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**Cyclobutane Scaffold in Bolaamphiphiles: Effect of
Diastereoisomerism and Regiochemistry on Their Surface Activity
Aggregate Structure**

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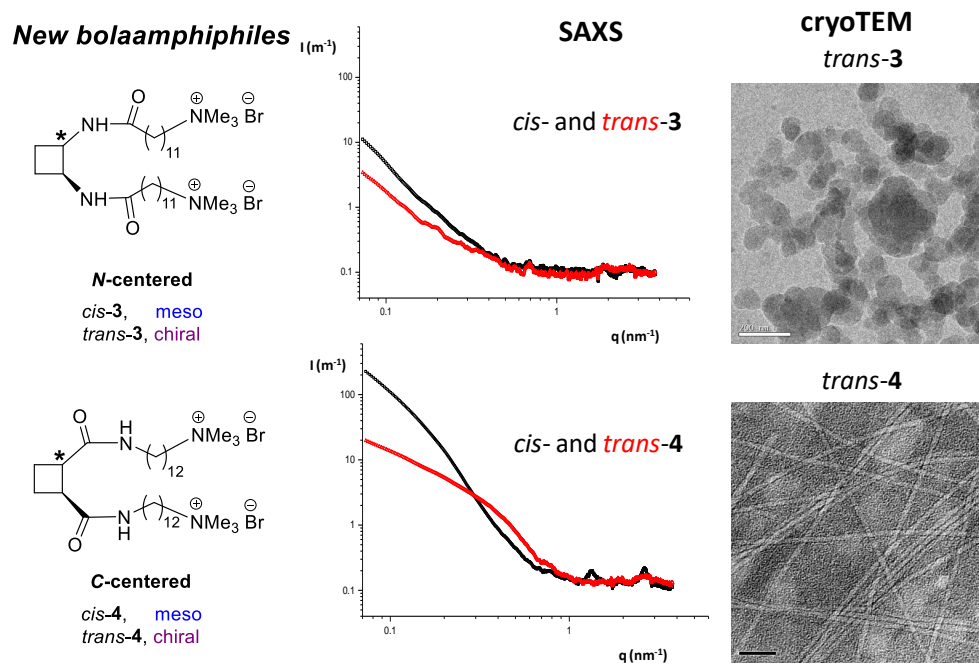
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ABSTRACT: Cationic bolaamphiphiles have been synthesized starting from meso *cis*- or chiral *trans*- 1,2-difunctionalized cyclobutane derivatives. They include *cis/trans* pairs of diastereoisomers, of *N*- or *C*- centered bisamides. The goal of this work was to investigate the influence of the stereochemistry and the regiochemistry on their abilities as surfactants and self-assembly. It is remarkable the very large differences in surface coverage (twofold), cmc (up to two orders of magnitude) and aggregate structure (from lamellae to fibers) for the four molecules due to regio- and stereochemistry differences. Computational calculations were carried out to rationalize the experimental findings and a new methodology has been developed to calculate the structure of these bolaamphiphiles at the surface. Although the four surfactants adopt a wicket-like conformation, for *N*-centered *trans* the distance between polar heads is much larger than for the other three molecules, as suggested by calculations. We have shown that the interplay between the regiochemistry and stereoisomerism, enhanced by rigidity of the cyclobutane ring, affects different physico-chemical properties quite differently. Namely, the cmc value is mainly governed by stereochemistry, with regiochemistry only modulating this value. On the other hand, regiochemistry definitely governs the morphology of the supramolecular aggregates (*i.e.* long fibers versus plates or spherical assemblies), with stereochemistry finely modulating their structural parameters. All these results must help in the rational design of new bolaamphiphiles with predictable properties and useful potential applications.

Keywords: Surfactant, SAXS, Cyclobutane, Bolaamphiphile, Aggregation,
Diastereoisomerism, Regiochemistry

TOC graphic



Introduction

Bola-type surfactants, also called bolaamphiphiles, or bolytes if they are charged,¹ are bipolar amphiphiles with two polar heads connected by one or two long hydrophobic spacers. They owe their name to *bolas*, the Argentinian throwing weapon made of weights on the ends of an interconnecting cord, which is designed to capture animals by entangling their legs. The aggregation morphologies of bolaamphiphiles are as variable as their molecular structures.² For instance, Nagarajan described the effect of the polar head and the molecular structure of the amphiphile on the morphology of its aggregates.³

Bolaamphiphiles are found in the membrane of *archaebacteria* conferring it with a high stability in harsh environments involving high salt concentrations, extreme temperatures or very acidic media.⁴ Due to the difficulty to isolate natural bolaamphiphiles from archeal membranes, synthetic analogs have been prepared trying to reproduce the unusual structure and properties of such membranes.⁵ For example, Sanders and co-workers have shown that bolamphiphiles can be used to stabilize a solubilized membrane protein, e.g. Escherichia Coli diacylglycerol kinase, when mixed with conventional detergents. In addition, they observed that certain bolaamphiphiles can be considered as lipid-like by providing activation of the enzyme catalytic activity. This work represents the first documentation of the potential of bolaamphiphiles for their use in biochemical and biophysical studies of membrane proteins.⁶

On the other hand, the ability of amphiphiles to form self-assembled structures has been used to template the synthesis of mesoporous inorganic materials (e.g. silica and zeolites), as well as of a variety of nano- and micro- objects from silica or metal oxides. One of the most important cases of soft-templating in the synthesis of mesoporous materials is when amphiphile aggregates and silica units interact in a non-

covalent way. In this case, the silica precursors organize around the structure-directing agent, *i.e.* the supramolecular assemblies, and then react with each other to generate the inorganic material. Afterwards, the organic amphiphiles are removed by calcination, which leaves the mesoporous material.^{7,8}

From the supramolecular point of view, bolaamphiphiles show an interesting behavior. They can adopt different geometries both at the air-water interface and inside their aggregates in solution, depending on their molecular structure, and in particular on the length and rigidity of the hydrophobic spacer. In some cases, they adopt a folded structure at the interfaces. However, some studies suggest that elongated conformations can be advantageous. The peculiar properties of their assemblies make bolaamphiphiles of relevant use in targeted drug delivery for the fabrication of nanosized carriers for drugs and genes.⁹

Hanabusa and co-workers have studied bisamide-based carbocyclic bolaamphiphiles as organogelators, showing their interesting ability to form chiral supramolecular aggregates, which have been used as soft-templates for helical inorganic nanostructures.¹⁰⁻¹² In our laboratories, different chiral bisamide- or bisurea-based carbocyclic compounds have been synthesized and studied as organobridged silsesquioxanes¹³ or as organogelators.¹⁴

We have also developed new chiral cyclobutane β -amino acid-based single head/single tail amphiphiles, whose aggregation behavior was extensively studied (Figure 1). In the case of anionic derivatives **1**, we have investigated the influence of *cis/trans* stereochemistry on solution self-aggregation and recognition. In particular, we have found that the two diastereoisomers **1** feature a markedly different morphology and kinetics of formation of their aggregates. In addition, their spherical micelles show dramatically diverse enantioselection ability for bilirubin enantiomers. Moreover, they

form heteroaggregates with bilirubin at submicellar concentrations but with a different expression of supramolecular chirality.¹⁵ Concerning diastereoisomeric nonionic amphiphiles **2**, a strong influence of the *cis/trans* stereochemistry on the physicochemical behavior, molecular organization, and morphology of their Langmuir monolayers and dry solid states was observed.¹⁶ Overall, these differential features make cyclobutane-containing amphiphiles promising building blocks for the design of new soft materials with predictable and tunable properties.

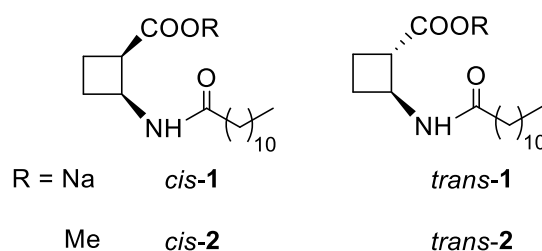


Figure 1. Structure of cyclobutane containing amphiphiles previously studied in our laboratories.

The influence of *cis/trans* stereochemistry has also been evidenced for closely related cycloalkane-based bisamide organogelators.¹⁴ The differences observed were attributed, in part, to the facility for intramolecular hydrogen-bonding of *cis* derivatives whereas this bonding is not favored for *trans* diastereoisomers.

Herein, we describe the preparation and investigation of four novel bolaamphiphiles containing a cyclobutane-bisamide scaffold connected with two symmetric chains, namely *N*-centered *cis*- and *trans*-**3**, and *C*-centered *cis*- and *trans*-**4** (Figure 2). *cis* Diastereoisomers are meso compounds (achiral), whereas *trans* ones are chiral and have been prepared in an enantioselective way. Note that **4** contains two methylene groups more than 3 in the hydrophobic chain. Thus, special attention has been focused on studying the influence that regiochemistry and relative stereochemistry,

joined to the rigidity of the cyclobutane ring, exert on their surfactant behavior and aggregation properties.

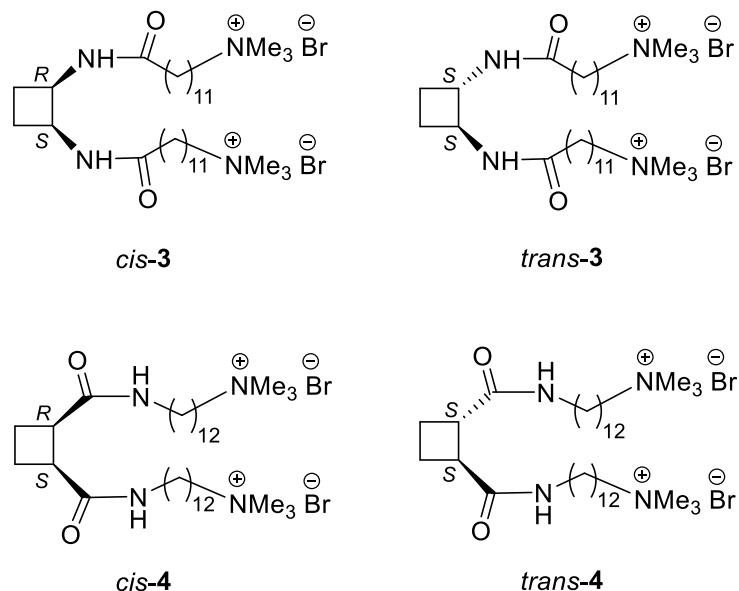


Figure 2. Structures of the bolaamphiphiles synthesized and studied in this work

EXPERIMENTAL SECTION **Synthesis of Bolaamphiphiles 3 and 4.** The detailed synthesis and characterization of surfactants *cis*- and *trans*-**3**, and *cis*- and *trans*-**4** and their precursors is reported in the Supporting Information.

Surface Tension Measurements. Solutions at different known concentrations of the previously synthesized amphiphiles were prepared using an analytical balance. Deionized water Milli-q was used. All of them generated foam at concentrations of few mM, qualitatively showing surfactant behavior.

The surface tension was measured using a home-made pendant drop tensiometer where the analysis is based on the application of the Young–Laplace equation to the drop profile.²⁵¹⁷ A drop of the surfactant solution was created at the end of a straight cut Teflon tube, with internal/external diameters of 0.8/1.6 mm. The droplet profile was extracted from spherical aberration corrected images. The profiles were fitted to the

Young-Laplace equation with a home-made software. More details can be found in reference 15. The temperature was maintained at 25.0 ± 0.5 °C in a near saturated atmosphere to prevent water evaporation.

The surface tension (γ) of the amphiphile solutions decreased progressively upon increasing concentration until a plateau was reached. The break at the onset of the plateau is identified as the critical aggregation concentration (cac) or critical micellar concentration if the aggregates are micelles (cmc). Surface tension was followed as a function of time until equilibration was reached (*i.e.* no appreciable variation of γ), which happened within 3-4 h from the drop formation for diluted samples below the cmc, while it was faster at higher concentrations.

The area per surfactant molecule at the interface was calculated from the slope of the plot of surface tension versus the logarithm of surfactant concentration, just before the cmc, using the Gibbs adsorption isotherm:

$$\Gamma_{\max} = (-1/nRT) \cdot (d\gamma/d\ln C) \quad [1]$$

where Γ_{\max} is the surface excess, γ is the surface tension in Nm^{-1} , C is the surfactant concentration, T is the absolute temperature, $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$, and n the effective number of dissociated species per molecule. From which the area per molecule at the surface can be determined according to Equation [2]:

$$A_m = 10^{18} / (N_A \Gamma_{\max}) \quad [2]$$

where, A_m is the minimum surface area expressed in nm^2 and N_A is the Avogadro's number. We are aware of the problems associated with the use of the Gibbs isotherm close to the cmc to determine the maximum surface adsorption and the concomitant minimum area per molecule.^{18,19} In particular, it has been shown that a frequent situation with cationic surfactants is that of reaching the cmc before surface saturation with the subsequent overestimation in the minimum area per molecule.²⁰ By using the

pendant drop method, the problems associated with imperfect wetting are avoided, and no evidence of minimum were observed which suggests the absence of substantial nonionic impurities.²¹ Also, the formation of premicellar aggregates and the presence of ionic impurities have been pointed out as possible source for error. Therefore, the values of area per molecule obtained from surface tension should be always used with caution. Even with these limitations in mind, it is useful to provide the calculated A_m as a comparison with the published values for other related surfactants, as well as a limiting maximum value of the minimum area per molecule to be compared for the bolaamphiphiles **3** and **4**.

Effectiveness, Π_{cmc} , can be determined by measuring the reduction of the surface tension value from water to the value at the cmc for each surfactant.

Cryogenic Transmission Electron Microscopy (CryoTEM). A drop of the surfactant solution with a known concentration was placed on a carbon-coated copper grid. Then, the sample-grid assembly was rapidly frozen using liquid ethane and kept at -180 °C during the imaging using liquid nitrogen. The images were acquired with Hitachi H-7000 microscope operating at 200 kV.

Small Angle X-ray Scattering (SAXS). SAXS was measured at the NDC beamline at ALBA Synchrotron, Barcelona. A sample-detector distance of 2.982 m was used with 0.10 nm wavelength coupled with a Q315r detector from ADSC. The q scale was calibrated with a silver behenate sample; data reduction was performed with the software provided by the beam line.

The intensity is shown as a function of scattering vector q expressed in nm^{-1} (Equation 3).

$$q = (4\pi/\lambda) \sin (\theta/2) \quad [3]$$

where θ is the scattering angle and λ the wavelength

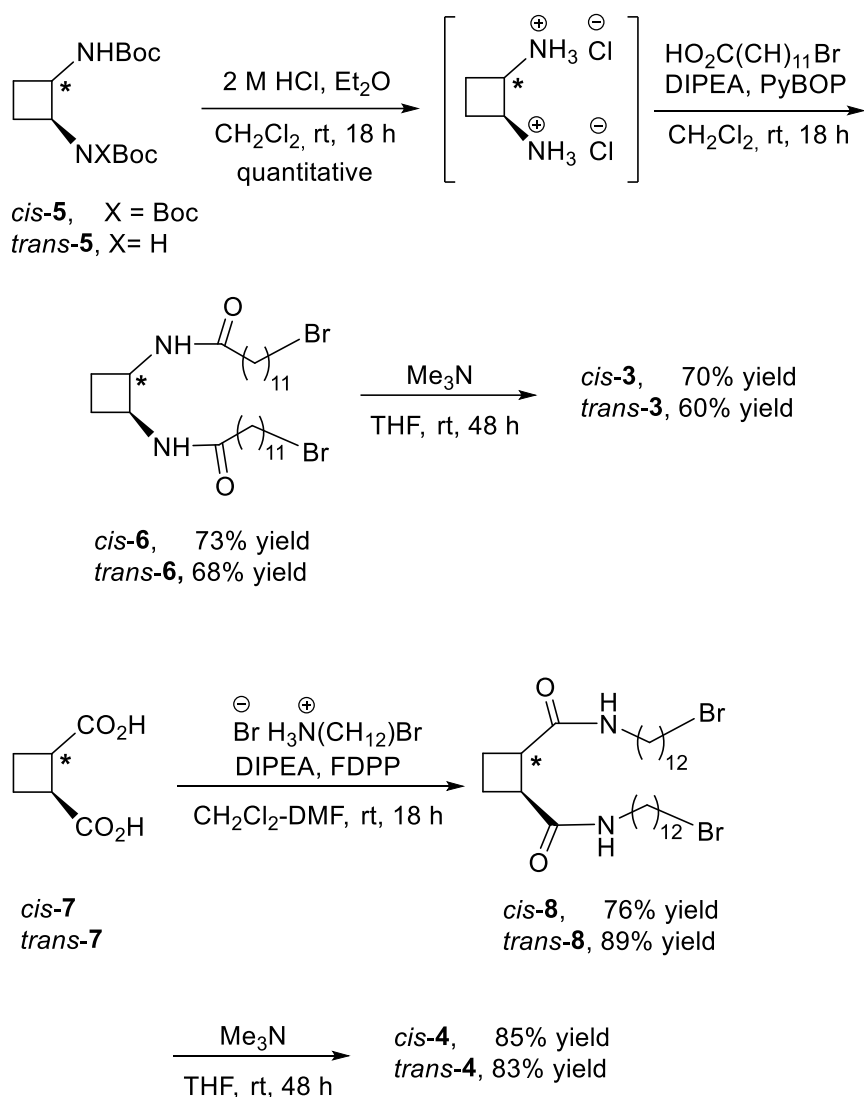
Model scattering curves have been built by Fourier transformation of the pair distribution function of real space models (see Figure S1 in the Supporting Information for sketches of those models). In those models, the volume and electronic density of the polar heads (trimethyl ammonium bromide) and hydrophobic tails have been preserved, an area per headgroup of 0.25 nm^2 has been used to restrict the length of the hydrophilic and lipophilic moieties. Allowing for some penetration of the first methylene groups (1-2) in the hydrophilic corona improves the overall fit.²²

Theoretical Calculations. Conformational searches of model systems were carried out in the gas phase using a mixed low mode/torsional sampling²³ with the OPLS-2005^{24,25} force field implemented in the MacroModel²⁶ program. The geometries of monomers, dimers and tetramers of the simplest models (I) were optimized by means of the M06-2X density functional²⁷ and the 6-31G(d) basis set. These calculations have been carried out using the Gaussian-09 program.²⁸ The aggregation pattern of the hydrophobic part of bolaamphiphiles has been studied from a neutral model (II). The geometries of octameric aggregates of the four systems have been optimized using the OPLS-2005 force field in the gas phase. One of the central molecules of each aggregate has been used to build the monomers of bolaamphiphiles. The geometries of these molecules have been optimized at the OPLS-2005 level of calculation in water solution. The solvent effect has been included using the GB/SA method.²⁹ The geometry parameters of the trimethylammonium heads necessary for the calculation of the surface area were obtained from a geometry optimization of tetramethylammonium at the M06-2X/6-31+G(d,p) level of calculation in water solution. The effect of the solvent was taken into account using the SMD method.³⁰

RESULTS AND DISCUSSION

Synthesis of the cyclobutane-based bolaamphiphiles 3 and 4. Compounds *cis*- and *trans*-**3** were easily synthesized following an analogous route (Scheme 1), which starts from the appropriate *N*-protected cyclobutane 1,2-diamines meso *cis*- and enantiomerically pure *trans*-**5**, respectively. These, in turn, were prepared in enantioselective manner as previously described.³¹ Deprotection of both amino groups followed by condensation with 12-bromolauric acid and subsequent alkylation with trimethylamine afforded *N*-centered quaternary salts *cis*- and *trans*-**3** in 41-51% overall yields.

Likewise, the synthesis of *C*-centered derivatives *cis*- and *trans*-**4** was achieved in good overall yields (65-74%) through condensation of previously known (1*R*,2*S*)- and (1*S*,2*S*)-cyclobutane-1,2-dicarboxylic acid, *cis*- and *trans*-**7**,³² respectively, with 12-bromododecylamine followed by exhaustive alkylation with trimethylamine.



Scheme 1. Synthesis of surfactants *cis*- and *trans*-**3**, and *cis*- and *trans*-**4**.

Aggregation behavior in dilute aqueous solution.

Surface tension measurements. With the aim of investigating the aggregation behavior of the two pairs of diastereoisomers, *cis*- and *trans*-**3**, and *cis*- and *trans*-**4** in the diluted regime, the surface tension *versus* concentration behavior of their diluted aqueous solutions was studied.

As shown in Figure 3, for all the investigated compounds the surface tension (γ) of water decreases progressively upon increasing concentration until a plateau is

reached. The existence of a plateau is normally associated to the formation of micelles.¹⁸ Therefore, all of them behave as typical surfactants. Interestingly, however, *trans*-**3** can only decrease the surface tension from 73 to 50 mN/m, so it cannot be considered as a good surfactant.

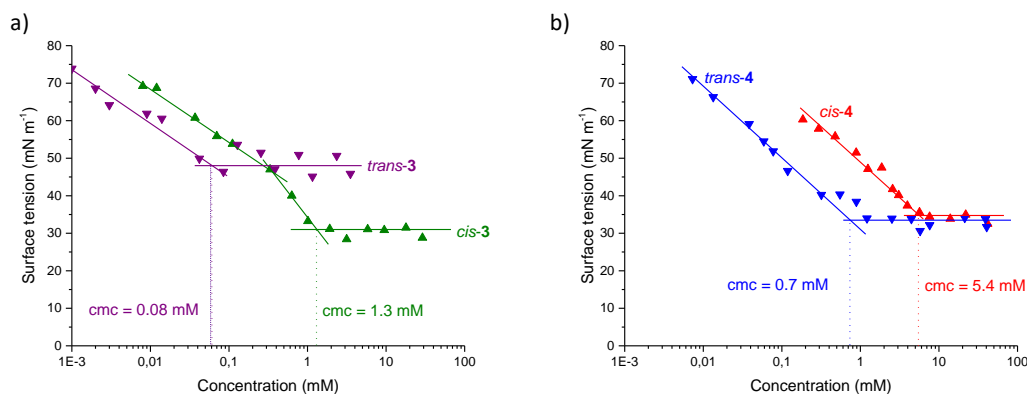


Figure 3. Plot of surface tension as a function of surfactant concentration for *cis*-**3** (green) and *trans*-**3** (pink) (a) and for *cis*-**4** (red) and *trans*-**4** (blue) (b) in water at 25 °C.

Otherwise, the other three compounds are good surfactants because they can decrease the surface tension (γ) under 40 mN/m, which has been taken as an indication of saturation of the water-air interface with hydrocarbon tails.³³ In Figure 3, the breaks correspond to the cmc values of the different surfactants that were determined as the intersection of two fitting straight lines in the plots of γ versus concentration. From the slope of the curve just before the cmc, the Gibbs surface excess (Γ_{\max}) at the air/solution interface corresponding to the cmc was obtained. From this value, the area per molecule (A_m) at the surface can be calculated using $n=3$ as the number of adsorbing species (namely, the surfactant cation plus two bromide anions). The precise value of n in equations 1 and 2 (See the Experimental Section) depends on the ionic conditions of the media and on the effective number of adsorbed species; for instance, a value of 2.5 have

been used for cationic Gemini surfactants.²⁰ The cmc and A_m values for the four surfactants are reported in Table 1.

Table 1. Surface Properties of Surfactants *cis*- and *trans*-3, and *cis*- and *trans*-4 in water at 25 °C.

Surfactant	Surface Excess Γ_{\max} ($\mu\text{mol}/\text{m}^2$)	Area A_m (nm^2)	Effectiveness Π_{cmc} (mN/m)	cmc (mM)
<i>cis</i> - 3	1.66±0.08	1.00±0.05	41.5±1	1.3±0.5
<i>trans</i> - 3	0.74±0.07	2.24±0.22	22.9±1	0.06±0.02
<i>cis</i> - 4	1.18±0.03	1.41±0.04	36.2±1	5.4±1.5
<i>trans</i> - 4	1.13±0.03	1.48±0.04	38.9±1	0.7±0.2

As stated before, the values of A_m obtained from surface tension measurements can only be taken as an upper estimate of the minimum area per molecule. However, we can still compare those values with other bolaamphiphilic surfactants in the literature. As observed before on the basis of surface tension, the less effective surfactant is *trans*-**3**. The higher value of surface tension at the plateau for the latter (lower Π_{cmc}) suggests that surface is far from being similar to a hydrocarbon surface, and the low slope agrees with low coverage (with all the due precautions on the true significance of the surface tension A_m). In addition, this surfactant showed some oscillations of the surface tension above the break that is wider than the reproducibility of the method; this could be a consequence of the low coverage and the difficulty of this molecule to effectively pack at the interface. The most effective surfactant is *cis*-**3**, followed by the two *C*-centered surfactants *cis* and *trans*-**4**. Although the literature on bolaamphiphiles is large, only few reports on water soluble compounds and their surface tensions can be found. We can compare the area per molecule with values from the literature obtained for linear bis(trialkylammonium)alkyl dibromides.^{34,35} Some values are $\text{C}_{12}\text{H}_{24}(\text{NMe}_3)_2\text{Br}_2$,

$A_m=1.07 \text{ nm}^2$; $C_{12}H_{24}(NBu_3)_2Br_2$, $A_m=1.46 \text{ nm}^2$; $C_{20}H_{40}(NEt_3)_2Br_2$, $A_m = 1.17 \text{ nm}^2$, which are similar to 1,12-disodium dodecyl disulfate, $A_m=1.18 \text{ nm}^2$.³⁶ These values were also obtained from surface tension measurements, that of $C_{20}H_{40}(NEt_3)_2Br_2$ is the closest to the present situation in terms of hydrophobic length (20 C-C bonds compared with 30 bonds in the present case) although the polar head corresponds to triethyl instead of trimethyl groups, which has been shown to correspond to bigger areas per molecule if we compare the triethyl with the trimethyl group. If the situation for the bola surfactants is similar to that encountered for gemini surfactants, the surface tension limiting area could be an overestimation of about 60% with respect to the true saturated surface.²⁰

Another, yet indirect, measurement of the surface degree of saturation is the effective surface pressure at the cmc (Π_{cmc}). For hydrocarbon chains the maximum value on water corresponds to nonionic surfactants with values close to 40 mNm^{-1} (48 for 1,2-decane diol or 44.1 for the maximum values recorded by Rosen for triethylene glycol monododecyl ether).³³ Therefore, *cis-3* at the cmc is likely to correspond to a saturated surface. It has been argued that the moderate reduction of surface tension achieved with polymethylene chains (27 mNm^{-1} for $C_{20}H_{40}(NEt_3)_2Br_2$)³⁵ is due to the absence of the terminal methyl groups; from our results it appears that the cyclobutane moiety has improved properties in this aspect. In this respect, *cis-4* and *trans-4* would be also close to present a saturated surface, making also the resulting surface tension limiting area plausible. Even taking into account the limitations of the surface tension technique both the surface tension at cmc shows, and the calculated area per molecule suggests, the increased difficulty in getting a good surface coverage with *trans-3* compared to the other molecules (see also the computational modeling results)

The cmc values are also noteworthy, with as much as two orders of magnitude difference observed, being the lowest the one corresponding to *trans*-**3** and the highest the one corresponding to *cis*-**4**. Similar differences in cmc need differences of about 6-7 methylene groups for classic monoalkyl surfactants.³³ Also the two extra methylene groups in the hydrophobic moiety of *C*-centered surfactants do not reflect on a reduction of cmc as compared to *N*-centered, enhancing even more the regiochemistry effect. The cmc value is a compromise between the water solubility and the tendency to self-aggregate. The presence of both intramolecular and intermolecular hydrogen bonding in the *trans* stereoisomers could influence those values by changing the energy of the monomers in water and in the aggregates compared to the *cis* molecules, which can only form intermolecular hydrogen bonds (See the Computational Studies below).

These results are closely related with the obtained experimental areas that these surfactants occupy at the surface. The larger the area at the surface, the less effective is the surfactant because fewer molecules can be placed there and, consequently, less saturated is the surface. In the case of the two *C*-centered surfactants **4**, relative stereochemistry shows no significant influence on the effectiveness, while referring to the *N*-centered surfactants, stereochemistry seems to play an important role.

CryoTEM Studies. The morphology of the aggregates formed by these amphiphiles above the cmc was studied by using cryoTEM, *i.e.* by freezing the sample using liquid ethane to avoid water crystallization and the possible modification of the aggregates. Selected cryoTEM images of the aggregates of these molecules are shown in Figure 4.

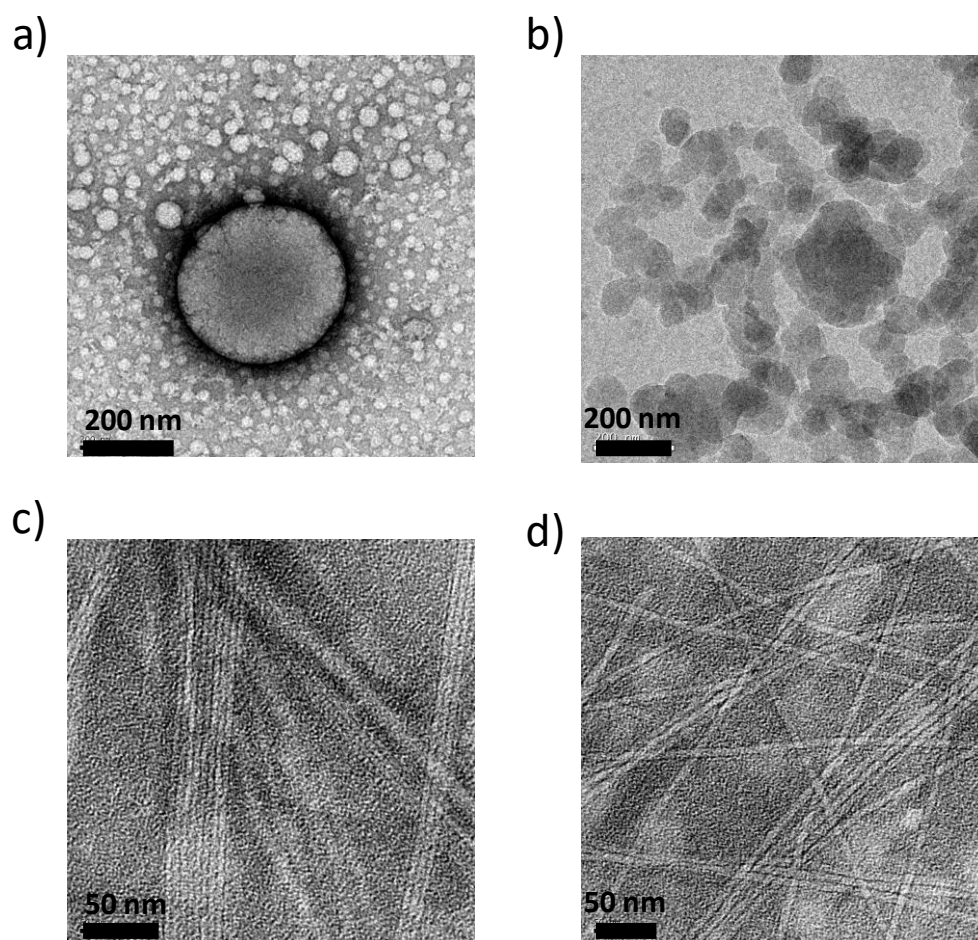


Figure 4. Representative cryo-TEM micrographs of 2.8 mM *cis-3* (a), 1.1 mM *trans-3* (b), 8.5 mM *cis-4* (c) and 8.5 mM *trans-4* (d) above the cmc. Bars correspond to different scales; a) and b) 200 nm, c) and d) 50 nm.

As shown in Figure 4, *cis-3* forms vesicles of different sizes ranging from 350 to 15 nm in diameter. *Trans-3* forms big and irregular aggregates which can correspond to platelet fragments. In contrast, both *cis* and *trans-4* form long and regular fibers with a width of 5 ± 1 nm and 8 ± 1 nm respectively. In addition, in the case of *cis-4*, fibers show a strong tendency to bundle into parallel sheets containing 4-15 elements in each, whereas in the case of *trans-4* single fibers are distinguishable with fewer cases of parallel bundling.

From these images, we can conclude that regiochemistry has a direct effect on the shape of the aggregates. While *N*-centered surfactants **3** form vesicles, *C*-centered surfactants **4** form fibers.

SAXS studies. Very poor information could be obtained for surfactants **3** due to their limited solubility in water and the absence of characteristic features in the scattering curves. SAXS profiles of 1.2 and 0.2 wt % aqueous solution of *cis*- and *trans*-**3**, respectively, are shown in Figure 5a. Only significant intensity is obtained at small q , this can mean that the intensity of other features is too weak to be detected or the absence of electronic density features in the scale from 1 to 20 nm. At low q values, the slope for *trans*-**3** is -2.2 which could be related to rough lamellar structures or polydisperse platelets and from -2.6 to -2.9 for *cis*-**3**, which could be related to a surface fractal structure or polydisperse vesicles. This information is compatible with what is observed in the cryoTEM images.

Figure 5b shows SAXS profiles of 1.6 and 3.0 wt % aqueous solutions of *cis* and *trans*-**4**, respectively. At low q values, *cis*-**4** show slopes around -2.2 that could be related to a rough lamellar structure which could be formed by different cylinders one next to the other. On the other hand, *trans*-**4** has a slope of -1.3, which could be related to cylinders with diameter about 8.2 nm, nicely coincident with what is observed by cryoTEM. Diastereoisomer *trans*-**4** does not show any peak at high q values, which means that any repetition in the structure of the aggregate is not found. A diameter of 8.2 nm cannot be formed by the *trans*-**4** molecule in an arrangement having the cyclobutane at the center and the surface decorated by the polar trimethyl ammonium heads. In fact, the fully extended molecule can hardly expand up to 4 nm; therefore, such cylinders must have a different structure.

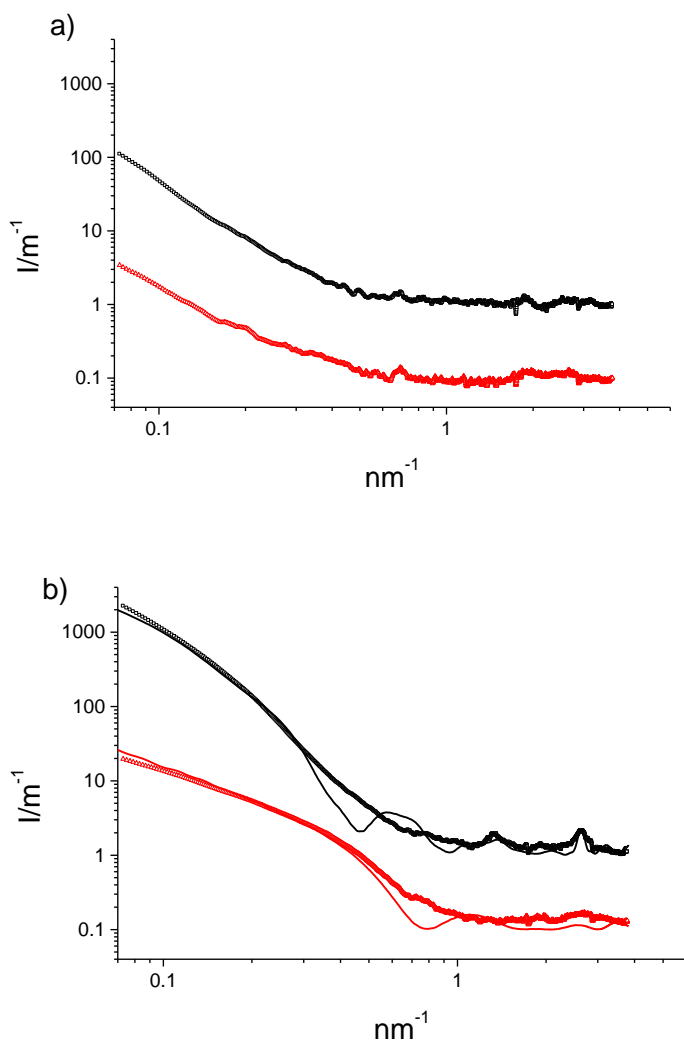


Figure 5. SAXS profiles for *cis-3* (black) and *trans-3* (red) at 1.2 and 0.2 % in weight, respectively (a); *cis-4* (black) and *trans-4* (red) at 1.6 and 3.0 % in weight, respectively, (b); the curves for the *cis* derivatives have been multiplied by a factor of 10 for clarity. The lines correspond to cylindrical models with parameters as described in the text.

The possibility of forming tubes, with the wall formed either by a bilayer (with u-configuration of the molecules) or a monolayer (with rather extended configuration of the molecules) should be considered. The second possibility does not seem too reasonable because of the large difference in the inner and outer surface of the tube, which would lead to a very large difference in area per polar head. A set of 7 tubes with

external diameter of 7.5 nm and internal diameter of 4.0 nm with 500 nm length with random directions and one point of near contact between different tubes at the middle of their length (the cylinders do not necessarily intersect but the centers were distributed with 4.7 nm spacing) fits this trend. This is shown as a line in figure 5b. In contrast, *cis-4* shows two distinct peaks, at $q = 1.3$ and 2.6 nm^{-1} suggesting the aggregation of cylinders. Similarly to the *cis* derivative, the diameter of the cylinders necessary to fit the most intense part of the curve (12 nm in diameter) is bigger than a fully extended surfactant molecule, also suggesting the formation of tubular structure. Moreover, the repetition distance corresponding to the observed peaks suggests that the tubes share the separation walls to make compatible the overall thickness with the repetition distance of 4.8 nm (see the description of the model in Figure S1 of the Supporting Information). The line in Figure 5b corresponds to this model. This analysis is in good agreement with the cryoTEM images from these surfactants.

Computational modeling of the aggregation mode. In order to gain insight into the structure and mode of aggregation of these bolaamphiphiles, theoretical calculations were carried out. The computational study of species present at the water-air interface is challenging because of the discontinuity between the two media. Surfactants dispose at the surface with the polar heads inside water, whereas the hydrophobic tails remain in the air. Bolaamphiphilic surfactants at interfaces may adopt a wicket structure as that shown in Figure 6.³⁴ The self-aggregation of the bolaamphiphiles considered in this work takes place through hydrogen bonds between the hydrophobic portions (among amide groups), which remain outside the aqueous phase. For this reason, we have focused our study on this part of the system.

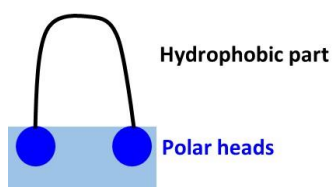


Figure 6. Schematic representation of a bolaamphiphile in the water-air interphase.

We have started by considering models of the four surfactants investigated in this work in which the $-(\text{CH}_2)_n\text{NMe}_3^+$ chains have been replaced by methyl groups (models I, in Figure 7). For these systems the formation of tetrameric aggregates has been studied at the M06-2X/6-31G(d) level of calculation. The two central molecules of each aggregate were used to build models II.

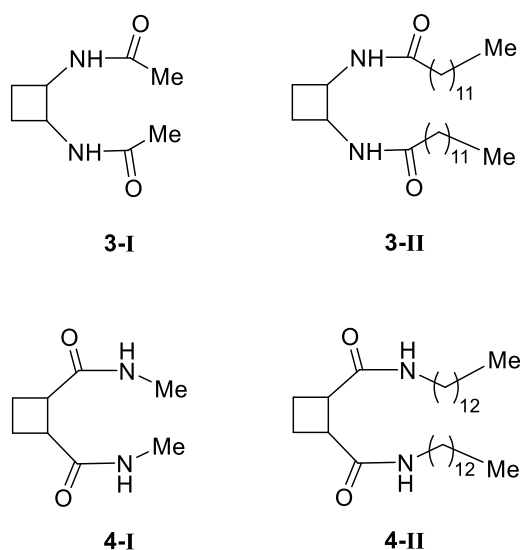


Figure 7. Models of surfactants **3** and **4** considered for the computational study.

The structures of octameric aggregates of models II have been optimized by molecular mechanics (see the Supporting Information for details). The geometry of the internal molecules inside an octameric aggregate can be a good representation of the

aggregation pattern in air. From one of these internal molecules, the full structure of the surfactant can be reconstructed by replacing the terminal methyl groups by the polar ones. The geometries of these “reconstituted” molecules were optimized through molecular mechanics in water solution and are shown in Figure 8. As we can see, the four amphiphiles present a wicket-like conformation, which results from the presence of the rigid cyclobutane ring. However, the distance between polar heads is similar for three of them (*cis-3*, *cis-4* and *trans-4*), whereas *trans-3* shows a much larger distance.

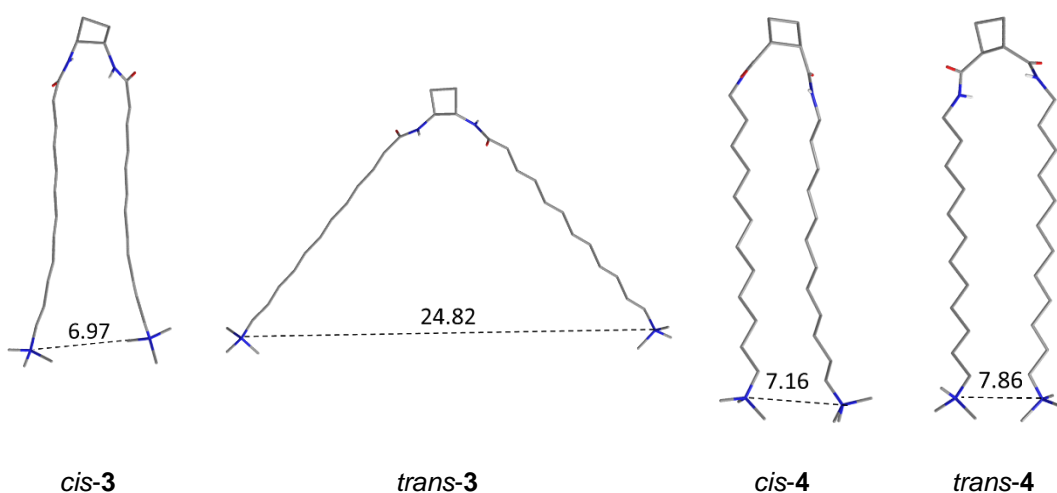


Figure 8. Calculated structures for the four bolaamphiphiles. Non-polar hydrogen atoms were omitted for clarity. Distances between polar heads (d_{NN}) in Å.

From these structures we have estimated the area per molecule at the surface for each amphiphile, A_p , and the contribution of the cationic part, A_p' , (see the Supporting Information for details). The results are shown in Table 2 and if we compare them with the experimental A_{cmc} values (Table 1) we can observe that they are in a qualitative agreement, in particular, for the maximum area per molecule of *trans-3*.

Table 2. Computed Surface Areas (in nm²) of Studied Amphiphiles

Surfactant	A _p ^a	A _p
<i>cis-3</i>	0.79	1.14
<i>trans-3</i>	1.88	2.22
<i>cis-4</i>	0.80	1.15
<i>trans-4</i>	0.85	1.19

^a Cationic part

Distance between polar heads can be related to the effectiveness of the surfactant since the longer is the distance, the smaller is the number of molecules that can be placed at the surface reducing the surface tension. From surface tension measurements (Table 1) we concluded that *trans-3* is the least surface active of all the amphiphiles synthesized (*i.e.* with the lowest effectiveness), and coherently it shows much larger distance between polar heads than the *C*-centered compounds, *cis-4*, and *trans-4*, and the *N*-centered one, *cis-3*. Thus, due to its structure, *trans-3* occupies the largest area at the surface (lowest slope of the curve of the surface tension plot), resulting in the lowest density of molecules at the surface. In addition, it aggregates at lower concentration (cmc).

Experimental results and calculations point out that regiochemistry (*N*- versus *C*-centered amphiphiles) plays a major role in the surface properties mainly for *trans* isomers. This different behavior is already observed in the calculations of models I. For *cis*- and *trans-3* there is a significant difference in the absolute values of the ϕ dihedral angles and in the angles between the two NCO planes which lead to distances between

C atoms of methyl groups of 4.2 Å (*cis-3*) and 7.2 Å (*trans-3*). On the other hand, absolute values of ψ dihedral angles and the angles between NCO planes are much more similar for *cis-4* and *trans-4* and, accordingly, the distances between methyl groups are 4.4 and 5.8 Å, respectively.

The computed distances between polar heads (Figure 8) are a consequence of the structures of model II aggregates. To assess the stability of these aggregates, restricted molecular dynamics calculations have been done (see the Supporting Information for more details). The mean distances between terminal methyl groups along the simulations are 7.2 Å (*cis-3*), 19.8 Å (*trans-3*), 5.3 Å (*cis-4*) and 7.8 Å (*trans-4*). The molecular dynamic calculations show that the structure of the calculated aggregates is rather stable with the maximum dispersion observed for *trans-3* (**figure S7**).

CONCLUSIONS

Four new bolaamphiphiles based on 1,2-disubstituted cyclobutane as a scaffold were synthesized and studied. We have shown that the interplay between the stereoisomerism (cis versus trans 1,2 disubstituted cyclobutane) and regiochemistry (N-centered versus C- centered amide bonds), enhanced by rigidity of the cyclobutane ring, affects different physico-chemical properties quite differently. Namely, the cmc value is mainly governed by stereochemistry, with regiochemistry only modulating this value. The adsorption effectiveness and area per molecule also show large differences that have been rationalized from theoretical results obtained by a new methodology of calculation of these bolaamphiphiles at the surface. On the other hand, regiochemistry definitely governs the morphology of the supramolecular aggregates (*i.e.* long fibers versus plates or spherical assemblies as determined by cryoTEM and SAXS), with stereochemistry finely modulating their structural parameters. As a consequence, the

information about the role that stereochemistry and regiochemistry play in the control of the surfactant abilities in solution and their aggregation must be useful for the rational design of new bolaamphiles with predictable and tunable properties with application in the development of new soft-materials or biomaterials.

ASSOCIATED CONTENT

Supporting information

The Supporting Information is available free of charge *via* the Internet at <http://pubs.acs.org>

Synthesis and characterization of the surfactants and their precursors, ¹H and ¹³C NMR spectra of new compounds. . Sketches of the structures modelled from SAXS. Theoretical study, details in Computational Calculations: Conformational search, determination of the calculated surface area and molecular dynamics.

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Notes

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