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Synthesis and Characterization of Highly Fluorinated Gemini Pyridinium Surfactants

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A series of fluorinated gemini pyridinium amphiphiles have been prepared, purified and fully characterized. The synthesis was performed using alkyl trifluoromethanesulfonates as the quaternizing agents, provided very good yields and overcame problems arising with other less powerful alkylating agents. The use of different characterization techniques, such as conductivity and surface tension measurements, shed light on the aggregation process. The title products showed interesting, unusual properties, which could be interpreted in view of a peculiar aggregating behaviour. This family of compounds is very interesting for their possible transfection activity and application in gene therapy.

Introduction

The field of fluorinated surfactants is very interesting, fascinating and complex, due to the particular chemistry of fluorinated molecules.[1] Many studies have been performed on chemistry, amphiphilic behaviour and biological activity of fluorinated amphiphiles.[2] An important characteristic of fluorinated compounds is that they have the ability to dissolve large quantities of oxygen, and this was exploited to formulate emulsions of perfluorocarbons in water. Some of these compounds received attention for clinical applications as oxygen carriers to substitute for blood in emergencies. They are normally known as "blood substitutes".[1,2b] Other important biological activities were discovered. Some members of a series of galactose-based fluorinated amphiphiles showed very interesting anti-HIV activity coupled to a very low toxicity, making them candidates for applications in AIDS therapy.[3] Moreover, thanks to their lyophobic character, fluorinated surfactants can be used for the in vitro synthesis of membrane proteins[4] because they can minimize the denaturing propensity of surfactants towards membrane proteins.[5]

In recent years, the synthetic challenge to find cationic structures good enough for applications in gene therapy and/or gene delivery produced a plethora of compounds, some of which are constituted by fluorinated surfactants.[6] The applications in this field were reserved to the monomeric type of surfactants, and only a few studies dealt with the synthesis and gene delivery application of gemini surfactants.[7] In the last decade, gemini surfactants were thoroughly studied from the physicochemical point of view.[8] Starting from the easier synthetic methods, very complex structures were recently prepared.[9] In the field of cationic gemini surfactants the more studied structures belong to the quaternary ammonium family.[10] Recently, we reported the synthesis of the first pyridinium gemini surfactants.[11] We studied the structure-activity relationships of these compounds with regard to their ability as DNA carriers for gene delivery. Other labs have reported that gemini pyridinium surfactants substantially enhance the kinetic rate of hydrolysis of organophosphoric esters in water, giving better results than alkylammonium gemini surfactants.[12] Among the surfactant structures for which biological activity or compatibility were demonstrated, those having a fluorocarbon moiety are emerging.[13] Fluorinated surfactants have been shown to be less toxic towards cells in culture and to have a lower haemolytic activity compared to their hydrocarbon analogues and tend to self-assemble more easily and to form better organized and more stable systems than their hydrocarbon counterparts.[13f] At the moment, only a few series of gemini surfactants with a fluorocarbon chain have been prepared.[13] The lower toxicity of fluorinated pyridinium compounds compared to that of alkyl ammonium compounds described in the literature justified our choice to study fluorinated gemini pyridinium surfactants.[13g] In this paper the synthesis and the amphiphilic behaviour of these compounds, as obtained from con-

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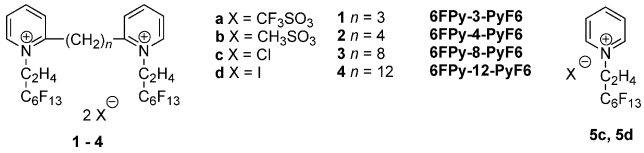
ductivity and surface tension measurements, will be presented. Peculiar results appeared from the characterization of these surfactants, the interpretation of which relies on a particular conformational arrangement and on the formation of premicellar aggregates. A very recent paper[14] describes the structure-activity relationships affecting membrane curvature stress in vivo, denoting the importance of a careful surfactant characterization. This is crucial for the application of these surfactants since the final goal of our study is to find structures capable of gene transfection, a well-assessed research field for which more suitable compounds are urgently needed.

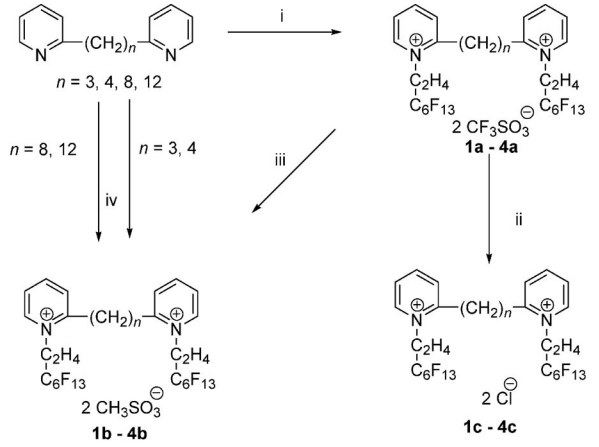
Results and Discussion

Synthesis

We synthesised twelve novel fluorinated gemini pyridinium surfactants (**1a**–**c** to **4a**–**c**) by varying both the length of the spacer (3, 4, 8, and 12 methylene groups) between the two cationic heads and the counterions (methanesulfonate, trifluoromethanesulfonate and chloride). The preparation of these products (Scheme 1) is outlined in Scheme 2. We prepared the 2,2′-(α,ω-alkanediyl)bis(pyridines) following our previously reported synthetic approach for the preparation of alkyl-substituted gemini pyridinium surfactants.[11]



Scheme 1. Synthesised compounds.



Scheme 2. Synthesis of the fluorinated gemini pyridinium surfactants **1**–**4**. i: $CF_3SO_3(CH_2)_2(CF_2)_5CF_3$, $CHCl_3$, reflux, 90 min; ii: Cl^- resin, CH_3OH; iii: $CH_3SO_3^-$ resin, CH_3OH; iv: $CH_3SO_3(CH_2)_2(CF_2)_5CF_3$, 140 °C, 6 h.

In order to have the monomeric reference status for fluorinated pyridinium gemini surfactants, we also prepared the pyridinium surfactants **5c**–**d**, by the simple quaternisation of pyridine with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl iodide and subsequent ionic exchange.[15] Analogously, we also treated the bis(pyridine) bases with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl iodide, but without success. During the reaction tars were produced in high quantity. This could result from a competitive pathway: an elimination reaction leading to an olefin bearing a fluorinated substituent may occur faster than the S_N2 reaction. Maybe the elimination reaction is favoured due to the steric hindrance on both the alkyl halide and the pyridine base.[15c] In an S_N2 reaction, the longer the alkyl chain is a substituent at the reactive carbon, the lower is the reactivity, due to steric hindrance. On the pyridine base, the presence of a substituent α to the nitrogen make the nucleophile nitrogen more hindered. To overcome the low reactivity, we chose a stronger leaving group on the tridecafluorooctyl moiety. In our next attempt, we reacted the bis(pyridine) bases at 140 °C without solvent with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methanesulfonate, which was previously prepared following a standard procedure.[16] The reaction yielded the expected products only in the case of short spacers (n = 3, 4), but the quantities of tars produced were substantial thus leading to difficult purification and low yields (a maximum of approximately 30–40%). Moreover, when working with the bases having longer spacers (n = 8, 12), the above procedure failed. We also performed the reaction in solvents, but without success. Finally, we used trifluoromethanesulfonates instead of methanesulfonates,[11] due to the excellent leaving group ability of the former. The inclusion of tridecafluorooctyl trifluoromethanesulfonate in the quaternisation reaction gave the expected products **1a**–**4a** in good to excellent yields (69–82%), allowing a reduction in temperature, reaction time and a simplification of the purification. We added dropwise the proper α,ω-di(2-pyridyl)alkane to the warmed perfluoroalkylethyl triflate using chloroform to ensure complete solubility of the quaternising agent. The correct addition order was crucial for the reaction to take the desired pathway, thus minimizing the occurrence of the elimination reaction. Upon reversing the addition order, the yield decreased to approximately 10–15%. Compounds **1a** and **2a** directly crystallized in the reaction mixture during cooling to room temperature, and we separated them by filtration and washed them with ethyl acetate to give white pure products. Compounds **3a** and **4a**, in the same conditions, separated as oils, and we centrifuged and washed them with ethyl acetate; a subsequent dissolution in methanol and evaporation of the solvent gave pure products.

Since bis(trifluoromethanesulfonate) gemini pyridinium amphiphiles are poorly soluble in water, the introduction of different counterions was compulsory. Gemini alkyl pyridinium surfactants show[11] low Kraft points (i.e they are able to form micelles in water at room temperature and are useful for biological applications) especially when they are coupled with chlorides and methanesulfonate counterions. In

order to determine the similarities and differences between classical alkyl and fluorinated gemini pyridinium surfactants, were obtained products **1b**–**4b** [= bis(methanesulfonates)] and **1c**–**4c** (= dichlorides) by ionic exchange on a strong ion-exchange resin, working in methanol to avoid foaming during the final solvent evaporation (which usually occurs with water as the solvent, reducing consistently the yield). The products thus obtained were chromatographically pure, and their elemental analyses were in good agreement with theoretical values (see the Exp. Section). Moreover, the purity can be assessed by surface tension measurements, a technique that shows the utmost sensitivity to impurities having higher a hydrophobic character than the compound under study.[17]

Conductivity Measurements

We obtained the critical micellar concentration (cmc) and degree of counterion binding (β) for **2b**–**4b**, **1c**–**4c**, **5c**, and **5d** as a result of conductivity measurements (Table 1).

Table 1. Amphiphilic properties of the surfactants **1**–**4b**,**c** and **5c**,**d** at 25 °C as determined by conductivity measurements.

Entry	Compd.	cmc[a] [mM]	β[a] [%]	cmc[b] [mM]	β[b] [%]
1	**2b**	1.93	51	1.71	51
2	**3b**	1.40	43	1.39	43
3	**4b**	1.05	54	1.11	55
4	**1c**	1.91	62	1.82	63
5	**2c**	1.86	63	1.76	62
6	**3c**	1.29	52	1.22	52
7	**4c**	1.10	55	1.04	54
8	**4c**[c]	0.33	6	0.30	4
9	**5c**[d]	20.31	68	20.09	67
10	**5c**[c]	4.51	21	4.61	16
11	**5d**[e]	9.64	84	10.24	84
12	**5d**[c]	0.34	16	0.33	16

[a] Determined from conductivity by the classical method. [b] Determined from conductivity by the non linear fit method. [c] First discontinuity (not a cmc) at very low surfactant concentration due to ion-couple formation. [d] Measured at 30 °C, Krafft point: 28.4 °C. [e] See also ref.[21]

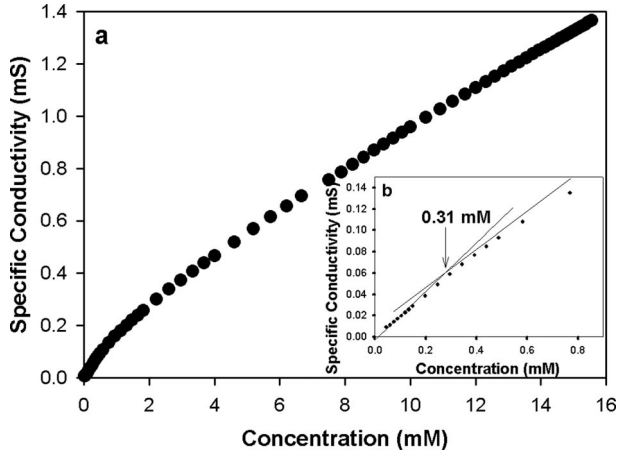


Figure 1. κ vs. C for **4c**: a) full concentration range, b) expanded region at low concentration; the arrows indicate peculiar points for the onset of ion pair formation.

Conductivity data are normally reported as a plot of the specific conductivity (κ) vs. surfactant concentration (C, see Figure 1) or as molar conductivity (Λ) vs. $C^{0.5}$, see Figure 2). In general, the cmc can be easily detected in the first plot, due to an evident discontinuity. However, the second approach can show peculiarities that can signal the formation of ion pairs and/or premicellar aggregates.[18]

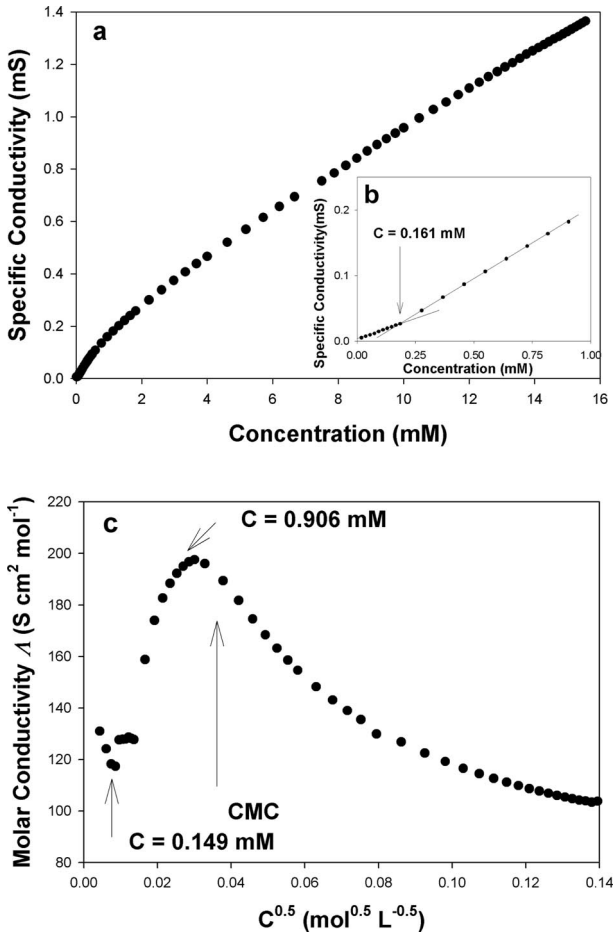


Figure 2. κ vs. C and Λ vs. $C^{0.5}$ plots for **2b**: a) κ vs. C, full concentration range, b) κ vs. C, low concentration range expansion, c) Λ vs. $C^{0.5}$.

Cmc

We obtained the cmc values from a plot of κ vs. surfactant concentration by the intersection of the lines fitted to the dilute and concentrated regions. As previously reported,[11,19] the extrapolation of two linear regimes in the premicellar and in the postmicellar region has been used for a long time to obtain the cmc from the abscissa of the intersection of these two lines. However, this approach is sometimes difficult to apply, and the problem can be overcome by using a recent, non linear, fitting approach.[19b,19c] The first application of this new method to the data obtained from gemini surfactants has given very good results,[11] avoiding the bias that the researcher can introduce using the first method. In our case cmc and β values are reported as obtained with both data analysis methods to allow for comparison with literature data for other surfac-

tants, usually obtained with the first method. The Krafft point of **1b** was 42.2 °C, showing that it is not completely soluble at room temperature (i.e. the cmc was not evaluated). We also determined the cmc of the monomers **5c**–**d**. Compound **5c** has a cmc approximately ten times higher than the corresponding gemini surfactants **1c**–**4c**, which agrees well with general findings in the gemini surfactants field.[8f,10c,20] This behaviour is due to the simultaneous transfer of two hydrophobic chains from water to the micellar environment.[8f,10c] Compound **5d** showed a cmc half of that for **5c**.[21] The cmc values of gemini bis(methanesulfonate) salts **2b**–**4b** were slightly higher than that of chlorides **1c**–**4c**. Both the methanesulfonates **2b**–**4b** and chlorides **1c**–**4c** showed a decrease of the cmc with increased spacer length. Interestingly, the cmc value was approximately halved in both series when the spacer increased in length from three to twelve methylenes. This effect of the spacer length on the cmc value is peculiar, since it is greater (approximately seven times for chlorides) for non fluorinated dodecyl gemini pyridinium surfactants.[11] Usually, the spacer folds towards the hydrophobic chains if it has both a hydrophobic nature and sufficient conformational freedom (i.e., a convenient length).[22] This smaller effect of spacer length on the cmc of fluorinated surfactants could be due to the particular nature of the fluoroalkyl chains, which are both hydrophobic and oleophobic.[23] Fluorinated chains are not compatible with normal hydrocarbon chains and do not interact with them (alkyl and perfluoroalkyl surfactants often do not form mixed micelles but two populations of fluorinated and normal micelles),[10c,20,24] and this should prevent the spacer from folding towards the chains and cause it to act as a partial third chain when the whole chain hydrophobicity is taken into account.

Degree of Counterion Binding

The degree of counterion binding, β, expresses the number of counterions tightly bound to the micelle in the Stern layer to counterbalance the coulombic repulsions that oppose the micellisation process. The evaluation of β is normally determined by the ratio of the slope of the lines graphically fitted in the premicellar and postmicellar ranges. It has been demonstrated that this method underestimates the micellar contribution to conductivity.[30] For this reason, in analogy with cmc data treatment, we obtained β values by the classical method (slopes of the two linear regimes) and the new one (non linear fitting approach),[22,23] allowing for a comparison with previously published literature data for other surfactants. As is evident from Table 1 the two methods agree well. The monomeric chloride and iodide, **5c** and **5d**, respectively, show β values in substantial agreement with that of other alkyl pyridinium halide surfactants.[25] In particular, **5d** shows a remarkably higher β value than that of its chloride counterpart. In the case of gemini pyridinium fluorinated surfactant, chlorides showed a higher degree of counterion binding than methanesulfonates. An opposite behaviour was observed for alkyl dodecyl gemini surfactants,[11] where the methanesulfonates had higher β values (as high as 74%) than those of the chlorides. These gemini pyridinium surfactants, having a short spacer, seem to bind tightly one of the two counterions between the charged pyridinium rings. These opposite behaviours should depend on the different nature of the hydrophobic chain. To our knowledge, data on the effect of methanesulfonate counterions of fluorinated amphiphiles are lacking. In the fluorinated chloride series, the value of β is lower for gemini structures (**1c**–**4c**) than it is for the monomeric one (**5c**) and dodecylpyridinium gemini surfactants.[11] In both the alkyl and fluorinated series, compounds having an octamethylenic spacer show the lowest β value. A low β value indicates poor packing of the surfactant molecules at both the air/water and micellar surface. The conformational arrangement the spacer adopts at the surfaces, modifying the surfactant headgroups compactness, gives a low surface charge density. These β values for fluorinated surfactants indicate that the spacer does not fold towards the fluorinated chains in micelles, which coincides with our interpretation of the cmc values above. The headgroups and counterions at the micellar surface do not give high compactness, in accordance with the usual low aggregation number of the micelles of the fluorinated surfactants. The exact reason for that behaviour and arrangement should be further investigated.

Conductivity Plot Shape

Formation of ion pairs and premicellar aggregates can be detected by inspecting the conductivity plots. Ion-pair occurrence can be detected by a deviation in the κ vs. C and the Λ vs. $C^{0.5}$ plots, which should show a curvature towards the C (or the $C^{0.5}$) axis well below the cmc. Formation of ion pairs anomalously reduces the conductivity of the solution, due to the neutralization of electric charges and the slower diffusion of the ion pairs with respect to the separated ions. The formation of ion pairs is also expected to increase with increasing surfactant concentration. Premicellar aggregates are defined as surfactant aggregates of very low aggregation number. Their formation (see the pictorial sketch of a dimer as an example in Figure 3) results in a curvature, in the low concentration range, towards the κ axis in the κ vs. C plot and in a maximum in the Λ vs. $C^{0.5}$ plot. Both **5c** and **5d** showed a first discontinuity at a concentration lower than that ascribed to the cmc (see Table 1, footnote d).[26] This behaviour is connected with the formation of ion pairs at low concentration. Gemini surfactant **4c** showed a discontinuity in the κ vs. C plot at a concentration approximately three times lower than the cmc (Figure 1, b) with curvature towards the C axis. This discontinuity and the appearance of a deviation from the conductivity predicted by Onsager's law have previously been found for monomeric pyridinium surfactants and other cationic surfactants.[18,26,27] The degree of counterion binding at this first discontinuity is low for **4c**, approximately 6%, and should be taken as evidence of the formation of tight ion pairs between the surfactant ion and one of its counterions.[26] However, the extent of ion-pair formation seems smaller than that is occurring in the case of the monomers **5c** and **5d**. In fact, the formation of these ion

pairs is normally favoured when the concentration is increased[18] and should be more evident, if occurring, for monomeric surfactants, since they have a higher cmc. As we[28] and others[18,29] have recently found for gluco-cationic surfactants, several surfactants (in particular those having high hydrophobic character)[30] show a tendency for premicellar aggregation. Compounds **1**–**4b**,**c** showed a maximum in a Λ vs. $C^{0.5}$ plot (as exemplified by **2b**, Figure 2, c). The maximum was more evident when the hydrophobicity of the molecule is enhanced and is clear evidence for the formation of premicellar aggregates in solution.[18,26b,28,31] As an example, for **2b** (Figure 2, c), this maximum in the Λ vs. $C^{0.5}$ plot occured at 0.906 mM, while the cmc for this surfactant was nearly twice that value at 1.71 mM. The formation of premicellar aggregates[28] was also evidenced by a slight curvature towards the κ axis in the κ vs. C plot in the low concentration range for **2b** at approximately 0.16 mM (see Figure 2, b). We found a minimum in the Λ vs. $C^{0.5}$ plot (Figure 2, c) at 0.149 mM, which agrees well with the discontinuity in the κ vs. C plot (0.149 mM vs. 0.161 mM). We observed this behaviour also for many other gemini surfactants under investigation, for which similar plots to those in Figure 2 are reported in the Supporting Information along with the concentrations at which discontinuities occured.

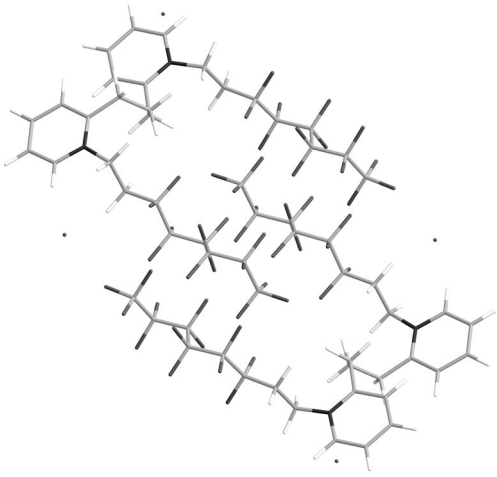


Figure 3. Pictorial view of the arrangement of surfactants in dimers. (See the Supporting Information for a deeper explanation).

Surface Tension Measurements

Surface tension measurements (γ vs. log$C$) are reported in Table 2 and Figures 4 and 5. Compound **1b** showed a remarkably high Krafft point (42.2 °C), and its surface tension was not recorded. Several parameters were determined from the γ vs. log$C$ plots: (i) cmc, taken as the concentration at the point of intersection of the two linear portions of the γ vs. log$C$ plots, (ii) maximum surface excess concentration (Γ_{max} [mol cm^{-2}]) calculated using the Gibbs adsorption Equation (1),[32] (iii) area per molecule at the interface (A_{min} [Å^2]) calculated using Equation (2),[32] (iv) efficiency in surface tension reduction, measured by C_{20} (or pC_{20}), which is the molar surfactant concentration required to reduce the surface tension of the solvent by 20 mN/m,[33] (v) effectiveness of the surface tension reduction, measured by the surface tension at the cmc (γ_{cmc}) and (vi) the cmc/C_{20} ratio, which is the measure of the tendency to form micelles relative to the tendency to adsorb at the air/water interface.

$$\Gamma_{max} = -\frac{1}{2.303nRT}\left(\frac{\partial\gamma}{\partial \log C}\right)_T \quad (1)$$

$$A_{min} = \frac{10^{16}}{N\Gamma_{max}} \quad (2)$$

Table 2. Amphiphilic properties of the surfactants **1**–**4b**,**c** and **5c**,**d** at 25 °C as determined by surface tension measurements.

Entry	Compd.	Cmc [mM]	γ_{cmc} [mN/m]	Γ [$\times 10^{-10}$ mol/cm^2]	A_{min} [Å^2]	pC_{20}	cmc/C_{20}
1	**2b**	0.16	31.6	2.9	57	4.41	4
2	**3b**	0.13	28.4	1.9	86	4.98	13
3	**4b**	0.089	31.2	1.5	110	5.17	13
4	**1c**	0.28	27.7	2.2	75	4.54	10
5	**2c**	0.50	27.7	2.1	79	4.40	11
6	**3c**	0.25	29.2	1.5	108	5.08	19
7	**4c**	0.12	30.9	1.4	119	5.15	21
8	**5c**[a]	22.40	28.3	2.6	63	2.38	5
9	**5d**[b]	7.99	18.5	3.3	50	2.99	8

[a] Determined from the first discontinuity (not a cmc) at very low surfactant concentration, due to ion-couple formation. [b] Measured at 30 °C, Krafft point: 28.4 °C.

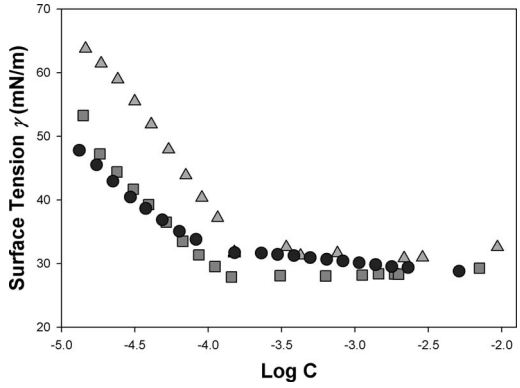


Figure 4. Surface tension vs. log C plots for **2b** (▲), **3b** (■) and **4b** (●).

We took the value of n (the number of ionic species whose concentration at the interface varies with the surfactant concentration in solution) as 2, although for divalent geminis (having one surfactant ion and two non-surfactant counterions), the values of both 2 and 3 have been proposed.[34a] For gemini surfactants, it was found that one of the two counterions is frequently firmly wedged in between the two charged headgroups, especially when the spacer is quite short.[34b] While the use of a different n does not affect the general trend for surface areas, this last finding enabled us to use n = 2 with some confidence.

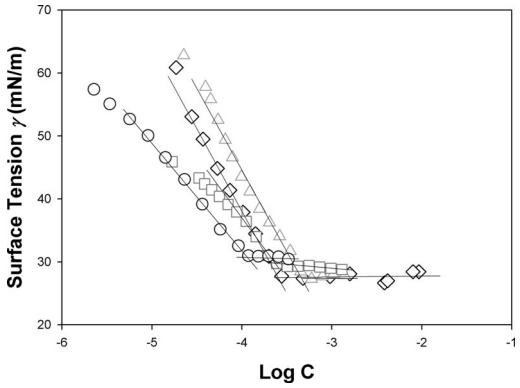


Figure 5. Surface tension vs. log C plots for **1c** (✧), **2c** (Δ), **3c** (□) and **4c** (○).

Cmc

Cmc values determined by surface tension (Table 2) showed a similar trend to cmc values determined by conductivity, but they are even lower by one order of magnitude. Rosen et al.[34] found that the occurrence of premicellar aggregates, especially for very short spacer gemini surfactants, caused the cmc, as determined by either surface tension or conductivity, to be substantially different in value. Similar results have been obtained by Pinazo et al.[31] and Esumi et al.[35] for hydrogenated gemini surfactants derived from arginine and for trimeric surfactants, respectively. They noticed that the cmc values obtained by fluorescence measurements and conductivity were similar to each other but larger than that determined by surface tension. This substantial difference in cmc values as determined by different techniques can be explained if we hypothesize that the small premicellar aggregates, here formed, are not surface-active. If premicellar aggregates are not surface-active, they cannot transfer at the air-water surface, and they do not contribute to the surface tension reduction. As is typical for fluorinated surfactants,[1] we observed the cmc "break" at a very low concentration and the occurrence of the surface tension plateau at around 30 mN/m.

The surface tension cmc is always found to fall in between the two concentrations determined from discontinuities in conductivity plots. As an example, **4b** showed the two conductivity discontinuities at 0.149 mM and 0.9 mM, and the surface tension cmc occured at 0.16 mM. We also observed this trend for all the geminis herein reported (see the figures and data in the Supporting Information). This requires that, after the premicellar aggregates have started to form, any further surfactant added to the solution is involved in the increase of the premicellar aggregate concentration and dimensions until the aggregates transform into regular micelles. Fluorinated surfactants give rise to micelles with small aggregation numbers; this fact may explain why the conductivity and surface tension measurements give such different results for cmc.[13e,36] For monomeric surfactants, the general trend is that β decreases with increasing A_{min}, due to the reduced charge density. This is strictly true for micelles having the same shape and aggregation number since the change from spherical to more complex shapes causes β to increase. The observed increase in β and A_{min} with increasing spacer length suggests the formation of non globular aggregates with lower aggregation numbers.[31]

Other Parameters Obtained by Surface Tension Measurements

The ability to reduce the surface tension is expressed by γ_{cmc} and pC_{20}. As a rule of thumb, the effectiveness in reducing the surface tension increases when the spacer is shortened (see **1c**–**4c**, for example). This would account for a better packing of molecules at the air-water surface. In fact, the incompatibility of alkyl and perfluoroalkyl chains[23c] does not permit the polymethylene spacer to fold towards the fluorinated chains, and it thus remains largely exposed to water. When the spacer is shortened the packing improves. This interpretation is supported by the trend of A_{min} as a function of the spacer length (Figure 6). Such behaviour is peculiar for fluorinated surfactants and is opposite that of alkanediyl-α,ω-bis(dodecyldimethyl)ammonium bromide surfactants,[32a] for which the value of A_{min} increases with increasing n, until n = 6–8. In fact, it has been suggested[32a] that this kind of dependence on n is due to the location of the spacer at the air-water interface. When the spacer is short, it lies more or less prone at the interface, but when it is longer, it changes its conformation and its increased hydrophobicity causes the spacer to fold into the air phase. In this way, the polar heads are allowed to get closer, thus reducing A_{min}. This does not happen for our fluorinated gemini pyridinium surfactants; the linear increase of A_{min} with n with a similar slope (Figure 6), both for methanesulfonates and chlorides, suggests that no change in the conformation of the molecule occurs at the interface, since the hydrogenated spacer can fold towards the fluorinated chains.[23c] A_{min} increases by approximately 6 Å^2 per CH_2 added in the spacer. The effect of counterions on the molecule packing at the interface is considerable. Methanesulfonate counterions always have lower A_{min} values than chlorides (Figure 6). This can be ascribed to the electronic structure of methanesulfonates, which enables an efficient reduction of the charge density of the micelle sur-

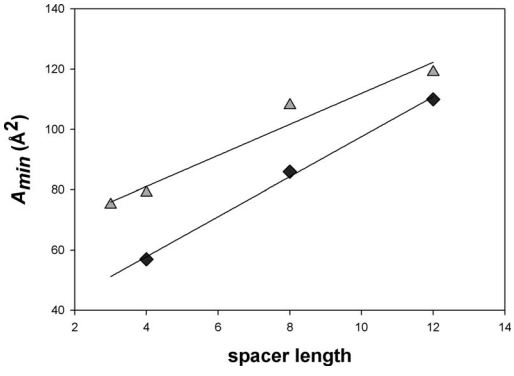


Figure 6. A_{min}, the minimum area per molecule at the interface, as a function of the spacer length, s: chlorides **1c**–**4c** (▲) and methanesulfonates **2b**–**4b** (♦).

face. This observation is in agreement with the generally lower β values for methanesulfonates relative to chlorides. This charge density reduction reduces the coulombic repulsion at the micellar surface and makes β lower than expected.

The pC_{20} value (Table 2) depends on the spacer length of **1c**–**4c**. Increasing the spacer length and hydrophobicity increases the C_{20} value and thus shows that higher molecular hydrophobicities result in an enhanced tendency to adsorb to the air–water surface. The cmc/C_{20} parameter gives some insights into the relative proneness to adsorb or to micellise. In particular, it is evident, once more, that the increase of the spacer length causes an increase in the cmc/C_{20} ratio; in other words, the compounds become more prone to adsorb to the surface than to micellise. This ability is approximately 2–4 times higher than that of the corresponding monomer for the chloride counterion (compare **1c**–**4c** vs. **5c**).

Conclusions

In this work the first series of fluorinated gemini pyridinium surfactants was prepared by quaternisation of α,ω-(2-pyridyl)alkanes. The reaction was performed by using alkyl trifluoromethanesulfonates as the quaternising agents, provided very good yields, and overcame problems arising with other less powerful alkylating agents. Surfactants were characterized by conductivity and surface tension techniques, which indicated the proneness of those molecules to give a more compact adsorbed layer with methanesulfonate counterions than with chlorides. The cmc decreased with spacer length, but the dependence of cmc on spacer length was lower for these compounds than it is for the corresponding alkyl-substituted gemini pyridinium surfactants. We interpreted this outcome as evidence for the difficulty of the long alkyl spacer to fold towards the fluorinated chains. The obtained A_{min} values indicated that these molecules did not change conformation at the air–water interface as a function of the spacer length, as was suggested for the alkanediyl-α,ω-bis(dodecyldimethyl)ammonium bromide surfactants, due to the non ideal mixing between fluorinated and hydrogenated moieties. Moreover increased formation of non globular aggregates of lower aggregation number with increasing spacer length was suggested. The surface tension attained at the cmc was as low as 27 mN/m, showing good surface properties. A peculiar issue of this work was the remarkable difference between the cmc as determined by conductivity and surface tension. This difference, along with the presence of peculiarities in the conductivity plots, could account for the formation of premicellar aggregates. Structure-activity relationships are of the utmost importance in this field, giving a key to the interpretation of biological results. Dymond and Attard[14] described how changes in both the headgroup and hydrocarbon chains affect cytotoxicity. Their considerations could also be true for our compounds. The compounds of the present series, which have short spacers, should, in principle, be less toxic and better candidates for transfection studies.

Experimental Section

General Procedure and Materials: Melting points were taken on a hot plate equipped with a microscope and are uncorrected. ^{1}H NMR (400 MHz) and ^{19}F NMR (376.2 MHz) spectra were recorded with a NMR spectrometer in [D$_6$]DMSO. The residual solvent signal and $CFCl_3$ were used as references for ^{1}H and ^{19}F NMR spectra, respectively. Final assignments for protons were confirmed by chemical shifts, peak multiplicity and a ^{1}H-^{1}H COSY experiment. FT-IR spectra were recorded in KBr with a FT-IR Spectrometer. UV spectra were recorded in 95% ethanol with a UV spectrometer. TLC were performed on silica gel 60 F_{254} or basic alumina plates using petroleum ether/ethyl acetate or BAW (butanol/acetic acid/water, 4:1:5) as eluents. Chromatographic purification was carried out by flash chromatography (FC) using silica gel (40–63 μm) or basic alumina. A strong anion exchanger was used. The ion exchange procedure was described elsewhere.[11] The conductivity measurements were performed as previously reported.[11] Glassware was carefully dried, assembled hot and purged with argon. 2,2′-(Alkanediyl)bis(pyridines) were prepared as previously described.[11] A detailed procedure[15,37] was applied for the synthesis of 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-8-(methylsulfonyl)octane and 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-8-(trifluoromethylsulfonyl)-octane. All characterization data agreed with those in the literature. Water having a conductivity of 0.05 μS and a surface tension of 72.8 mN/m at 20 °C was used for the anionic exchange steps and the conductivity and surface tension measurements.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-trimethylenebis(pyridinium) Ditrifluoromethanesulfonate (1a): 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl trifluoromethanesulfonate (5.45 g, 0.011 mol) was introduced into a three-necked flask with anhydrous chloroform (50 mL) under argon. The solution was warmed to 60 °C, and a chloroform solution of 1,3-di(2-pyridyl)propane (0.87 g, 0.0044 mol) was added dropwise. The reaction was stopped after 1.5 h. During the reaction, a white solid separated on the solution surface, which increased in quantity considerably upon cooling. The solid was filtered and washed with cold chloroform, giving a first crop of a white crystalline solid. A second crop was recovered by the concentration of the resulting chloroform solution to a smaller volume and cooling it in the refrigerator. The solid obtained was filtered and washed with ethyl acetate, which removed the last impurities and gave a white crystalline solid; yield 4.12 g, 79%; m.p. 203–206 °C; R_f = 0.28 on silica (BAW). UV (ethanol): λ_{max} = 269 nm, logε = 4.13. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.31 (m, 2 H, PyCH$_2$CH_2CH$_2$Py), 3.17 (m, 4 H, N$^+$CH$_2$CH_2), 3.26 (t, J = 7.9 Hz, 4 H, PyCH_2CH$_2$CH_2Py), 4.95 (t, J = 7.3 Hz, 4 H, N$^+$CH_2), 8.09 (t, J = 7.5 Hz, 2 H, H_{meta}), 8.16 (d, J = 7.5 Hz, 2 H, H'_{meta}), 8.62 (t, J = 7.8 Hz, 2 H, H_{para}), 9.12 (d, J = 5.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR: (376.2 MHz, [D$_6$]DMSO): δ = –78.30 (CF_3SO_3), –80.92 (CF_3), –113.34 (CF_2CH$_2$), –122.34, (CF_2), –123.37, (2 CF_2), –126.47 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3090, 1636, 1516, 1484, 1252, 1192, 790 cm^{-1}. MS-ESI: m/z = 1041 [M – CF_3SO_3]. $C_{31}H_{22}F_{32}N_2O_6S_2$ (1190.04): calcd. C 31.27, H 1.86, N 2.35; found C 31.34, H 1.83, N 2.29.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-tetramethylenebis(pyridinium) Ditrifluoromethanesulfonate (2a): The reaction was performed as for **1a** with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl trifluoromethanesulfonate (5.85 g, 0.0118 mol) and 1,4-di(2-pyridyl)butane (0.91 g, 0.0043 mol); yield 4.25 g, 82%; m.p. 137–139 °C; R_f = 0.15 on silica (BAW). UV (ethanol): λ_{max} = 269 nm, logε = 3.97. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.95 (m, 4 H, PyCH$_2$CH_2), 3.20 (m, 8 H, N$^+$CH$_2$CH_2 and PyCH_2), 4.94 (t, J = 7.6 Hz, 4 H, N$^+$CH$_2$), 8.06 (dt, J_{ortho} = 7.5, J_{meta} = 1.2 Hz,

2 H, H_{meta}), 8.10 (dd, J_{ortho} = 8.1, J_{meta} = 1.0 Hz, 2 H, H'_{meta}), 8.57 (dt, J_{ortho} = 7.7, J_{meta} = 1.4 Hz, 2 H, H_{para}), 9.11 (dd, J_{ortho} = 6.3, J_{meta} = 1.0 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]-DMSO): δ = –78.28 (CF_3SO_3), –80.86 (CF_3), –113.36 (CF_2CH_2), –122.30, (CF_2), –123.30, (CF_2), –123.45 (CF_2), –126.43 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 2930, 1636, 1537, 1483, 1288, 1155, 807 cm^{-1}. MS-ESI: m/z = 1055 [M – CF_3SO_3]. $C_{32}H_{24}F_{32}N_2O_6S_2$ (1204.06): calcd. C 31.91, H 2.01, N 2.33; found C 31.97, H 2.00, N 2.29.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-octamethylenebis(pyridinium) Ditrifluoromethanesulfonate (3a): The reaction was performed as for **1a** with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl trifluoromethanesulfonate (21.2 g, 0.043 mol) and 1,8-di(2-pyridyl)octane (4.60 g, 0.017 mol). The reaction was stopped after 3 h, and a viscous brown oil separated. The chloroform phase was carefully removed by centrifugation, the remaining oil was dissolved and recovered with methanol, and the solvents were evaporated under vacuum. A brown viscous oil was obtained; yield 19.85 g, 92%; R_f = 0.25 on silica (BAW). UV (ethanol): λ_{max} = 269 nm, logε = 4.16. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.40 (m, 8 H, 4 CH_2), 1.75 (m, J = 7.0 Hz, 4 H, PyCH$_2$CH_2), 3.12 (m, 8 H, N$^+$CH$_2$CH_2 and PyCH_2), 4.91 (t, J = 7.3 Hz, 4 H, N$^+$CH$_2$), 8.03 (t, J = 7.0 Hz, 2 H, H_{meta}), 8.08 (d, J = 7.9 Hz, 2 H, H'_{meta}), 8.55 (t, J = 8.0 Hz, 2 H, H_{para}), 9.10 (d, J = 6.1 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –78.44 (CF_3SO_3), –81.12 (CF_3), –113.43 (CF_2CH_2), –122.41, (CF_2), –123.41, (CF_2), –123.65 (CF_2), –126.60 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3090, 2931, 1632, 1514, 1484, 1274, 1168, 790 cm^{-1}. MS-ESI: m/z = 1111 [M – CF_3SO_3]. $C_{36}H_{32}F_{32}N_2O_6S_2$ (1260.12): calcd. C 34.30, H 2.56, N 2.22; found C 34.25, H 2.50, N 2.28.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-dodecamethylenebis(pyridinium) Ditrifluoromethanesulfonate (4a): The reaction was performed as for **1a** with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl trifluoromethanesulfonate (3.23 g, 0.0065 mol) and 1,8-di(2-pyridyl)octane (0.85 g, 0.0026 mol). The reaction was stopped after 3 h, and a viscous brown oil separated. The chloroform phase was carefully removed by centrifugation, the resulting oil was dissolved and recovered with methanol, and the solvents were evaporated under vacuum. A brown viscous oil was obtained; yield 3.11 g, 91%; R_f = 0.29 on silica (BAW). UV (ethanol): λ_{max} = 269 nm, logε = 3.97. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.42 (m, 16 H, 8 CH_2), 1.75 (m, J = 7.4 Hz, 4 H, PyCH$_2$CH_2), 3.12 (m, 8 H, N$^+$CH$_2$CH_2 and PyCH_2), 4.91 (t, J = 7.6 Hz, 4 H, N$^+$CH$_2$), 8.03 (dt, J_{ortho} = 6.9, J_{meta} = 1.2 Hz, 2 H, H_{meta}), 8.08 (dd, J_{ortho} = 8.0, J_{meta} = 1.0 Hz, 2 H, H'_{meta}), 8.55 (dt, J_{ortho} = 7.9, J_{meta} = 1.1 Hz, 2 H, H_{para}), 9.10 (d, J = 6.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –78.37 (CF_3SO_3), –80.97 (CF_3), –113.27 (CF_2CH_2), –122.29, (CF_2), –123.30, (CF_2), –123.54 (CF_2), –126.45 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3086, 2933, 1628, 1516, 1480, 1272, 1166, 802 cm^{-1}. MS-ESI: m/z = 1167 [M – CF_3SO_3]. $C_{40}H_{40}F_{32}N_2O_6S_2$ (1316.18): calcd. C 36.48, H 3.06, N 2.13; found C 36.51, H 3.02, N 2.11.

General Ion-Exchange Procedure to Obtain Products 1–4 with Different Counterions: The ion exchange was performed using a strong anion exchange resin (32 g for 1 g of product), conditioned by being suspended in water for 0.5 h, being packing in a glass column, and being treating in order with aq NaOH (4%), water (until the effluent was neutral), an aq solution (10%) of the sodium salt of the proper counterion (KCl or CH_3SO_3Na) and water to eliminate the residual salt. Before use, the resin was unpacked, filtered through a funnel and twice suspended in methanol for 0.5 h. Finally, the methanolic suspension was packed in the column and washed with 3 volumes of methanol. A methanolic solution of the product to be subjected to the ion exchange was added to the column and eluted with methanol. After evaporation of methanol, the product with the desired counterion was recovered and purified by crystallization.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-trimethylenebis(pyridinium) Dimethanesulfonate (1b): Procedure 1: Into a three-necked, round-bottomed flask, 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-8-(methylsulfonyl)octane (28.02 g, 0.063 mol) was introduced and warmed to 140 °C whilst stirring. The 1,3-di(2-pyridinyl)propane base (5.00 g, 0.025 mol) was introduced in small quantities over 2 h. As the reaction proceeded, the viscosity of the system increased, and stirring became progressively more difficult. The reaction was stopped after 6 h by rapid cooling (ice bath), and the crude reaction product was suspended a few times in ethyl acetate, giving a solid that was purified by crystallization from hot ethanol/ethyl acetate. A grey powder was obtained; yield 10.20 g, 37%. Procedure 2: Product **1a** was subjected to ion exchange following an previously described protocol.[11] Crystallization from the same solvent mixture used above gave a white solid; yield 95%; m.p. 295–303 °C; R_f = 0.26 on silica (BAW). UV (ethanol): λ_{max} = 204, 271 nm, logε = 4.16, 4.35. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.27 (t, J = 7.3 Hz, 2 H, PyCH$_2$CH_2CH$_2$Py), 2.34 (s, 6 H, CH_3SO_3), 3.22–2.97 (m, 8 H, PyCH_2CH$_2$CH_2Py, N$^+$CH$_2$CH_2), 4.96 (t, J = 7.2 Hz, 4 H, N$^+$CH$_2$), 8.07 (dt, J_{ortho} = 7.0, J_{meta} = 1.6 Hz, 2 H, H_{meta}), 8.17 (d, J = 8.1 Hz, 2 H, H'_{meta}), 8.61 (dt, J_{ortho} = 7.9, J_{meta} = 1.2 Hz, 2 H, H_{para}), 9.12 (d, J = 6.1 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR: (376.2 MHz, [D$_6$]DMSO): δ = –81.09 (CF_3), –113.26 (CF_2CH_2), –122.36, (CF_2), –123.43, (2 CF_2), –126.47 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3066, 2922, 2854, 1640, 1524, 1458, 1346, 1202, 1126, 1050, 780 cm^{-1}. MS-ESI: m/z = 987 [M – CH_3SO_3]. $C_{31}H_{28}F_{26}N_2O_6S_2$ (1082.10): calcd. C 34.39, H 2.61, N 2.59; found C 34.35, H 2.56, N 2.63.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-trimethylenebis(pyridinium) Dichloride (1c): The product obtained by the ion exchange of **1a** was freeze-dried, providing a white-grey powder. Crystallization from ethanol/ethyl acetate gave white-grey crystals; yield 89%; m.p. 208–210 °C; R_f = 0.29 on silica (BAW). UV (ethanol): λ_{max} = 203, 270 nm, logε = 3.02, 3.18. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.32 (m, J = 7.3 Hz, 2 H, PyCH$_2$CH_2CH$_2$Py), 3.22–3.00 (m, 8 H, N$^+$CH$_2$CH_2, PyCH_2CH$_2$CH_2Py), 4.97 (t, J = 7.3 Hz, 4 H, N$^+$CH$_2$), 8.07 (dt, J_{ortho} = 6.9, J_{meta} = 1.2 Hz, 2 H, H_{meta}), 8.18 (d, J_{ortho} = 8.1 Hz, 2 H, H'_{meta}), 8.61 (dt, J_{ortho} = 8.0, J_{meta} = 1.4 Hz, 2 H, H_{para}), 9.13 (d, J = 6.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –81.35 (CF_3), –113.63 (CF_2CH_2), –122.36, (CF_2), –123.69, (2 CF_2), –126.86 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3042, 2926, 2858, 1632, 1514, 1240, 1186, 1122, 1030, 786 cm^{-1}. MS-ESI: m/z = 927 [M – Cl]. $C_{29}H_{22}Cl_2F_{26}N_2$ (962.07): calcd. C 36.16, H 2.30, N 2.91; found C 36.10, H 2.35, N 2.93.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-tetramethylenebis(pyridinium) Dimethanesulfonate (2b): Procedure 1: Synthesis was performed with 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-8-(methylsulfonyl)octane under conditions similar to those used for product **1b**; yield 58%. Procedure 2: By ion exchange; yield 89%; m.p. 295 °C (dec.); R_f = 0.17 on silica (BAW). UV (ethanol): λ_{max} = 271 nm, logε = 4.16. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.00 (m, 4 H, PyCH$_2$CH_2), 2.34 (s, 6 H, CH_3SO_3), 3.25–3.00 (m, 8 H, N$^+$CH$_2$CH_2, PyCH_2CH$_2$), 4.96 (t, J = 7.5 Hz, 4 H, N$^+$CH$_2$), 8.07 (dt, J_{ortho} = 7.6, J_{meta} = 1.3 Hz, 2 H, H_{meta}), 8.17 (dd, J_{ortho} = 8.1, J_{meta} = 1.1 Hz, 2 H, H'_{meta}), 8.61 (dt, J_{ortho} = 7.9, J_{meta} = 1.2 Hz, 2 H, H_{para}), 9.15 (d, J = 6.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR

(376.2 MHz, [D$_6$]DMSO): δ = –81.13 (CF_3), –113.32 (CF_2CH_2), –122.41, (CF_2), –123.40 and –123.56 (2 CF_2), –126.39 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3066, 2932, 2864, 1640, 1522, 1468, 1236, 1160, 810 cm^{-1}. MS-ESI: m/z = 1001 [M – CH_3SO_3]. $C_{32}H_{30}F_{26}N_2O_6S_2$ (1096.11): calcd. C 35.05, H 2.76, N 2.55; found C 35.13, H 2.72, N 2.52.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-tetramethylenebis(pyridinium) Dichloride (2c): The product was obtained by the ion exchange of **2a**; yield 87%; m.p. 238–240 °C; R_f = 0.19 on silica (BAW). UV (ethanol): λ_{max} = 270 nm, logε = 4.11. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.95 (m, 4 H, $PyCH_2CH_2$), 3.32–3.00 (m, 8 H, $N^+CH_2CH_2$, and $PyCH_2$), 4.97 (t, J = 7.6 Hz, 4 H, N^+CH_2), 8.07 (dt, J_{ortho} = 7.5, J_{meta} = 1.3 Hz, 2 H, H_{meta}), 8.13 (dd, J_{ortho} = 8.1, J_{meta} = 1.0 Hz, 2 H, H'_{meta}), 8.59 (dt, J_{ortho} = 7.8, J_{meta} = 1.1 Hz, 2 H, H_{para}), 9.15 (d, J = 6.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –80.99 (CF_3), –113.16 (CF_2CH_2), –122.20, (CF_2), –123.33 and –123.48 (2 CF_2), –126.39 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3070, 2928, 2869, 1642, 1520, 1465, 1235, 1157, 820 cm^{-1}. MS-ESI: m/z = 941 [M – Cl]. $C_{30}H_{24}Cl_2F_{26}N_2$ (976.09): calcd. C 36.87, H 2.47, N 2.87; found C 36.83, H 2.49, N 2.85.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-octamethylenebis(pyridinium) Dimethanesulfonate (3b): The ion exchange of **3a** gave a slightly brown viscous oil; yield 7.90 g, 87%; R_f = 0.22 on silica (BAW). UV (ethanol): λ_{max} = 269 nm, logε = 4.12. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.43 (m, 8 H, 4 CH_2), 1.73 (m, J = 7.5 Hz, 4 H, $PyCH_2CH_2$), 2.29 (s, 6 H, CH_3SO_3), 3.25–3.00 (m, 8 H, $N^+CH_2CH_2$, and $PyCH_2$), 4.92 (t, J = 7.5 Hz, 4 H, N^+CH_2), 8.04 (dt, J_{ortho} = 7.6, J_{meta} = 1.2 Hz, 2 H, H_{meta}), 8.10 (dd, J_{ortho} = 8.1, J_{meta} = 1.0 Hz, 2 H, H'_{meta}), 8.57 (dt, J_{ortho} = 7.9, J_{meta} = 1.3 Hz, 2 H, H_{para}), 9.13 (dd, J_{ortho} = 6.3, J_{meta} = 1.0 Hz 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –81.26 (CF_3), –113.47 (CF_2CH_2), –122.47, (CF_2), –123.48, –123.67 (2 CF_2), –126.69 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3072, 2933, 2874, 1640, 1520, 1468, 1238, 1161, 800 cm^{-1}. MS-ESI: m/z = 1057 [M – CH_3SO_3]. $C_{36}H_{38}F_{26}N_2O_6S_2$ (1152.18): calcd. C 37.51, H 3.32, N 2.43; found C 37.54, H 3.27, N 2.40.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-octamethylenebis(pyridinium) Dichloride (3c): The ion exchange of **3a** gave a white crystalline solid; yield 1.33 g, 57%; m.p. 123–126 °C; R_f = 0.19 on silica (BAW). UV (ethanol): λ_{max} = 271 nm, logε = 4.10. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.40 (m, 8 H, 4 CH_2), 1.75 (m, J = 7.3 Hz, 4 H, $PyCH_2CH_2$), 3.25–3.00 (m, 8 H, $N^+CH_2CH_2$ and $PyCH_2$), 4.95 (t, J = 7.1 Hz, 4 H, N^+CH_2), 8.05 (t, J = 6.6 Hz, 2 H, H_{meta}), 8.10 (d, J = 8.1 Hz, 2 H, H'_{meta}), 8.57 (t, J = 8.3 Hz, 2 H, H_{para}), 9.19 (d, J = 6.5 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –80.90 (CF_3), –113.27 (CF_2CH_2), –122.30, (CF_2), –123.30 and –123.52 (2 CF_2), –126.43 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3066, 2931, 2881, 1647, 1528, 1468, 1244, 1154, 826 cm^{-1}. MS-ESI: m/z = 997 [M – Cl]. $C_{34}H_{32}Cl_2F_{26}N_2$ (1032.15): calcd. C 39.51, H 3.12, N 2.71; found C 39.56, H 3.09, N 2.67.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-dodecamethylenebis(pyridinium) Dimethanesulfonate (4b): The ion exchange of **4a** gave a slightly brown viscous oil; yield 80%; m.p. 137–139 °C; R_f = 0.06 on silica (BAW). UV (ethanol): λ_{max} = 270 nm, logε = 4.19. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.27 (br. s, 12 H, 6 CH_2), 1.43 (m, 4 H, 2 CH_2), 1.73 (m, J = 7.2 Hz, 2 H, $PyCH_2CH_2$), 2.29 (s, 6 H, CH_3SO_3), 3.25–3.00 (m, 8 H, $N^+CH_2CH_2$, and $PyCH_2$), 4.92 (t, J = 7.4 Hz, 4 H, N^+CH_2), 8.04 (dt, J_{ortho} = 6.9, J_{meta} = 1.2 Hz, 2 H, H_{meta}), 8.09 (dd, J_{ortho} = 8.1, J_{meta} = 1.1 Hz, 2 H, H'_{meta}), 8.54 (dt, J_{ortho} = 7.8, J_{meta} = 1.1 Hz, 2 H, H_{para}), 9.13 (d, J = 6.3 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –81.05 (CF_3), –113.31 (CF_2CH_2), –122.34, (CF_2), –123.35 and –123.54 (2 CF_2), –126.53 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3063, 2928, 2878, 1638, 1526, 1465, 1242, 1152, 826 cm^{-1}. MS-ESI: m/z = 1113 [M – CH_3SO_3]. $C_{40}H_{46}F_{26}N_2O_6S_2$ (1208.24): calcd. C 39.74, H 3.84, N 2.32; found C 39.72, H 3.87, N 2.36.

1,1′-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2′-dodecamethylenebis(pyridinium) Dichloride (4c): The ion exchange of **4a** gave a white solid; yield 88%; m.p. 108–110 °C; R_f = 0.05 on silica (BAW). UV (ethanol): λ_{max} = 270 nm, logε = 4.17. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.31 (m 16 H, 8 CH_2), 1.73 (m, J = 7.2 Hz, 4 H, $PyCH_2CH_2$), 3.25–3.00 (m, 8 H, $N^+CH_2CH_2$, and $PyCH_2$), 4.94 (t, J = 7.2 Hz, 4 H, N^+CH_2), 8.02 (dt, J_{ortho} = 6.9, J_{meta} = 1.2 Hz, 2 H, H_{meta}), 8.09 (dd, J_{ortho} = 8.2, J_{meta} = 1.1 Hz, 2 H, H'_{meta}), 8.54 (dt, J_{ortho} = 8.0, J_{meta} = 1.1 Hz, 2 H, H_{para}), 9.15 (d, J_{ortho} = 6.3, J_{meta} = 1.0 Hz, 2 H, H_{ortho}) ppm. ^{19}F NMR (376.2 MHz, [D$_6$]DMSO): δ = –80.82 (CF_3), –113.18 (CF_2CH_2), –122.23, (CF_2), –123.23 and –123.46 (2 CF_2), –126.36 (CF_3CF_2) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3068, 2931, 2882, 1640, 1525, 1470, 1244, 1154, 826 cm^{-1}. MS-ESI: m/z = 1053 [M – Cl]. $C_{38}H_{40}Cl_2F_{26}N_2$ (1088.22): calcd. C 41.89, H 3.70, N 2.57; found C 41.94, H 3.64, N 2.53.

1-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)pyridinium Iodide (5d) and Chloride[15,38] (5c): To a three-necked flask, pyridine was added (88.75 g, 93.4 mL, 1.12 mol) and heated at reflux under magnetic stirring. 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl iodide (96.17 g, 0.203 mol) was slowly added dropwise, and the reaction was continued for another 6 h. After the reaction was cooled, a yellow precipitate separated, which was filtered and crystallized a few times from acetone. Yellow crystals of the iodide **5d** were obtained; yield 85.3 g, 76%; m.p. 234–236 °C; R_f = 0.32 on silica (BAW). UV (ethanol): λ_{max} = 260 nm, logε = 3.68. ^{1}H NMR (400 MHz, CD_3OD): δ = 3.31 (m, 2 H, $N^+CH_2CH_2$), 5.19 (t, J = 7.4 Hz, 2 H, N^+CH_2), 8.29 (t, J = 8.1 Hz, 2 H, H_{meta}), 8.79 (t, J = 7.9 Hz, 1 H, H_{para}), 9.28 (d, J = 6.5 Hz, 2 H, H_{ortho}) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3068, 2931, 2882, 1640, 1525, 1470, 1244, 1154, 826 cm^{-1}. MS-ESI: m/z = 426 [M – I]. $C_{13}H_9F_{13}IN$ (552.96): calcd. C 28.23, H 1.61, N 2.53; found C 28.25, H 1.57, N 2.51. After the usual ionic exchange, **5c** was obtained as white crystals. Caution was taken to freeze-dry the product before crystallization from acetone/methanol since traces of water caused a consistent decrease in the crystallization yield. Very hygroscopic product; yield 80%; R_f = 0.32 on silica (BAW). UV (ethanol) λ_{max} = 260 nm, logε = 3.66. ^{1}H NMR (400 MHz, CD_3OD): δ = 3.26 (m, 2 H, $N^+CH_2CH_2$), 5.16 (t, J = 7.5 Hz, 2 H, N^+CH_2), 8.27 (t, J = 8.0 Hz, 2 H, H_{meta}), 8.77 (t, J = 8.0 Hz, 1 H, H_{para}), 9.23 (d, J = 6.5 Hz, 2 H, H_{ortho}) ppm. FT-IR (KBr): $\tilde{\nu}$ = 3068, 2931, 2882, 1640, 1525, 1470, 1244, 1154, 826 cm^{-1}. MS-ESI: m/z = 426 [M – Cl]. $C_{13}H_9ClF_{13}N$ (461.02): calcd. C 33.82, H 1.97, N 3.03; found C 33.79, H 1.99, N 3.08.

Conductivity Measurements: Conductivity measurements were performed on an conductivity meter equipped with a conductivity cell having a cell constant of 0.943 cm^{-1}, as previously reported.[26a] The addition of concentrated surfactant solutions by a titrator and the collection of the conductivity data were performed by using a computer-controlled automated system, working with a home-made program, written in Quick Basic, available from the author.

Krafft Point Measurements: Krafft points were first estimated by visual inspection of the clearing point of surfactant suspensions (50 mg in 2 mL of water). When values different from 0 °C were observed, a careful procedure was applied using conductivity measurements.[39]

Surface Tension Measurements: The surface tension was measured using a digital tensiometer. Measurements were made at 25 ± 0.1 °C using the Du Noüy ring [Pt/Ir alloy (80:20); 60 ± 0.2 mm circumference, 0.4 mm wire diameter, 1.6 g weight]. Sample temperature was controlled within 0.1 °C using a circulating water thermostatic bath. The data were corrected according to the Zuidema and Waters[40] method. The instrument was calibrated against double-distilled (and previously deionised) water and equilibrated against atmospheric CO_2 each time measurements were performed. Because the dicationic gemini surfactants adsorb onto negatively charged glass surfaces, all glassware was thoroughly soaked with the solution to be measured; soaking solutions were discarded. The fresh solution was aged for several hours before surface tension measurement. Sets of measurements were taken at 15 min intervals until no significant change occurred using a very slow ring rising velocity. These tactics ensure that the ring is completely wetted. The standard deviation of the surface tension measurements was less than 0.15 mN/m. The absence of a minimum in the surface tension vs. log C plot in the post-cmc region showed that there was very little or no surface-active impurity present in the final products.

Supporting Information (see also the footnote on the first page of this article): ^{1}H NMR experiments for new compounds, κ vs. C plots and Λ vs. $C^{0.5}$ plots for all soluble surfactants and further comments on the parameters obtained by surface tension measurements are reported.

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