

Spontaneous vesicles formation of a catanionic association involving a head and tail functionalized amino-calix[6]arene

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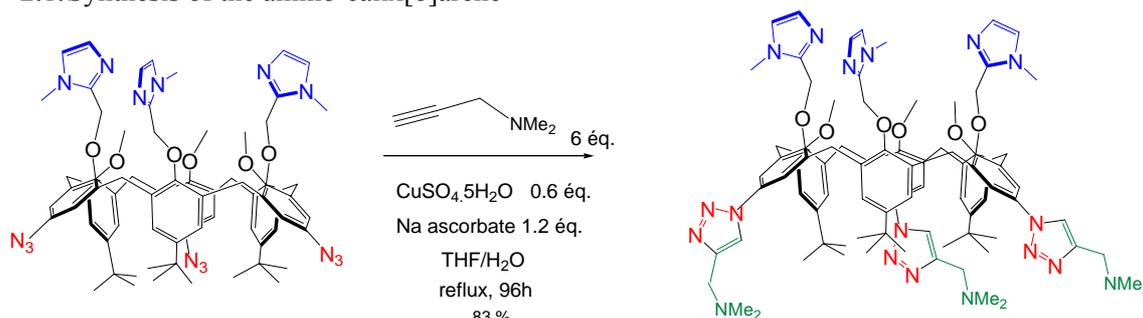
1. Characterization Methods

^1H and ^{13}C NMR spectra of the amino-calix[6]arene were recorded on Bruker ARX 250 spectrometer at nominal frequencies of, respectively, 250 MHz for ^1H and 62.5 MHz for ^{13}C . ^1H and ^{13}C NMR spectra of the sugar-based surfactant and of the catanionic association were recorded on Bruker Avance 300 spectrometer at nominal frequencies of, respectively, 300.18 MHz for ^1H and 75.48 MHz for ^{13}C . Chemical shifts (δ) are denoted in ppm using residual solvent peaks as internal standard.

A Waters Q-ToF Ultima API mass spectrometer with an electrospray ionisation source (ESI) in positive mode was used for HRMS measurement. The capillary, cone and Rf lens potentials were set to 3 kV, 35 V and 40 V respectively. The source and desolvation temperature were set to 100 and 120°C and the collision energy was 10 eV in TOF-MS mode. Analyses were monitored with MassLynx 4.0 software. To measure the exact mass, the spectrometer was calibrated with H_3PO_4 clusters.

2. Synthesis

2.1. Synthesis of the amino-calix[6]arene



The tris-azido calixarene derivative¹ (100mg, 80 μmol) was dissolved in THF (5.3mL). N,N-dimethylpropargylamine (52 μL , 480 μmol), sodium ascorbate (19mg, 96 μmol) and CuSO₄·5H₂O (650 μL) were added to the solution. The reaction mixture was refluxed under argon for 96 h and the concentrated. The crude product was dissolved in CH₂Cl₂ (20mL) and washed with water (20mL) containing 10 drops of 28% aq. ammonia. The organic layer was separated, washed with brine (20mL), dried over Na₂SO₄, filtered and concentrated. The brown solid was triturated with Et₂O (2x20mL). After centrifugation, 100mg (83%) of amino-calix[6]arene were obtained as a tan solid.

¹ Colasson, B., Save, M., Milko, P., Roihovà, J., Schröder, D., Reinaud, O. *Org. Lett.*, **2007**, 9, 4987-4990

ESI-MS (MeOH) m/z : 1501.9 $[M+H]^+$, 752.1 $[M+2H]^{2+}$. RMN 1H (250 MHz, CD_3CN , in the presence of 1 equiv. of $Zn(H_2O)_6(ClO_4)_2$, 300 K) δ ppm: 1.36 (s, 27H, *t*Bu); 2.35 (br s, 18H, NMe_2); 3.56-3.73 (m, 30H, NCH_3 , OCH_3 , CH_2NMe_2 and $ArCH_2$); 4.10 (d, $J = 16$ Hz, 6H, $ArCH_2$); 5.12 (br s, 6H, CH_2 , Im); 6.50 (s, 6H, $H_{Ar\ tria}$); 6.94 (s, 3H, H_{Im}); 7.42 (s, 6H, $H_{Ar\ tBu}$); 7.46 (s, 3H, H_{Im}); 7.58 (br s, 3H, $H_{triazole}$). RMN ^{13}C (62.5 MHz, CD_3CN , in the presence of 1 equiv. of $Zn(H_2O)_6(ClO_4)_2$, 300K) δ ppm: 26.3, 31.2, 31.7, 35.3, 45.2, 53.1, 54.4, 61.2, 64.9, 68.4, 118.3, 119.5, 120.9, 123.4, 125.0, 128.2, 130.2, 132.4, 133.7, 137.2, 144.0, 148.1, 149.2, 155.3, 156.1. IR (ATR) $\nu\ cm^{-1}$: 2953.7, 1601.6, 1363.1, 1285.1, 1229.8, 1045.2, 1006.8. Anal. Calcd $C_{87}H_{108}N_{18}O_6 \cdot 5/3CH_2Cl_2$: C, 64.80; H, 6.83; N, 15.34. Found: C, 65.15; H, 7.09; N, 14.71.

Note: in the absence of $Zn(II)$, the 1H NMR spectrum presents extremely broad and ill-defined resonances, which makes its interpretation impossible. This is due to conformational moves that occur at the same rate as the NMR time scale analysis. Upon the addition of just one equiv. of $Zn(II)$, the whole structure is rigidified and the spectrum displays sharp resonances characteristic of the mono-nuclear complex, the metal ion being bound at the tris-imidazole core.¹ This $Zn(II)$ complex was further characterized by ESI-MS (MeOH) m/z : 603 $[M+3H+Zn+2(ClO_4)+CH_3CN]^{3+}$, 570 $[M+2H+Zn+ClO_4+CH_3CN]^{3+}$, 536 $[M+H+Zn+CH_3CN]^{3+}$.

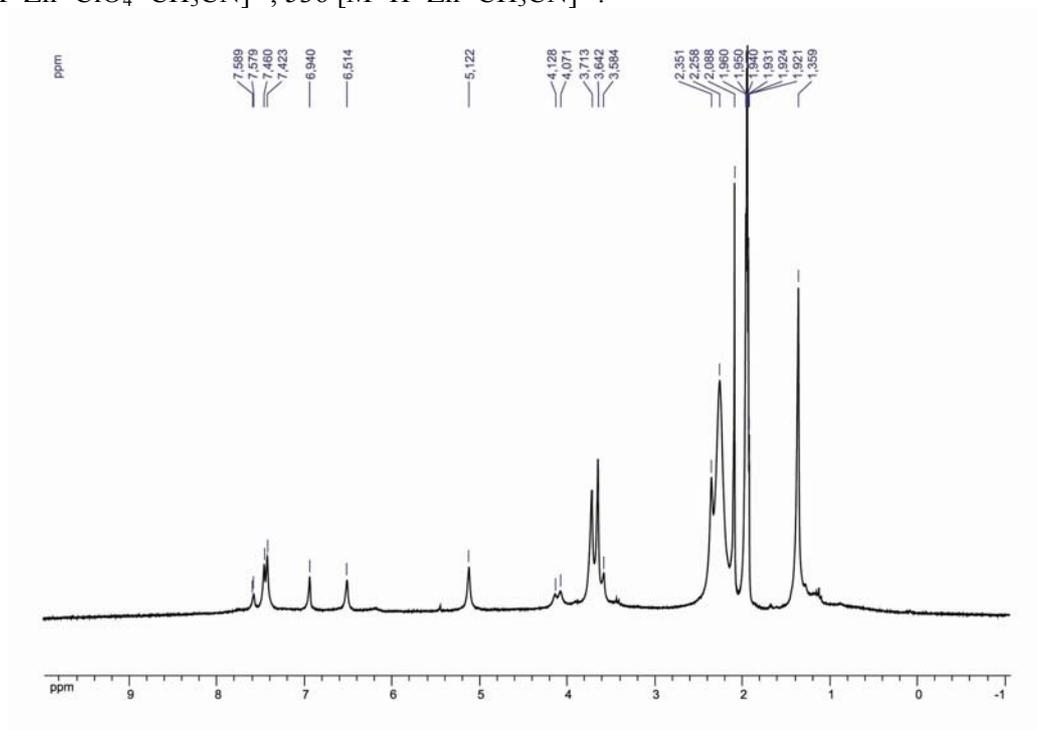


Figure S1: 1H NMR spectrum (250 MHz, CD_3CN , 300 K) of the amino-calix[6]arene in the presence of 1 equiv. of $Zn(H_2O)_6(ClO_4)_2$.

2.2. Synthesis of the sugar-based surfactant

1,7-lactobioamidoheptanoate de sodium

A mixture of sodium hydroxide (224mg, 5.59mmol), 7-aminoheptanoic acid (810mg, 5.59mmol) and lactobionic acid (2,00g, 5.59mmol) in 50mL of methanol was heated to 50°C for 24h. Then the resulting mixture was concentrated to dryness. Flash chromatography of the residue ($CHCl_3/CH_3OH/H_2O$: 2.5/7/0.5, $R_f=0.6$) was performed to give a white powder (64%).

ESI-MS (H₂O): 508 [M+H]⁺, 530 [M+Na]⁺, 484 [M-Na]⁻. RMN ¹H (300 MHz, D₂O, 298 K) δ ppm : 1.25 (m, 4H, CH₂); 1.47 (m, 4H, CH₂CH₂COO⁻ and CH₂CH₂CONH); 2.18 (t, *J*=7.5Hz, 2H, CH₂COO⁻); 3.14 (m, 2H, CH₂CONH); 3.46-3.30 (m, 12H, CH and CH₂ of the sugar moiety); 4.28 (d, *J*=9Hz, 1H, anomeric H). RMN ¹³C (75 MHz, D₂O, 298 K) δ ppm : 25.05, 25.67, 28.06, 28.22, 35.85, 39.06, 61.06, 61.93, 68.59, 70.36, 71.03, 71.40, 72.33, 72.44, 75.35, 80.96, 103.47, 173.88, 182.02. IR (KBr) ν cm⁻¹: 1635(s, HNC=O); 1571 (s, C=O_{asym}); 1406 (s, C=O_{sym}).

1,7-lactobioamidoheptanoic acid

To a suspension of 1,7-lactobioamidoheptanoate de sodium (0.88mmol, 450mg) in 50mL of methanol was added about 2g of proton-exchange resin (Amberlite IR 120-H⁺). The resulting mixture was stirred at room temperature for 3h and filtered to eliminate the resin. The organic layer was concentrated under reduced pressure. The residue was purified by flash chromatography (acetone/H₂O: 9/1, R_f=0.4) and then freeze-dried to give a white powder (90%).

ESI-MS (H₂O): 508 [M+Na]⁺, 484 [M-H]⁻. RMN ¹H (300 MHz, D₂O, 298 K) δ ppm : 1.27 (m, 4H, CH₂); 1.49 (m, 4H, CH₂CH₂COO⁻ and CH₂CH₂CONH); 2.31 (t, *J*=6Hz, 2H, CH₂COO⁻); 3.17 (t, *J*=6Hz, 2H, CH₂CONH); 3.46-4.31 (m, 12H, CH and CH₂ of the sugar moiety); 4.48 (d, *J*=9Hz, 1H, anomeric H). RMN ¹³C (75 MHz, D₂O, 298 K) δ ppm : 24.16, 25.56, 27.76, 28.18, 33.71, 39.00, 61.06, 61.92, 68.59, 70.37, 71.02, 71.40, 72.33, 72.44, 75.35, 80.96, 103.47, 173.89, 179.26. IR (KBr) ν cm⁻¹: 1644 (s, HNC=O); 1724 (s, COOH). Anal. Calcd C₁₉H₃₅NO₁₃: C, 47.01; H, 7.22; N, 2.89; Found : C, 46.88; H, 7.11; N, 2.60.

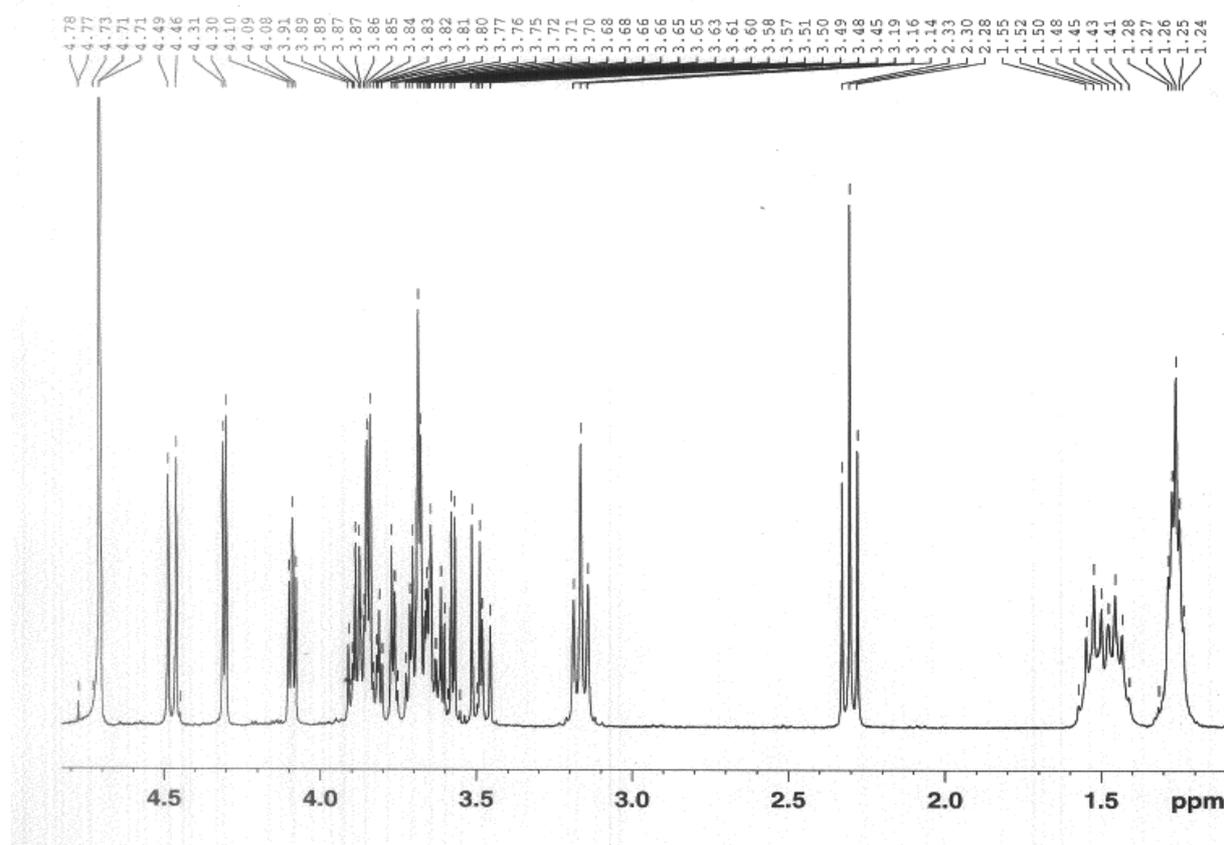


Figure S2: ¹H NMR spectrum (300 MHz, D₂O, 298 K) of the 1,7-lactobioamidoheptanoic acid

2.3. Association 3:1 between the polyamino-calix[6]arene and the sugar-derived surfactant

To 0.10mmol of 1,7-lactobioamidoheptanoic acid (48.4mg) in 30mL of distilled water was added 0.22mmol of calixarene (50mg). After 24h of stirring at room temperature, a homogeneous solution was obtained giving quantitatively a tan powder after water removal by freeze-drying.

ESI-HRMS (H₂O): 986. 5796 [M+3H]³⁺. RMN ¹H (300 MHz, DMSO, 298 K) δ ppm : 0.74 (m, 4H, CH₂); 1.22 (s, 27H, tBu); 1.40 (m, 4H, CH₂CH₂COO⁻ and CH₂CH₂CONH); 2.20 (m, 20H, NMe₂ and CH₂COO⁻); 3.04-4.07 (m, 50H, NCH₃, OCH₃, CH₂NMe₂, ArCH₂, CH₂CONH, CH and CH₂ of the sugar moiety); 4.26 (d, ³J_{HH}=6Hz, 1H, anomeric H); 4.98 (br s, 6H, CH₂Im); 6.63 (s, 6H, H_{Ar} tria); 6.88 (s, 3H, H_{Im}); 7.21 (s, 3H, H_{Im}); 7.56 (s, 6H, H_{Ar} tBu); 7.90 (br s, 3H, H_{triazole}); 8.63 (br s, 1H, NHCO). RMN ¹³C (75 MHz, DMSO, 298 K) δ ppm : 183.70 (COO⁻). IR (KBr) ν cm⁻¹: 1647 (s, HNC=O); 1560 (s, C=O_{asym}); 1413 (s, C=O_{sym}).

Note: ¹H NMR spectra of the 3:1 cationic association showed a loss of resolution comparatively to the sugar-based surfactant under the same conditions of analysis. This modification in the relaxation time of the protons was attributed to a self-assembling phenomenon where protons were enclosed in a less dynamic and more structured environment. In this regard, it is also very difficult to obtain ¹³C NMR spectra that can be fully interpreted.

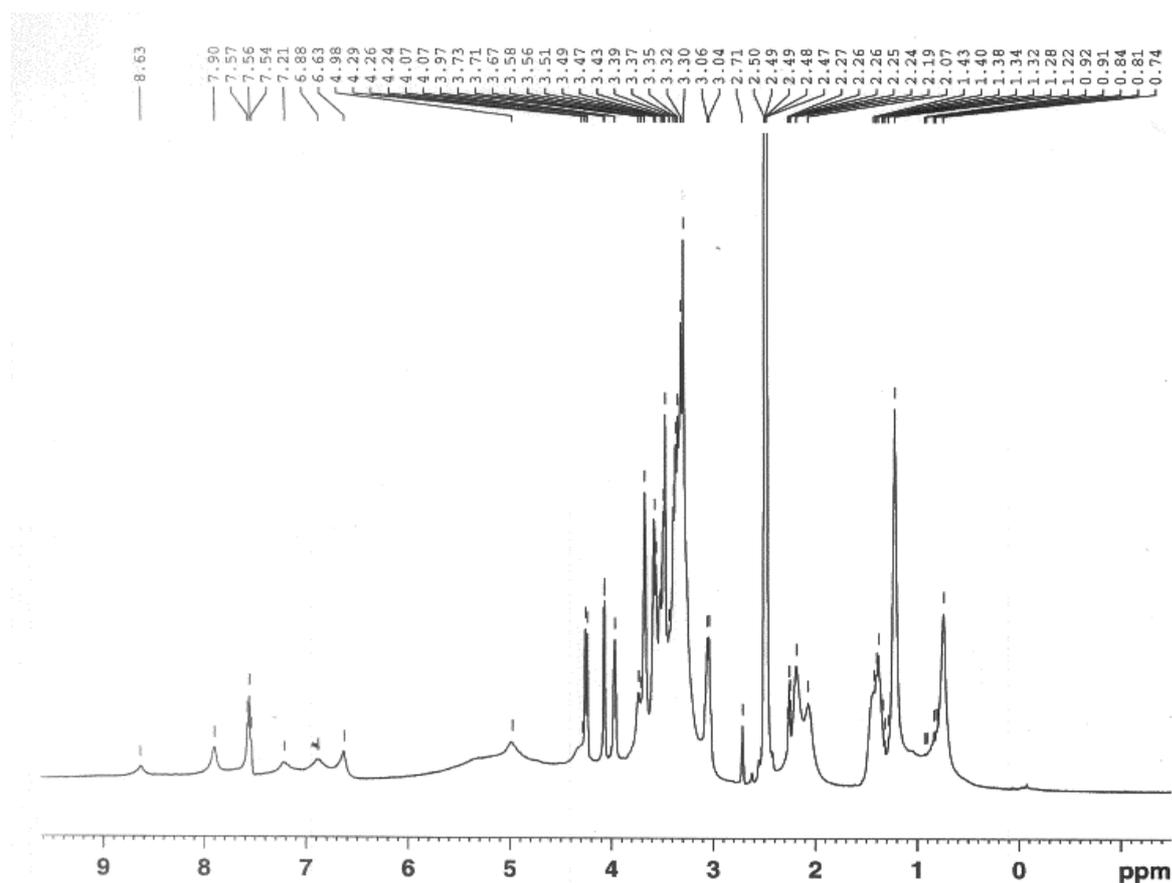


Figure S3: ¹H NMR spectrum (300 MHz, DMSO, 298 K) of the cationic association

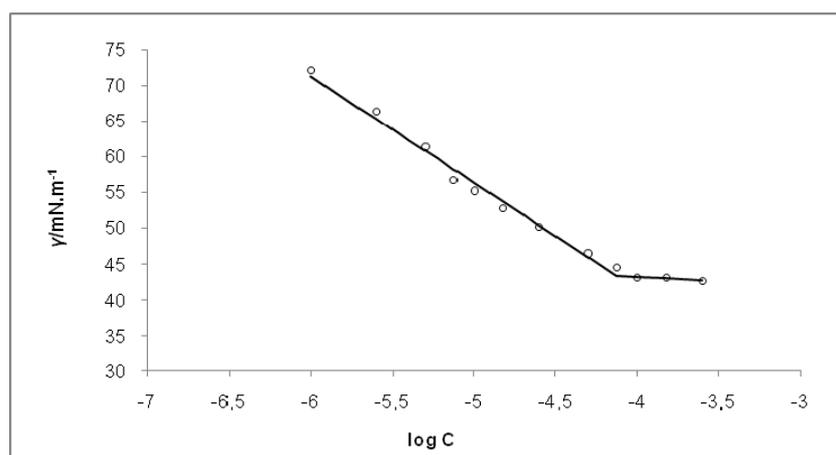


Figure S4: Plot of the surface tension versus logarithm of the concentration for the cationic assemblies at 25°C

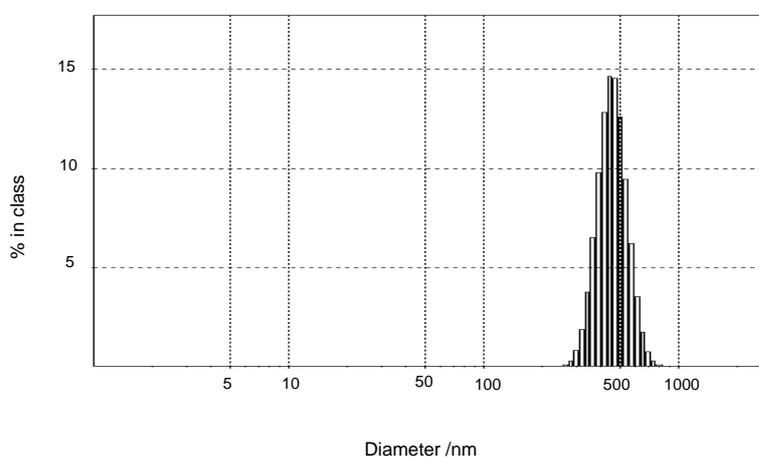


Figure S5: Size distribution of the aggregates formed by the cationic association at pH=6.8

pH	0	5 days	15 days	30 days
4	450 (0.21)	530 (0.20)	600 (0.18)	580 (0.21)
5	440 (0.12)	550 (0.23)	515 (0.14)	535 (0.18)
6	475 (0.11)	530 (0.15)	565 (0.10)	510 (0.18)
6.8	460(0.10)	460(0.22)	490(0.13)	570(0.24)
7	450 (0.26)	510 (0.13)	525 (0.21)	620 (0.25)
8	450 (0.20)	450 (0.18)	400 (0.20)	440 (0.16)
9	665 (0.30)	475 (0.30)	420 (0.32)	450 (0.28)
10	970 (0.88)	660 (0.50)	665 (0.28)	570 (0.20)
11	1000 (1.00)	700 (0.60)	700 (0.19)	570 (0.16)
12	1200 (1.00)	1530 (1.00)	1500 (1.00)	1500 (1.00)

Table S1: Mean hydrodynamic diameter and polydispersity index (into the brackets) for aggregates spontaneously form in aqueous solution, determined by DLS at 25°C