From unsymmetrical bolaamphiphiles to supermolecules

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This paper describes the synthesis and self-assembly properties of bolaamphiphiles with two different head groups: a urocanic moiety and another group such as an active function (hydroxyl, carboxylic acid or methyl ester) or a sugar (arbutin). The morphologies of the aggregates formed by these amphiphiles in aqueous solutions were studied by light scattering and transmission electron microscopy and found to be strongly dependent on three parameters (pH, structure of the amphiphile and position of the connecting links on the imidazole moiety). These investigations reveal that the form of the aggregates is mainly affected by the presence and the number of hydrogen-bonding functions in the amphiphilic structure (from vesicles to flexible fibers then rigid fibers).

Organized molecular assemblies are very common in biological systems,[1] for example in proteins, DNA and cellular membranes. The aim of reaching the unique specificity of the functions performed by these biological structures has led chemists to develop ways to design and prepare molecules to obtain nanoscale molecular assemblies through molecular recognition-directed self-assembly processes based on intermolecular noncovalent interactions.[2,3] Many approaches have been successful, particularly those concerning amphiphiles. These molecules are known to self-organize in water to form molecular aggregates[4] such as micelles, multi-layered sheets, vesicles, rings, and fibers (rods,[5] ribbons,[6] helices,[7,5a] and tubules[8]). On the one hand, their amphiphilicity holds the molecules together and, on the other hand, repulsive hydration and steric effects prevent the formation of three-dimensional crystals. Other parameters are also important: the structural features of the amphiphiles such as the length of the hydrophobic chain,[9] even–odd carbon number,[10] nature of the counter-ion,[11] chiral centers, secondary amide functions,[12] spatial geometry of the molecule and also media conditions such as pH,[13] ionic strength and temperature.[14]

Molecular aggregates have been studied with the dual objectives, of preparing "designer assemblies"[15] and of developing potential biological applications. So, it is interesting to understand the relationship that connects amphiphiles and aggregate structures with the aim of predicting the shape of the aggregates and even their applications.

After preliminary results obtained in our laboratory with micellar media from monopolar amphiphile derivatives of urocanic acid,[16] we wanted to obtain a greater diversity of assemblies so we chose to study symmetrical urocanic bolaamphiphiles,[17] which are molecules with two identical head groups connected by one chain. The morphologies of the aggregates formed by these bolaamphiphiles (micelles, vesicles or fibers) have been found to be strongly dependent on three parameters: pH, the structure of the head group and the position of the connecting link.[18] With unsymmetrical bolaamphiphiles, the possibilities of modulating the intermolecular interactions would increase.

The present work concerns unsymmetrical bolaamphiphiles with two head groups of different sizes. One polar head is urocanic acid (UCA), a chemically intriguing molecule of biophysiological importance[19–21] (Scheme 1), the other polar head is a group bearing a hydroxyl, carboxylic acid or methyl ester function or a sugar (arbutin). The latter polar head was chosen owing to its size, shape and ability to participate in hydrogen-bonded networks. A glucosidic head group was selected because sugars have many interesting properties; they are naturally occurring chiral molecules, they have multiple and directional hydroxyl groups that are able to participate in effective and strong hydrogen bonding to generate self-organized structures in water; they are biocompatible.

In the present paper, we shall first describe the synthesis of unsymmetrical bolaamphiphiles (Scheme 2) and then the aggregates formed by these compounds in water at various pH values.

Experimental

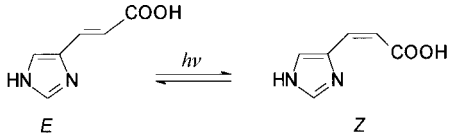
Materials and methods

Commercial products (Aldrich Chemical Co.) were used without further purification. Solvents were freshly distilled and dried before use according to standard procedures.

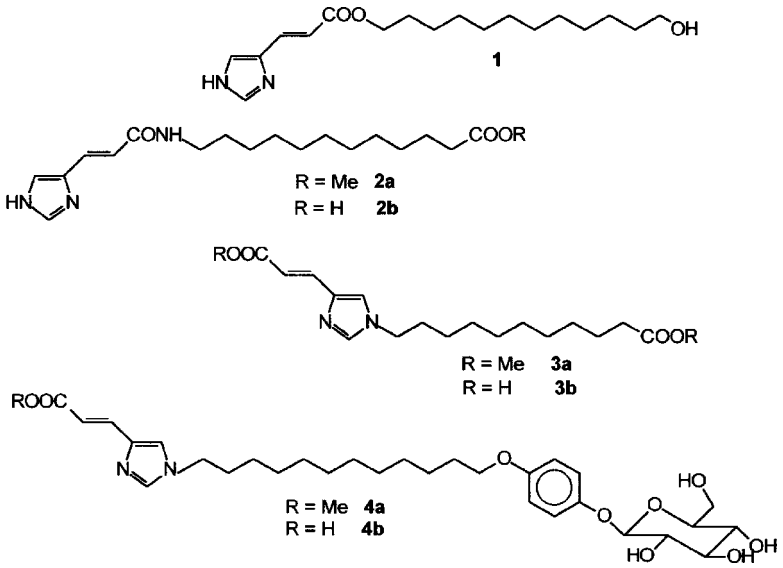
^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 200 and 250 spectrometers at nominal frequencies of 200 and 250 MHz for ^{1}H and 50 and 63 MHz for ^{13}C, *J* are given in hertz. Mass spectra were recorded on a Nermag R10-10DCI instrument for direct chemical ionization (DCI, with NH_3) and a ZAB–HS instrument (WG–Analytical, Manchester, UK) using the FAB (fast atom bombardment) mode with a glycerol matrix or using the ESI (electro spray ionization) mode. Melting points were determined on an Electrothermal apparatus (capillary tubes). Microanalyses were carried out on a Carlo Erba 1106 at the ENSCT (Toulouse, France).

Syntheses

Compound 1. To a stirred suspension of *E*-urocanic acid (2 g, 14.8 mmol) in 80 mL of anhydrous toluene was added a mixture of monohydrated *p*-toluene sulfonic acid (3.3 g, 17.3 mmol) and dodecan-1,12-diol (8.8 g, 43.4 mmol). The mixture was refluxed for 7 h, then the precipitate was recovered by



Scheme 1 *E* and *Z* isomers of urocanic acid.



Scheme 2 Unsymmetrical bolaamphiphiles studied.

warmed filtration. Washing with warm diethyl oxide eliminated the excess of dodecan-1,12-diol. The precipitate was recovered and washed three times with warm ethyl acetate–methanol 5 : 1 v/v. The filtrate was concentrated to obtain the *p*-toluene sulfonate salt of **1** (3.65 g, 50%) as a solid, mp 90 °C. ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 1.24 [m, 16H, $(CH_2)_8$], 1.62 (t, 4H, $J = 6.4$, CH_2CH_2OOC and CH_2CH_2OH), 2.28 (s, 3H, CH_3), 3.36 (t, 2H, $J = 6.4$, CH_2OH), 4.14 (t, 2H, $J = 6.6$, CH_2OOC), 6.68 (AB system, 1H, $J_{AB} = 16.2$ Hz, CH=C*H*COOR), 7.11 (AB system, 2H, $J_{AB} = 6.5$, 2 H$_{ortho}$), 7.50 (AB system, 2H, $J_{AB} = 6.5$, 2H$_{meta}$), 7.55 (AB system, 1H, $J_{AB} = 16.2$ Hz, ImC*H*=), 8.08 (s, 1H, H$_5$Im), 9.19 (s, 1H, H$_2$Im); ^{13}C NMR (50 MHz, DMSO-d$_6$): δ 20.7, 25.3–32.4, 60.6, 64.3, 120.1, 121.6, 125.4, 128.0, 128.5, 129.2, 137.7, 145.3, 165.3; ESI MS (>0): $m/z = 323$ [M − PTS]$^+$.

The pH of the suspension of the *p*-toluene sulfonate salt of **1** (2 g, 4.05 mmol) in 100 mL of water was adjusted to ~8 with 0.1 mol L^{-1} NaOH. The reaction mixture was stirred at room temperature for 3 h. The precipitate was filtered off and thoroughly washed with water. The resulting product was recrystallized from acetone and a few-drops of pentane to give 731 mg of **1** (56%) as a white solid, mp 95 °C. ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 1.25 (m, 16H, 8 CH_2), 1.61 (m, 4H, CH_2CH_2OH and CH_2CH_2OOC), 3.37 (t, $J = 6.3$, 2H, CH_2OH), 4.10 (t, $J = 6.6$, 2H, CH_2OOC), 6.34 (AB system, $J_{AB} = 15.7$ Hz, 1H, CH=CH_ACOOR), 7.54 (AB system, $J_{AB} = 15.7$ Hz, 1H, CH_B=CHCOOR), 7.55 (s, 1H, H$_5$im), 7.83 (s, 1H, H$_2$im); ^{13}C NMR (50 MHz, DMSO-d$_6$): δ 25.3–32.4 (CH_2), 60.7 (CH_2OH), 63.6 (CH_2OOC), 114.0 (=CHCOOR), 123.5, 127.9, 133.9, 135.4, 166.5; Anal. calc. for $C_{18}H_{30}N_2O_3 \cdot 12H_2O$: C, 65.17; H, 9.35; N, 8.44%. Found: C, 65.20; H, 8.95; N, 8.14%; DCI MS (NH_3): $m/z = 323$ [MH]$^+$ (100%).

Compound 2a. Methyl 12-aminododecan-1-oate hydrochloride was synthesized according to an esterification method described elsewhere.[22] It was purified by recrystallization in *n*-hexane with a few drops of diethyl oxide and methanol (yield 90%), mp 156 °C. ^{1}H NMR (80 MHz, $CDCl_3$): δ 1.26 (m, 18H, 9 CH_2), 2.29 (t, $J = 7.2$ Hz, 2H, CH_2COOCH_3), 2.95 (m, 2H, $CH_2NH_3^+$), 3.65 (s, 3H, CH_3OOC), 8.25 (m, 3H, NH_3^+); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.9–29.4, 34.1, 40.0, 51.5, 174.3; Anal. calc. for $C_{13}H_{28}NO_2Cl$: C, 58.68; H, 10.53; N, 5.26%. Found: C, 58.33; H, 10.59; N, 5.26%; ESI MS: $m/z = 230$ [M − Cl]$^+$.

To methyl 12-aminododecan-1-oate hydrochloride (962 mg, 3.62 mmol) in 20 mL of anhydrous chloroform was added dropwise freshly distilled triethylamine (0.52 mL) at room temperature. To this solution cooled to 0 °C, *E*-urocanic acid (0.5 g, 3.62 mmol) and dicyclohexylcarbodiimide (747 mg, 3.62 mmol) in 40 mL of anhydrous dimethylacetamide was added. The mixture was stirred for an additional 3 days at room temperature. After removal of the precipitate by filtration, the filtrate was concentrated and the residue purified by flash chromatography on silica gel (eluent: CH_2Cl_2–EtOH 95 : 5 v/v) to yield **2a** (190 mg, 15%) as a white solid, mp 118 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (m, 14H, 7 CH_2), 1.55 (tt, $J_1 = J_2 = 7.6$, 4H, $CH_2CH_2NHCOR + CH_2CH_2COOCH_3$), 2.29 (t, $J_1 = 7.6$, 2H, CH_2COOCH_3), 3.33 (td, $J_1 = 7.6$, $J_2 = 6.7$, 2H, CH_2NHCOR), 3.65 (s, 3H, CH_3OOC), 6.06 (t, $J_2 = 6.7$, 1H, NHCOR), 6.52 (AB system, $J_{AB} = 15.2$ Hz, 1H, =CH_ACONH), 7.15 (s, 1H, H$_5$im), 7.50 (AB system, $J_{AB} = 15.2$ Hz, 1H, CH_B=CH$_A$CONH), 7.62 (s, 1H, H$_2$im); Anal. calc. for $C_{19}H_{31}N_3O_3 \cdot 14H_2O$: C, 64.49; H, 8.91; N, 11.88%. Found: C, 64.23; H, 8.55; N, 11.35; DCI MS (NH_3): $m/z = 350$ [MH]$^+$ (100%).

Compound 2b. Bolaamphiphile **2a** was hydrolyzed according the previously described method[23] to give **2b** in 70% yield, mp 180 °C. ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 1.25 (m, 14H, 7 CH_2), 1.46 (m, 4H, $CH_2CH_2NHCOR + CH_2CH_2COOH$), 2.18 (t, $J = 7.2$, 2H, CH_2COOH), 3.13 (td, $J_1 = J_2 = 5.9$, 2H, CH_2NHCOR), 6.48 (AB system, $J_{AB} = 15.2$, 1H, =CH_ACONH), 7.29 (AB system, $J_{AB} = 15.7$, 1H, CH_B=CH$_A$CONH), 7.35 (s, 1H, H$_5$im), 7.70 (s, 1H, H$_2$im), 7.95 (t, $J_2 = 5.9$ Hz, 1H, NHCOR), 12.13 (s, 1H, COOH); ^{13}C NMR (50 MHz, DMSO-d$_6$): δ 24.4–33.6, 40.3, 42.2, 118.5, 136.8, 165.4, 174.4; DCI MS (NH_3): $m/z = 336$ [MH]$^+$ (100%).

Compounds 3a, 3b. The synthesis of compounds **3a** and **3b** has already been published.[24]

Compound 4a. N-Alkylation of *E*-methyl urocanate was carried out according to a previously published method[25] from a mixture of *E*-methyl urocanate (1 g, 6.57 mmol), 1,12-dibromododecane (6.46 g, 19.7 mmol), K_2CO_3 (9.08 g, 65.7 mmol) and 18-crown-6 (173 mg, 0.657 mmol) in 50 ml of anhydrous THF. The N_τ-12-bromododecyl urocanic methyl ester was purified by flash chromatography (silica gel, dichloromethane–ethanol 99 : 1) to yield a solid (1.33 g, 51%), mp 82 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (m, 18H, 9 CH_2), 1.83 (m, 4H, CH_2CH_2N and CH_2CH_2Br), 3.39 (t, $J = 6.8$, 2H, CH_2Br), 3.75 (s, 3H, CH_3OOC), 3.90 (t, $J = 7.1$, 2H, CH_2N), 6.53 (AB system, 1H, $J_{AB} = 15.6$, CH=CH_A-$COOCH_3$), 7.07 (s, 1H, H$_5$im), 7.45 (s, 1H, H$_2$im), 7.54 (AB system, 1H, $J_{AB} = 15.6$ Hz, CH_B=CHCOOCH$_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.5–32.8, 34.1, 47.4, 51.5, 115.4, 121.5,

136.4, 138.3, 138.5, 168.2; Anal. calcd for $C_{19}H_{31}BrN_2O_2$: C, 57.14; H, 7.82; N, 7.01%. Found: C, 57.27; H, 7.82; N, 6.71%; DCI MS (NH_3): $m/z = 399$ $[M + H]^+$ (100%).

The etherification was carried out according to a method already published[26] from a mixture of arbutin (0.64 g, 2.37 mmol), N_τ-12-bromododecyl urocanic methyl ester (0.87 g, 2.37 mmol), K_2CO_3 (8.2 g, 59.2 mmol) and 18-crown-6 (187 mg, 0.71 mmol) in 50 ml of anhydrous THF. **4a** was purified by chromatography (silica gel, dichloromethane–ethanol 90 : 10) to give a white solid (210 mg, 15%), mp: 96 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (m, 18H, 9 CH_2), 1.67 (m, 4H, CH_2CH_2N and $CH_2CH_2OC_6H_4$), 3.38–3.74 (m, 12H, CH_3OOC, $CH_2OC_6H_4$, CH_2N, H2, H3, H4, 2 H6), 4.72 (m, 1H, H1), 6.51 (AB system, 1H, $J_{AB} = 15.6$, $CH{=}CH_ACOOCH_3$), 6.68 (AB system, $J_{AB} = 8.8$, 2H, H2′, H6′), 6.89 (AB system, $J_{AB} = 8.8$, 2H, H3′, H5′), 7.02 (s, 1H, H_5im), 7.45 (s, 1H, H_2im), 7.51 (AB system, 1H, $J_{AB} = 15.6$ Hz, $CH_B{=}CHCOOCH_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.6–30.9, 47.3, 51.5, 61.9, 68.3, 69.4, 73.3, 75.8, 102.0, 115.2, 115.5, 118.4, 121.5, 136.3, 138.0, 138.7, 151.1, 154.0, 168.2; Anal. calc. for $C_{31}H_{46}N_2O_9 \cdot 12H_2O$: C, 62.03; H, 7.84; N, 4.67%. Found: C, 62.19; H, 7.86; N, 4.12%; FAB MS: $m/z = 591$ $[M + H]^+$.

Compound 4b. Compound **4a** was hydrolyzed according to the previously described method[23] and gave **4b** in 80% yield, mp 80 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.24 (m, 18H, 9 CH_2), 1.67 (m, 4H, CH_2CH_2N and $CH_2CH_2OC_6H_4$), 3.19–3.37 (m, 9H, H2, H3, H4, 2 H6, 4 OH), 3.80 (m, 4H, CH_2N and $CH_2OC_6H_4$), 4.70 (d, $J = 7.2$, 1H, H1), 6.27 (AB system, 1H, $J_{AB} = 15.5$, $CH{=}CH_ACOOH$), 6.83 (AB system, $J_{AB} = 9.1$, 2H, H2′, 6′), 6.96 (AB system, $J_{AB} = 9.1$, 2H, H3′, 5′), 7.41 (AB system, 1H, $J_{AB} = 15.5$ Hz, $CH_B{=}CHCOOH$), 7.59 (s, 1H, H_5im), 7.73 (s, 1H, H_2im), 12.07 (s, 1H, COOH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 25.4–30.2, 46.2, 60.7, 67.7, 69.7, 73.2, 76.5, 76.9, 101.4, 113.1, 114.9, 117.5, 122.8, 138.7, 138.8, 139.1, 151.3, 153.6 (C1′), 167.9 (COOH); Anal. calcd for $C_{30}H_{44}N_2O_9 \cdot H_2O$: C, 60.58; H, 7.79; N, 4.71%; Found: C, 60.52; H, 7.45; N, 4.32%; FAB MS: $m/z = 575$ $[M - H]^-$.

Molecular aggregation of the balaamphiphiles in aqueous solution

As bolaamphiphiles are only slightly soluble, aqueous suspensions containing 5×10^{-3} mol L^{-1} were prepared. The supramolecular aggregates were obtained by sonication of these suspensions (sample concentrations were $\leqslant 5 \times 10^{-3}$ mol L^{-1}) with a titanium probe (High Intensity Ultrasonic Processor 600 watt model), at 110 watt and 0 °C, for 15 min using an 80% duty cycle. The aqueous suspensions were filtered through a 0.8 μm Millipore membrane only for the dynamic light scattering method that used a Malvern Instruments Zetasize 3000 apparatus. The unfiltered aqueous suspensions were observed by transmission electronic microscopy (TEM) using a JEOL JEM 200 CX electron microscope operating at 200 kV. Aliquots were applied to carbon-coated Formvar grids, negatively stained with 2% uranyl acetate to reveal the aggregates, including particles larger than 0.8 μm.

Results and discussion

Synthesis

A hydrocarbon chain can be fixed on the urocanic moiety either by *N*-alkylation or by *O*-alkylation. With *O*-alkylation, the spacer can be fixed *via* ester (compound **1**) or amide (compounds **2**) functions. Compound **1** was synthesized according to the esterification conditions described elsewhere[16a] and compounds **2** were obtained from the reaction of urocanic acid with methyl 12-aminododecanoate in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC). Synthesis of compounds **3** was published elsewhere.[24] Compounds **3** and **4** were formed by *N*-alkylation of urocanic methyl ester using solid-liquid phase transfer catalysis.[25] Two *N*-alkylation sites (N_τ and N_π) exist because of the presence of two tautomeric forms of the imidazole ring but results described in a preceding work indicate that the *N*-alkylation occurs essentially on the N_τ nitrogen. So, in compounds **3** and **4**, the alkyl chain was attached on the N_τ nitrogen.

Amphiphile aggregation behavior

The organization of the unsymmetrical bolaamphiphiles in aqueous solution was studied at different pH values. The formation of aggregates was checked by dynamic light scattering. Before sonication, no objects were detected, showing that these bolaamphiphiles did not organize spontaneously. After sonication, aggregates were observed. This behavior is usual for synthetic amphiphiles. The sizes and distributions (scattering-intensity weighted fractions) of aggregates are indicated in Table 1, but the dynamic light scattering method gives only an average of the dimensions of the particles probed by the laser beam. These values only give an indication of size for spherical aggregates. So, the morphology of the aggregates was determined by TEM using 2% uranyl acetate as contrast agent (Table 2). The experiments were reproduced

Table 1 Sizes (nm) and distributions (%, scattering-intensity weighted fractions) of aggregates of bolaamphiphiles **1**, **2a**, **2b**, **3a**, **3b**, **4a** and **4b** at various pH values,[a] observed by dynamic light scattering

	pH 2		pH 6.3		pH 9.9	
	Diameter	%	Diameter	%	Diameter	%
1	84	28.3	186	100	166	100
	347	62.2				
	686	9.5				
2a	147	93.4	304	100	23	44
	699	6.6			280	53
2b	495	100	547	100	223	100
3a	495	100	547	100	ND[b]	ND
3b	249	100	136	38	282	100
			369	62		
4a	70	96.8				
			ND	ND	ND	ND
	437.5	3.2				
4b	15	23	240	100	90	52
	121	77			216	48

[a] Acidic pH = 1 mM HCl; neutral pH-distilled water; basic pH = 0.1 mM NaOH. Samples were filtered through a 0.8 μm Millipore membrane. [b] ND: not determined.

Table 2 Size (nm) and morphology[a] of aggregates formed by the bolaamphiphiles **1**, **2a**, **2b**, **3a**, **3b**, **4a** and **4b** at various pH values,[b] observed by TEM[c]

	pH 2	pH 6.3	pH 9.9
1	$\mathbf{F}_1$: Tubules (w ~ 13.3)	$\mathbf{F}_2$: Rods (l ~ 900, w ~ 65) Vesicles: sm (d ~ 20–100); lg (d ~ 500)	Vesicles (d ~ 30–50 and ~200–800)
2a	$\mathbf{F}_3$: Slightly twisted ribbons (w ~ 300)	Multi-layered sheets (l ~ 300–1000, w ~ 300–1000) Vesicles (d ~ 100–325)	Multi-layered sheets (l ~ 250–1400, w ~ 1000–1250) Vesicles (d ~ 150)
2b	Multi-layered sheets (l ~ 2450–5000, w ~ 300–800)	$\mathbf{F}_{4A}$: Multi-layered sheets (l ~ 2400–4000, w ~ 1500) Cotton-like aggregates (l ~ 500–1500) $\mathbf{F}_{4B}$: Twisted fibers (d ~ 10) Vesicles (d ~ 100–230)	Multi-layered sheets (l ~ 3000, w ~ 1500) Vesicles (d ~ 30–60)
3a	Vesicles: sm (d ~ 120); lg (d ~ 1200)	Pluri-lamellar vesicles (d ~ 150–400; lamellar thickness ~ 3) Lamellar structure (lamellar thickness ~ 8)	ND[d]
3b	Vesicles (d ~ 40–80) Groups of vesicles	Vesicles (d ~ 100–250)	$\mathbf{F}_5$: Vesicles (d ~ 35–200) Few rods (l ~ 1900, w ~ 300)
4a	$\mathbf{F}_{6A}$: Hair-like fibers (d ~ 5) $\mathbf{F}_{6B}$: Tubules (d ~ 80) Few vesicles (d ~ 100–300)	ND	ND
4b	$\mathbf{F}_{8A}$: Flexible tubules (d ~ 50) Geometric structures with right angles	$\mathbf{F}_{8B}$: Fibers rolled up in rings (d ~ 20) Thin fibers Vesicles (d ~ 30–60)	$\mathbf{F}_{7A}$ and $\mathbf{F}_{7B}$: Geometric structures with right angles and spherical objects showing 2 opposite excrescencies

[a] d-diameter, l-length, w-width, lg-large. [b] Acidic pH-1 mM HCl; neutral pH-distilled water; basic pH-0.1 mM NaOH. [c] **F**: figures. [d] ND: not determined.

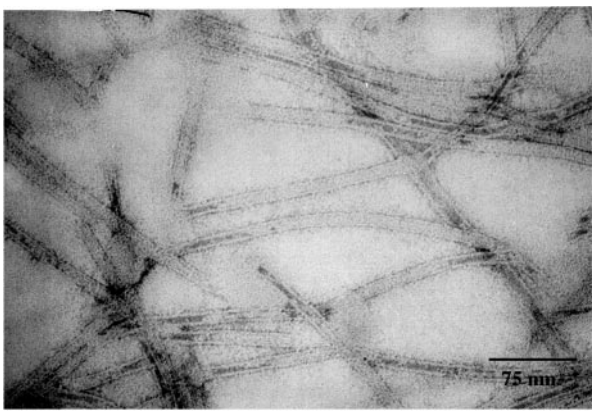


Fig. 1 Transmission electron micrograph (negative stain, 2% uranyl acetate) of tubular aggregates formed from bolaamphiphiles **1** in aqueous medium at pH 2.

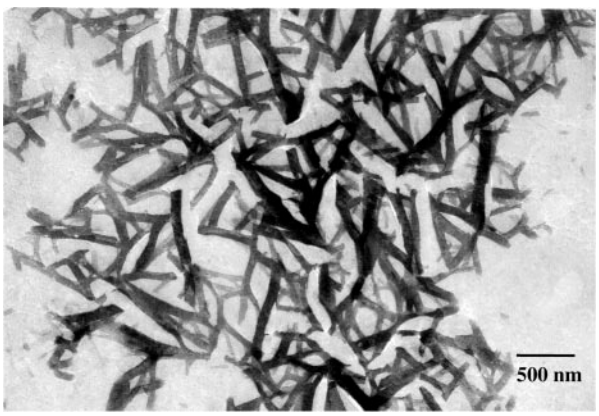


Fig. 2 Transmission electron micrograph (negative stain, 2% uranyl acetate) of branched rods formed from bolaamphiphiles **1** in aqueous medium at pH 6.3.

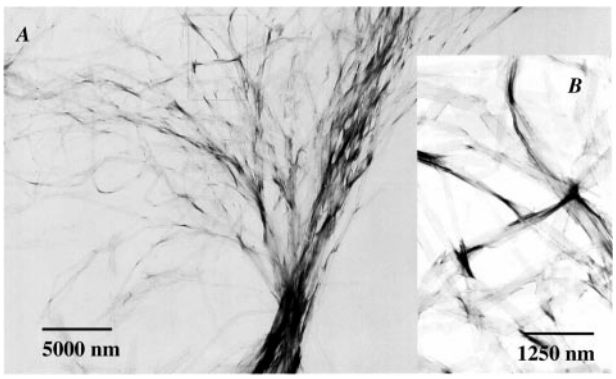


Fig. 3 Transmission electron micrograph (negative stain, 2% uranyl acetate) of lightly twisted ribbons formed from bolaamphiphiles **2a** in aqueous medium at pH 2. Micrograph B is a part of the sample (indicated in A by a dotted triangle) taken with a higher magnification.

twice and the reproducibility was checked and found to be excellent.

In acidic media, compound **1** gave long tubules of 13 nm cross section (Fig. 1). The white color appearing in the middle of the tubule and also the dimension of the cross section indicate that the aggregates seem to be vesicular water-filled tubules. The dissymmetric structure of the bolaamphiphile suggests that the hydroxyl head group, which is the smaller head group, points mainly inwards in vesicular tubules, in which hydrogen-bonded networks could exist between hydroxyl and inner water molecules. In neutral media, two aggregation forms were discerned: small and large vesicles, and also rods, which look like branches of a tree (Fig. 2). In basic media, many small and a few larger vesicles were formed.

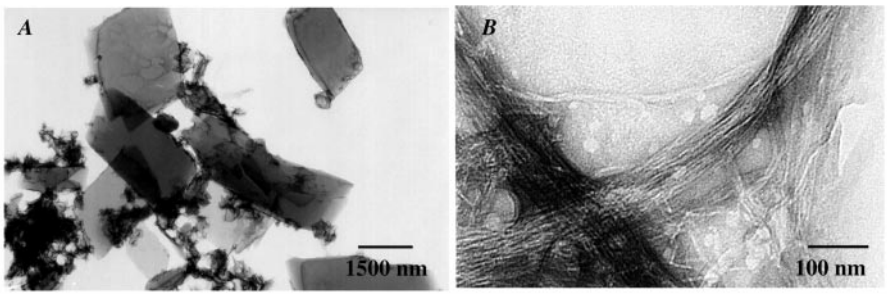


Fig. 4 Transmission electron micrograph (negative stain, 2% uranyl acetate) of aggregates formed from bolaamphiphiles **2b** in aqueous medium at pH 6.3. (A) multilayer sheets and aggregates looking like cotton; (B) twisted fibers made up of double strands.

For bolaamphiphile **2a**, the aggregates that formed at basic and neutral pH were similar: multi-layered sheets (larger in basic medium) and vesicles. In acidic media, TEM pictures revealed the presence of twisted ribbon-like structures (Fig. 3).

Multi-layered sheets were formed from **2b** whatever the pH. Nothing else was detected in acidic media. In basic media, many small vesicles were also present. In neutral media, various aggregates were observed: multi-layered sheets, aggregates looking like cotton (Fig. 4A), vesicles and twisted fibers made up of double-strands (Fig. 4B).

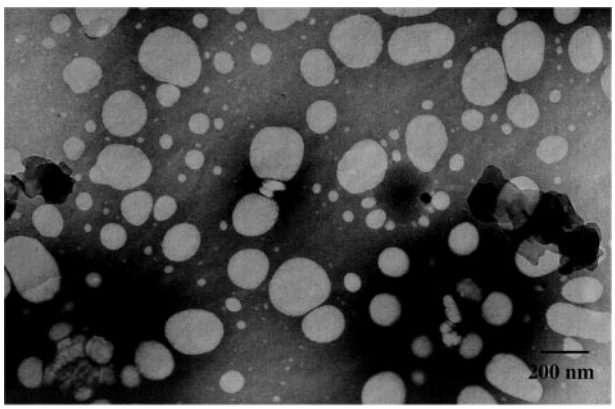


Fig. 5 Transmission electron micrograph (negative stain, 2% uranyl acetate) of vesicles formed from bolaamphiphiles **3b** in aqueous medium at pH 9.9.

At pH 6.3, the difference observed in the aggregation modes of compounds **2a** and **2b** could be related to the protonation states of the carboxylic function. In compound **2b**, the coexistence of carboxylic/carboxylate forms would favor the formation of twisted fibers.

An elegant organized structure reminiscent of a biological cell was observed by TEM with bolaamphiphile **3a** (at neutral and basic pH).[24] In this structure, pluri-lamellar vesicles occurred in which the thickness of the lamellae (~3 nm) corresponds to the length of the amphiphilic molecule, and also thicker (~8 nm) lamellar structures. In acidic media, only vesicles were observed.

For bolaamphiphile **3b**, vesicles were formed at the three pH values (Fig. 5), but they were smaller in acidic medium. A very few rods were also present in basic medium.

In acidic media compound **4a** gave rare vesicles and many varied fibers: long and thin hair-like fibers (Fig. 6A) and some fibers with a larger cross-section, which seem to pair up to form tubules (Fig. 6B).

Surprising geometric structures were observed for compound **4b** in basic medium (Fig. 7) and also in acidic medium but in smaller quantities. In these structures, vesicles showing two opposite excrescencies are present. In acidic medium, flexible tubules like neuron networks (Fig. 8A) are also present. In neutral media, different aggregates were discerned: small vesi-

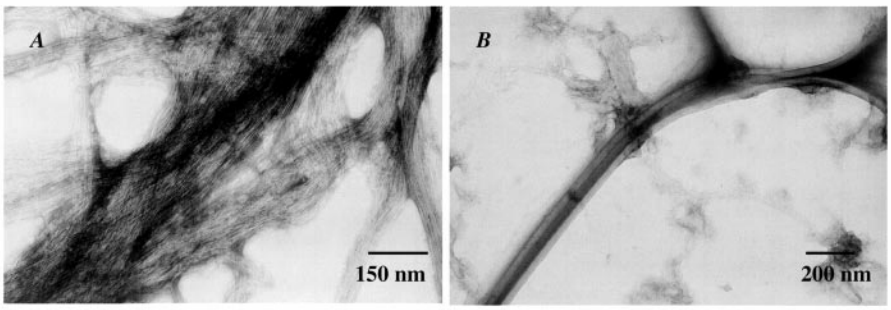


Fig. 6 Transmission electron micrograph (negative stain, 2% uranyl acetate) of fibers formed from bolaamphiphiles **4a** in aqueous medium at pH 2: (A) long thin hair-like fibers; (B) larger fibers that seem to pair up to form tubules.

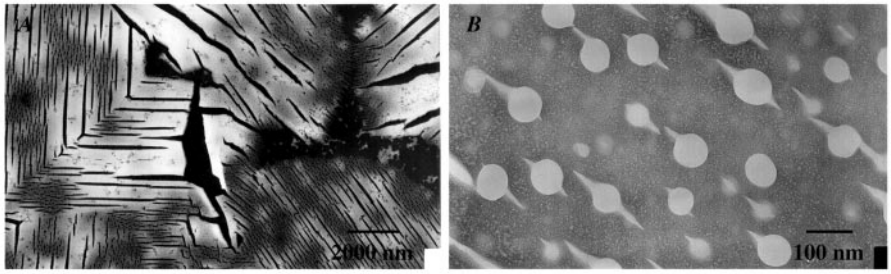


Fig. 7 Transmission electron micrograph (negative stain, 2% uranyl acetate) of geometric structures formed from bolaamphiphiles **4b** in aqueous medium at pH 9.9. Micrograph B is an enlargement of part of the bottom right hand side of A.

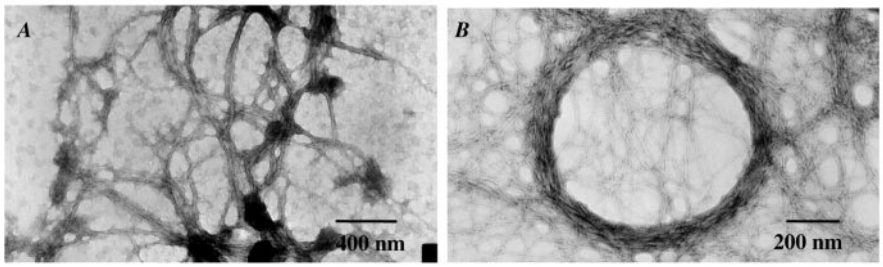


Fig. 8 Transmission electron micrograph (negative stain, 2% uranyl acetate) of aggregates formed from bolaamphiphiles **4b**: (A) flexible tubules in neuron-like networks in aqueous medium at pH 2; (B) fibers rolled up into rings in aqueous medium at pH 6.3.

cles (d ~ 30–60 nm), fibers rolled into rings (Fig. 8B) and thin twisted fibers. So, if the presence of a glucosidic head group seems to favor the formation of fibers, it does not induce aggregation to give regular helices. This could be explained by the predominance of aromatic ring stacking over the chiral effect of the carbohydrate head. This hypothesis is supported by the work of Nolte *et al.* concerning amphiphilic gluconamides bearing a pyridinium ring on C_6 of the sugar.[13b]

Conclusion

The results presented here support the conclusions of our previous work concerning symmetrical amphiphiles: the importance of pH, the structure of the amphiphile, the positions of the connecting links and the ability to form hydrogen-bonded networks. Concerning this last point, the shape of the aggregates given by *O*-alkylated compounds at low pH are related to the existence in the amphiphile of several groups (amide, carboxyl, hydroxyl and protonated imidazole) allowing hydrogen bonding between these molecules. Indeed, for amphiphiles with only an imidazolium group, polar head hydration predominates, leading to vesicle formation. When another group responsible for hydrogen bonds also exists in the molecules, they become organized into flexible fibers (ribbons or tubules). The addition of a third group (imidazolium + amide + hydroxyl or imidazolium + amide + carboxyl) induces the formation of rigid fibers (rods).

The importance of hydrogen bonding is also demonstrated by the fact that the bolaamphiphile with an arbutin head group gives fibers. This emphasizes the importance of the hydrogen bonding. Indeed, all other *N*-alkylated urocanic compounds give vesicles.

These results will allow the design of urocanic amphiphiles in view of further applications of their aggregates.

References

1 (*a*) J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1304; (*b*) D. S. Lawrence, T. Jiang and M. Levett, *Chem. Rev.*, 1995, **95**, 2229; (*c*) D. B. Amabilino and J. F. Stoddart, *Chem.Rev.*, 1995, **95**, 2725.

2 J.-M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, 1995.

3 (*a*) G. M. Whitesides, E. E. Simanek, J. P. Mathias, C. T. Seto, D. N. Chin, M. Mammen and D. M. Gordon, *Acc. Chem. Res.*, 1995, **28**, 37; (*b*) J. S. Lindsey, *New J. Chem.*, 1991, **15**, 153; (*c*) J. Rebek, Jr., *Chem. Soc. Rev.*, 1996, **25**, 255; (*d*) M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee and K. Khazanovich, *Nature* (*London*), 1993, **366**, 324; (*e*) F. Garcia-Tellado, S. J. Geib, S. Goswani and A. D. Hamilton, *J. Am. Chem. Soc.*, 1991, **113**, 9265.

4 D. D. Lasic, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 1685.

5 (*a*) B. Pfannmueller and W. Welte, *Chem. Phys. Lipids*, 1985, **37**, 227; (*b*) H. Yanagawa, Y. Ogawa, H. Furuta and K. Tsuno, *J. Am. Chem. Soc.*, 1989, **111**, 4567; (*c*) Y. Itojima, Y. Ogawa, K. Tsuno, N. Handa and H. Yanagawa, *Biochemistry*, 1992, **31**, 4757; (*d*) J. H. Fuhrhop, D. Spiroski and C. Boettcher, *J. Am. Chem. Soc.*, 1993, **115**, 1600.

6 (*a*) T. Shimizu, M. Mori, H. Minamikawa and M. Hato, *J. Chem. Soc., Chem. Commun.*, 1990, 183; (*b*) T. Imae, Y. Takahashi and H. Muramatsu, *J. Am. Chem. Soc.*, 1992, **114**, 3414; (*c*) C. Endisch, C. Boettcher and J. H. Fuhrhop, *J. Am. Chem. Soc.*, 1995, **117**, 8273.

7 (*a*) G. K. Newkome, G. R. Baker, S. Arai, M. J. Saunders, P. S. Russo, K. J. Theriot, C. N. Moorefield, L. E. Rogers, J. E. Miller, T. R. Lieux, M. R. Myrray, B. Phillips and L. Pascal, *J. Am. Chem. Soc.*, 1990, **112**, 8458; (*b*) J. H. Fuhrhop, P. Schnieder, J. Rosenberg and E. Boekema, *J. Am. Chem. Soc.*, 1987, **109**, 3387.

8 (*a*) A. Harada, J. Li and M. Kamachi, *Nature* (*London*), 1993, **364**, 516; (*b*) J. D. Hartgering, J. R. Granja, R. A. Milligan and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1996, **118**, 43; (*c*) M. Kogiso, S. Ohnishi, K. Yase, M. Masuda and T. Shimizu, *Langmuir*, 1998, **14**, 4978.

9 (*a*) S. Franceschi, N. de Viguerie, E. Perez, M. Rivière and A. Lattes, *J. Dispersion Sci. Technol.*, 1999, **20**, 1523; (*b*) F. Brisset, R. Garelli-Calvet, J. Azéma, C. Chebli, A. Moisand and I. Rico-Lattes, *New J. Chem.*, 1996, **20**, 595; (*c*) R. Nagarajan, *Chem. Eng. Commun.*, 1987, **55**, 251; (*d*) F. M. Menger and S. Wrenn, *J. Phys. Chem.*, 1974, **98**, 1387.

10 (*a*) T. Shimizu and M. Masuda, *J. Am. Chem. Soc.*, 1997, **119**, 2812; (*b*) M. Masuda and T. Shimizu, *Chem. Commun.*, 1996, 1057; (*c*) T. Shimizu, M. Masuda and M. Shibakami, *Chem. Lett.*, 1997, 267.

11 J. H. Fuhrhop, C. Fritsch, B. Tesche and H. Schmiady, *J. Am. Chem. Soc.*, 1984, **106**, 1998.

12 (*a*) J. H. Fuhrhop and W. Helfrich, *Chem. Rev.*, 1993, **93**, 1565, and references therein; (*b*) J. M. Schnur, *Science*, 1993, **262**, 3414.

13 (*a*) D. A. Jaeger, G. Li, W. Subotkowski and K. T. Carron, *Langmuir*, 1997, **13**, 5563; (*b*) R. J. H. Hafkamp, M. C. Feiters and R. J. M. Nolte, *J. Org. Chem.*, 1999, **64**, 412.

14 (*a*) N. Nakashima, S. Asakuma, J. M. Kim and T. Kunitake, *Chem. Lett.*, 1984, 1709; (*b*) N. Nakashima, S. Asakuma and T. Kunitake, *J. Am. Chem. Soc.*, 1985, **107**, 509.

15 J. H. Fuhrhop and J. Köning, *Membranes and Molecular Assemblies: The Synkinetic Approach*, Royal Society of Chemistry, Cambridge, UK, 1994.

16 (*a*) M. C. Monje, A. Lattes and M. Rivière, *Bull. Soc. Chim. Fr.*, 1990, **127**, 292; (*b*) J. Sirieix, N. Lauth-de Viguerie, M. Rivière and A. Lattes, *New J. Chem.*, 1999, **23**, 103.

17 S. Franceschi, V. Andreu, N. de Viguerie, M. Rivière and A. Lattes, *New J. Chem.*, 1998, **22**, 225.

18 J. Sirieix, N. Lauth-de Viguerie, M. Rivière and A. Lattes, *Langmuir*, 2000, in press.

19 (*a*) A. Zenisek and J. A. Kral, *Biochim. Biophys. Acta*, 1953, **12**, 479; (*b*) W. Schwartz, K. Langer, H. Schell and A. Schonberger, *Photodermatology*, 1986, **3**, 239; (*c*) P. M. Krein and D. Moyal, *Photochem. Photobiol.*, 1994, **60**, 280.

20 A. Zenisek, I. M. Hais and E. Marklova, *Parfums Cosmétiques Arômes*, 1978, **24**, 79; Cosmetic Ingredient Review Expert Panel, *J. Am. Coll. Toxicol.*, 1995, **14**, 387.

21 (*a*) E. C. De Fabo and F. P. Nooman, *J. Exp. Med.*, 1983, **158**, 84; (*b*) M. Norval, N. K. Gibbs and J. Gilmour, *Photochem. Photobiol.*, 1995, **62**, 209.

22 J. Mc Nulty and I. W. J. Still, *Synth. Commun.*, 1992, **22**, 979.

23 E. J. Corey, A. Marfat, G. Goto and F. Brion, *J. Am. Chem. Soc.*, 1980, **102**, 7984.

24 J. Sirieix, N. Lauth-de Viguerie, M. Rivière and A. Lattes, *Heterocycles*, 2000, in press.

25 N. Lauth-de Viguerie, N. Sergueeva, M. Damiot, H. Mawlawi, M. Rivière and A. Lattes, *Heterocycles*, 1994, **37**, 1561.

26 J. W. Leon, M. Kawa and M. J. Frechet, *J. Am. Chem. Soc.*, 1996, **118**, 8847.