Monolayer lipid membrane-forming dissymmetrical bolaamphiphiles derived from alginate oligosaccharides†

M. Roussel,‡[a] V. Lognoné,§[b] D. Plusquellec[a] and T. Benvegnu*[a]

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New dissymmetrical neutral–cationic or anionic–cationic α,ω-diamido bolaamphiphiles have been synthesized in which the polar headgroups are derived from alginate and glycine betaine and which exhibit monolayer lipid membrane vesicles, large lamellae and rods.

Bolaamphiphiles, which are composed of two hydrophilic headgroups connected by one or several hydrophobic polymethylene chains, represent a novel class of surface-active components which are supposed to self-assemble into stable monolayer lipid membranes (MLMs) reproducing the unusual architecture of natural archaebacterial bipolar lipids.[1] The uniqueness of these atypical molecules has already boosted interest in their potential uses as drug or gene delivery systems,[2] coatings of smooth solid materials or machinery based on molecular recognition processes.[3] Several past studies have focused on the self-assembly of synthetic amphiphilic compounds possessing two similar ionic or non-ionic polar head groups.[1,4] In contrast, the properties of dissymmetrical bolaamphiphiles with one neutral head group and one cationic head group or with one anionic head group and one cationic head group have not been widely investigated.[5] Here we report on the preparation and the self-organizing properties of a new series of nonsymmetrical α,ω-diamido bolaamphiphiles **1–2** (Fig. 1) with one tetraalkylammonium chloride headgroup derived from glycine betaine and one neutral monosaccharidic or anionic unsaturated disaccharidic headgroup derived from alginate oligosaccharides. An additional alkyl group with a variable length (C_8 or C_4) is linked to the sugar ring by a stereocontrolled *O*-glycosidic bond at the anomeric site in order to modulate the hydrophobic–hydrophilic balance (HLB) of the surfactants with the dodeca- or docosamethylene bridging chain length (C_{12} or C_{22}).

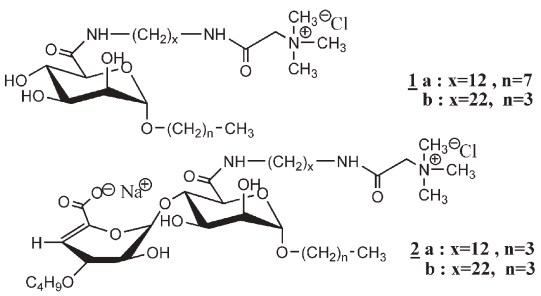
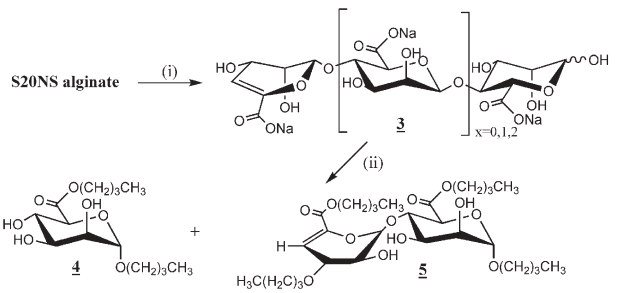


Fig. 1 Dissymmetrical α,ω-diamido bolaamphiphiles **1–2**.

Alginate, a heteropolysaccharide from brown algae, is composed of (1,4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units in the form of homopolymeric (MM- or GG-blocks) and heteropolymeric sequences (MG- or GM-blocks).[6] Alginate lyases[7] depolymerize alginate through a β-elimination reaction that releases unsaturated oligosaccharides containing a 4-deoxy-L-*erythro*-hex-4-ene pyranosyluronate moiety at their non-reducing terminal uronate residues.[8] The enzyme specificity towards poly-M, poly-G or poly-MG blocks is frequently difficult to control and complex mixtures of M- and/or G-containing uronides of various lengths are usually provided by enzymatic alginate hydrolysis.[8] Taking into account this problem, an enzymatic method, utilizing commercially available S20NS alginate from *Laminaria digitata* algae (high M/G ratio, low composition in MG blocks) and the enzyme (AL951)[9] produced by *Pseudomonas alginovora*, was developed to prepare oligomannuronates (degree of polymerization, DP < 4) incorporating 4,5-unsaturated non-reducing terminal residues. The use of this alginate lyase possessing a double M- and G-lyase specificity needs a perfect control of the ionic strength (< 0.05 mol/L) of the reaction medium in order to inhibit its guluronate-lyase activity. The enzymatic degradation of alginate was performed in an aqueous solution (50 g/L) containing enzyme (AL951) (enzyme/alginate = 0.5%, w/w) at 22 °C for 24 h and at pH 7.5 (aq. 1 N NaOH) in order to avoid the depolymerization of GG- and MG-/GM- blocks (Scheme 1). A purification by an ultrafiltration process with a 3500 D cell-membrane cut-off permitted both elimination of the guluronic

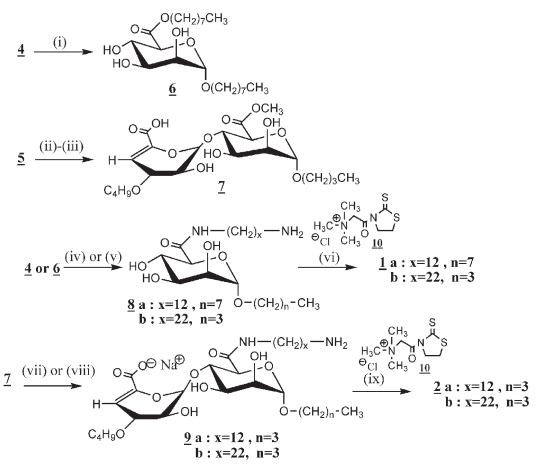


Scheme 1 *Reagents and conditions*: (i) enzyme (AL951),[9] pH 7.5, 22 °C, 40%; (ii) MSA, BuOH, 50–55 °C , **4**: 35%; **5**: 25%.

[a]*ENSCR, UMR CNRS 6226 Sciences Chimiques de Rennes, Equipe ''Synthèse Organique et Systèmes Organisés'', Avenue du Général Leclerc, 35700 Rennes, France. E-mail: thierry.benvegnu@ensc-rennes.fr*
[b]*Centre d'Etude et de Valorisation des Algues (CEVA), Presqu'île de Pen-lan, BP 3, 22610 Pleubian, France*
† Electronic supplementary information (ESI) available: Optical textures of bolaamphiphiles **1a–1b** and **2b** (crossed polarizers). See DOI: 10.1039/b607368a
‡ *Present address*: R&D COGNIS France, Z.A., 19 rue Pierre Brasseur, 77109 Meaux cedex, France.
§ *Present address*: Codif International, 61 rue du Commandant l'Herminier, BP 40, 35404 Saint-Malo cedex, France.

homopolymeric and heteropolymeric sequences and the isolation of DP 2–4 unsaturated mannuronates **3** (ΔMM, 40% yield) in the presence of salts (Scheme 1). Treatment of mannuronate oligomers **3** with methanesulfonic acid (MSA, 4 equiv.) in butanol at 50–55 °C with several successive azeotropic co-evaporations (H_2O/BuOH) under reduced pressure, provided *n*-butyl (*n*-butyl α-D-mannopyranosiduronate) **4**[10] and *n*-butyl [*n*-butyl 4-deoxy-β-D-*threo*-hex-4-ene pyranosiduronate-(1→4)-*n*-butyl α-D-mannopyranosid] uronate **5**, in 35% and 25% yield, respectively. These two derivatives are the result of four *one pot* reactions: acid hydrolysis of glycosidic linkages, esterification, glycosylation and allylic ether formation reactions. They could be easily separated by column chromatography (CH_2Cl_2–CH_3OH: 98/2, v/v) and the half-chair conformation $^1H_2$ of the 4,5-unsaturated residue of **5** which is derived from the altrose series was evidenced by 2D-COSY ^1H NMR, ^1H–^{13}C correlation spectra and t-ROESY experiments [$J_{H1'H2'}$ = 2.3 Hz, $J_{H2'H3'}$ = 6.9 Hz, distance between H1′ and H3′, d(H1′–H3′) ≈ d(H1–H5) and d(H2′–H3′) ≈ d(H3–H4)], demonstrating the inversion of the allylic alcohol C3′ configuration during the reaction of BuOH with the carbocation intermediate.

The introduction of the octyl chain into the anomeric position of monosaccharide **4** was carried out through simultaneous transesterification and transglycosidation to yield double-tailed amphiphile **6** (Scheme 2). Compound **5** was saponified (aq. 0.1 N NaOH, acetone, 0 °C) and after acidification of the carboxylate functions (aq. 5% HCl), selective esterification of the nonconjugated carboxyl group under Anand's conditions[11] (Amberlyst A-15 resin, CH_3OH) furnished monoester **7** in addition to the corresponding diester. The major derivative **7** was efficiently isolated in 65% yield after column chromatography (CH_2Cl_2–CH_3OH: 8/2, v/v). Reaction of monosaccharidic esters **4** or **6** with 1.2 equiv. of 1,12-diaminododecane or 1,22-diaminodocosane at rt or at 60 °C in isopropyl alcohol provided the pyranosiduronamides **8a** (90% yield) and **8b** (26% yield), resulting from monoacylation of the diamines. The same strategy was used for the transformation of monoester **7** into uronamides **9a** and **9b** in the presence of triethylamine followed by treatment with IR 120 (Na^+) resin. *N*-acylation of the glycosylated monoamines **8a–8b** and **9a–9b** was efficiently performed with *N*-acyl thiazolidine-2-thione derivative **10**[12] of glycine betaine in DMF. The required bolaamphiphiles **1** and **2** were isolated in 50–77% yield after chromatography and gel filtration on Sephadex G-10.



Scheme 2 *Reagents and conditions*: (i) MSA, $CH_3(CH_2)_7OH$, 65 °C, 2 mbar, 67%; (ii) aq. 0.1 N NaOH, acetone, 0 °C, then aq. 5% HCl, 0 °C, 95%; (iii) CH_3OH, Amberlyst A-15 resin, rt, 65%; (iv) $H_2N(CH_2)_{12}NH_2$, *i*PrOH, rt, **8a**: 90%; (v) $H_2N(CH_2)_{22}NH_2$, *i*PrOH, 60 °C, **8b**: 26%; (vi) **10**, DMF, **1a**: 77%; **1b**: 70%; (vii) $H_2N(CH_2)_{12}NH_2$, NEt_3, *i*PrOH, then IR 120 (Na^+) resin, **9a**: 60%; (viii) $H_2N(CH_2)_{22}NH_2$, NEt_3, *i*PrOH, then IR 120 (Na^+) resin, **9b**: 30%; (ix) **10**, DMF, **2a**: 74%; **2b**: 50%.

The lyotropic phase behaviour of bolaamphiphiles **1–2**, and in particular their supramolecular structures in aqueous media, was studied by means of freeze-fracture electron microscopy (FFEM) and/or differential polarized optical microscopy (POM) and dynamic light scattering (DLS). Firstly, identification of the lyotropic mesophases of compounds **1a–1b** was carried out by POM on cooling from the isotropic liquid (55–60 °C). The contact preparations between bipolar lipids **1a–1b** and water revealed myelins and similar bands of lamellar mesophase between water and the thermotropic mesophase (see ESI†). Our aim was not to describe complete phase behaviours, rather, we focused upon the morphology of the assemblies and the stretched or U-shaped conformation of the bolalipids within the aggregates. At a 10 mg/mL concentration, unsonicated aqueous dispersions of bolaamphiphile **1a** gave at room temperature rods whose dimension did not exceed 100 nm (Fig. 2a). The absence of a fracture plane within the membrane indicates that most of the lipids are in a transmembrane conformation, and only a part of the molecules adopt a U-bent shape to form the edges of the structures.[12] Temperature variation did not alter the aggregates formed since similar assemblies could be visualized when the probe was frozen at higher starting temperatures (up to 60 °C). In more concentrated aqueous media (40 mg/mL), compound **1a** assembled into various phases depending on temperature. At rt, FFEM revealed a complex behaviour: large lamellae, unilamellar vesicles and rods were obtained (Fig. 2b). No membrane splitting along the mid-planes of the membranes was found, and only fractures across the membranes giving rise to curved lines and circles for lamellae and monolayered vesicles, were visualized as in the case of natural archaeal lipids.[13] The structures observed at 60 °C were somewhat different. Large lamellae were still present but unilamellar vesicles and rods completely disappeared (Fig. 2c). In contrast, when the temperature was lowered, only uni- and multilamellar vesicles were observed at 10 °C (Fig. 2d) whereas a mixture of unilamellar vesicles and lamellar crystallites resulting from a Lβ phase where the chains are in a solid state, was produced at 4 °C (Fig. 2e). These results clearly demonstrated the concentration and temperature dependence of the preferred supramolecular structures of bolaamphiphile **1a**. At the lowest temperatures, headgroup hydration enhances solvophobic forces that could enforce the curvature of the monolayers to form vesicles. When the temperature increases, the hydration of charged and neutral polar heads decreases, thus favouring the formation of large lamellae instead of spherical aggregates. Upon dilution, electrostatic interactions between chloride counterions and positive quaternary ammonium groups of the glycine betaine residues are weaker, which probably facilitates the transition from a stretched to a folded molecular conformation in the rod formation.

Interestingly, bolaamphiphile **1b** possessing a longer docosamethylene bridging chain and a shorter butyl chain at the anomeric site of the mannuronamide moiety, furnished large

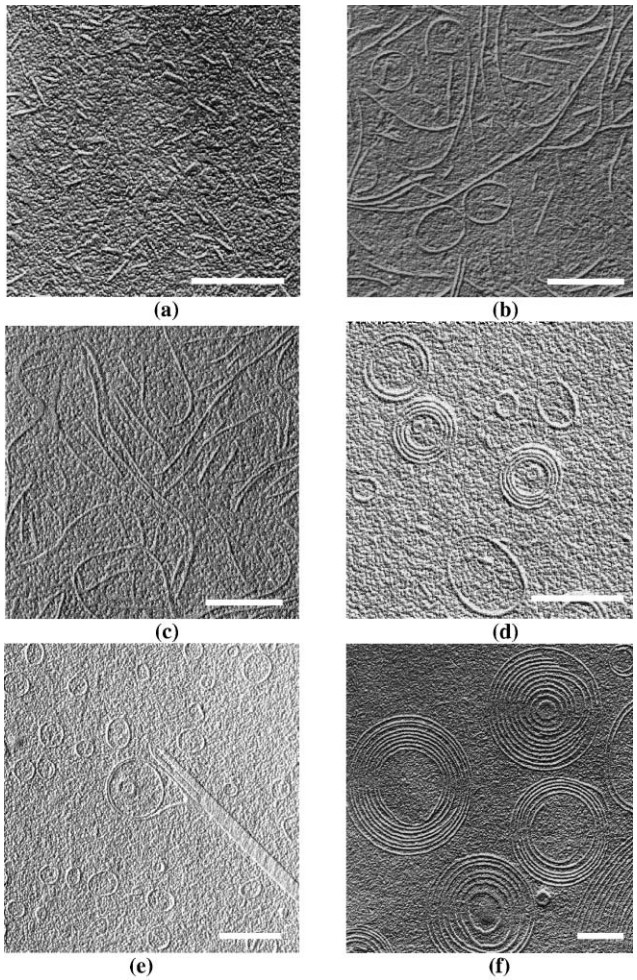


Fig. 2 FFEM of bolaamphiphiles **1a** and **1b** after gentle hydration by vortexing (1–5 min) at a 10 or 40 mg/mL concentration and at various temperatures. The fractured samples were etched before shadowing in order to visualize the propagation path much more clearly. (a) Rods, **1a**, 10 mg/mL, rt. (b) Mixture of cross-fractured large lamellae, rods and unilamellar vesicles, **1a**, 40 mg/mL, rt. (c) Cross-fractured large lamellae, **1a**, 40 mg/mL, 60 °C. (d) Cross-fractured uni- and multilamellar vesicles, **1a**, 40 mg/mL, 10 °C. (e) Mixture of cross-fractured unilamellar vesicles and lamellar crystallite, **1a**, 40 mg/mL, 4 °C. (f) Cross-fractured multilamellar vesicles, **1b**, 40 mg/mL, 25–30 °C. Bar is 200 nm.

multilamellar liposomes of 0.4–1 μm diameter (Fig. 2f) upon vortexing at 25–30 °C (Lβ → Lα transition phase temperature ≈ rt). Here again, there was no fracture plane within the membranes, giving evidence for a trans membrane organization of the monomers. It is important to note that the smallest vesicles (350–400 nm) were still stable at 60 °C, as demonstrated by FFEM and by the size distribution obtained from dynamic light scattering measurements (not shown). The lower sensitivity to temperature of the aggregates formed by compound **1b** may be due to its longer bridging chain which certainly plays a role in membrane curvature and facilitates the formation of more stable vesicles. Preliminary studies on the lyotropic properties of disaccharidic derivatives **2a**–**2b** were carried out by POM. On cooling from the isotropic liquid (80 °C), only bolalipids **2b** produced Maltese crosses (see ESI†) which were indicative of the presence of large multilamellar vesicles.[14] Further experiments based upon FFEM are in progress in order to evaluate the influence of pH variation on bolaamphiphile **2b** aggregation and on the stretched or folded lipid membrane organization of this original anionic–cationic bolalipid containing a pH-sensitive carboxylate function.

In conclusion, the results presented here show the ability of new synthetic dissymmetrical archaeal bolaamphiphile analogues derived from alginate to self-assemble into monolayer membranes. The corresponding physico-chemical studies allowed us to evaluate the influence of the bridging chain length and the monomer concentration in water on the morphology of the aggregates and their sensitivity towards temperature. Applications of these monolayer systems for the encapsulation of negatively or positively charged materials including drugs and nucleic acids are under investigation.

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