

Synthesis and aggregation behaviour of symmetric glycosylated bolaamphiphiles in water

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This work is dedicated to the Professor Armand Lattes for his great contribution to the organic and supramolecular chemistry

Abstract

The synthesis of a new family of symmetric bolaamphiphiles derived from tris(hydroxymethyl)aminomethane (Tris) is described. The specific glycosylation of Tris hydroxyl groups allows modulation of their polar head volumes. Symmetric bolaamphiphiles were prepared by grafting the Tris moieties onto a hydrophobic chain through urea or amide bonds and a glyceryl linker. Moreover, the introduction of phenyl groups or a perfluoroalkyl chain afforded increased rigidity of the hydrophobic spacer arm. A diluted water suspension of these amphiphiles did not show a supramolecular organisation any larger than micellar-type aggregates

Keywords: Bolaamphiphile, vesicles, micelles, aggregation behavior, supramolecular chemistry

Introduction

Bolaamphiphilic compounds consist of a hydrophobic chain with two polar head groups at its extremities.¹ By virtue of their chemical structure, these compounds can form monolamellar membranes, vesicles or fibrous organisations in aqueous solution. These synthetic surfactants are in fact a copy of the lipids which form the cytoplasmic membranes of the archaebacteria, microorganisms which live in extreme conditions of temperature, pressure or pH.^{2,3} In order to withstand these extreme living conditions, the hydrophobic chains which form the lipid bilayer of their cell membranes are fused, creating macrocyclic molecules not easily synthesised in a

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laboratory.⁴ For 30 years, the scientific community has been trying to synthesise lipid analogues capable of forming non-merging vesicle membranes. From this point of view, considering the slower rate of the membrane fusion phenomenon in monolamellar membranes, the bolaform surfactants represent a good alternative and archaeal membranes have begun to be used in the pharmaceutical preparation of liposomes.⁵

In the past 20 years, many papers have been published on this subject and numerous simplified models of archaeal lipid molecules have been synthesised.⁶ Indeed, the preparation of these macrocyclic molecules is a real challenge for chemists. In most cases, chemists contented themselves with preparing molecules consisting of only one hydrocarbon chain ended with polar heads. These monostrand compounds often formed fibrous networks in aqueous solution, due to the setting up of intermolecular hydrogen bonds within the membranes.⁷⁻¹³ On the other hand, few examples were reported describing the formation of vesicle systems by bolaform surfactants. One of the main concerns in the formation of monolamellar vesicle systems from bolaform surfactants is the nature of the spacer arm and its ability to not fold up again (figure 1).

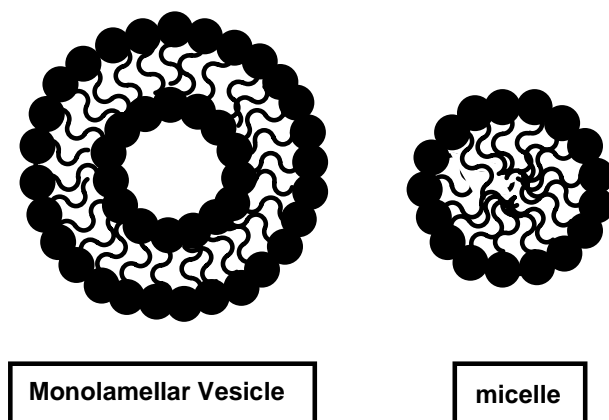
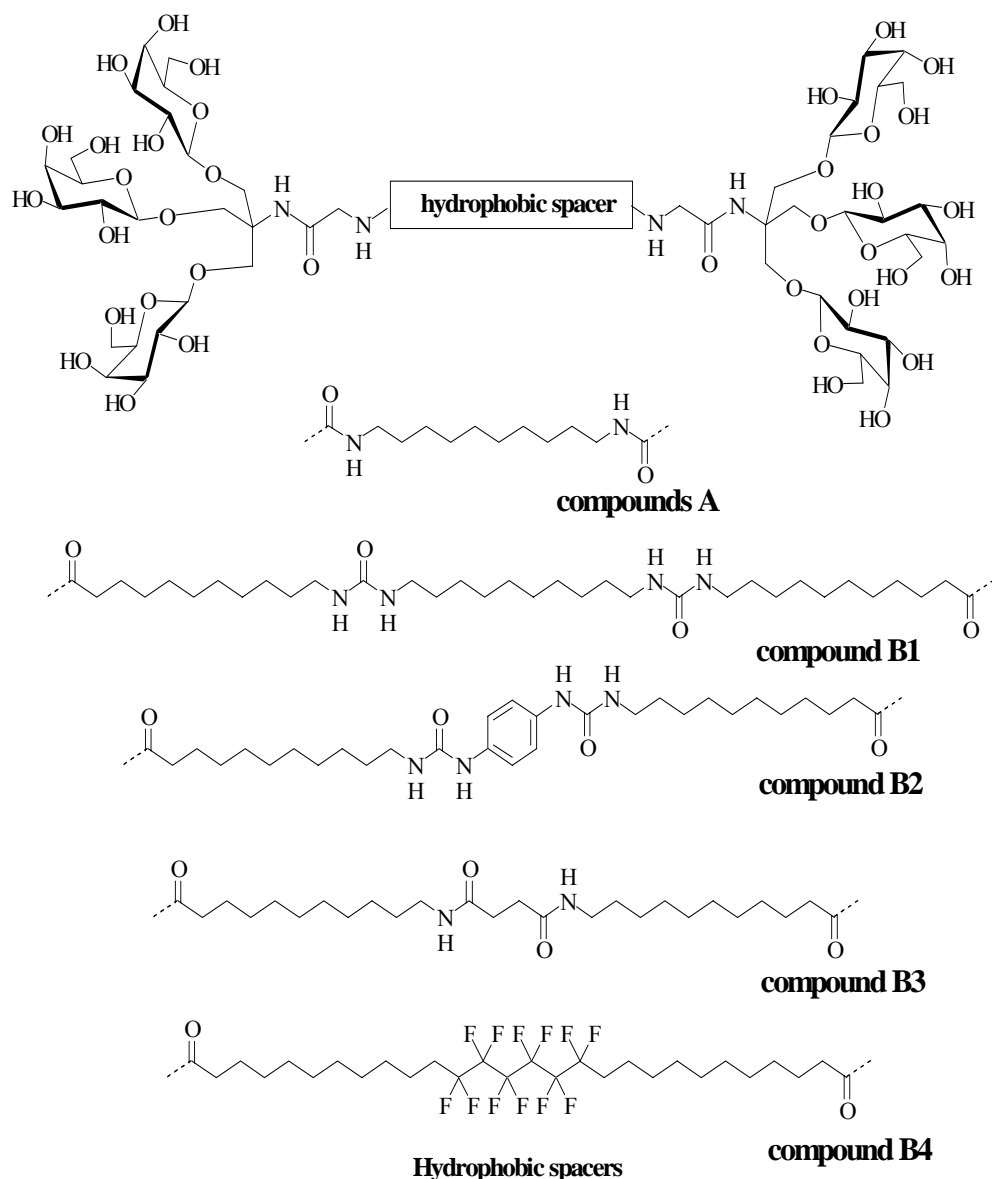


Figure 1. Structures of micelles and vesicles formed by bolaamphiphiles in aqueous solution.

Munoz *et al*¹⁴ and Brisset *et al*¹⁵ reported a significant impact of the length of the hydrophobic linkage on the aggregation properties of bolaamphiphiles with crown ether or sugar heads. These authors suggested a bending of chains consisting of 12 methylene groups or more. The aim of the work presented here is to specify the impact of various structural parameters, the length and rigidity of the hydrophobic chain and/or volume of the two polar heads, on the ability of monostrand bolaform surfactants to form vesicle-type or fibrous-type supramolecular organisations in aqueous solution. In order to modulate these different structural parameters, Tris(hydroxymethyl)aminomethane (Tris) was chosen as starting material.

Results and Discussion

Tris is the basic component of the polar heads of these new bolaform compounds. The use of such a polyfunctional molecule permits modulation of the HLB (Hydrophilic Lipophilic Balance) of bolaamphiphiles by the grafting one, two or three galactopyranose moieties onto its hydroxyl groups. The hydrophilic head groups were linked to a hydrocarbon chain through urea or amide bonds and a glyceryl linker. The Tris amine function ensures the junction with the hydrophobic component (scheme 1).

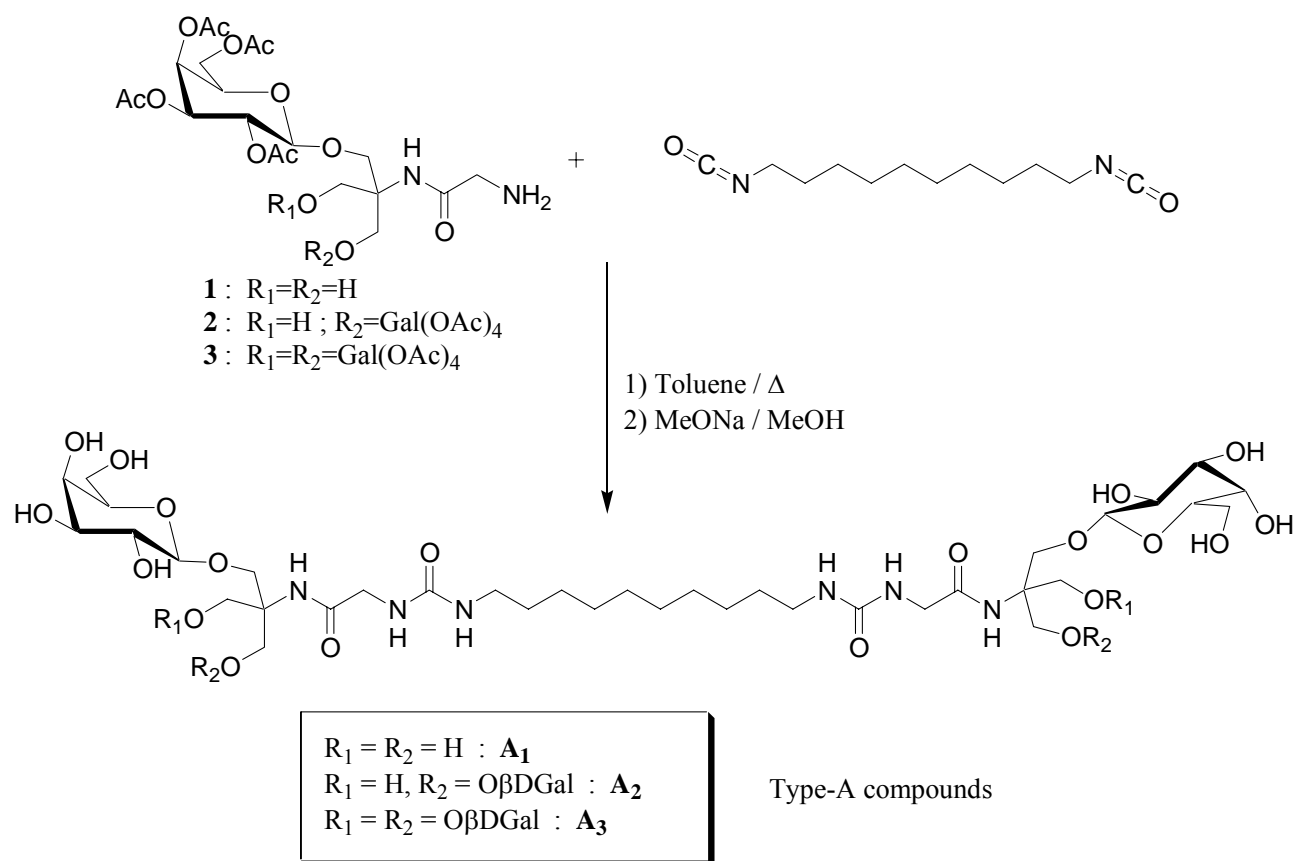


Scheme 1. Structure of Tris bolaamphiphile compounds

In order to determine the impact of the hydrophobic linker on the aqueous behaviour of these new bolaamphiphiles, we used different alkyl chain lengths, bearing 10 to 30 carbon atoms (type-**A** compounds and compounds **B1**, **B3**). We tried to rigidify the hydrophobic portion by including a phenyl group (compound **B2**) or a perfluorocarbon chain (compound **B4**). Such rigid moieties should theoretically inhibit the bending of the chain.

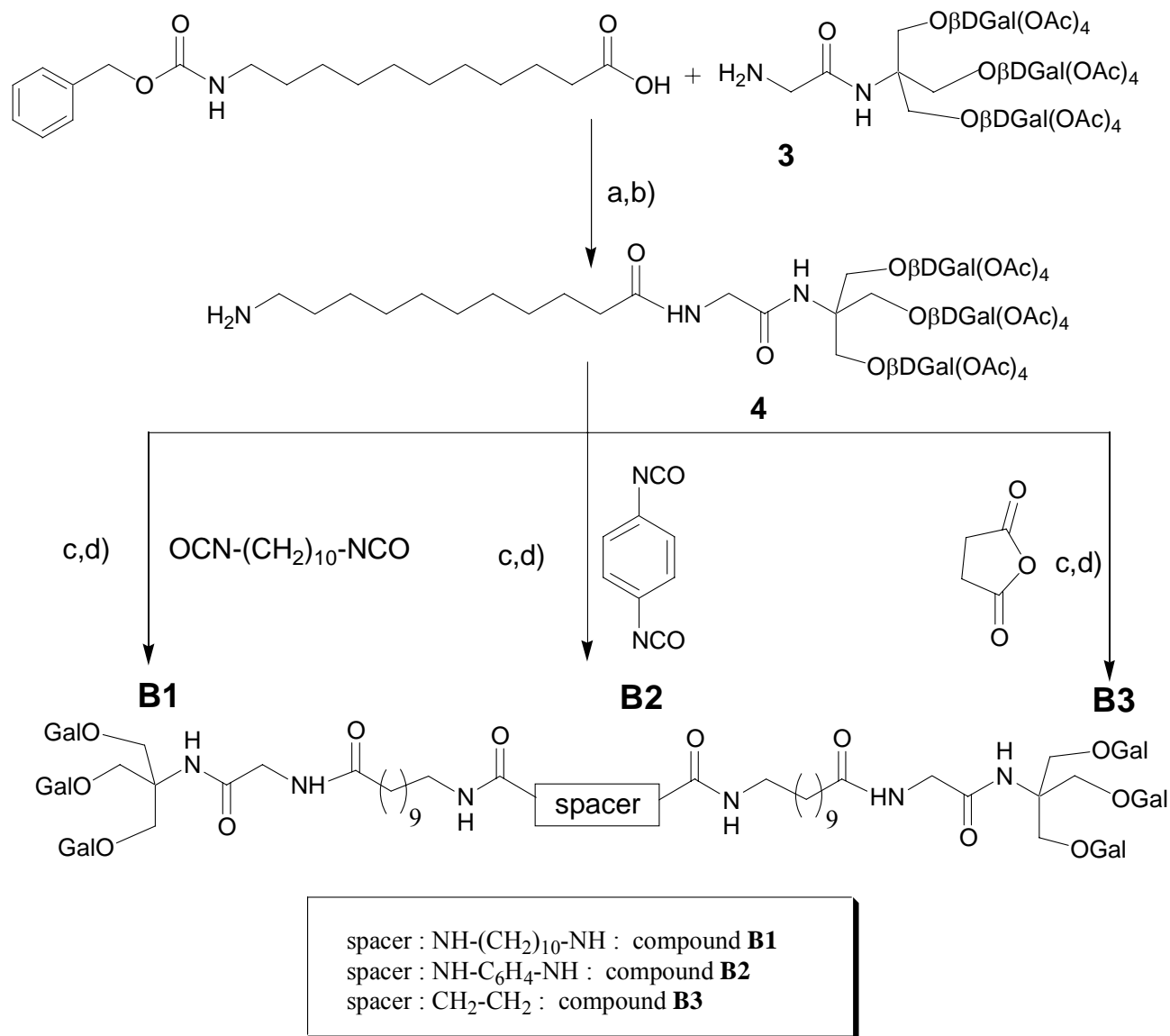
Synthesis of type-A and type-B compounds

The glycosylated polar heads **1-3** were synthesised according to a general procedure (scheme 2) previously described.¹⁶



Scheme 2. Synthesis of tupe A compounds

Commercially available 1,10-diisocyanate was chosen as a hydrophobic linker of type-**A** compounds in order to avoid bending of the chain as previously described by Brisset et al.¹⁵ The grafting of the diisocyanate and polar portions was achieved in dry toluene at room temperature in the presence of catalytic amounts of diazabicyclooctane (DABCO). This reaction produced the final **A**-type compounds in yields ranging from 50 to 80% after column chromatography on silica gel. Compounds **B1-B3** were synthesized in 4 steps (Scheme 3).



Scheme 3. Synthesis pathway of Tris hydrocarbon B-type bolaamphiphiles

The reaction of benzylloxycarbonyl-amino-11-undecanoic acid with compound **3** was catalyzed by a mixture of dicyclohexylcarbodiimide and dimethyl aminopyridine (DCC/DMAP). Subsequent hydrogenation on palladium on charcoal (Pd/C) provided the deprotected synthon **4**. Decyl diisocyanate and phenyl diisocyanate were then treated with an excess of synthon **4** in toluene to give acetylated bolaamphiphiles **B1** and **B2** in a good overall yield (30-38%). Reaction of synthon **2** with succinic anhydride, in the presence of DCC/DMAP as coupling reagent, provided compound **B3** with an overall yield of 23 %. Pure compounds **B1-B3** were

Pd/C in ethanol. The diacid **7** thus obtained reacted with synthon **1** in the presence of DCC/DMAP as a coupling reagent, with a low yield (23%). The type-**A** and **B** bolaamphiphiles were deacetylated according to the Zemplen method, by transesterification in methanol with a catalytic amount of sodium methoxide. All compounds were isolated after gel filtration on sephadex G-25, freeze-dried and were fully characterized by NMR spectroscopy and mass spectroscopy (see experimental section).

Aggregation properties of Tris derived bolaamphiphiles

The physico-chemical features and aggregation properties of these compounds are summarised in Table 1. Dilute dispersions of all these compounds were obtained by vortex mixing at 50 °C and sonication for 10 min at 25 °C using a 3 mm probe. They were then studied by Transmission Electron Microscopy (TEM), after negative staining with uranyl acetate, and Freeze-Fracture Electron Microscopy (FFEM). The critical aggregation concentrations (CAC) were determined either by measuring their superficial tension *vs* concentration, or by the dye method in spectrofluorimetry (with pyrene) or in colorimetry, according to Menger's method with pinacyanol chloride.¹⁸

Table 1. Physico-chemical data of compounds **A-B**

Entries	Overall yield %	F ⁽¹⁾ (° C)	$[\alpha]_D^{20(1)}$	CAC ³ (mM)	Aggregate diameter (nm)	Aggregati on State ⁴
A1	22	63	-2.2°	ND		-
A2	22	90	-10.1°	50		-
A3	28	102	-10.4°	ND		-
B1	38	88	-8.3°	0.066	9	micelles
B1cs ²²				0.013 ⁵		
B2	30	83	-7.1°	ND ²		micelles
B3	23	101	-9.3°	1.2		micelles
B3cs ²¹				1 ⁵		
B4	6	96	-10.7°	0.0049	6.8	micelles

¹Acetylated compounds ²Not Determined ³Determined by pyrene method ⁴Determined by ffem ⁵Determined by surface tension measurement

Type **A** compounds bearing a ten carbon atom hydrophobic portion did not show any visible objects on the images obtained by FFEM, regardless of the number of galactosylated units grafted on the polar head (figure not shown). This observation indicates the absence of supramolecular organisations larger than very small micelle-like aggregates.

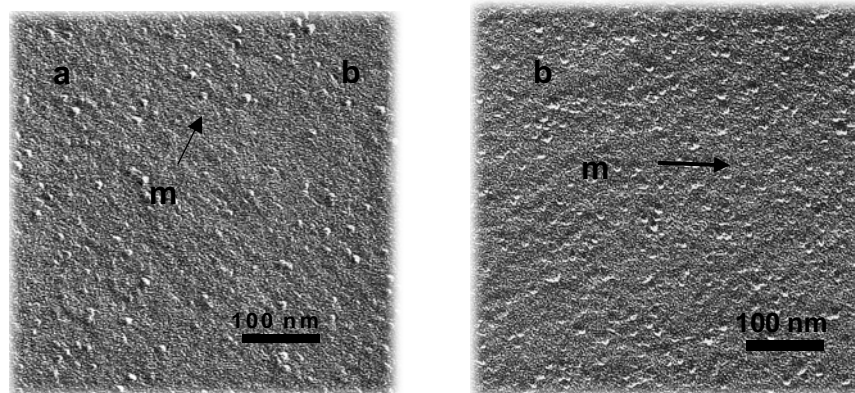


Figure 2. Electron micrographs after freeze-fracture of compound **B1** at 100 mg/ml (a) and compound **B4** at 100 mg/ml (b) in water.

It should be emphasized that the micrographs obtained by TEM, after negative staining by uranyl acetate, showed vesicle-like aggregates, at variance with the FFEM results (picture not shown). This result reveals a difficulty in properly analyzing supramolecular organisations by TEM alone; in many cases artifacts were observed. Thus, all results obtained by TEM should be confirmed with other techniques such as FFEM or cryoTEM.

Apparently, for short hydrophobic spacers, the HLB does not play a key role on the aggregation behaviour of these bolaamphiphilic compounds. It seems that the hypothesis of the impact of the spacer length previously proposed can not be applied to all kinds of bolaform surfactants.^{14,15} The formation of unilamellar vesicles (ULV) from mono-strand surfactants seems to depend on other parameters than spacer length.

Thus, in order to specify the association ability of these compounds in aqueous solutions, we tried to highlight and measure their possible critical aggregation concentration. The surface tension measurements did not allow us to determine a CAC up to 0.1 M. Indeed, with this method, equilibrium surface tension values were difficult to obtain. The CAC determinations performed with compound **A2**, showed an atypical behaviour with the dye methods (figure 3), quite different from other surfactants.¹⁸ Indeed, if an aqueous solution of pinacyanol chloride containing, for example, the perfluorocarbon bolaform surfactant presented a constant absorbance up to the Critical Micellar Concentration (CMC) at 610 nm and a marked decrease of the absorbance below the CMC, one observed a continuous decrease of the absorbance vs dilution with the bolaform surfactant **A2**. One can assume that, unlike the micelle-like aggregates which fully dissociated at concentrations lower than the CMC, compound **A2** aggregates broke up slowly upon dilution.

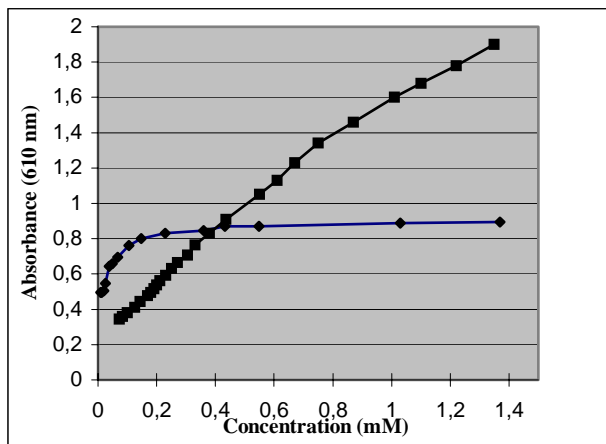


Figure 3. Plot of the absorbance of 1.10^{-5} M pinacyanol chloride at 610 nm versus concentration of fluorinated **B4** (◆) and hydrocarbon **A2** (■) bolaamphiphile.

The critical micelle concentration of compound **A2** was also determined by spectrofluorimetry with pyrene as a probe (figure 4a). The fluorescence emission spectra were obtained with aerated solutions at very low pyrene concentration (2.10^{-6} M) at an excitation wavelength of 335 nm. The spectra were used to specify the I_1/I_3 ratio of the intensities of the first and third vibronic peaks of pyrene monomer. The I_1/I_3 ratio is sensitive to the microenvironment of pyrene and can be used to highlight any change in polarity sensed by the pyrene probe while, upon micelle or aggregate formation, the pyrene probe becomes partly or totally solubilised within the aggregates. The change of I_1/I_3 ratio vs surfactant concentration can thus be used to obtain the CMC of bolaamphiphilic compounds. The CAC values were determined at the point of the initial break in the I_1/I_3 value vs log C curve (figure 4a).

This very sensitive procedure allowed us to measure a CAC for the compound **A2** (5.10^{-2} M). Thus, we observed a gradual decline in the I_1/I_3 ratio after the initial drop was reached, meaning a slow equilibrium in aggregate formation.

The increase in the number of methylene groups (compounds **B1** and **B3**) or the insertion of phenyl groups or fluor atoms into the hydrophobic chain (compounds **B2** and **B4**) led to the formation of small (less than 100 Å) monodisperse particles at high concentration (100 mg/ml), as observed by FFEM (figures 2a and 2b). This type of freeze fracture image was in good agreement with the presence of micelles. The surface density of these particles was proportional to the concentration of the compounds. Indeed, dilution of the solutions resulted in a strong decrease in micelle density.

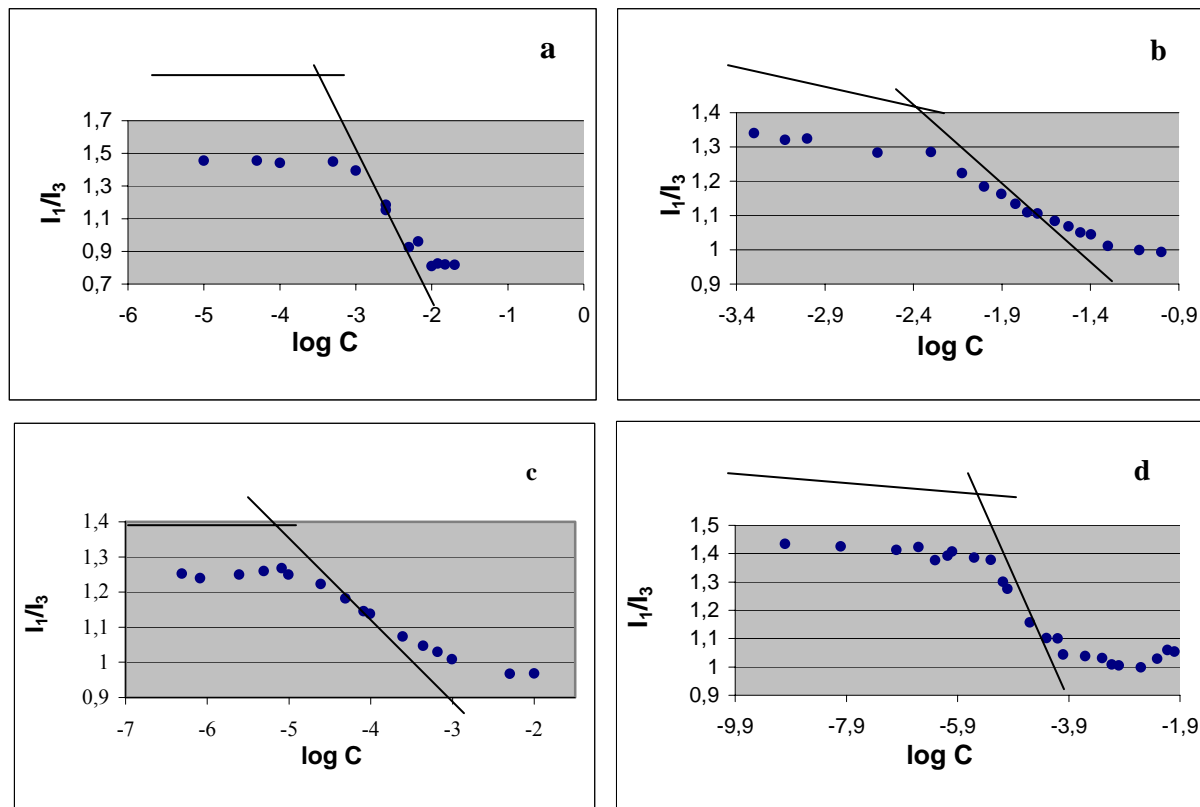


Figure 4. Variation of pyrene monomere fluorescence I_1/I_3 ratio in water as a function of **A2** (a), **B4** (b), **B3** (c) and **B1** (d) concentration.

Thus, we measured the CMC of some representative compounds of these surfactants by the pyrene method.¹⁹ The relative CMC values were coherent. The compound **B3** (figure 4c), having 20 methylene groups in the spacer arm, exhibited a CMC (1.2 mM) 20 times higher than compound **B1** (0.066 mM), in which the hydrophobic chain included 30 methylene groups (figure 4d).

Due to the high hydrophobicity of perfluorocarbon chains, fluorinated compound **B4** presented a CMC (0.0049 mM) 10 times lower than **B1** (figure 4b). It is generally acknowledged that a CF_2 group is roughly 1.7 times more hydrophobic than a CH_2 group.²⁰ Furthermore, we observed that the CMC of compound **B3** is very close to that of its analogous surfactant **B3cs** (1 mM), bearing a dodecylthioether chain as the hydrophobic component (figure 5).

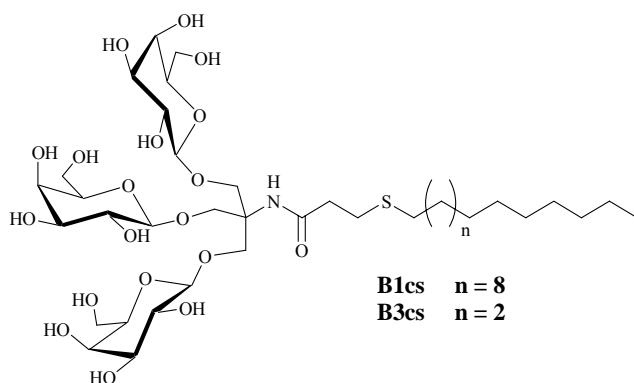


Figure 5. Structure of B1cs and B3cs compounds

In the same way, compounds **B1** and **B1cs**, bearing a hexadecylthioether chain, exhibited comparable Critical Micellar Concentrations, 0.066 mM and 0.013 mM, respectively (the synthesis and physico-chemical properties of surfactants **B1cs** and **B3cs** were previously reported.²¹ Such results are in very good agreement with the hypothesis that the hydrophobic segment folds up when bolaform surfactants bear a long hydrocarbon spacer arm.^{14,15} However, it is noteworthy that the rigidity of the linker in compounds **B2** and **B4**, afforded by a biphenyl or fluorocarbon chain, did not prevent micelle formation and thus, probably, the folding up of the spacer group.

Conclusions

The preliminary results reported here show that synthetic bolaforms derived from a polyol, such as a Tris moiety, are not good candidates for vesicle formation whatever the length or the rigidity of the spacer arm. The insertion of a short hydrophobic linker between the two polar heads (type-A compounds) did not lead to vesicle or membrane systems. Such bolaform surfactants formed atypical associations which did not look like classic micellar systems. These compounds were probably unable to form micelles by a suitable folding up of their hydrophobic chain, because of their short hydrophobic segments. The accurate nature of the aggregates they formed will have to be determined by other physico-chemical techniques. The lengthening of the hydrophobic linker led to its folding up and the formation of spherical micelles, which could be studied by the FFEM technique. An increase in the rigidity of the hydrophobic linker arm, through the introduction of phenyl groups or a perfluorocarbon chain, did not prevent the folding up phenomenon. It seems that monostrand bolaform surfactants are not able to form unilamellar membrane vesicles. The most suitable and easiest means to form vesicles now seems to be the use of acyclic doublestrand bolaform surfactants. These are easily synthesised by grafting a lateral hydrophobic chain close to the polar heads (Figure 6). Such a method was successfully carried out by Guilbot et al.²²

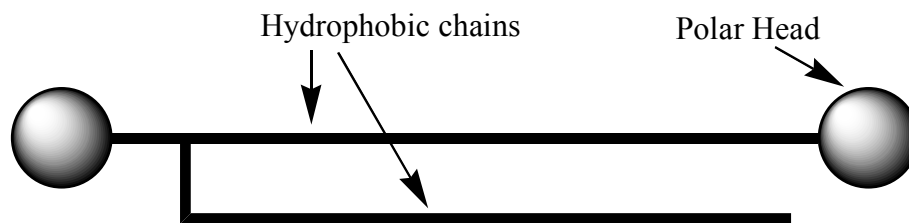


Figure 6. General structure of biantennary bolaamphiphiles according to Guilbot et al.²²

Experimental Section

General Procedures. The progress of the reactions and the homogeneity of the compounds were monitored by thin layer chromatography (TLC Merck 254). Compound detection was achieved by exposure to UV light (254 nm), by spraying a 5% sulfuric acid solution in methanol, or 5% ninhydrin solution in ethanol (in order to detect the amine-containing compound), then heating at 150°C. Purifications were achieved by column chromatography over silica gel (Merck 60). Melting points were measured on an electrothermal machine and have not been corrected. The ¹H NMR, ¹³C NMR and ¹⁹F NMR spectra were recorded using a BRUKER AC 250 at a frequency of 250 MHz for ¹H, 62.86 MHz for ¹³C and 235 MHz for ¹⁹F spectra. The spectrum settings of the glycosylated compounds were taken from the acetylated compounds before deprotection (in CDCl₃), as this facilitates taking a reading. Chemical shifts are given in ppm relative to tetramethylsilane using the deuterium signal of the solvent (CDCl₃) as a heteronuclear reference for ¹H and ¹³C. For the ¹⁹F-NMR spectra, the internal reference is CFCl₃. Mass spectra were recorded on a DX 300 Jeol apparatus. Sonication was performed by pulsing (power 100%) with a titanium probe, 3 mm in diameter, on a Vibracell sonicator 72412. Reactions were carried out in anhydrous conditions under dry nitrogen. All the solvents were distilled and dried according to standard procedure.

Preparation of aqueous suspensions. Type A and B compounds were dispersed in deionized water (10 mg/ml) as a following procedure. The compound was dissolved in methanol and the solvent removed under vacuum. The thin film obtained was thus dispersed in deionized water and stirred with a vortex for 5 min. Then the dispersion was sonicated for 15 min (titanium probe of 3 mm, power 40%, 1/1 pulse method).

Identification of vesicles by Transmission Electron Microscopy and freeze-fracture electron microscopy. The formation of vesicles was observed by Transmission Electron Microscopy (TEM) according to the negative staining method and by Freeze fracture Electron Microscopy (FFEM) as previously described.²³ The samples were examined using a Philips CM 10 microscope (TEM) or a Zeiss EM 912 electron microscope (Zeiss, Iena, Germany) (FFEM).

Steady-state fluorescence measurements. Fluorescence spectra were obtained on a SPEX-Fluoromax 2 instrument from Jobin Yvon. Emission spectra were taken at an excitation

wavelength of 338 nm with a band-pass of 4 nm. I_1/I_3 values obtained from emission spectra were reproducible within 0.02. Pyrene was used as a probe at the very low concentration of 1-2 mM which ensures the absence of excimer. This ratio is sensitive to the microenvironment of pyrene. The decline of the I_1/I_3 ratio with surfactant concentration corresponds to the beginning of the aggregation process. The solutions were prepared as follows: to a series of dry flasks were added aliquots of a methanol solution of pyrene. A thin layer of dye was formed in each flask after the solvent was evaporated in a stream of nitrogen. Surfactant solutions of different concentrations were added and the probe was solubilised in the resulting solutions in an ultrasonic bath. The solutions were stabilised for 12h00 before the fluorescence measurements were made.

Pinacyanol chloride absorption. A glass cuvette was filled with 3 ml of a $1.03 \cdot 10^{-5}$ M pinacyanol chloride solution in distilled water. Small volumes of bolaamphiphiles solution were injected into this solution with a micropipette to obtain the desired concentration. The mixture was stirred with a vortex mixer and rested 5 min before measuring the absorption at 610 nm using a Spectronic 20D+ (Milton Roy Company) spectrophotometer.

Compound characterization

Compound A1. 1,10-decanediisocyanate (82 mg, 0.36 mmole), DABCO (20 mg) and compound **1** (500 mg, 0.83 mmole)²¹ were stirred at room temperature in dry toluene for 8h00. The solution was concentrated under vacuum and the residue directly applied at the top of a silica-gel column (ethyl acetate/ acetonitrile / triethyl amine 80 : 19 : 1 v/v) to afford the pure product **A1** as an amorphous solid (400 mg, 60 %, Mp: 63°C). $[\alpha]_D^{25} = -2.2$ (c, 1, CH_2Cl_2). $^1\text{H NMR}$ (250 MHz, CDCl_3) δ 7 (2H, t, NH tris), 5.9 (2H, t, NH), 5.4 (4H, m, NH, H_4), 5.1-5.0 (4H, m, H_3, H_2), 4.5 (2H, d, H_1), 4.4 (8H, m, CH_2OAc), 4.2 (8H, m, CH_2tris , CH_2gly), 4.0-3.8 (6H, m, $\text{H}_5, \text{H}_6, \text{H}_6'$), 3.2 (4H, m, CH_2NH), 2.2-2.0 (36H, m, CH_3CO), 1.5-1.3 (16H, m, CH_2chain). $^{13}\text{C NMR}$ (62.86 MHz, CDCl_3) δ 171.3-169.8 (CO), 158.6 (CO urea), 101.2 (C anomer β), 70.7-67.0 (CH_2gly , CH glycoside), 62.6-58.2 (CH_2tris), 44.6 (Ctris), 40.4-26.7 (CH_2NH , CH_3CO , CH_2 chain), 21.0-20.5 (CH_3). FAB^+MS : $m/z = 1409$ ($\text{M}+\text{H}$)⁺.

Compound A2. Prepared according to the above procedure, starting from compound **2** (456 mg, 0.517 mmole),¹⁶ DABCO (20 mg) and 1,10-decanediisocyanate (50 mg, 0.225 mmole), to afford compound **A2** after purification by flash chromatography (ethyl acetate/acetonitrile /triethyl amine 90 : 9 : 1 v/v/v) as an amorphous solid (214 mg, 49 %, Mp: 90°C). $[\alpha]_D^{25} = -10.1$ (c, 1, CH_2Cl_2). $^1\text{H NMR}$ (250 MHz, CDCl_3) δ 6.5 (2H, t, NH tris), 5.4 (4H, d, H_4), 5.1-5.0 (10H, m, $\text{H}_3, \text{H}_2, \text{NH Gly}$), 4.9 (2H, t, NH urea), 4.4 (4H, m, H_1), 4.2-3.7 (28H, m, CH_2Oac , CH_2tris , CH_2gly , $\text{H}_5, \text{H}_6, \text{H}_6'$), 3.2 (4H, m, CH_3NH), 2.2-1.9 (54H, m, CH_3CO), 1.5-1.3 (16H, m, CH_2chain). $^{13}\text{C NMR}$ (62.86 MHz, CDCl_3) δ 170.8-169.8 (CO), 158.0 (CO urea), 101.4-101.2 (C anomer β), 70.8-68.1 (CH glycoside), 62.9-58.8 (CH_2tris , CH_2gly), 44.3 (Ctris), 40.5-26.7 (CH_2NH , CH_3CO , CH_2 chain), 20.8-20.5 (CH_3). FAB^+MS : $m/z = 1985$ ($\text{M}+\text{H}$)⁺.

Compound A3. Prepared according to the above procedure starting from compound **3** (897 mg, 0.765 mmole),¹⁶ DABCO (20 mg) and 1,10-decanediisocyanate (78 mg, 0.348 mmole) to afford

compound **A3** after purification by flash chromatography (ethyl acetate / triethyl amine 99 : 1 v/v) as an amorphous solid (450 mg, 60 %, Mp: 102°C). $[\alpha]_D^{25} = -10.4$ (c, 1, CH₂Cl₂). ¹H NMR (250 MHz, CDCl₃) δ 6.3 (2H, t, NH tris), 5.4 (6H, d, H₄), 5.2-5.0 (14H, m, H₃, H₂, NH Gly), 4.9 (2H, t, NH urea), 4.4 (6H, m, H₁), 4.2-3.8 (34H, m, CH₂tris, CH₂gly, H₅, H₆, H_{6'}), 3.2 (4H, m, CH₃NH), 2.2-2.0 (72H, m, CH₃CO), 1.5-1.3 (16H, m, CH₂chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.5 (CO), 158.7 (CO urea), 101.3 (C anomer β), 70.8-59.3 (CH glycoside, CH₂tris, CH₂gly), 44.0 (Ctris), 40.7-26.8 (CH₂NH, CH₃CO, CH₂ chain), 20.7-20.5 (CH₃). FAB⁺MS : m/z = 2561 (M+H)⁺.

Benzyloxycarbonyl amino-11-undecanoic acid. Amino-11-undecanoic acid (8.0 g, 39.0 mmoles) was stirred in a mixture of acetonitrile / water (500 ml, 50/50 : 1 / 1) at 0° C. Benzyl chloroformate (10.15 g, 59.7 mmoles) was added dropwise. The pH was adjusted to 7-8 by adding dropwise 3N NaOH solution. The mixture was stirred at room temperature for 24h and acidified (pH 3). The precipitate was filtered and dissolved in dichloromethane, washed with water, dried with sodium sulfate and concentrated under vacuum. Pure compound **4** was obtained as a white amorphous powder after crystallization in hot ethyl acetate (8.1 g, 62 %). ¹H NMR (250 MHz, CDCl₃) δ 7.3 (5H, m, Ph), 5.08 (2H, s, CH₂-Ph), 4.7 (1H, t, NH), 3.1 (2H, t dd, CH₂-NH), 2.4 (2H, t, CH₂-CO), 1.59-1.27 (16H, m, CH₂ chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 178.9 (CO), 142.1 (C₁ Phenyl), 128.4-128.1 (C Phenyl), 66.6 (CH₂NH), 41.1 (CH₂Ph), 33.9-24.6 (CH₂ chain).

Compound 4. Benzyloxycarbonyl amino-11-undecanoic acid (4.55 g, 3.9 mmoles), compound **4** (1.44 g, 4.28 mmoles), DCC (1.043 g, 5 mmoles) and DMAP (0.522 g, 4.28 mmoles) were stirred in dry dichloromethane (25 ml) at room temperature for 12h. The mixture was filtered and washed with 1N HCl (30 ml) and 10 % NaHCO₃ (30ml). Organic layer was dried with sodium sulfate and concentrated under vacuum.

The crude product was subjected to column chromatography on silicagel (ethyl acetate / hexane 85 / 15 v / v) to afford the pure product **4** as a white amorphous powder (3.6 g, 62%). ¹H NMR (250 MHz, CDCl₃) δ 7.4 (5H, m, Ph), 6.34 (1H, s, NH tris), 6.2 (1H, t, NH gly), 5.3 (3H, dd, H₄), 5.0 (8H, m, CH₂Ph, H₃ et H₂), 4.8 (1H, t, NH₂), 4.4 (3H, d, H₁), 4.1-3.7 (17H, m, H₅, H₆, CH₂O, H_{6'}, CH₂ gly), 3.1 (2H, tdd, CH₂NH), 2.2 (4H, m, CH₂CO), 2.1-1.9 (36H, s, CH₃CO), 1.6-1.4 (2H, m, CH₂CH₂NH), 1.2 (16H, m, CH₂ chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.6 (CO amide), 153.9 (CO carbamate), 137.3-128.5 (Ph), 101.9 (C anomer β), 71.4-59.7 (CH₂O, CH₂Z, CH₂ gly, CH Gal, CH₂ tris), 43.2 (C tris), 36.9-25.4 (CH₂ chain), 21.1 (CH₃).

Deprotection of benzyloxycarbonyl group by catalytic hydrogenation of compound 4 Compound **4** (1.02 g, 0.686 mmole) was stirred in ethanol at 0°C. Palladium on charcoal (60 mg / mmoles) was added slowly to the solution. The mixture was hydrogenated for 4 h, then filtered over celite. The filtrate was concentrated under vacuum to obtain the deprotected compound as a white powder (0.927 g, 100%).

Compound B1. After hydrogenolysis of benzyloxycarbonyl group with hydrogen on palladium on charcoal, compound **4** (0.536 g, 0.397 mmole), decyl diisocyanate (0.042 g, 0.189 mmole) and DABCO (10 mg) were stirred in acetonitrile (25 ml) for 12h at room temperature. The

mixture was concentrated under vacuum, dissolved in ethyl acetate and washed with 1N HCl, sat. NaHCO₃ and dried with sodium sulfate. After concentration under vacuum the residue was directly applied at the top of Sephadex LH20 column (CH₂Cl₂ / MeOH 1 / 1 : v/v) to afford the pure product as a white amorphous powder (0.400 g, 65%, Mp = 88-90° C). $[\alpha]_D^{20} = -8.31$ (c, 1, CH₂Cl₂). ¹H NMR (250 MHz, CDCl₃) δ 6.43 (2H, t, NH gly, 6.22 (2H,s, NH tris); 5.4 (6H, dd, H₄); 5.3-5.0 (16H, NH(CH₂)₁₀, H₃ et H₂), 4.4 (6H, d, H₁), 4.2-3.6 (34H, m, CH₂O, H₅, H₆, H_{6'} et CH₂ gly), 3.1 (8H, td, CH₂NH), 2.3 (4H, t, CH₂CO), 2.2-2.0 (72H, s, CH₃), 1.6-1.2 (48H, m, CH₂ chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.4-168.8 (CO), 158.3 (CO urea), 101.3 (C₁ anomer β), 70.8-59.2 (CH glycoside, CH₂O gal, CH₂NH, CH₂ gly), 42.6 (C tris), 40.4 (CH₂-CH₂NH), 34.2-30.2 (CH₂CO), 29.6-25.5 (CH₂ chain), 20.7 (CH₃). TOF MS ES⁺ (1918 g/mole) : m/z = 1942.93 (M+Na+H)⁺, 1957 (M+K)⁺

Compound B2. Prepared according to the above procedure starting from compound **4** (0.955 g, 0.707 mmole), phenyl diisocyanate (0.054 g, 0.336 mmole), DABCO (100 mg) to afford compound **B3** after purification by flash chromatography (CH₂Cl₂/MeOH 9/1 : v/v) and sephadex LH 20 (CH₂Cl₂ / MeOH : 1/1) as an amorphous solid (0.280 g, 51%, Mp = 82.4-84° C). $[\alpha]_D^{20} = -7.1$ (c, 1, CH₂Cl₂). ¹H NMR (250 MHz, CDCl₃) δ 7.23 (4H, s, Ph), 6.4 (2H, t, NH gly), 6.3 (2H, s, NH tris), 6.2 (2H, s, NH urea), 5.4 (6H, dd, H₄), 5.1-5.0 (14H, m, NH(CH₂)₁₀, H₃ et H₂) 4.4 (6H, d, H₁), 4.2-3.8 (34H, m, CH₂O, H₅, H₆, H_{6'}, CH₂ gly), 3.2 (4H, t, CH₂NH), 2.2 (4H, t, CH₂CO), 2.1-1.9 (72H, s, CH₃CO), 1.2 (32H, m, CH₂ chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.4-168.9 (CO), 134.4-122.0 (Ph), 101.3 (C anomer β), 70.7-59.2 (CH₂O gal, CH glycoside, CH₂ gly), 42.7 (C tris), 40.0 (CH₂CO), 36.2 (CH₂NH), 30-26.7 (CH₂ chain), 20.9-20.6 (CH₃). TOF MS ES⁺ (1854 g/mole) : m/z = 1878 (M+Na+H)⁺.

Compound B3. Compound **4** (0.910g, 0.674 mmole), BOP (0.313 g, 0.707 mmole), DMAP (0.0863 g, 0.707 mmole) and succinic anhydride (0.033 g, 0.337 mmole) were stirred in dichloromethane dry for 12h at room temperature. After concentration under vacuum the crude product was purified by flash chromatography (AcOEt / MeOH 95/5 : v/v) and sephadex LH 20 (CH₂Cl₂/MeOH : 1/1) to afford compound **B3** as an amorphous solid (0.370 g, 40 %, Mp = 100-102° C). $[\alpha]_D^{20} = -9.3$ (c, 1, CH₂Cl₂). ¹H NMR (250 MHz, CDCl₃) δ 6.36 (2H, t, NH amide), 6.2 (2H, s, NH tris), 5.3 (6H, dd, H₄), 5.0 (14H, m, H₃, H₂, NH carbamate), 4.4 (6H, d, H₁), 4.1-3.7 (30H, m, CH₂O, H₅, H₆ et H_{6'}, CH₂gly), 3.2 (4H, t dd, CH₂NH), 2.4 (4H, t, CH₂ succinate), 2.2 (4H, t, CH₂CO), 2.1-1.9 (72H, m, CH₃), 1.5-1.2 (32H, m, CH₂ chain). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.5-169.0 (C=O), 101.5 (C anomer β), 70.9-59.4 (CH₂ gly, CH₂Ogal, CHO glycoside), 39.7 (C tris), 36.5 (CH₂NH), 32.1 (CH₂CO), 29.6-25.6 (CH₂ chain), 20.9-20.6 (CH₃). TOF MS ES⁺ (1776 g/mole) : m/z = 1799.74 (M+Na)⁺, 1800.61 (M+Na+H)⁺.

Compound 5. Ethyl undecenoate (9.6 ml, 39.7 mmoles) and diiodo perfluorohexane (2 g, 3.61 mmoles) were stirred under argon for 15 min at 90 °C. AIBN (0.018 g, 0.109 mmoles) was added and the mixture stirred for 12h at 90 °C in the dark. Some excess ester was eliminated under high vacuum and the crude product was purified by flash-chromatography (hexane / ethyle acetate 9 / 1 : v/v) to afford compound **5** as a colourless oil (1.8 g, 51%). ¹H NMR (250 MHz, CDCl₃) δ 4.3 (2H, tt, CH-I), 4.1 (4H, q, CH₂CH₃), 2.8 (4H, m, CH₂CF₂), 2.3 (4H, t, CH₂CO),

1.8-1.7 (4H, td, ICH-CH₂-(CH₂)₆), 1.6-1.3 (30H, m, CH₂ chain, CH₃). ¹⁹F NMR (235 MHz, CDCl₃) δ -113.3 (4F, dd, CF₂-CH₂), -121.9 (4F, s, CH₂CF₂CF₂), -123.9 (4F, s, CH₂CF₂CF₂CF₂). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.1 (CO), 120.3-110.8 (CF₂), 65.8 (CH₂O), 60.3 (CH₂CHI), 40.5 (CHI), 36.4 (CH₂CO), 34.0 (ICHCH₂(CH₂)₇), 29.8-26.0 (CH₂ chain), 20.6 (CH₃).

Compound 6. Compound **5** (1.8 g, 1.84 mmol) was stirred in 1.5 N KOH-methanol mixture (50 ml) for 12h at room temperature. The mixture was acidified with 3N HCl (pH = 2) and concentrated under vacuum. The crude product was dissolved in ethyl acetate, washed with 1N HCl and sat Na₂S₂O₄ and dried with sodium sulfate. The solvent was removed under vacuum and the compound **6** was obtained as a colourless oil (0.910 g, 76 %). ¹H NMR (250 MHz, CDCl₃) δ 6.4-6.0 (1.5H, 0.5H, m, CHC₆F₁₂ trans and cis respectively), 5.6-5.3 (2H, m, CH=CHC₆F₁₂), 2.3-2.0 (8H, m, CH₂CO, CH₂CH=CH), 1.6-1.3 (24H, m, CH₂chain). ¹⁹F NMR (235 MHz, CDCl₃) δ -108.68 (1F, CH=CHCF₂ cis), -113.58 (3F, CH=CHCF₂ trans), -123.81 (4F, CF₂-CF₂CH), -126.08 (4F, CF₂CF₂CF₂CH). ¹³C NMR (62.86 MHz, CDCl₃) δ 172.8 (CO), 142.15 (CF₂CH=CH), 139.1 (CF₂CH=CH), 120.3-110.8 (CF₂), 34.49 (CH₂CO), 32.4 (CH₂CH=CH), 29.6-21.1 (CH₂ chain).

Compound 7. Compound **6** (0.910g, 1.37 mmol) was dissolved in toluene at 5°C. Palladium on charcoal (60 mg/mmol) was added slowly under vigorous stirring. The mixture was hydrogenated (P=5 bars) for 2h. Palladium was removed by filtration over celite. The filtrate was concentrated under vacuum to afford compound **7** as a white powder (0.6 g, 66 %). ¹H NMR (250 MHz, DMSO-d₆) δ 2.3-2.2 (8H, m, CH₂CF₂, CH₂CO), 1.56-1.53 (4H, t, CH₂CH₂CF₂), 1.3 (28H, m, CH₂ chain). ¹⁹F NMR (235 MHz, DMSO-d₆) δ -123.1 (CH₂CF₂), -121.4 (CF₂CF₂CH₂), -122.8 (CF₂CF₂CF₂CH₂). ¹³C NMR (62.86 MHz, DMSO-d₆) δ 174.0 (CO), 120-110 (CF₂), 54.86 (CH₂CF₂), 33.62 (CH₂CO), 29.8-24 (CH₂ chain).

Compound B4. Compound **7** (0.3g, 0.447 mmol), compound **3** (1.099 g, 0.941 mmol) DCC (0.243 g, 1.177 mmol) and HOBT (100 mg) were stirred in DMF for 12h at room temperature. DCU was removed by filtration. The filtrate was concentrated under vacuum and the crude product was dissolved in ethyl acetate. The organic layer was washed with sat NaHCO₃, water and dried over sodium sulfate. The solvent was eliminated under vacuum and the crude product flash-chromatographed over silica-gel (ethyl acetate/hexane 96/4 : v/v then ethyl acetate) to afford the pure compound **B4** as a white powder (600 mg, 43%, Mp = 95-97°C). [α]_D²⁰ = -10.7 (c, 1, CH₂Cl₂). ¹H NMR (250 MHz, CDCl₃) δ 6.3 (2H, t, NH gly), 6.2 (2H, s, NH tris), 5.4 (6H, dd, H₄), 5.1-5.0 (12H, m, H₃ et H₂), 4.4 (6H, d, H₁), 4.2-3.7 (34H, m, CH₂Ogal, CH₂gly, H₅, H₆ et H_{6'}), 2.25-2.0 (80H, m, CH₃, CH₂CO, CH₂CF₂), 1.6-1.3 (32H, m, CH₂ chain). ¹⁹F NMR (235 MHz, CDCl₃) δ -114.7 (4F, CF₂CH₂), -122.16 (4F, CF₂CF₂CH₂), -124.0 (CF₂CF₂CF₂CH₂). ¹³C NMR (62.86 MHz, CDCl₃) δ 170.5-169 (CO), 136.8-126.2 (CF₂), 101.5 (C₁ anomer β), 70.9-59.4 (CH₂gly, CH₂Ogal, CH glycoside), 42.8 (C tris), 36.5 (CH₂CO), 29.5-25.7 (CH₂ chain), 20.9-20.7 (CH₃). TOF MS ES⁺ (1962 g/mole) : m/z = 1985.73 (M+Na)⁺, 1986.66 (M+Na+H)⁺.

Deacetylation of compounds A1-A3 and B1-B4. Type-A and -B compounds were de-O-acetylated overnight by Zemplen method with a catalytic amount of sodium methylate in methanol (40 ml). After treatment with a H⁺-type resin (Amberlite IRC 50) followed by filtration

and evaporation of the solvent, pure type-**A** and **B** compounds were isolated in quantitative yield after filtration on sephadex G 25 column with water as eluent.

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