ^{13}C NMR (DMSO-d_6, δ); 174.18 (NHCO); 170.45 (COO^-); 49.6 (CH); 35.70 ($CH_2\alpha CO$); 28.78 ($CH_2\beta CO$); 28.67–25.29 (CH_2); 19.33 (CH_3). IR $\upsilon(cm^{-1})$: 3400 (OH); 3320–3280 (NH); 2920–2910 (CH_2); 2840 (CH_3); 1630 (COO^-); 1580 (*CO*NH). MS (positive FAB, NBA matrix): $m/z = 487$, MK^+ (100%); 449, MH^+; 411, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{18}H_{30}K_2N_2O_6 \cdot 3\ H_2O$: 43.01; H 7.22; N 5.57. Found C 43.69; H 7.04; N 5.57.

D-1. Yield = 49%; $[\alpha]_D^{25} = +15°$ (water, $c = 10^{-3}$ g ml^{-1}). ^1H NMR (DMSO-d_6, δ): 1.11 (d, $J_1 = 6.9$ Hz, 6H, CH_3); 1.22 (m, 12H, CH_2); 1.44 (m, 4H, $CH_2\beta CO$); 2.04 (t, $J = 7.3$ Hz, 4H, $CH_2\alpha CO$); 3.64 (qd, $J_1 = 6.9$ Hz, $J_2 = 6.5$ Hz, 2H, CH); 7.20 (d, $J_2 = 6.5$ Hz, 2H, NHCO). ^{13}C NMR (DMSO-d_6, δ): 173.79 (NHCO); 170.37 (COO^-); 49.97 (CH); 35.75 ($CH_2\alpha CO$); 28.81 ($CH_2\beta CO$); 28.70–25.31 (CH_2); 19.35 (CH_3). IR $\upsilon(cm^{-1})$: 3420 (OH); 3310–3280 (NH); 2920–2910 (CH_2); 2840 (CH_3); 1620 (COO^-); 1580 (*CO*NH). MS (positive FAB, NBA matrix): $m/z = 487$, MK^+ (100%); 449, MH^+; 411, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{18}H_{30}K_2N_2O_6 \cdot 3\ H_2O$; C 43.01; H 7.22; N 5.57. Found C 42.49; H 6.75; N 5.37.

L-2. Yield = 52.%; $[\alpha]_D^{25} = -23°$ (water, $c = 10^{-2}$ g ml^{-1}). ^1H NMR (DMSO-d_6, δ): 1.11 (d, $J_1 = 6.9$ Hz, 6H, CH_3); 1.22 (m, 16H, CH_2); 1.44 (m, 4H, $CH_2\beta CO$); 2.04 (t, $J = 7.3$ Hz, 4H, $CH_2\alpha CO$); 3.65 (qd, $J_1 = 6.9$ Hz, $J_2 = 6.7$ Hz, 2H, CH); 7.22 (d, $J_2 = 6.7$ Hz, 2H, NHCO). ^{13}C NMR (DMSO-d_6, δ): 173.82 (NHCO); 170.37 (COO^-); 49.97 (CH); 35.75 ($CH_2\alpha CO$); 28.87 ($CH_2\beta CO$); 28.72–25.32 (CH_2); 19.35 (CH_3). IR $\upsilon(cm^{-1})$: 3420 (OH); 3340–3300 (NH); 2920 (CH_2); 2850 (CH_3); 1630 (COO^-); 1585 (*CO*NH). MS (positive ESI): $m/z = 515$, MK^+ (100%); 477, MH^+; 439, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{20}H_{34}K_2N_2O_6 \cdot \frac{5}{2}\ H_2O$; C 45.87; H 7.89; N 5.35. Found C 46.09; H 7.40; N 5.29.

D-2. Yield = 48%; $[\alpha]_D^{25} = +22°$ (water, $c = 10^{-2}$ g ml^{-1}). ^1H NMR (DMSO $-$ d_6, δ): 1.11 (d, $J_1 = 6.7$ Hz, 6H, CH_3); 1.22 (m, 16H, CH_2); 1.44 (m, 4H, $CH_2\beta CO$); 2.04 (t, $J = 7$ Hz, 4H, $CH_2\alpha CO$); 3.64 (qd, $J_1 = 6.7$ Hz, $J_2 = 6.6$ Hz, 2H, CH); 7.22 (d, $J_2 = 6.6$ Hz, 2H, NHCO). ^{13}C NMR (DMSO-d_6, δ): 173.81 (NHCO); 170.37 (COO^-); 49.96 (CH); 35.75 ($CH_2\alpha CO$); 28.87 ($CH_2\beta CO$); 28.73–25.31 (CH_2); 19.35 (CH_3). IR $\upsilon(cm^{-1})$: 3420 (OH); 3330–3300 (NH); 2920 (CH_2); 2850 (CH_3); 1630 (COO^-); 1585 (*CO*NH). MS (positive ESI): $m/z = 515$, MK^+; 477, MH^+ (100%); 439, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{20}H_{34}K_2N_2O_6 \cdot \frac{5}{2}\ H_2O$: C 45.87; H 7.89; N 5.35. Found C 46.06; H 7.38; N 5.29.

L-3. Yield = 30%; $[\alpha]_D^{25} = -10°$ (water, $c = 10^{-2}$ g ml^{-1}). ^1H NMR (CD_3OD, δ): 1.28 (m, 32H, CH_2); 1.33 (d, $J = 7.1$ Hz, 6H, CH_3); 1.60 (m, 4H, $CH_2\beta CO$); 2.21 (t, $J = 7.5$ Hz, 4H, $CH_2\alpha CO$); 4.19 (q, $J = 7.1$ Hz, 2H, CH). ^{13}C NMR (CD_3OD, δ): 180.09 (NHCO); 175.20 (COO^-); 51.95 (CH); 37.36 ($CH_2\alpha CO$); 30.86 ($CH_2\beta CO$); 30.71–27.01 (CH_2); 19.43 (CH_3). IR $\upsilon(cm^{-1})$: 3400 (OH); 3320–3290 (NH); 2910 (CH_2); 2840 (CH_3); 1620 (COO^-); 1575 (*CO*NH). MS (positive ESI): $m/z = 627$, MK^+ (100%); 611, MNa^+; 589, MH^+ (100%); 551, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{28}H_{50}K_2N_2O_6 \cdot 3\ H_2O$: C 52.31; H 8.78; N 4.36. Found C 52.45; H 8.57; N 4.21.

D-3. Yield = 26%; $[\alpha]_D^{25} = +11°$ (water, $c = 10^{-2}$ g ml^{-1}). ^{1}NMR (CD_3OD, δ): 1.28 (m, 32H, CH_2); 1.33 (d, $J = 7.1$ Hz, 6H, CH_3); 1.60 (m, 4H, $CH_2\beta CO$); 2.21 (t, $J = 7.6$ Hz, 4H, $CH_2\alpha CO$); 4.19 (q, $J = 7.1$ Hz, 2H, CH). ^{13}C NMR (CD_3OD, δ): 180.09 (NHCO); 175.20 (COO^-); 51.90 (CH); 37.36 ($CH_2\alpha CO$); 30.85 ($CH_2\beta CO$); 30.71–27.00 (CH_2); 19.43 (CH_3). IR $\upsilon(cm^{-1})$: 3400 (OH); 3320 (NH); 2920 (CH_2); 2860 (CH_3); 1640 (COO^-); 1590 (*CO*NH). MS (positive ESI): $m/z = 627$, MK^+ (100%); 611, MNa^+; 589, MH^+ (100%); 551, $[M - K + 2H]^+$. Anal. calcd (%) for $C_{28}H_{50}K_2N_2O_6 \cdot 3\ H_2O$: C 52.31; H 8.78; N 4.36. Found C 52.20; H 8.88; N 4.07.

4. Yield = 25%; $[\alpha]_D^{25} = +14.5°$ (water, $c = 10^{-2}$ g ml^{-1}). ^1H NMR (D_2O, δ): 1.09 (m, 12H, CH_2); 1.38 (m, 4H, $CH_2\beta CO$); 2.15 (t, $J = 7.1$ Hz, 4H, $CH_2\alpha CO$); 3.18 (dd, AB system, $J_{AB} = 15.3$ Hz, 4H, CH_2his); 4.43 (dd, X, $J_{ax} = 9.4$ Hz, $J_{BX} = 4.7$ Hz, 2H, CH); 7.10 (s, 2H, H5im); 8.25 (s, 2H, H2im). ^{13}C NMR (D_2O, δ): 179.79 (CONH); 179.13 (COO^-); 136.48 (C2im); 133.55 (C4im); 119.56 (C5im); 56.78 (CH); 38.33 (CH_2his); 31.12 ($CH_2\alpha CO$); 30.95 ($CH_2\beta CO$); 30.65–27.86 (CH_2). MS (positive ESI): $m/z = 549$, $[M - 2HCl + H]^+$; 527, $[M - 2HCl - Na + 2H]^+$ (100%); 505, $[M - 2HCl - 2Na + 3H]^+$. Anal. calcd (%) for $C_{24}H_{36}N_6O_6Na_2Cl_2$: C 46.38; H 5.84; N 13.5. Found C 46.53; H 5.87; N 13.61.

5. Yield = 30%; $[\alpha]_D^{25} = +15°$ (water, $c = 10^{-2}$ g ml^{-1}). ^1H NMR (D_2O, δ): 1.11 (m, 16H, CH_2); 1.39 (qt, $J_1 = 7.2$ Hz, $J_2 = 7.1$ Hz, 4H, $CH_2\beta CO$); 2.14 (t, $J_1 = 7.2$ Hz, 4H, $CH_2\alpha CO$); 2.92 (dd, AB system, $J_{AB} = 15.2$ Hz, 4H, CH_2his); 4.41 (dd, X, $J_{AX} = 9.2$ Hz, $J_{BX} = 4.6$ Hz, 2H, CH); 7.00 (s, 2H, H5im); 8.03 (s, 2H, H2im). ^{13}C NMR (D_2O, δ): 180.10 (CONH); 178.84 (COO^-); 137.07 (C2im); 134.27 (C4im); 119.85 (C5im); 56.99 (CH); 38.41 (CH_2his); 31.53 ($CH_2\alpha CO$); 31.43 ($CH_2\beta CO$); 31.21–27.95 (CH_2). MS (positive ESI): $m/z = 577$, $[M - 2HCl + H]^+$; 555, $[M - 2HCl - Na + 2H]^+$ (100%); 533, $[M - 2HCl - 2Na + 3H]^+$ (100%). Anal. calcd (%) for $C_{26}H_{40}N_6O_6Na_2Cl_2 \cdot H_2O$: C 46.78; H 6.34; N 12.59. Found C 46.85; H 6.37; N 12.65.

6. Yield = 23%; $[\alpha]_D^{25} = +8°$ (water, $c = 3.7 \times 10^{-3}$ g ml^{-1}). ^1H NMR (D_2O, δ): 1.25 (m, 32H, CH_2); 1.51 (m, 4H, $CH_2\beta CO$); 2.22 (t, $J = 7.1$ Hz, 4H, $CH_2\alpha CO$); 3.06 (dd, AB system, $J_{AB} = 15.3$ Hz, 4H, CH_2his); 4.70 (dd, X, $J_{AX} = 9.4$ Hz, $J_{BX} = 4.7$ Hz, 2H, CH); 6.93 (s, 2H, H5im); 7.70 (s, 2H, H2im). ^{13}C NMR (D_2O, δ): 180.64 (CONH); 178.09 (COO^-); 135.67 (C2im); 119.94 (C5im); 57.48 (CH); 38.62 (CH_2his); 32.41 ($CH_2\alpha CO$); 32.14 ($CH_2\beta CO$); 32.00–28.24 (CH_2). MS (positive ESI): $m/z = 689$, $[MH]^+$; 667, $[M - Na + 2H]^+$ (100%); 645, $[M - 2Na + 3H]^+$. Anal. calcd (%) for $C_{34}H_{54}N_6Na_2O_6 \cdot 2H_2O$: C 56.34; H 8.07; N 11.59. Found C 56.53; H 7.65; N 11.61.

Molecular aggregation of the bolaforms in aqueous solution

Surface tension measurements were made with a Prolabo tensiomat 3 (equipped with a thermostated water bath; $T = 30°C$) using a platinum stirrup. Conductivity measurements were made at 30°C with a conductivity–resistivity CDRV62 (Tacussel Electronique) meter with a platinum electrode. Vesicles and fibers were prepared by sonication with a titanium probe (High Intensity Ultrasonic Processor 600 watt Model) of 5×10^{-3} mol l^{-1} solutions, at 110 watts, at 0 °C (ice bath), for 15 min using an 80% duty cycle. Titanium (from the probe) and dust were removed by centrifugation (3000 rpm for 10 min) and filtration through a Millipore 0.45 μ filter.

The samples were observed by light scattering (Coulter N4MD) and transmission electron microscopy using the negative staining method or platinum shadowing. For the negative staining method, a drop of the sample was placed on a carbon-coated grid, the excess was removed, a drop of 2% uranyl acetate was added then dried. The grid was immediately examined under a EM-301 Philips transmission electron microscope. For platinum shadowing, a drop of the sample was placed on a carbon-coated grid and was shaded

with platinum. The samples were observed under a CM-12 Philips transmission electron microscope.

Bolaamphiphiles **1** in ethanol (0.1 mol l^{-1}) form white gels. Micrographs of gel coated with a conductive gold layer were obtained under the scanning electron microscope (Cambridge Instrument 250 MK3). Micrographs of gel were also observed on a transmission electron microscope (3 MV at CEMES in Toulouse, France). With this second observation method, the sample was placed on a carbon-coated grid and observed immediately.

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