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Efficient Synthesis of Unsymmetrical Bolaamphiphiles for Spontaneous Formation of Vesicles and Disks with a Transmembrane Organization

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Unsymmetrical bolaamphiphiles **1a**−**1d** bearing a neutral glycosidic polar head and an electropositive ammonium group at opposite ends of a polymethylene bridging spacer were efficiently synthesized. An additional alkoxy group was introduced at the anomeric site of the carbohydrate in order to yield anomerically defined compounds, to allow the modulation of hydrophobic−hydrophilic balance (HLB), and to increase the dissymmetry of the monomers. The self-assembling properties of the bolaphiles **1a**−**1d** were investigated in both concentrated and more diluted aqueous media and were characterized in a variety of ways. An appropriate modulation of the HLB allowed us to obtain spontaneous formation of stable vesicles characterized by a transmembrane conformation of the lipids. Freeze fracture electron microscopy clearly demonstrated an unusual transformation of the vesicular systems into disks upon heating.

Introduction

In recent years, bolaamphiphiles have progressively gained importance because of their abilities to provide original supramolecular structures and advanced biomaterials.[1−3] Bolaamphiphiles consist of two polar headgroups connected to each other by one or more hydrophobic spacer groups.[4] These amphiphiles are supposed to self-assemble into monolayer lipid membranes (MLMs), reproducing the unusual architecture of natural archaeal macrocyclic bolaamphiphilic lipids.[5−7] Such bipolar lipids offer several advantages for the construction of advanced liposomes that are characterized by high mechanical and thermal stabilities due to the organization of the membrane.[8] Fusion process of MLMs with surface charges are particularly slow because charged headgroups cannot cross the hydrophobic membranes.[9] However, despite the growing development of synthetic symmetrical bolaamphiphiles,[10−25] surprisingly few transmembrane vesicle systems have clearly been evidenced from molecular structures. Furthermore, studies on bolaamphiphiles bearing two different polar heads at opposite ends of the lipophilic core are less common mainly due to synthetic difficulties.[26−28] Monolayer membranes formed from such compounds may therefore be characterized by an outer surface varying chemically from the inner one, as in biological membranes where the glycoproteins are exclusively anchored onto the outer surface.

We herein describe a new series of unsymmetrical bolaforms **1a**−**1d** (Figure 1) and their self-assembling properties in aqueous media. These amphiphiles are characterized by a dodecamethylene bridging chain linked through amide bonds to (i) a neutral saccharidic polar head in a D-glucofuranosidurono configuration at one end

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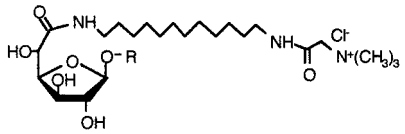

1a: R = CH_3, **1b**: R = CH_2-CH_3
1c: R = $(CH_2)_7$-CH_3, **1d**: R = $(CH_2)_{11}$-CH_3

Figure 1. Structural formula for bolaamphiphiles **1a**−**1d**.

and (ii) an electropositive ammonium group derived from glycine betaïne at the opposite end. An additional alkyl group with a variable length is linked to the furanose ring by a *O*-glycosidic bond at the anomeric site. The incorporation of this alkyl chain into one side of the bolaphile backbone was envisaged for (i) obtaining anomerically pure compounds, (ii) allowing the modulation of the hydrophobic−hydrophilic balance (HLB) of the surfactants without having to change the bridging chain length, and (iii) increasing the dissymmetry of the monomers. Furthermore, the presence of amide and sugar hydroxyl groups should contribute to the stabilization of the supramolecular aggregates through cooperative hydrogen bonding, as observed in natural archaeal glycolipids.[7]

Materials and Methods

Materials. All solvents were reagent grade and distilled before use. Chemicals were purchased from Acros or Fluka chemika Co. Alkyl D-glucofuranosidurono-6,3-lactones **2a**−**2d** were prepared according to the recently described procedure from this laboratory.[29,30] ^{1}H NMR spectra were recorded at 400 MHz, and ^{13}C NMR spectra were recorded at 100 MHz.

1-(Alkyl-*β*-D-glucofuranosiduronamido)-12-aminododecane (3a−3d). To a solution of 1,12-diaminododecane (1.5 equiv) in dry CH_3OH was added slowly over 45 min alkyl *β*-D-glucofuranosidurono-6,3-lactones **2a**−**2d**[29,30] dissolved in dry CH_3OH. After stirring for 2.5 h, the solution was concentrated and was then flash chromatographed over silica gel with CH_3OH/NH_3 (9:1). The desired products were isolated as solids typically in 70% yields. **3a:** R_f = 0.6 (CH_3OH/NH_3). Mp 89 °C. $[\alpha]^{20}_D$ −50.4 (c 1.03, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 1.21 (s, 16H, CH_2), 1.35−1.45 (m, 4H, CH_2), 2.51 (t, 2H, C*H*$_2$NH$_2$, *J* = 7.1 Hz), 3.14 (m, 2H, NHC*H*$_2$), 3.27 (s, 3H, CH_3), 3.90 (s, 1H, H-2 sugar), 3.99 (dd, 1H, H-3 sugar, *J* = 1.5, 4.6 Hz), 4.23 (d, 1H, H-5 sugar, *J* = 5.6 Hz), 4.33 (t, 1H, H-4 sugar, *J* = 5.6 Hz), 4.66 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 27.9−33.9 (CH_2), 40.2 ($NHCH_2$), 42.6 (CH_2NH_2), 55.8 (OCH_3), 72.3 (C-5 sugar), 77.5 (C-3 sugar), 81.7 (C-2 sugar), 84.2 (C-4 sugar), 111.0 (C-1 sugar), 174.5 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + H]^+$ 391.2808, found 391.2907. **3b:** R_f = 0.3 (CH_3OH/NH_3). Mp 68 °C. $[\alpha]^{20}_D$ −46.8 (c 1.03, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 1.10 (t, 3H, CH_3), 1.22 (s, 16H, CH_2), 1.38−1.44 (m, 4H, CH_2), 2.53 (t, 2H, C*H*$_2$NH$_2$, *J* = 7.1 Hz), 3.15 (m, 2H, NHC*H*$_2$), 3.35 (q, 2H, CH_2), 3.91 (s, 1H, H-2 sugar), 3.99 (dd, 1H, H-3 sugar, *J* = 2.0, 5.1 Hz), 4.25 (d, 1H, H-5 sugar, *J* = 5.1 Hz), 4.33 (t, 1H, H-4 sugar), 4.77 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 15.3 (CH_3), 27.9−33.7 (CH_2), 40.2 ($NHCH_2$), 42.5 (CH_2NH_2), 64.7 (OCH_2), 72.3 (C-5 sugar), 77.6 (C-3 sugar), 81.9 (C-2 sugar), 84.0 (C-4 sugar), 109.7 (C-1 sugar), 174.5 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + H]^+$ 405.2965, found 405.2966. **3c:** R_f = 0.4 (CH_3OH/NH_3). Mp 65 °C. $[\alpha]^{20}_D$ −38.1 (c 1.03, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 0.93 (CH_3), 1.21 (s, 24H, CH_2), 1.41 (m, 8H, CH_2), 2.50 (t, 2H, C*H*$_2$-NH$_2$), 3.07 (m, 2H, NHC*H*$_2$), 3.16 (t, 2H, OCH_2), 3.77 (s, 1H, H-2 sugar), 3.85 (d, 1H, H-3 sugar, *J* = 3.6 Hz), 4.14 (d, 1H, H-5 sugar), 4.15 (t, 1H, H-4 sugar), 4.66 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 14.1 (CH_3), 21.9−31.1 (CH_2), 38.1 ($NHCH_2$), 41.2 (CH_2NH_2), 67.5 (OCH_2), 70.3 (C-5 sugar), 75.6 (C-3 sugar), 80.6 (C-2 sugar), 83.1 (C-4 sugar), 108.6 (C-1 sugar), 172.2 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + H]^+$ 489.3904, found 489.3906. **3d:** R_f = 0.3 (CH_3OH/NH_3). Mp 133 °C. $[\alpha]^{20}_D$ −24.6 (c 1.03, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 0.81 (t, 3H, CH_3), 1.21 (s, 32H, CH_2), 1.42 (m, 8H, CH_2), 2.52 (t, 2H, C*H*$_2$NH$_2$, *J* = 6.6 Hz), 3.20 (m, 2H, NHC*H*$_2$), 3.30 (t, 2H, OCH_2), 3.91 (s, 1H, H-2 sugar), 3.98 (dd, 1H, H-3 sugar, *J* = 2.0, 5.1 Hz), 4.25 (d, 1H, H-5 sugar), 4.32 (t, 1H, H-4 sugar), 4.75 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 14.5 (CH_3), 23.7−33.8 (CH_2), 40.1 ($NHCH_2$), 42.5 (CH_2NH_2), 69.5 (OCH_2), 72.2 (C-5 sugar), 77.5 (C-3 sugar), 81.8 (C-2 sugar), 84.1 (C-4 sugar), 109.9 (C-1 sugar), 174.4 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M + H]^+$ 545.4530, found 545.4531.

***N*-(*N*′,*N*′,*N*′-Trimethylammonium acetyl) Thiazolidine-2-thione Chloride (4).** To a solution of glycine betaïne (1.17 g, 10.0 mmol) in dry acetonitrile (5 mL) was added dropwise a solution of thionyl chloride (1.8 g, 15 mmol)) in acetonitrile (20 mL). The mixture was stirred at 35 °C for 1 h before the solvent and the thionyl chloride in excess were removed under reduced pressure. To a suspension of the resulting acyl chloride in dichloromethane (10 mL) were then added at 0 °C 2-mercaptothiazoline (1.31 g) and a solution of triethylamine (1 g) in dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 30 min. After removal of the solvent, the residue was stirred in dichloromethane under reflux for an additional 30 min in order to solubilize trimethylammonium salts. The residue was filtered, and after repeating this procedure twice, the desired product was isolated as a yellow solid (2.20 g, 86%). R_f = 0.7 (CH_3OH). Mp 200 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 3.31 (s, 9H, CH_3), 3.49 (t, 2H, $CON(CH_2)$, *J* = 7.6 Hz), 4.57 (t, 2H, CH_2S, *J* = 7.6 Hz), 5.27 (s, 2H, CH_2CO). ^{13}C NMR (100 MHz, DMSO-d_6) 29.2 [$CON(CH_2)$], 53.2 (CH_3), 55.7 (CH_2S), 66.8 (CH_2-CO), 165.3 (CO), 202.4 (CS).

Bolaamphiphiles 1a−1d. *N*-(*N*′,*N*′,*N*′-trimethylammonium acetyl) thiazolidine-2-thione chloride **(4)**[31] (1.5 equiv) was added to a solution of glucofuranosiduronamides **3a**−**3d** in dry DMF under nitrogen. The reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was flash chromatographied upon elution with ethyl acetate/2-propanol/water (6/3/1). Bolaforms **1a**−**1d** were then purified on Sephadex G-10 using water as eluent. **1a:** Yield 67%. R_f = 0.1 ($AcOEt/CH_3OH/H_2O$). Mp 85 °C. $[\alpha]^{20}_D$ −30.2 (c 1.0, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 1.22 (s, 16H, CH_2), 1.45 (m, 4H, CH_2), 3.18 (m, 4H, NHC*H*$_2$), 3.26 [s, 9H, $(CH_3)_3N$], 3.30 (s, 3H, CH_3), 3.93 (s, 1H, H-2 sugar), 4.02 (dd, 1H, H-3 sugar, *J* = 2.0, 4.6 Hz), 4.04 (s, 2H, CH_2CO), 4.32 (m, 2H, H-4 and H-5 sugar), 4.69 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 27.9−30.6 (CH_2), 40.2 ($NHCH_2$), 40.3, 40.5 (CH_2NH), 54.7 [$(CH_3)_3N$], 56.0 (OCH_3), 65.8 ($COCH_2$), 72.1 (C-5 sugar), 77.4 (C-3 sugar), 81.6 (C-2 sugar), 84.2 (C-4 sugar), 111.2 (C-1 sugar), 164.5 ($COCH_2$), 174.5 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M - Cl]^+$ 490.3492, found 490.3492. **1b:** Yield 65%. R_f = 0.2 ($AcOEt/CH_3OH/H_2O$). Mp 50 °C. $[\alpha]^{20}_D$ −33.5 (c 1.0, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 1.10 (t, 3H, CH_3), 1.21 (s, 16H, CH_2), 1.43 (m, 4H, CH_2), 3.17 (m, 4H, NHC*H*$_2$), 3.23 [s, 9H, $(CH_3)_3N$], 3.35 (q, 2H, CH_2), 3.91 (s, 1H, H-2 sugar), 3.98 (dd, 1H, H-3 sugar, *J* = 1.8, 3.9 Hz), 3.99 (s, 2H, CH_2CO), 4.27 (d, 1H, H-5 sugar, *J* = 5.4 Hz), 4.31 (t, 1H, H-4 sugar), 4.77 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 15.4 (CH_3), 27.9−30.7 (CH_2), 40.2, 40.5 (CH_2-NH), 54.7 [$(CH_3)_3N$], 64.7 (OCH_2), 65.7 ($COCH_2$), 72.2 (C-5 sugar), 77.5 (C-3 sugar), 81.8 (C-2 sugar), 84.0 (C-4 sugar), 109.7 (C-1 sugar), 164.5 ($COCH_2$), 174.5 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M - Cl]^+$ 504.3649, found 504.3651. **1c:** Yield 78%. R_f = 0.2 ($AcOEt/CH_3OH/H_2O$). Mp 60 °C. $[\alpha]^{20}_D$ −25.8 (c 0.97, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD) 0.79 (t, 3H, CH_3), 1.20 (s, 24H, CH_2), 1.45 (m, 8H, CH_2), 3.11 (m, 4H, NHC*H*$_2$), 3.22 [s, 9H, $(CH_3)_3N$], 3.27 (t, 2H, CH_2), 3.90 (s, 1H, H-2 sugar), 3.97 (dd, 1H, H-3 sugar, *J* = 1.5, 4.6 Hz), 4.00 (s, 2H, CH_2CO), 4.25 (d, 1H, H-5 sugar, *J* = 5.1 Hz), 4.29 (t, 1H, H-4 sugar, *J* = 4.6 Hz), 4.75 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 14.5 (CH_3), 23.7−33.0 (CH_2), 40.2, 40.5 (CH_2NH), 54.7 [$(CH_3)_3N$], 65.7 ($COCH_2$), 69.5 (OCH_2), 72.2 (C-5 sugar), 77.5 (C-3 sugar), 81.8 (C-2 sugar), 84.1 (C-4 sugar), 110.0 (C-1 sugar), 164.6 ($COCH_2$),

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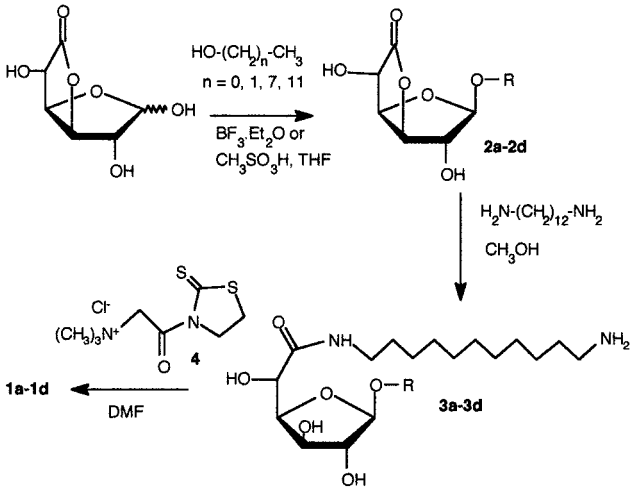
Scheme 1. Synthesis Pathway to Unsymmetrical Bolaamphiphiles 1a−1d



174.5 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M - Cl]^+$ 588.4588, found 588.4585. **1d:** Yield 65%. $R_f = 0.2$ ($AcOEt/CH_3OH/H_2O$). Mp 70 °C. $[\alpha]^{20}_D$ −25.3 (c 1.0, CH_3OH). ^1H NMR (400 MHz, CD_3OD) 0.82 (t, 3H, CH_3), 1.22 (s, 32H, CH_2), 1.45 (m, 8H, CH_2), 3.14 (m, 4H, $NHCH_2$), 3.26 [s, 9H, $(CH_3)_3N$], 3.32 (t, 2H, CH_2), 3.93 (s, 1H, H-2 sugar), 4.00 (dd, 1H, H-3 sugar, $J = 1.5$, 4.6 Hz), 4.04 (s, 2H, CH_2CO), 4.28 (d, 1H, H-5 sugar, $J = 5.6$ Hz), 4.33 (t, 1H, H-4 sugar, $J = 5.1$ Hz), 4.77 (s, 1H, H-1 sugar). ^{13}C NMR (100 MHz, CD_3OD) 14.5 (CH_3), 23.7−33.1 (CH_2), 40.2, 40.5 (CH_2NH), 54.7 [$(CH_3)_3N$], 65.7 ($COCH_2$), 69.5 (OCH_2), 72.1 (C-5 sugar), 77.5 (C-3 sugar), 81.8 (C-2 sugar), 84.1 (C-4 sugar), 109.9 (C-1 sugar), 164.5 ($COCH_2$), 174.4 (C-6 sugar). FABMS (*m*-nitrobenzyl alcohol matrix) calcd for $[M - Cl]^+$ 644.5214, found 644.5208.

Methods. Melting points reported were uncorrected. The aqueous preparations were obtained by simple mixing and gentle stirring without sonication. X-ray scattering experiments were performed with a focusing Guinier temperature-controlled camera using monochromatic Cu $K\alpha_1$ radiation ($\lambda = 1.54$ Å) and linear collimation. The samples were placed between two mica windows in vacuum-tight cells. For freeze fracture electron microscopy, a small drop of the preparation containing glycerol as a cryoprotectant (30/70, glycerol/water) was deposited on a thin copper planchett, rapidly frozen in liquid propane, and kept in liquid nitrogen. The samples were frozen from various temperatures according to specific requirements. Freeze fracture was performed with a Balzers 301 freeze etch unit. The samples were fractured at −125 °C in a vacuum lower than 10−6 Torr and subsquently shadowed with Pt−C; when necessary the samples were heated to −105 °C, etched for 2 min, and cooled to −125 °C prior to shadowing. The replicas were washed with a SDS solution, rinsed with water, and examined in a Philips 410 electron microscope. ζ-potential measurements and particle size distributions were measured using a 3000 Zetasizer Malvern Instrument, and ^1H and ^{13}C NMR spectra were obtained in CD_3OD or in D_2O using a Bruker AMX 400-MHz spectrometer. Fast atom bombardment (FAB) mass spectra were acquired on a MS/MS ZabSpec TOF Micromass spectrometer using metanitrobenzylic alcohol as matrix. Tensiometry measurements were performed using the ring method with a Kruss K10T tensiometer.

Results

Synthesis. Bolaamphiphiles **1a−1d** were prepared by sequential introduction of the saccharidic headgroup followed by the cationic moiety onto unprotected 1,12-diaminododecane. D-Glucofuranurono-6,3-lactone (D-glucurone) was converted to the corresponding alkyl D-glucofuranosidurono-6,3-lactones **2a−2d** (Scheme 1) in satisfactory yields (70−85%) and with good stereoselectivity (α,β ratio 1:9) according to a previously described procedure from this laboratory.[29,30] The pure β-anomer was easily isolated by chromatography. Treatment of this lactone with 1.5 equiv of 1,12-diaminododecane at room temperature in methanol specifically provided the glucofuranosiduronamides **3a−3d** resulting from monoacylation of the diamine, typically in 70% yields. *N*-Acylation of the resulting glycosylated monoamines **3a−3d** was efficiently performed with the *N*-acyl thiazolidine-2-thione derivative **4** of glycine betaïne in DMF and under neutral conditions in order to prevent any hydrolysis of the glycosidic bond. The former *N*-acyl thiazolidine-2-thione derivative **4** was prepared by reacting 2-mercaptothiazoline with the acyl chloride derived from glycine betaïne.[31] The required bolaamphiphiles **1a−1d** were isolated in 65−78% yields after chromatography and gel filtration on Sephadex G-10. The structures of **1a−1d** were fully substantiated by NMR spectroscopy and high-resolution mass spectrometry. The methodology described herein therefore represents an efficient three-step procedure for producing unsymmetrical bolaamphiphiles from natural compounds. It is noteworthy that targeted monomers could be produced on a multigram scale at a low overall cost.

Concentrated Systems. X-ray Diffraction. The self-assembling properties of bolaamphiphiles **1a−1d** were first investigated in concentrated aqueous media. Compounds **1a** and **1b** were miscible with water. Upon the addition of 10% water, the X-ray diffraction pattern of **1a** consisted of only a broad reflection at 1/25.6 Å and that of bolaform **1b** showed a large diffusion at 1/24 Å upon addition of 20% water. With more added water, both samples **1a** and **1b** formed a transparent solution. Tensiometry measurements did not allow the determination of any micellar concentration for a concentration range between 10^{-5} and 10^{-2} M.

The increase of the *O*-glycosidic alkyl chain length modified the aggregation properties of the surfactants in water. Indeed, both compounds **1c** and **1d**, characterized by an octyl and a dodecyl chain at the anomeric position of the sugar head, respectively, exhibited lamellar phases. For the present X-ray scattering experiments, the amount of water added to the surfactant did not exceed 60% water. With such an amount of water and after a short preheating, compound **1c** formed a lamellar phase L_α at 20 °C of 59 Å dimension characterized by two reflections ($h = 1,2$) in the low-angle region and a broad diffuse band in the wide-angle region (1/4.5 Å) which is ascribed to the presence of disordered chains. With much less water incorporated within the lamellar phase, we could observe a L_α phase at 20° of 33 Å dimension and an increase of the dimension upon heating, 35 Å at 26°and 42 Å at 40° and 60°, whereas the small angle reflections became slightly less thin. This variation is at variance with the usual variation of lamellar dimension of L_α lipid phases with temperature. This unexpected result may be attributed either to an increase of the water content within the lamellar phase and/or to some rearrangement at the polar interface or within the lipid lamellae, according to temperature variation.

The lyotropic behavior of compound **1d** containing a dodecyl chain at the anomeric site was also examined. At room temperature it remained in a crystalline form. The lyotropic phase formation required a first heating above 45 °C in order to hydrate the compound. Then a lamellar phase L_β was observed at 20 °C, characterized by two sharp reflections ($h = 1,2$) in the low-angle region and a sharp reflection at 4.2 Å which is characteristic of ordered chains. The dimension of the lamellar phase varied according to the amount of water within the phase. Above the transition temperature (45 °C), the scattering pattern displayed two small angle reflections ($h = 1,2$) and a broad diffusion band around 1/4.5 Å, indicating the presence of a L_α phase. However, the small angle reflections of the L_α

phase became extremely faint upon increasing the amount of water. These observations may be correlated to the morphological changes obtained in diluted systems at high temperatures (vide infra).

Diluted Systems: Supramolecular Assemblies. *1. Freeze Fracture Electron Microscopy.* The self-assembling properties were evaluated with more dispersed systems containing 4−10% of bolamphiphiles **1a**−**1d** using freeze fracture electron microscopy (FFEM). No supramolecular aggregate was visualized for compounds **1a** and **1b**, although they might form small micelles which are not detectable by FFEM. Noteworthy, unsonicated suspension of bolaamphiphile **1c** gave at room temperature vesicles which were not fractured in the usual way along the midplane of the membrane. These vesicles were cross-fractured; that-is-to-say, the fracture propagated across the membrane, generating circles instead of the usual convex and concave surfaces as in the case of bolaamphiphiles developed by Fuhrhop[30] and others[32] or as found for natural tetraether-type lipids.[33] The fractured samples were etched before shadowing in order to visualize much more clearly the propagation path (Figure 2a). The absence of a fracturable midplane in the membrane indicates that the bolaamphiphile does not adopt a U-bent shape but spans the membrane to form a monolayer. Depending on the surfactant concentration, mixing the suspension at room temperature, or preheating the mixture for obtaining a transparent suspension, multilamellar and/or unilamellar vesicles could be obtained. These vesicular aggregates remained stable at room temperature for several months, as checked by FFEM. In some cases a few open disk-type aggregates were observed (Figure 2a). Remarkably, the proportion of the disks increased when the preparations were frozen from 60 °C and only some unilamellar closed vesicles were still present (Figure 2b).

Aqueous suspensions of bolaamphiphile **1d** bearing a dodecyl chain at the anomeric site (4−10 wt %) revealed, without preheating, 3D crystals with large fracture planes of the lamellar type. The material could be dispersed in water after heating the suspension at 45 °C, and large lamellae were observed by FFEM at 20 °C. Here again, there was no fracture plane within the membrane, giving evidence for a transmembrane organization of the lipids (Figure 2c). Above the transition temperature, the same type of fracture was observed, but only small disks whose dimension did not exceed 50 nm could be visualized (Figure 2d).

2. Dynamic Light Scattering and ζ-Potential Determination. Dynamic light scattering (DLS) of a suspension of **1c** at 20 °C indicated the presence of two populations having hydrodynamic diameters of 50 and 400 nm, respectively, that probably correspond to unilamellar and multilamellar vesicles visualized with FFEM. To determine a possible unsymmetrical organization of bipolar lipids within the membrane, the ζ-potential of the aggregates of bolaform **1c** was determined. The ζ-potential calculated from the electrophoretic mobility of the aggregates formed from compound **1c** showed a positively charged surface density (ζ-potential = +40 mV), giving evidence for the presence of cationic headgroups on the exterior membrane side of the aggregates.

3. ^1H NMR Studies. ^{1}H NMR spectra of aggregates (Figure 3) in D_2O prepared from bolaform **1c** gave at ambient temperature nonresolved signals except for that

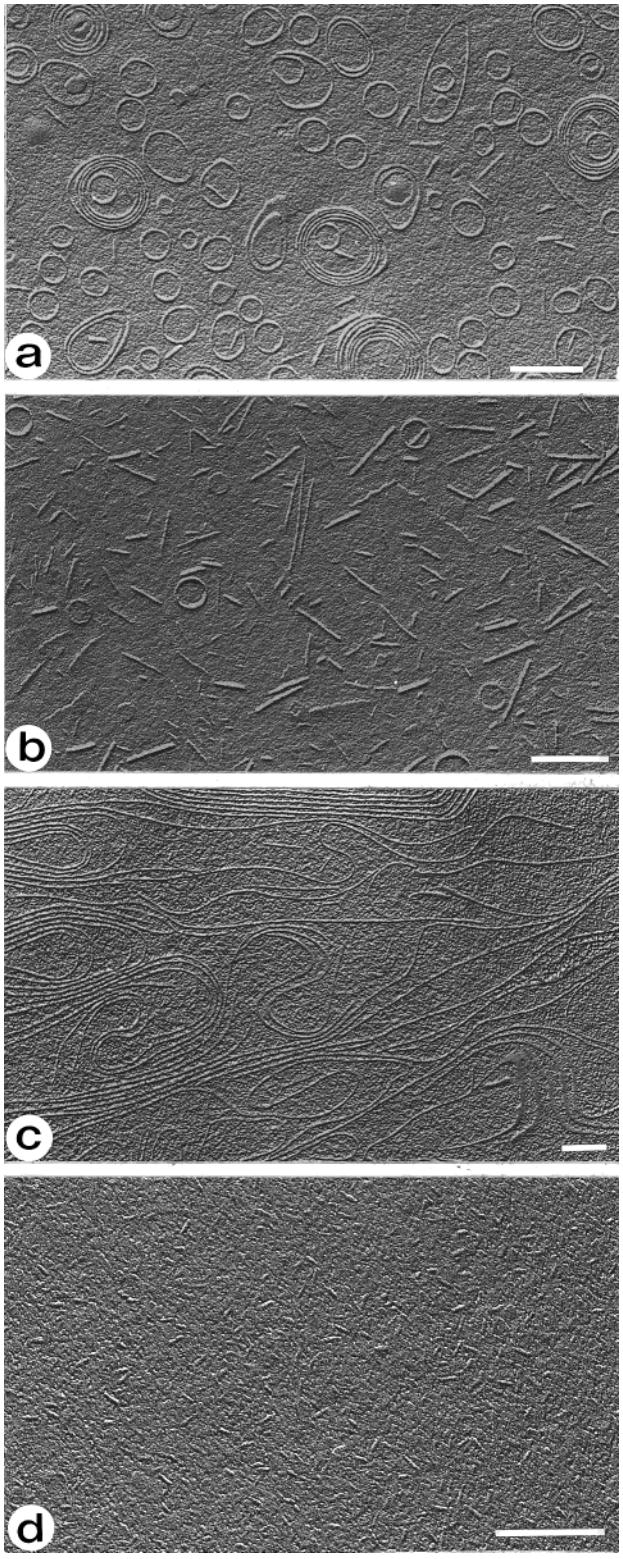


Figure 2. Freeze fracture electron microscopy of bolaform dispersions. **1c**: (a) 20 °C; (b) 60 °C. Bolaform **1d**: (c) 20 °C; (d) 45 °C. For a better visualization, the fractured samples were etched for 2 min at −105 °C. Bar is 200 nm.

for the trimethylammonium protons ($\delta = 3.26$ ppm). With increasing temperature, the width of the peaks decreased and an additional signal at higher field appeared for the $N^+(CH_3)_3$ protons. The intensity of this second peak increased with increasing temperature whereas the signal observed at room temperature became less and less intense. The intense higher field signal observed at 60 °C may result from the presence of cationic headgroups

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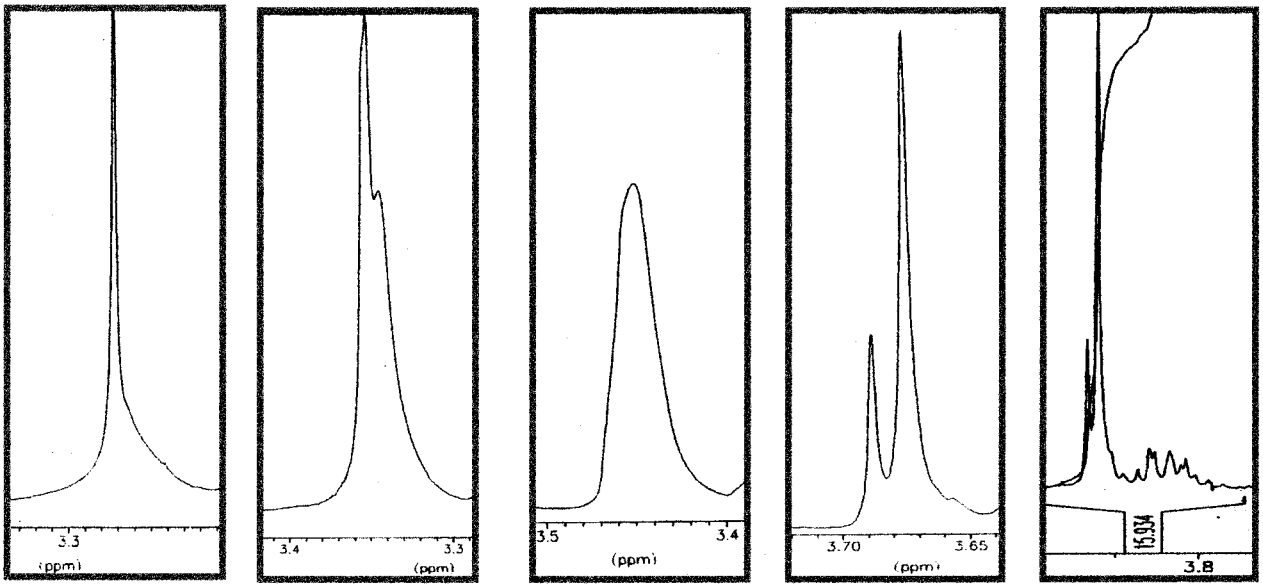

Figure 3. ^{1}H NMR signals for the trimethylammonium moieties of a 6.4×10^{-2} M solution of bolaform **1c** in D_2O with increasing temperature: (a) 25 °C; (b) 32 °C; (c) 42 °C; (d) 62 °C; (e) 92 °C.

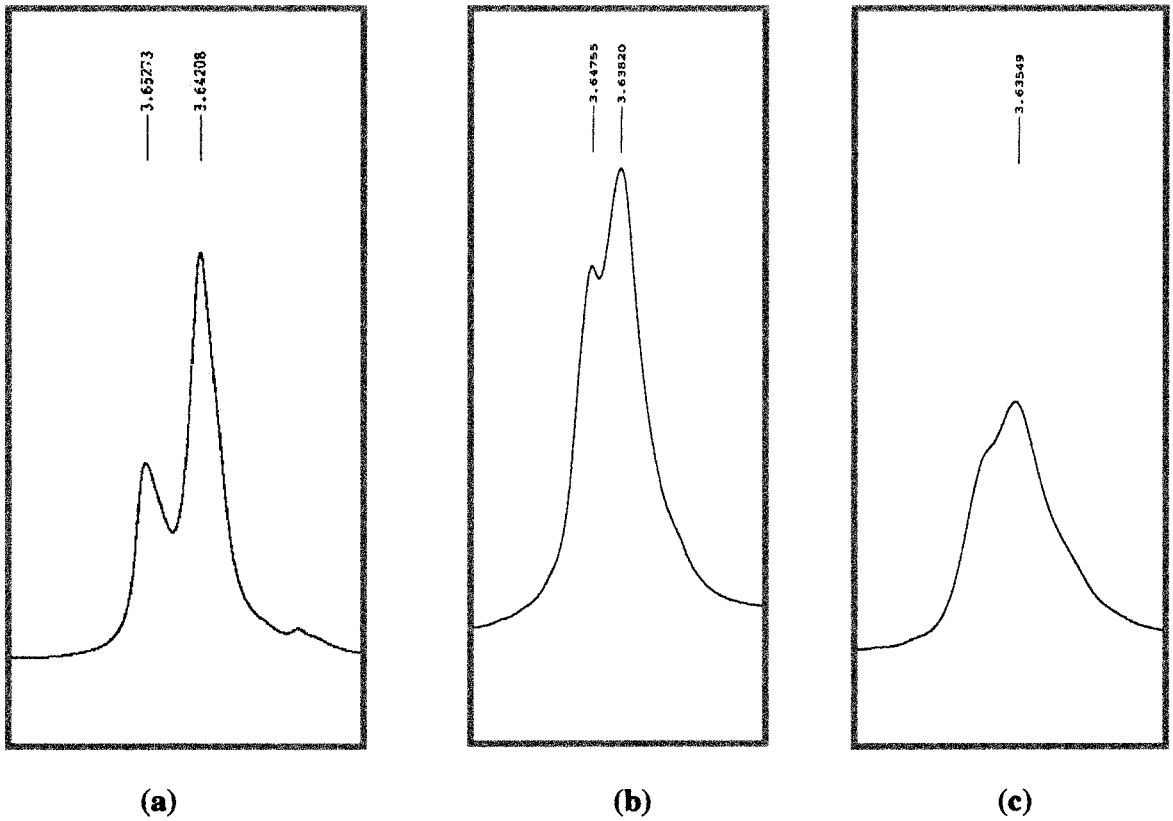

Figure 4. ^{1}H NMR signals for the trimethylammonium moieties of a 6.4×10^{-2} M solution of bolaform **1c** in D_2O at 60 °C with increasing addition of $Gd(NO_3)_3 \cdot 6H_2O$: (a) without addition of $Gd(NO_3)_3 \cdot 6H_2O$; (b) after addition of 20 μL of a 1.3 10^{-2} M solution of $Gd(NO_3)_3 \cdot 6H_2O$ in D_2O; (c) after addition of 40 μL of a 1.3 10^{-2} M solution of $Gd(NO_3)_3 \cdot 6H_2O$ in D_2O.

located on the outer surface of both disks and unilamellar vesicles whereas the lower field signal may correspond to endovesicular $N^+(CH_3)_3$ moieties.[34] The addition of paramagnetic gadolinium(III) ions[34] into the dispersion of **1c** in D_2O at 60 °C caused a broadening effect on both signals (Figure 4). Supposing that these ions do not penetrate the hydrophobic vesicle membranes (not checked), one might attribute this result to a thermally driven flip-flop of the lipids involving endovesicular/exovesicular exchanges, that could also be associated with a gradual formation of disks. The detailed kinetics of flip-flop dynamics have already been evaluated in liposomes formed by bolaam-

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phiphilic macrocyclic lipids, showing a significant decrease of the resistance to flip-flop at high temperature.[35] In the case of bolaamphiphile **1d**, ^{1}H NMR spectra of a dispersion of the lipid in D_2O displayed no proton signal at 20 °C, probably due to the presence of the gel state observed by FFEM whereas a single signal for the $N^+(CH_3)_3$ protons was observed at 60 °C, that-is-to-say, at temperature conditions leading exclusively to disk formation.

Discussion and Conclusion

By an appropriate modulation of the HLB of the bolaamphiphiles, it was possible to obtain lamellar phases in concentrated systems with compounds **1c** and **1d**. At room temperature, the alkyl chains of **1c** are disordered whereas a $L_\beta \rightarrow L_\alpha$ transition at 45 °C was observed for compound **1d**. In diluted conditions, bolaamphiphile **1c** exhibited vesicles where most of the molecules adopted a transmembrane conformation as revealed by FFEM. Surprisingly, upon heating, the vesicles disappeared progressively and disks became predominant. Compound **1d** did not form vesicles above the transition temperature, but it tended to self-assemble into small disks with a transmembrane conformation of the hydrophobic chains. However, the existence of disks requires that a part of the molecules adopt a U-bent shape on the edges of the disks in order to prevent the contact of the hydrophobic chains with the aqueous environment. Moreover, the proportion of this configuration increases as the size of the disks decreases; clearly this configuration is more frequent for compound **1d** than for compound **1c**. It is probable that at lower water content or in the gel state the sugar residues do not hydrate as easily as the charged groups and might be partly embedded in the hydrophobic matrix. At higher temperature, the glucurone residues might be more readily hydrated, therefore favoring the transition from a stretched to a folded molecular conformation. It is possible that the initial monolayers are forced to rearrange into small pieces of lamellae or disks when many molecules adopt a U-shape.

The results presented here show that transmembrane systems can readily be obtained without providing energy from simple unsymmetrical bolaamphiphiles. These molecules are characterized by the presence of neutral and positively charged polar heads possessing different hydration properties. By an appropriate balance between the length of the bridging chain and the second alkyl chain, it was possible to obtain a bolaamphiphile, referred to as **1c**, able to form spontaneously monolayer vesicles, reproducing the self-organization of natural archaeal macrocyclic lipids. Upon increasing temperature, some of the molecules adopted a U-shape, allowing the formation of disks. This transformation, which could occur within the domain where the chains are disordered, appears unusual and will be further investigated. Besides, applications of these monolayer systems for the encapsulation and the controlled release of negatively charged materials including nucleic acids are under investigation.

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