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Synthesis and molecular aggregation of new sugar bola-amphiphiles

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Abstract

Novel bola-amphiphilic compounds have been synthesized with D-galactose or lactose as polar head groups. The sugar groups are attached to the six-, ten- or twelve-polymethylene bridging chain by a carbamate function. Vesicles are obtained for some of these derivatives. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Bola-amphiphile surfactants; Sugar head group; Aggregation properties

1. Introduction

A bola-amphiphile is defined as a molecule in which two or more hydrophilic groups are connected by a hydrophobic bridging chain [1]. Over recent years there has been increasing interest in such surfactants from both fundamental and applied points of view. Most of these compounds have been studied for their polymorphic behavior in water as a function of their structures [2–4]. When dispersed in aqueous medium, upon sonication, these molecules often self-organize into monolayer vesicles (monolayer lipid membranes), which are stable over a long period of time to variations of temperature or to ionic strength changes and could be used as membrane models.

Bola-amphiphile surfactants involving sugar at both ends have been synthesized, because of their ability to form vesicles and supramolecular arrangements, and because of their potential application in pharmacy and chemistry [5–7]. The use of sugar as the polar head can offer greater potential for interaction at the cell surface by molecular recognition.

We describe here the synthesis and aggregation properties of new sugar-based bolaforms with different polar heads and alkyl chain having the following general formula:

[sugar]–Y^1–X–Y^2–[sugar]

where Y^1 = –OC(O)NH– and Y^2 = –NHC(O)O–; Y^1 = –NHC(S)NH– and Y^2 = –C(O)NH–; X is a polymethylene bridging chain

The polar head is derived from D-galactose or lactose. A variety of compounds were prepared in order to determine the relationship between molecular structure and the nature of the aggregates.

2. Results and discussion

*Synthesis of bis(*O*-galactopyranosyl) and bis(*O*-lactosyl) bis(carbamate)* **7–10**.—These derivatives were obtained by reaction of one

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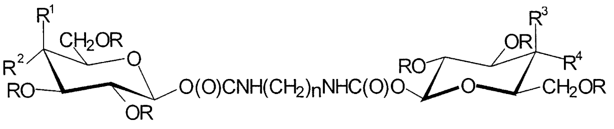
molar equivalent of a α,ω-polymethylene diisocyanate with two equivalents of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose or 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-lactose [8]. The reaction was performed in toluene in the presence of 1,4-diazabicyclo[2.2.2]octane at room temperature for 8 h. Such reaction conditions allowed us to obtain the β anomer with complete diastereoselectivity [9,10]. The expected compounds (**1**–**4**) were obtained in good yield (ca. 70%) after flash chromatography. Their structure was confirmed by NMR spectroscopy. The single resonance observed for C-1 (92–93 ppm) demonstrates that only one anomer is present. The β anomeric configuration is clearly established by the ^{1}H NMR spectrum, which demonstrates the 1,2-trans relationship between H-1 and H-2 (5.6–5.7 ppm, $J_{1,2}$ 8 Hz).

These bola-amphiphiles were O-deacetylated using the Zemplén procedure to give the expected compounds **7**–**10** in quantitative yields.



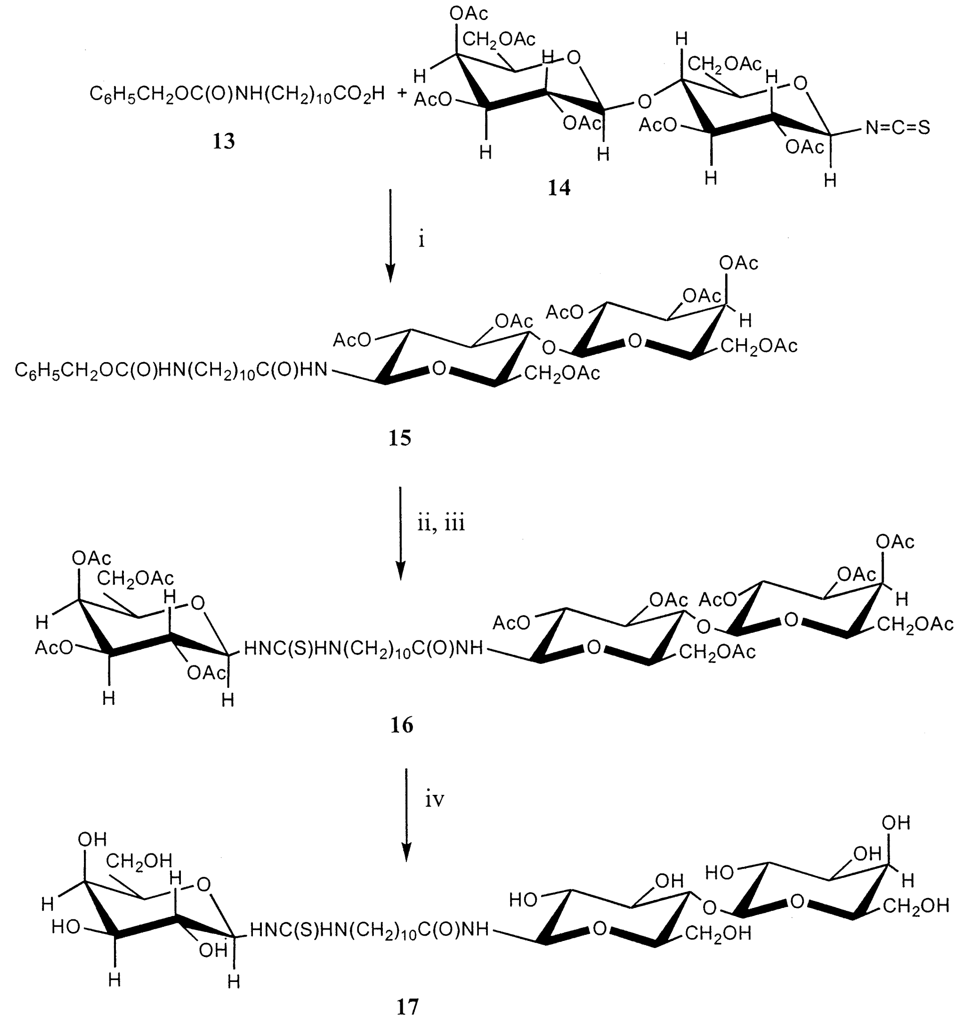
1 n = 6, R =Ac, R^1 = R^3 = OAc, R^2 = R^4 = H
2 n = 12, R =Ac, R^1 = R^3 = OAc, R^2 = R^4 = H
3 n = 6, R = Ac, R^1 = R^3 = H, R^2 = R^4 = 2,3,4,6-tetra-*O*-acetyl-β-D-Gal*p*
4 n = 12, R = Ac, R^1 = R^3 = H, R^2 = R^4 = 2,3,4,6-tetra-*O*-acetyl-β-D-Gal*p*
5 n = 6, R = Ac, R^1= OAc, R^2 = R^3 = H, R^4 = 2,3,4,6-tetra-*O*-acetyl-β-D-Gal*p*
6 n = 12, R = Ac, R^1 = OAc, R^2 = R^3 = H, R^4 = 2,3,4,6-tetra-*O*-acetyl-β-D-Gal*p*
7 n = 6, R = R^2 = R^4 = H, R^1 = R^3 =OH
8 n = 12, R = R^2 = R^4 = H, R^1 = R^3 =OH
9 n = 6, R = R^1 = R^3 = H, R^2 = R^4 = β-D-Gal*p*
10 n = 12, R = R^1 = R^3 = H, R^2 = R^4 = β-D-Gal*p*
11 n = 6, R = R^2 = R^3 = H, R^1 = OH, R^4 =β-D-Gal*p*
12 n = 12, R = R^2 = R^3 = H, R^1 =OH, R^4 =β-D-Gal*p*

Synthesis of O-*galactopyranosyl* O-*lactosyl bis(carbamate)* **11** *and* **12**.—These compounds were obtained, in a one-pot sequence, by successive addition of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose and 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-lactose [8] to a long-chain alkyl diisocyanate in toluene. The expected compounds (**5** and **6**) were obtained in 40% yield after flash chromatography on silica gel. The structures of these compounds were confirmed by ^{13}C NMR spectroscopy. The single resonance observed for each anomeric carbon demonstrates that a single isomer is present. The β configuration was confirmed by the chemical shifts of the anomeric carbon (92 and 93 ppm). Zemplén O-deacetylation afforded the bola-amphiphiles **11** and **12** in almost quantitative yield.

Synthesis of the galactopyranosylthiourea derivative **17**.—The complete reaction scheme for the preparation of this bola-amphiphile is shown in Scheme 1. The amino group of 11-amino decanoic acid was first protected with a *N*-benzyloxycarbonyl group. This product was reacted with the per-*O*-acetyl-β-lactosyl isothiocyanate **14** [11] in toluene in the presence of 1,4-diazabicyclo[2.2.2]octane at room temperature. The structure of the resulting compound **15** was confirmed by NMR spectroscopy. Deprotection of the amino group was performed by hydrogenation with palladium-on-carbon in methanol and the resulting amino compound was reacted with 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isothiocyanate [11] to obtain the bola-amphiphile **16** in 50% yield after purification by flash chromatography. The structure of **16** was confirmed by NMR spectroscopy. The chemical shifts observed for C-1 (90.09 ppm) and C-1′ (101.03 ppm) of the lactose moiety, and C-1 (89.21 ppm) of the galactose part confirmed the β anomeric configuration at both sites. Compound **16** was finally O-deacetylated using the Zemplén procedure to afford **17** in almost quantitative yield.

Molecular aggregation in aqueous solution.—In a preliminary study, the solubility in water of these compounds was measured at 20 °C. Compounds **7**, **9** and **11**, with a six-methylene chain, exhibited a solubility of about 10 g/L. Compounds **8**, **10** and **12**, with a chain of twelve methylene groups, exhibited a solubility of about 5 g/L. As expected, the elongation of the hydrophobic chain reduces the solubility. The bolaform **17** with a chain of ten methylene groups exhibited a solubility of about 10 g/L, similar to that of **7**, **9** and **11** with six methylene groups. This is probably due to introduction of the amide linkage.

Surface tension measurements for compounds **8**, **10** and **12** showed the absence of micellization, which was attributed to the inability of the alkyl chain to bend [12].



Scheme 1. (i) DABCO, toluene, rt; (ii) Pd–H_2, MeOH; (iii) 2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl isothiocyanate, DABCO, toluene, rt; (iv) NaOMe–MeOH.

The formation of vesicles and supramolecular arrangements were specified by quasi-elastic light-scattering measurements. Aggregates were observed after sonication of various bola-amphiphiles solutions at 1.1 to 1.9×10^{-2} M and 25 °C. The stability and the diameter of the aggregates are reported in Table 1.

We also examined the particles by the electron microscopy technique. After sonication of the bolaform solutions, the dispersion was placed on a grid, stained with phosphotungstic acid and dried. The presence of vesicles was observed for compounds **9**, **10**, **12** and **17**, whereas compounds with a short chain length and galactose as polar head did not form vesicles. Compound **9** gave vesicles (Fig. 1) with a diameter of 28 nm for the smaller one and of 408 nm for the larger vesicle. Bola-amphiphile **10** presented a agglomeration of vesicles (Fig. 2), with a size of 114 nm for the smaller vesicle and of 408 nm for the larger vesicle. Compound **12** gave vesicles and tubules (Figs. 3 and 4). The vesicles had a diameter of 51 nm for the smaller one and of

Table 1
Stability and diameter of the aggregates in bola-amphiphiles solutions

Compound	Stability of solution [a] (days)	QLS mean diameter (nm)
9	2	420
10	1	246
11	5	37
12	2	833
17	10	45

[a] Measured at 25 °C in water.

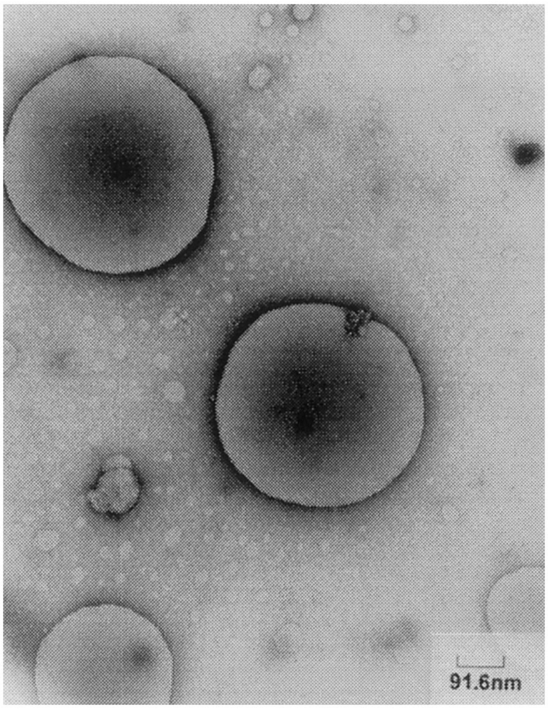


Fig. 1. Electron micrograph of compound **9** (negative staining by phosphotungstic acid).

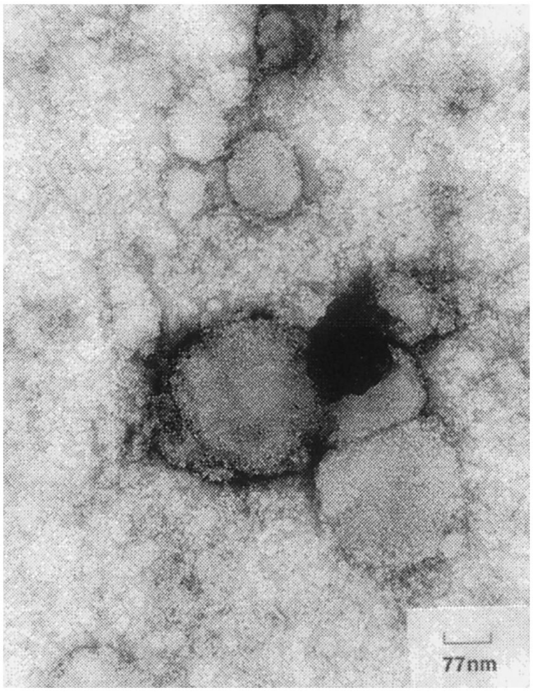


Fig. 2. Electron micrograph of compound **10** (negative staining by phosphotungstic acid).

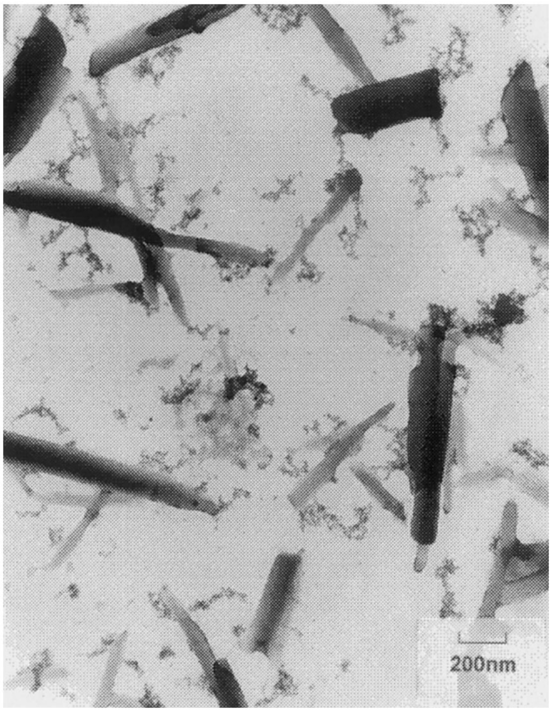


Fig. 3. Electron micrograph of compound **12** (negative staining by phosphotungstic acid).

152 nm for the larger one. Aggregation with subsequent fusion was observed. Study of compound **17** by electron microscopy showed the formation of vesicles (Fig. 5). These vesicles were not stable and fused. The electron microscopic dispersions supported the light-scattering results. The importance of the length of the hydrophobic chain and of the

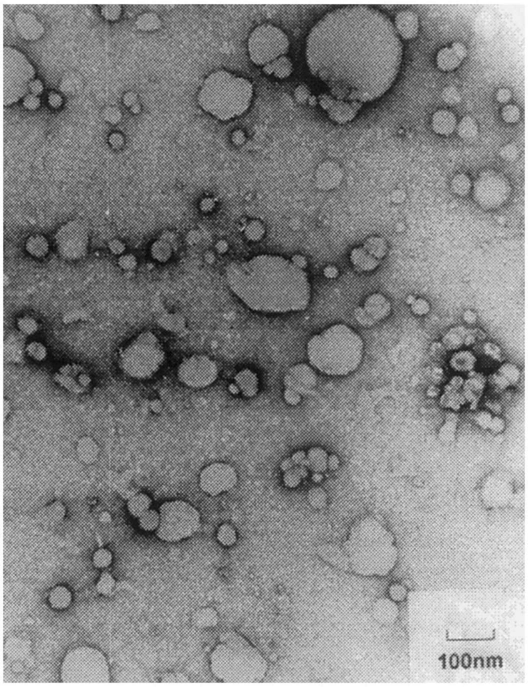


Fig. 4. Electron micrograph of compound **12** (negative staining by phosphotungstic acid).

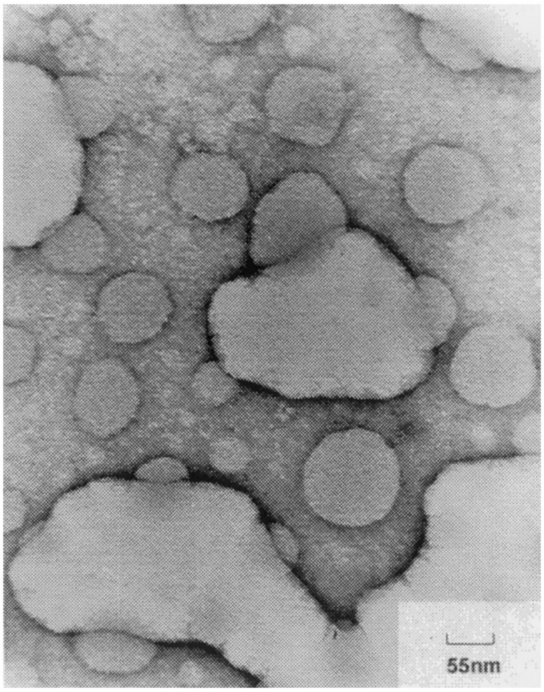


Fig. 5. Electron micrograph of compound **17** (negative staining by phosphotungstic acid).

polar head in the formation of vesicles is to be noticed. A short chain or a short head do not form vesicles.

3. Experimental

General methods.—Melting points were determined on an electrothermal 9100 apparatus and were uncorrected. TLC analysis was performed on aluminum sheets coated with Silica Gel 60 F 254 (E. Merck). Compounds were visualized by spraying the TLC plates with dilute 10% H_2SO_4 in EtOH followed by charring at 150 °C for a few minutes. Column chromatography was performed on Silica Gel Si 60. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter in a 1 dm cell. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer working at 250 and 80 MHz, respectively, with Me_4Si as internal reference. Elemental analyses were performed by the 'Service Central de Microanalyse de Montpellier'.

All reagents were of commercial quality and were purchased from Aldrich.

The critical micelle concentration (cmc) was determined by measuring the surface tension in aq soln at 20 °C, using the ring method with a Tensiometer Kruss K-12.

The compounds were weighed out to make 1.1 to 1.9×10^{-2} M suspensions in deionized water, which were then sonicated for 30 min using a Branson B-30 cell disruptor working at a frequency of 20 kHz with a maximum power of 350 W. The suspensions were filtered through Dynagard 0.45 μm. The quasi-elastic light-scattering measurements were performed with a Semathec F60 apparatus.

The formation of vesicles was also observed by transmission electron microscopy according to the negative staining method. The dispersion was applied on a grid covered with a Formvar membrane, using the drop method: a drop of the liquid dispersion was placed on the grid for 1 min, and the excess was then removed with a filter paper. The sample was colored by depositing a drop of phosphotungstic acid (2%, pH adjusted to 7) for 1 min, the excess being removed using a filter paper. The grid was then dried in an oven at 60 °C. The sample was examined using a Philips microscope (CM/2 model) at 80 kV.

General procedure for the synthesis of symmetrical bola-amphiphiles.—A mixture of the reducing sugar (2 mmol), alkyl diisocyanate (1 mmol) and 1,4-diazabicyclo[2.2.2]octane (DABCO) in toluene was stirred at room temperature (rt) for 18 h. After evaporation to dryness, the mixture was chromatographed on silica gel with 3:2 EtOAc–hexane.

*Bis[*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)] 1,6-hexanediylbis(carbamate)* (**1**). Needles from EtOAc–hexane (71%); mp 47–50 °C; $[\alpha]_D^{20}$ + 14.3° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.65 (d, 2 H, $J_{1,2}$ 8.22, H-1), 5.43 (d, 2 H, H-4), 5.30 (dd, 2 H, H-2), 5.10 (dd, 2 H, H-3), 4.98 (t, 2 H, NH), 4.06–4.24 (m, 6 H, H-5, H-6, H-6′), 3.16–3.21 (m, 4 H, CH_2), 2.06 (s, 6 H, CH_3), 2.05 (s, 6 H, CH_3), 2.04 (s, 6 H, CH_3), 2.00 (s, 6 H, CH_3), 1.51 (m, 4 H, CH_2), 1.33 (m, 4 H, CH_2); ^{13}C NMR ($CDCl_3$): 170.31, 170.12, 169.94 and 169.93 (CH_3*C*O), 153.78 (CONH), 93.26 (C-1), 71.41 (C-5), 70.89 (C-3), 67.98 (C-2), 66.88 (C-4), 60.95 (C-6), 40.90, 29.53 and 26.10 (CH_2), 20.73, 20.64, 20.61 and 20.52 (*C*H_3CO). Anal. Calcd for $C_{36}H_{52}N_2O_{22}$: C, 50.00; H, 6.06; N, 3.24. Found: C, 49.62; H, 6.14; N, 3.18.

*Bis[*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)] 1,12-dodecanediylbis(carbamate)* (**2**). White powder (71%); mp 57–58 °C; $[\alpha]_D^{20}$ + 17.0° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.65 (d, 2 H, *J* 8.25, H-1), 5.43 (d, 2 H, H-4), 5.30 (dd, 2 H, H-2), 5.08 (dd, 2 H, H-3), 4.97 (t, 2 H, NH), 4.06–4.19 (m, 6 H, H-5, H-6, H-6′), 3.16–3.20 (m, 4 H, CH_2), 2.10 (s, 6 H, CH_3), 2.05 (s, 6 H, CH_3), 2.04 (s, 6 H, CH_3), 2.00 (s, 6 H, CH_3), 1.42 (m, 4 H, CH_2), 1.26 (m, 16 H, CH_2); ^{13}C NMR ($CDCl_3$): 170.31, 170.12, 169.91 and 169.62 (CH_3*C*O), 153.73 (CONH), 93.19 (C-1), 71.37 (C-5), 70.90 (C-3), 67.98 (C-2), 66.91 (C-4), 60.97 (C-6), 41.18, 29.63, 29.48, 29.16, 26.91 and 26.67 (CH_2), 20.70, 20.63, 20.60 and 20.52 (*C*H_3CO). Anal. Calcd for $C_{42}H_{64}N_2O_{22}$: C, 53.16; H, 6.80; N, 2.95; O, 37.09. Found: C, 53.20; H, 7.19; N, 2.68; O, 37.00.

*Bis[*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)] 1,6-hexanediylbis(carbamate)* (**3**). White powder (60%); mp 45–46 °C; $[\alpha]_D^{20}$ + 3.9° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.61 (d, 2 H, $J_{1,2}$ 8.32, H-1), 5.34 (d, 2 H, H-4′), 5.24 (t, 2 H, H-2), 4.91–5.14 (m, 6 H, H-2′, H-1′, H-3′), 4.88 (t, 2 H, NH), 4.43–4.49 (m, 4 H, H-3, H-5), 4.03–4.17 (m, 6 H, H-4, H-6′, H-6″), 3.76–3.89 (m, 6 H, H-5′, H-6, H-6′), 3.13–3.18 (m, 4 H, CH_2), 2.11 (s, 6 H, CH_2), 2.07 (s, 6 H, CH_3), 2.05 (s, 6 H, CH_3), 2.04 (s, 6 H, CH_3), 2.02 (s, 6 H, CH_3), 2.01 (s, 6 H, CH_3), 1.97 (s, 6 H, CH_3), 1.48 (m, 4 H, CH_2), 1.31 (s, 4 H, CH_2); ^{13}C NMR ($CDCl_3$): 170.22, 170.14, 170.00, 169.80, 169.56 and 169.00 (CH_3*C*O), 153.73 (CONH), 100.95 (C-1′), 92.63 (C-1), 71.02 (C-2), 75.73 (C-3), 73.31 (C-5), 72.71 (C-4), 70.75 (C-5′), 70.66 (C-3′), 69.08 (C-2′), 66.66 (C-4′), 61.81 (C-6), 60.82 (C-6′), 40.92, 29.43 and 26.14 (CH_2), 20.79, 20.76, 20.67, 20.63, 20.61, 20.55 and 20.49 (*C*H_3CO). Anal. Calcd for $C_{60}H_{84}N_2O_{38}$: C, 50.01; H, 5.87; N, 1.94. Found: C, 49.73; H, 5.75; N, 1.89.

*Bis[*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)] 1,12-dodecanediylbis(carbamate)* (**4**). White powder (50%); mp 60–61 °C; $[\alpha]_D^{20}$ + 34.5° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.61 (d, 2 H, $J_{1,2}$ 8.32, H-1), 5.34 (d, 2 H, H-4′), 5.24 (t, 2 H, H-2), 4.91–5.14 (m, 6 H, H-2, H-1′, H-3′), 4.43–4.49 (m, 4 H, H-3, H-5), 4.03–4.17 (m, 6 H, H-4, H-6′, H-6″), 3.76–3.89 (m, 6 H, H-6, H-6′, H-5′), 3.13–3.18 (m, 4 H, CH_2), 2.11 (s, 6 H, CH_3), 2.07 (s, 6 H, CH_3), 2.05 (s, 6 H, CH_3), 2.04 (s, 6 H, CH_3), 2.02 (s, 6 H, CH_3), 2.01 (s, 6 H, CH_3), 1.97 (s, 6 H, CH_3), 1.48 (m, 4 H, CH_2), 1.31 (s, 16 H, CH_2); ^{13}C NMR ($CDCl_3$): 170.35, 170.15, 170.06, 170.01, 169.93, 169.83 and 169.56 (CH_3*C*O), 153.66 (CONH), 100.94 (C-1′), 92.65 (C-1), 75.77 (C-3), 73.32 (C-5), 72.73 (C-4), 71.04 (C-2), 70.73 (C-5′), 70.66 (C-3′), 69.13 (C-2′), 66.71 (C-4′), 61.91 (C-6), 60.90 (C-6′), 40.20, 29.70, 29.63, 29.49, 29.21 and 26.14 (CH_2), 20.76, 20.65, 20.61, 20.57, 20.48, 20.44 and 20.39 (*C*H_3CO). Anal. Calcd for $C_{66}H_{96}N_2O_{38}$: C, 51.96; H, 6.34; N, 1.34. Found: C, 52.01; H, 6.41; N, 1.26.

General procedure for the synthesis of asymmetrical bola-amphiphiles.—A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (0.5 mmol) in toluene was added drop by drop to alkyl diisocyanate (1 mmol) and DABCO in toluene. The mixture was stirred at rt for 48 h, then 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactose was introduced. The mixture was stirred at rt for 48 h. After evaporation to dryness, the mixture was chromatographed on silica gel with 3:2 EtOAc–hexane.

O*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl)-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl) 1,6-hexanediylbis(carbamate)* (**5**). White powder (38%); mp 79–81 °C; $[\alpha]_D^{20}$ + 8.6° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.64 (d, 1 H, $J_{1,2}$ 8.3), 5.62 (d, 1 H, *J* 8.6), 5.35 (d, 1 H), 5.43 (d, 1 H), 4.93–5.29 (m, 8 H), 4.44–4.49 (m, 2 H), 4.03–4.17 (m, 6 H), 3.78–3.90 (m, 3 H), 3.13–3.20 (m, 4 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.97 (s, 3 H), 1.42 (m, 4 H), 1.26 (s, 4 H); ^{13}C NMR ($CDCl_3$): 170.24, 170.14, 170.12, 170.06, 169.93, 169.79, 169.65, 169.56, 169.45, 169.02 and 169.00 (CH_3*C*O), 153.82 (CONH), 153.72 (CONH), 100.95 (C-1′), 93.24 (C-1), 92.64 (C-1), 75.74 (C-3), 73.30 (C-5), 72.70 (C-4), 71.39 (C-5), 71.02 (C-2), 70.91 (C-3), 70.75 (C-5′), 70.75 (C-3′), 69.08 (C-2′), 67.99 (C-2), 66.90 (C-4), 66.68 (C-4′), 61.83 (C-6), 60.97 (C-6), 60.86 (C-6′), 40.89, 29.50 and 26.08 (CH_2), 20.94, 20.85, 20.79, 20.75, 20.72, 20.66, 20.64, 20.62, 20.52, 20.50 and 20.48 (*C*H_3CO), Anal. Cald for

$C_{48}H_{68}N_2O_{30}$: C, 50.00; H, 5.94; N, 2.43. Found: C, 49.71; H, 5.87; N, 2.31.

O-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galactopyranosyl)* O-*(2,3,6,2′,3′,4′,6′-hepta*-O-*acetyl-β-lactosyl) 1,12-dodecanediylbis(carbamate)* (**6**). White powder (20%); mp 90–92 °C; $[\alpha]_D^{20}$ + 26.4° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.65 (d, 1 H, $J_{1,2}$ 8.5), 5.63 (d, 1 H, $J_{1,2}$ 8.5), 5.43 (d, 1 H), 5.34 (d, 1 H), 4.88–5.26 (m, 8 H), 4.42–4.52 (m, 2 H), 4.03–4.23 (m, 6 H), 3.74–3.88 (m, 3 H), 3.16–3.20 (m, 4 H), 2.11 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.42 (m, 4 H), 1.27 (s, 16 H); ^{13}C NMR ($CDCl_3$): 170.34, 170.22, 170.14, 170.07, 170.00, 169.80, 169.65, 169.56, 169.45, 169.01 and 169.00 ($\mathrm{CH_3}\mathit{C}\mathrm{O}$), 153.70 (CONH), 153.61 (CONH), 100.95 (C-1′), 93.22 (C-1), 92.60 (C-1), 75.77 (C-3), 73.27 (C-5), 72.69 (C-4), 71.39 (C-5), 71.02 (C-2), 70.91 (C-3), 70.75 (C-5′), 70.65 (C-3′), 69.05 (C-2′), 67.97 (C-2), 66.87 (C-4), 66.64 (C-4′), 61.87 (C-6), 60.95 (C-6), 60.84 (C-6′), 41.19, 38.76, 30.38, 29.70, 29.63, 29.48, 29.20, 28.94, 26.67, 25.36, 23.77 and 22.98 (CH_2), 20.96, 20.86, 20.79, 20.76, 20.73, 20.67, 20.63, 20.61, 20.55, 20.50 and 20.49 ($\mathit{C}\mathrm{H_3CO}$). Anal. Calcd for $C_{54}H_{80}N_2O_{30}$: C, 52.42; H, 6.51; N, 2.26. Found: C, 52.35; H, 6.60; N, 2.21.

N-*(2,3,6,2′,3′,4′,6′-Hepta*-O-*acetyl-β-lactopyranosyl) 11-benzyloxycarbonylamino-undecanamide* (**15**). A mixture of ZNH(CH_2)$_{10}$$CO_2H$ (**13**) (0.169 g, 0.5 mmol), 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl isothiocyanate (**14**) (0.340 g, 0.5 mmol) and DABCO in toluene was stirred 8 h at rt. The solvent was removed and the residue was then chromatographed on silica gel with 3:2 hexane–EtOAc. It was obtained as a white powder (40%); mp 75–77 °C; $[\alpha]_D^{20}$ +29.2° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 7.14–7.35 (m, 5 H), 6.26 (d, 1 H), 5.43–5.51 (m, 2 H), 5.35 (d, 1 H), 4.79–5.23 (m, 4 H), 4.65 (d, 1 H, $J_{1,2}$ 7.67), 4.42–4.52 (m, 2 H), 3.76–4.29 (m, 6 H), 3.13–3.16 (m, 1 H), 2.31 (m, 1 H), 2.10 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.62 (m, 2 H), 1.46 (m, 2 H), 1.26 (m, 12 H); ^{13}C NMR ($CDCl_3$): 178.16 (CONH), 170.50, 170.35, 170.27, 169.89, 169.78, 169.36 and 169.23 (CH_3CO), 128.49 and 128.06 (Ph), 100.91 (C-1′), 89.93 (C-1), 76.40 (C-3), 74.47 (C-5), 73.21 (C-4), 71.53 (C-2), 71.05 (C-5′), 70.58 (C-3′), 69.22 (C-2′), 66.57 (C-4), 61.01 (C-6), 60.47 (C-6′), 41.09, 33.93, 29.88, 29.39, 29.28, 29.16, 29.01, 26.99, 26.68 and 24.72 (CH_2), 20.90, 20.83, 20.74, 20.66, 20.63, 20.56 and 20.51 ($\mathit{C}\mathrm{H_3CO}$). Anal. Calcd for $C_{45}H_{64}N_2O_{20}$: C, 56.72; H, 6.76; N, 2.94. Found: C, 56.51; H, 6.82; N, 3.01.

N-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galactopyranosyl)*-N′-*[10-(2,3,6,2′,3′,4′,6′-hepta*-O-*acetyl-β-lactosylaminocarbonyl)decan-1-yl]thiourea* (**16**). To a mixture of **15** (0.63 g, 0.66 mmol) in MeOH was added Pd–C (40 mg). The solution was saturated with hydrogen at 8×10^5 Pa. After 4 h, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The syrup obtained was reacted with isothiocyanate 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl (0.56 g, 0.66 mmol) in the presence of DABCO in toluene. The solution was stirred 18 h at rt. The solvent was removed and the residue was then chromatographed on silica gel with 3:2 EtOAc–hexane. It was obtained as a white powder (47%); mp 74–76 °C; $[\alpha]_D^{20}$ +44.9° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): 5.85 (d, 1 H), 5.36–5.77 (m, 6 H), 4.94–5.24 (m, 3 H), 4.78–4.89 (m, 5 H), 4.65 (d, 1 H), 4.43–4.52 (m, 2 H), 4.03–4.23 (m, 6 H), 3.74–3.88 (m, 3 H), 3.16–3.20 (m, 2 H), 2.35 (m, 2 H), 2.11 (s, 3 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H), 1.42 (m, 4 H), 1.26 (s, 12 H); ^{13}C NMR ($CDCl_3$): 178.70 (CONH), 170.34, 170.22, 170.13, 170.07, 170.00, 169.76, 169.65, 169.26, 169.19, 169.07 and 169.00 ($\mathrm{CH_3}\mathit{C}\mathrm{O}$), 165.34 (NHCSNH), 101.03 (C-1′), 90.09 (C-1), 89.21 (C-1), 76.26 (C-3), 74.51 (C-5), 73.08 (C-4), 71.41 (C-2), 71.39 (C-5), 70.91 (C-3), 70.75 (C-5′), 70.65 (C-3′), 69.05 (C-2′), 67.97 (C-2), 66.87 (C-4), 66.64 (C-4′), 61.87 (C-6), 60.95 (C-6), 60.84 (C-6′), 20.96, 20.86, 20.79, 20.76, 20.73, 20.67, 20.63, 20.61, 20.55, 20.50 and 20.49 ($\mathit{C}\mathrm{H_3CO}$), 41.19, 38.76, 30.38, 29.70, 29.63, 29.20, 26.67, 25.36, 23.77 and 22.98 (CH_2). Anal. Calcd for $C_{52}H_{77}N_3O_{27}S$: C, 51.16; H, 6.37; N, 3.47. Found: C, 50.98; H, 6.62; N, 3.30.

General O-deacetylation procedure.—The fully protected glycoside was dissolved in dry MeOH and treated with a catalytic amount of NaOMe. After 2 h at rt, the mixture was neutralized with Amberlite IRC 50 (H^+ form), filtered and evaporated. The compounds obtained were analytically pure, in almost quantitative yield.

*Bis[*O*-(β-*D*-galactopyranosyl)] 1,6-hexanediylbis(carbamate)* (**7**). White hygroscopic powder; $[\alpha]_D^{20}$ + 6.9° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.25 (d, 2 H, $J_{1,2}$ 7.9), 3.83 (d, 2 H), 3.56–3.70 (m, 10 H), 3.31 (dd, 2 H), 3.11 (t, 4 H), 1.50 (m, 4 H), 1.31 (s, 4 H); ^{13}C NMR (CD_3OD): 157.31 (CONH), 97.25 (C-1), 77.12 (C-5), 74.84 (C-3), 71.47 (C-2), 70.06 (C-4), 62.30 (C-6), 41.93, 32.76 and 27.65 (CH_2). Anal. Calcd for $C_{20}H_{36}N_2O_{14}$: C, 45.40; H, 6.81; N, 5.30. Found: C, 45.21; H, 7.02; N, 5.15.

*Bis[*O*-(β-*D*-galactopyranosyl)] 1,12-dodecanediylbis(carbamate)* (**8**). White hygroscopic powder; $[\alpha]_D^{20}$ + 9.6° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.25 (d, 2 H, $J_{1,2}$ 7.9), 3.83 (d, 2 H), 3.56–3.70 (m, 10 H), 3.31 (dd, 2 H), 3.11 (t, 4 H), 1.50 (m, 4 H), 1.31 (s, 16 H); ^{13}C NMR (CD_3OD): 157.26 (CONH), 97.31 (C-1), 77.15 (C-5), 74.81 (C-3), 71.44 (C-2), 70.10 (C-4), 62.27 (C-6), 41.98, 33.16, 32.63, 32.48, 27.91 and 27.67 (CH_2). Anal. Calcd for $C_{26}H_{36}N_2O_{14}$: C, 52.00; H, 6.04; N, 4.66. Found: C, 51.76; H, 6.14; N, 4.58.

*Bis[*O*-(β-lactosyl)] 1,6-hexanediylbis(carbamate)* (**9**). White hygroscopic powder; $[\alpha]_D^{20}$ + 2.5° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.35 (d, 2 H, $J_{1,2}$ 8.2), 4.36 (d, 2 H), 3.29–3.95 (m, 26 H), 3.10 (t, 4 H), 1.46 (m, 4 H), 1.29 (s, 4 H); ^{13}C NMR (CD_3OD): 157.61 (CONH), 104.95 (C-1′), 96.60 (C-1), 79.87 (C-4), 77.18 (C-5′), 77.05 (C-3), 76.42 (C-5), 74.89 (C-2), 73.64 (C-3′), 72.53 (C-2′), 70.30 (C-4′), 62.60 (C-6′), 61.46 (C-6), 41.92, 32.33 and 27.24 (CH_2). Anal. Calcd for $C_{32}H_{56}N_2O_{24}$: C, 45.07; H, 6.62; N, 3.28. Found: C, 44.80; H, 6.82; N, 3.15.

*Bis[*O*-(β-lactosyl)] 1,12-dodecanediylbis(carbamate)* (**10**). White hygroscopic powder; $[\alpha]_D^{20}$ + 17.0° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.30 (d, 2 H, $J_{1,2}$ 8.2), 3.29–3.95 (m, 28 H), 3.10 (t, 4 H), 1.46 (m, 4 H), 1.29 (s, 16 H); ^{13}C NMR (CD_3OD): 157.66 (CONH), 104.94 (C-1′), 96.61 (C-1), 79.87 (C-4), 77.19 (C-5′), 77.06 (C-3), 76.44 (C-5), 74.88 (C-2), 73.64 (C-3′), 72.53 (C-2′), 70.35 (C-4′), 62.60 (C-6′), 61.46 (C-6), 41.91, 32.80, 32.71, 32.59, 32.31 and 27.22 (CH_2). Anal. Calcd for $C_{38}H_{68}N_2O_{24}$: C, 48.69; H, 7.26; N, 2.99. Found: C, 48.78; H, 7.71; N, 3.02.

O*-(β-*D*-Galactopyranosyl)* O*-(β-lactosyl) 1,6-hexanediylbis(carbamate)* (**11**). White hygroscopic powder; $[\alpha]_D^{20}$ + 6.6° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.35 (d, 1 H, $J_{1,2}$ 7.9), 5.30 (d, 1 H, $J_{1,2}$ 8.6), 3.30–4.32 (m, 21 H), 3.10 (t, 4 H), 1.46 (m, 4 H), 1.29 (s, 4 H); ^{13}C NMR (CD_3OD): 157.80 and 157.75 (CONH), 104.94 (C-1′ Lac), 97.31 (C-1 Gal), 96.61 (C-1 Lac), 79.87 (C-4 Lac), 77.19 (C-5′ Lac), 77.15 (C-5 Gal), 77.06 (C-3 Lac), 76.44 (C-5 Lac), 74.88 (C-2 Lac), 74.81 (C-3 Gal), 73.64 (C-3′ Lac), 72.53 (C-2′ Lac), 71.44 (C-2 Gal), 70.35 (C-4′ Lac), 70.10 (C-4 Gal), 62.60 (C-6′ Lac), 62.27 (C-6 Gal), 61.46 (C-6 Lac), 41.89, 32.50 and 27.08 (CH_2). Anal. Calcd for $C_{26}H_{46}N_2O_{19}$: C, 45.19; H, 6.66; N, 4.05. Found: C, 45.6; H, 6.80; N, 4.15.

O*-(β-*D*-Galactopyranosyl)* O*-(β-lactosyl) 1,12-dodecanediylbis(carbamate)* (**12**). White hygroscopic powder; $[\alpha]_D^{20}$ + 14.7° (*c* 1.0, MeOH); ^1H NMR (CD_3OD): 5.35 (d, 1 H, $J_{1,2}$ 7.9), 5.30 (d, 1 H, $J_{1,2}$ 8.6), 3.28–4.35 (m, 21 H), 3.10 (t, 4 H), 1.46 (m, 4 H), 1.29 (s, 16 H); ^{13}C NMR (CD_3OD): 157.70 and 157.61 (CONH), 104.94 (C-1′ Lac), 97.33 (C-1 Gal), 96.63 (C-1 Lac), 79.86 (C-4 Lac), 77.19 (C-5′ Lac), 77.17 (C-5 Gal), 77.09 (C-3 Lac), 76.44 (C-5 Lac), 74.85 (C-2 Lac), 74.80 (C-3 Gal), 73.64 (C-3′ Lac), 72.53 (C-2′ Lac), 71.43 (C-2 Gal), 70.35 (C-4′ Lac), 70.07 (C-4 Gal), 62.60 (C-6′ Lac), 62.21 (C-6 Gal), 61.41 (C-6 Lac), 41.89, 40.76, 32.38, 31.70, 31.63, 31.48, 31.20, 30.64, 27.67, 26.36, 24.77 and 23.98 (CH_2). Anal. Calcd for $C_{32}H_{58}N_2O_{19}$: C, 49.58; H, 7.49; N, 3.61. Found: C, 49.75; H, 7.61; N, 3.75.

N*-(β-*D*-Galactopyranosyl)-*N′*-[10-(β-lactosylaminocarbonyl)decan-1-yl]thiourea* (**17**). White powder; mp 133–135 °C; $[\alpha]_D^{20}$ + 19.6° (*c* 1.0, CH_2Cl_2); ^1H NMR (CD_3OD): 3.29–4.9 (m, 26 H), 3.20 (t, 2 H), 3.10 (t, 4 H), 1.46 (m, 4 H), 1.29 (s, 12 H); ^{13}C NMR (CD_3OD): 177.75 (CONH), 171.59 (NHCSNH), 104.94 (C-1′ Lac), 85.82 (C-1 Gal), 81.60 (C-1 Lac), 78.86 (C-4 Lac), 78.17 (C-2 Gal), 77.19 (C-5′ Lac), 77.09 (C-3 Lac), 76.44 (C-5 Lac), 75.91

(C-3 Gal), 74.85 (C-2 Lac), 73.64 (C-3′ Lac), 72.53 (C-2′ Lac), 71.65 (C-4 Gal), 70.80 (C-5 Gal), 70.35 (C-4′ Lac), 62.95 (C-6 Gal), 62.60 (C-6′ Lac), 61.41 (C-6 Lac), 41.90, 40.75, 33.31, 32.76, 32.62, 32.20, 27.57, 26.26, 24.47 and 23.28 (CH_2). Anal. Calcd for $C_{30}H_{55}N_3O_{16}S$: C, 48.34; H, 5.95; N, 3.76. Found: C, 47.99; H, 5.86; N, 3.74.

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