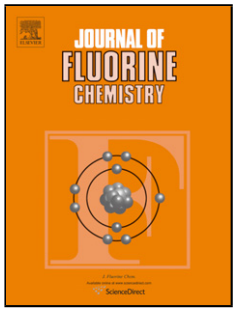

Title: Novel fluorinated dialkylphosphonatocholines: synthesis, physicochemical properties and antiprotozoal activities against *Acanthamoeba* spp.

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Highlights

- synthesis of fluorinated gemini zwitterionic surfactants
- the physicochemical properties expressed as micellar and solubilization properties
- biological activities of prepared compounds tested against *Acanthamoeba* spp.

Novel fluorinated dialkylphosphonatocholines: synthesis, physicochemical properties and antiprotozoal activities against *Acanthamoeba* spp.

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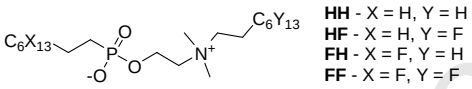
Graphical abstract



Abstract – The synthesis of four new organophosphorous gemini surfactants is presented. They belong to the class of dialkylphosphonatocholines. Tails are represented with two octyl groups both non or partially fluorinated. The synthesis was performed by reaction of alkylphosphonic acids with choline derivatives in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride. The micellar, surface active, and solubilization properties of new surfactants were studied. Antiprotozoal activities were tested against *Acanthamoeba lugdunensis* and *Acanthamoeba quina*.

Keywords: critical micelle concentration, fluorinated surfactants, miltefosine, solubilization, trophocidal activity

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1. Introduction

Alkylphosphocholines are compounds with interesting physicochemical [1-5] and biological properties [6,7]. These amphiphilic compounds are classified as pH non-sensitive zwitterionic surfactants. They contain two charges: the negative one, located at the phosphate anion, and the positive one represented by the ammonium cation. Ions are connected with a spacer, mainly ethylene. Critical micelle concentration (CMC) is one of the most important physicochemical parameters describing the properties of surfactants. Alkylphosphocholines as representatives of zwiterionic amphiphilic compounds have lower values of CMC than ionic surfactants with similar structure [8]. They are more related to non-ionic surfactants than to ionic ones. Antiprotozoal and anticancer activities of alkylphosphocholines are the most interesting and the most studied biological properties of this class of compounds. Hexadecylphosphocholine (HPC, miltefosine) is the main representative of alkylphosphocholines. It is used in treatment of skin metastases of breast cancer and visceral leishmaniosis [6].

Fluorinated surfactants have several advantages in comparison with surfactants containing only hydrocarbon alkyl chains. Their physicochemical and biological activities are improved in many cases in comparison with conventional surfactants [9]. They posses lower values of CMCs [10,11], decreasing of surface tension of surfactants solution is more effective, the values are lower in many cases than 20 $mN.m^{-1}$ [12-14] or they form interesting aggregates [12,15-17]. Fluorinated surfactants also received attention for their interesting biological activities. They are used e.g. as stabilizers of fluorocarbon-in-water emulsions for the preparation of blood substitutes [18]. They have lower haemolytic activities [18,19] or enhanced activities against some microorganisms [20,21] compared to classical hydrocarbon

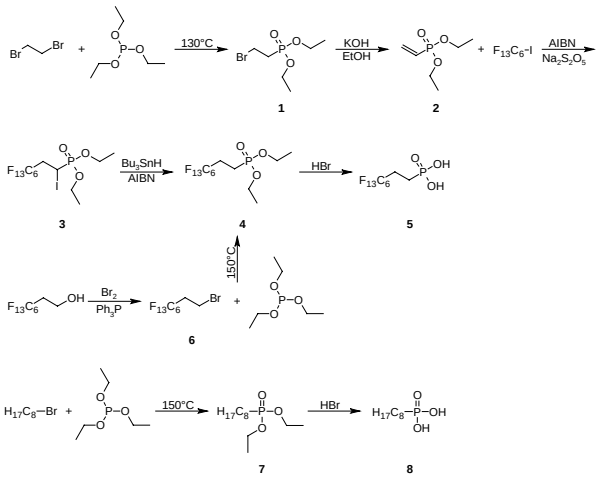
surfactants. Furthermore, they are also studied as antibody binding [22] or nucleic acids transfer agents [23].

The main goal of this work is the preparation and the study of physicochemical and biological properties of dialkylphosphonatocholines with hydrocarbon and highly fluorinated tails. The compounds are representatives of new types of organophosphorus heterogemini surfactants. Optimization of the synthesis of the compounds was also studied.

2. Results and discussion

2.1. Chemistry

Altogether four new non-fluorinated and partially fluorinated dialkyphosphonatocholines were synthesised. The common structural motif of the prepared compounds is the presence of a phosphonate anion connected with an ammonium cation by an ethylene spacer and two eight carbon atom length alkyl chains (C_8), which are attached to the phosphonate anion and to the quaternary nitrogen. Differences in the molecules are in alkyl chains. Compounds are possessing an octyl and/or 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-tridecafluorooctyl groups. One dialkylphosphonatocholine was prepared with two octyl groups (**HH**), two others with one partially fluorinated alkyl chain and one octyl group (**HF**, **FH**) and one with two partially fluorinated alkyl chains (**FF**).



Scheme 1. Preparation of alkylphosphonic acids

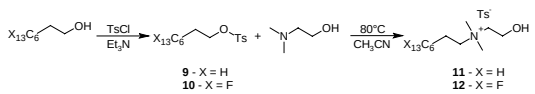
The synthesis started with the preparation of alkylphosphonic acids. *1H,1H,2H,2H*-Perfluorooctylphosphonic acid (**5**) was prepared with two synthetic approaches (Scheme 1). The main step in the first approach was the coupling of diethyl vinylphosphonate (**2**) with perfluorohexyl iodide. Diethyl vinylphosphonate was prepared by a general procedure [24,25]. The Michaelis-Arbuzov reaction of 1,2-dibromoethane with triethyl phosphite yielded diethyl 2-bromophosphonate (**1**). This reaction was performed with excess of 1,2-dibromoethane because tetraethyl ethylene-1,2-bisphosphonate was concurrently formed. 1,2-Dibromoethane was in 4 fold excess to triethylphosphite. However, the yield of **1** was only 62% in spite of the excess of dibromide used. Higher yields can be obtained using microwave synthesis. Jansa et al. [26] described microwave synthesis of dialkyl ω-bromoalkylphosphonates using an equivalent amount of dibromide to phosphite and with higher yields and shorter reaction times. Hydrogen bromine was eliminated from **1** with potassium hydroxide and diethyl vinylphosphonate (**2**) was obtained in high yield after distillation.

The addition of perfluorohexyl iodide to vinylphosphonate (**2**) was catalyzed by aza-bis-*iso*-butyronitrile (AIBN). **3** was used in the next synthetic step without purification. Iodine in its molecule was reduced by tri-*n*-butyltin hydride. The yield of **4** was very low (13%). This could be caused by a competitive reaction of **3** with diethyl vinylphosphonate. Ester **4** was hydrolyzed by concentrated HBr. The syntheses were performed according adopted procedures [27,28]. Similar addition reaction of perfluorohexyl chloride to diethyl vinylphosphonate was performed by Huang and Chen [29]. The reaction was carried out in the presence of zinc, nickel dichloride and triphenylphosphine. Finally the compound **4** was obtained. The advantage of this synthetic approach is in good yield of the product (71%) and in the preparation of **4** in one synthetic step.

In view of the fact that **5** was prepared in low yield by the previously described procedure, a second approach was performed. *1H,1H,2H,2H*-perfluorooctanol was firstly brominated. Triphenylphosphine dibromide, which was generated *in situ* in the reaction mixture from bromine and triphenylphosphine, was used as brominating agent [30]. Bromide **6** was used in the following Michaelis-Arbuzov reaction. Without isolation the product **4** was hydrolyzed with HBr.

The comparison of the overall yields of both synthetic approaches favored the second one. **5** was prepared in 24% overall yield when the second approach was used instead of 5% overall yield in the first approach.

The octylphosphonic acid (**8**) was obtained in two steps (Scheme 1): its dietyl ester **7** was synthesized by Michaelis-Arbuzov reaction of 1-bromooctane with triethyl phosphite and hydrolyzed by hydrobromic acid.



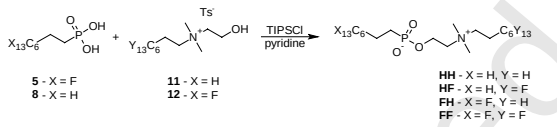
9 - X = H
10 - X = F

11 - X = H
12 - X = F

Ts = tosylate

Scheme 2. Preparation of choline derivatives

The choline derivatives **11** and **12** needed in the last step of the synthesis of dialkylphosphonatocholines were prepared by quaternization of 2-(dimethylamino)ethanol with alkyl tosylates **9** or **10**, respectively (Scheme 2). An attempt was made to prepare fluorinated derivatives of choline by quaternization of a tertiary amine with bromide **6**, however, we were unsuccessful to isolate the product in solid state. Therefore, we prepared tosylate **10** from *1H*,*1H*,*2H*,*2H*-perfluorooctanol and tosylchloride according to the procedure described by Elshani et al. [31] and then 2-(dimethylamino)ethanol was quaternised with tosylate **10**.



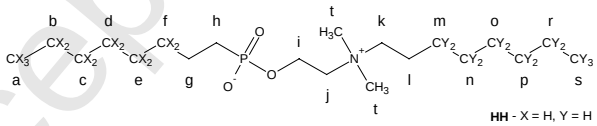
Scheme 3. Preparation of dialkylphosphonatocholines

The last step, the synthesis of non-fluorinated and fluorinated dialkylphosphonatocholines (Scheme 3), was performed by the general method used in the preparation of alkylphosphocholines [32,33] or dialkylphosphocholines [34]. Alkylphosphonic acids **5, 8** and choline derivatives **11, 12** were coupled in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TIPSCl) in anhydrous pyridine. Compounds **HH**, **HF**, **FH** and **FF** were obtained in moderate or good yields.

The influence of fluorine on chemical shifts of some atomic groups of dialkylphosphonatocholines in ^{1}H, ^{13}C and ^{31}P NMR is shown in Table 1. Substitution of

hydrogen by fluorine in the alkyl chains of dialkylphosphonatocholines shifted the signals of methylene and methyl groups of partially fluorinated dialkylphosphonatocholines (except of methylene groups of alkyl chain bounded to phosphate group of **HF**) observed in the ^{1}H NMR to higher values in comparison with nonfluorinated dialkylphosphonatocholine. However, **HF**, **FH** and **FF** had the signals of carbon atoms of methylene groups present at partially fluorinated alkyl chains observed in ^{13}C NMR at lower values in comparison with values of the same methylene groups present at nonfluorinated alkyl chains. Similar situation was observed in ^{31}P NMR spectra. The chemical shifts of phosphorus of **FH** and **FF** with partially fluorinated alkyl chain connected to phosphonate group were shifted to lower values in comparison with dialkylphosphonatocholines which contained hydrocarbon alkyl chain bounded to the phosphate group.

Table 1. Chemical shifts of the signal of some groups of dialkylphosphonatocholines in ^{1}H, ^{13}C, ^{19}F, ^{31}P NMR



HH - X = H, Y = H
HF - X = H, Y = F
FH - X = F, Y = H
FF - X = F, Y = F

Comp	H_g	H_h	H_i	H_j	H_k	H_l	H_t	C_g[a]	C_h[a]	C_k	C_l[a]	F_f	F_m	P
HH	1.55	1.55	4.19	3.32	3.50	1.73	3.14	31.4 d *J*=17.1	27.6 d *J*=136.0	66.5	26.4	-	-	28.0
HF	1.55	1.55	4.22	3.37	3.76	2.79	3.24	31.3 d *J*=17.3	27.5 d *J*=136.3	58.2	25.1 t *J*=21.6	-	-113.9	29.0
FH	2.38	1.81	4.23	3.33	3.54	1.75	3.15	26.1 t *J*=22.1	18.1 d *J*=138.8	66.5	26.4	-115.7	-	23.5
FF	2.37	1.81	4.24	3.63	3.77	2.79	3.24	26.1 t *J*=22.4	18.1 d *J*=139.3	58.0	25.1 t *J*=21.8	-115.9	-114.0	24.0

[a] d = doublet, t = triplet; values of interaction constant are expressed in Hz

2.2. Surface active properties

The micellar and the surface-active properties of the investigated compounds **HH**, **HF**, **FH** and **FF** were studied by measurements of surface tension of surfactant aqueous solutions by the Wilhelmy plate technique. The plots of surface tension *vs.* log concentration curves of the surfactants are shown in Figure 1. Critical micelle concentration (CMC), surface tension at the CMC (γ_{CMC}), and the surface area at the surface saturation per head group (A_{CMC}) of the surfactants are shown in Table 2.

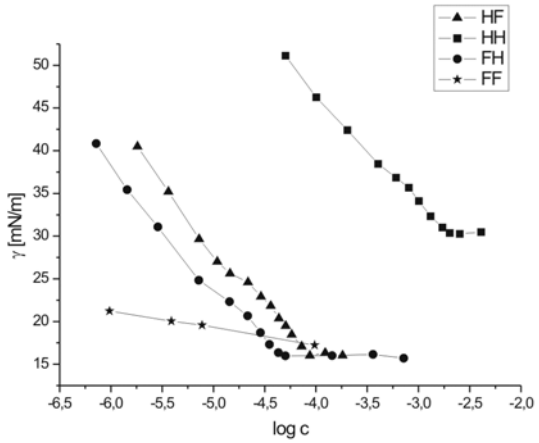


Figure 1. Plots of surface tension *vs.* log concentration curves of dialkylphosphonatocholines

Table 2. Physicochemical properties of dialkylphosphonatocholines

Comp	c_1 [mol.dm^{-3}]	CMC [mol.dm^{-3}]	γ_{CMC} [mN.m^{-1}]	A_{CMC} [Å^2]
HH	$(3.7 \pm 0.4) \times 10^{-4}$	$(1.9 \pm 0.1) \times 10^{-3}$	30.3	66 ± 3
HF	$(1.2 \pm 0.1) \times 10^{-5}$	$(8.5 \pm 0.1) \times 10^{-5}$	16.1	65 ± 1
FH	$(6.9 \pm 0.3) \times 10^{-6}$	$(4.4 \pm 0.1) \times 10^{-5}$	16.1	65 ± 3
FF	–	$< 1 \times 10^{-6}$	> 21.2	–

The CMC value of **HH** is comparable with similar compound (octyl 2-[dimethyl(octyl)ammonio]ethyl phosphate) which was investigated by Peresypkin and Menger [35]. They obtained the CMC value for it equal to 1×10^{-3} mol.dm^{-3}. The replacement

of octyl group with partially fluorinated one led to a decrease of the CMC. The compounds with one partially fluorinated carbon chain possessed the CMC of about 1.5 orders of magnitude lower than **HH** and the compound with both partially fluorinated carbon chains had the CMC lower than 1×10^{-6} mol.dm^{-3}, only the postmicellar part of the curve was obtained (Figure 1).

A very interesting feature of the compounds investigated is the multilinear relationship between the surface tension and the concentration of the surfactant showing clearly two breaks in the curve (Figure 1). We assume that the first break (the break in the premicellar region of the curve, Figure 1) indicated premicellar aggregation. The formation of small aggregates from surfactant molecules is expected. We estimated that the values of surfactant concentrations when premicellar aggregates start to form (c_1) are 5 to 7 times lower than the CMCs. This unusual phenomenon was previously observed in the case of some types of surfactants [36-38].

The measured values of surface tension at the CMC showed that the compounds with fluorine in the alkyl chains had lower values than the compound with hydrocarbon alkyl chains. However, it is interesting that the replacement of one hydrocarbon alkyl chain with partially fluorinated alkyl chains caused a decrease of the γ_{CMC} but the compound with two partially fluorinated alkyl chains (**FF**) had the γ_{CMC} higher ($\gamma_{CMC} > 21.2$ mN.m^{-1}) than **HF** and **FH**. The most fluorinated surfactant did not have the lowest γ_{CMC}. A similar effect of increasing the γ_{CMC} with decreasing lipophilicity (decrease of the CMC) in the ranges of partially fluorinated surfactants was also described in the literature [12,39]. However, when the value of surface tension ($\gamma = 17.2$ mN.m^{-1}) of **FF** at a concentration ($c = 9.7 \times 10^{-5}$ mol.dm^{-3}) similar to CMCs of **HF** ($\gamma_{CMC} = 16.1$ mN.m^{-1}) and **FH** ($\gamma_{CMC} = 16.1$ mN.m^{-1}) is compared, the differences are not high.

198 The values of A_{CMC} showed that partial fluorination of one alkyl chain of **HH** did not have an
199 influence on the surface area of the surfactant at the water/air interface.
200
201 **2.3. Solubilization properties**
202
203 Solubilization properties of the prepared surfactants were investigated by the solubilization of
204 a poorly soluble drug, griseofulvin. This compound is a representative solute because it is
205 solubilized almost exclusively in the hydrophobic micelle core [40]. Its solubility in water
206 was estimated at 3.34×10^{-5} mol.dm^{-3}. The changes in the solubilities of griseofulvin with
207 varying concentrations of surfactants solutions are shown in Figure 2. It is apparent that **HH**
208 had the best solubilization properties. It has a molar solubilization ratio (MSR) of 6.4×10^{-3}.
209 Surfactants with one partially fluorinated alkyl chain were poorer solubilizers than **HH**
210 ($MSR_{\mathbf{HF}} = 2.0 \times 10^{-3}$, $MSR_{\mathbf{FH}} = 4.2 \times 10^{-3}$) and **FF** was capable of solubilizing the lowest
211 amount of griseofulvin ($MSR_{\mathbf{FF}} = 1.2 \times 10^{-3}$). One can say that increasing the number of
212 fluorine atoms in the tails decreased the solubility of the solute. Fluorinated alkyl chains are
213 more hydrophobic than hydrogenated alkyl chains and griseovulvin with many of its polar
214 groups is more difficult to incorporate into the more hydrophobic perfluorinated micelle core.
215 Matsuoka et al. [41] obtained similar results. Alkylbenzenes were better solubilized in
216 hydrogenated surfactant micelles than the fluorinated ones. Solubilization of pyrene with
217 tetraalkylammonium perfluorooctanoates also showed that the solute interacted better with
218 hydrocarbon chains than with fluorinated chains. Pyrene was localised in the counterion layer
219 of the micelle containing tetraalkylammonium ions and not in the micelle core containing
220 perfluorinated alkyl chains of the perfluorooctanoates [42].
221

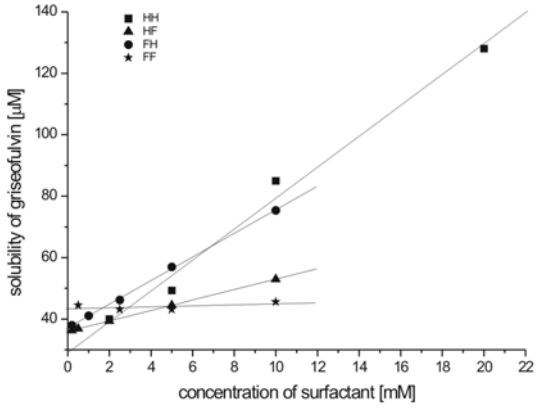


Figure 2. Plots of solubility of griseofulvin *vs.* concentration of dialkylphosphonatocholines

2.4. Antiprotozoal activities

The biological activities of dialkylphosphonatocholines were tested against opportunistic parasites of the genus *Acanthamoeba* which are the causative agents of frequently fatal granulomatous amoebic encephalitis and a serious sight threatening amoebic keratitis. Strains of *A. lugdunensis* and *A. quina* were chosen because of their higher resistance to action of a standard, HPC, than the other species, e.g., *A. polyphaga* or *A. lenticulata* [43,44]. The values of minimal trophocidal concentrations (MTC) of the compounds against parasites after 24 hours of incubation are shown in Table 3. **HF** was the most active compound, as its MTCs were four and two times lower than the MTCs of HPC against *A. lugdunensis* and *A. quina*, respectively. The same value of activity against *A. quina* was reached also by **FH**. **HF** and **FH** have optimal physicochemical properties (CMC), which is also a measure of the lipophilicity of the surfactant, for the antiprotozoal activity. The activities of alkylphosphocholines could be mainly caused by a non-specific mode of action, namely the biophysical destruction of the cell membrane [45]. Their optimal CMCs caused that they are capable to solubilize cell

membranes of the parasites most efficiently among the investigated dialkyphosphonatocholines. As we discussed previously [46], the bilinear type of cut-off effect, well known in the case of surfactants [47], was observed in the amoebicidal activities of alkylphosphocholines and therefore **HH** with high CMC and **FF** with low CMC were inactive against parasites.

Table 3. Minimal trophocidal concentration of dialkylphosphonatocholines after 24 hours of incubation

Compound	*A. lugdunensis* [μM]	*A. quina* [μM]
HH	> 500	> 500
HF	125	125
FH	> 500	125
FF	> 500	> 500
HPC[43]	500	250

3. Conclusion

In summary, we synthesised four new dialkylphosphonatocholines with two octyl groups, one with hydrocarbon alkyl chains (**HH**) and three with partially fluorinated tails (**HF**, **FH**, **FF**). The compounds were prepared by coupling the alkylphosphonic acids with choline derivatives in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride. Partially fluorinated alkylphosphonic acids were prepared by two methods. The first approach used radical addition of perfluorohexyl iodide to diethyl vinylphosphonate as a key step of synthesis and the second one employed the Michaelis-Arbusov reaction of partially fluorinated octylbromide with triethyl phosphite. Concerning the yields the second approach was more effective. The physicochemical properties were expressed as micellar, surface-active, and solubilization properties. Increasing numbers of fluorine atoms in the molecules caused a decrease of the values of CMC. The second break in the curve of surface tension *vs.* log c of **HH**, **HF** and **FH** was also observed. One can suppose that the break indicated the formation

of small aggregates from surfactant molecules. The lowest values of the surface tension at the CMC were shown by compounds with one partially fluorinated tail. The best solubilization properties were obtained with **HH.** The reason is that the fluorinated alkyl chains of **HF**, **FH** and **FF** are more hydrophobic than the hydrogenated alkyl chains of **HH** and griseovulvin with many of its polar groups is more difficult to incorporate into the more hydrophobic perfluorinated micelle core. The antiprotozoal activities were tested against *A. lugdunensis* and *A. quina*. **HF** was the most effective compound. The cut-off effect was observed in antiprotozoal activities of series of dialkylphosphonatocholines. According to expectations the **HH** with high CMC and **FF** with low CMC were inactive against parasites.

4. Experimental part

4.1. Chemistry

4.1.1. Materials

All chemicals used for the synthesis were purchased from commercial suppliers. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra were recorded on a Varian Mercury plus spectrometer operating at 300, 75.5, 282 and 121.5 MHz, respectively, ^{13}C, ^{19}F and ^{31}P spectra being recorded with proton-decoupling. The spectra were measured in $CDCl_3$, CD_3COCD_3, CD_3SOCD_3, or a mixture of $CDCl_3/CD_3OD$ relative to the internal standard TMS for ^{1}H and ^{13}C NMR spectra, CCl_3F for ^{19}F NMR spectra and to the external standard 85% H_3PO_4 for ^{31}P NMR spectra. Infrared spectra were recorded on a FT-IR Impact 400 D spectrophotometer as potassium bromide discs or liquid films on potasium bromide discs. Molecular masses of final compounds were measured by high resolution spectrometer ESI-LTQ Orbitrap XL Thermo Scientific. Melting points were measured using a Koffler hot-stage apparatus and are uncorrected.

4.1.2. Preparation of diethyl 2-bromoethylphosphonate

Triethylphosphite (35 ml, 0.2 mol) was mixed with 1,2-dibromoethane (69 ml, 0.8 mol) in a round-bottom flask equipped with a reflux condenser, heated at 160°C and stirred for 4 h. The reaction mixture was cooled and purified by distillation at reduced pressure.

Diethyl 2-bromoethylphosphonate (**1**): colourless liquid, b.p. 133–135°C at p = 2 kPa, n_D^{20} = 1,4596, yield = 30.39 g (62%). FT-IR (liquid film): υ_{max} = 2981, 1443, 1392, 1368, 1285, 1243, 1163, 1054, 1020, 961, 787 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 4.20–4.05 (m, 4H), 3.53 (q, *J* = 8.5 Hz, 2H), 2.47–2.32 (m, 2H), 1.34 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 62.1 (d, *J* = 6.1 Hz), 30.8 (d, *J* = 133.5 Hz), 23.9, 16.4 (d, *J* = 6.1 Hz). ^{31}P NMR (125.5 MHz, $CDCl_3$): δ = 26.3.

4.1.3. Preparation of diethyl vinylphosphonate

Diethyl 2-bromoethylphosphonate (24.5 g, 0.1 mol) was slowly added to a cold solution of KOH (5.6 g, 0.1 mol) in 150 ml of ethanol. The reaction mixture was stirred at room temperature for 1 h then heated to reflux for 15 min. The formed solid was filtered off and washed with ethanol. Ethanol was removed under vacuum and the remaining oil was distilled under reduced pressure.

Diethyl vinylphosphonate (**2**): colourless liquid, b.p. 94–96°C at p = 2.4 kPa, n_D^{20} = 1,4283, yield = 14.07 g (86%). FT-IR (liquid film): υ_{max} = 1445, 1393, 1243, 1164, 1050, 1022, 958, 785 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.38–5.97 (m, 3H), 4.18 – 4.03 (m, 4H), 1.34 (t, *J*

= 7.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 135.6 (d, *J* = 2.4 Hz), 126.0 (d, *J* = 183.6 Hz), 61.9 (d, *J* = 6 Hz), 16.4. ^{31}P NMR (125.5 MHz, $CDCl_3$): δ = 18.0.

4.1.4. Preparation of diethyl 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonate

Diethyl vinylphosphonate (8.2 g, 50 mmol) was mixed with 1-iodo-1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane (22.3 g, 50 mmol) and heated at 60°C. AIBN (0.33 g, 2 mmol) was then added and the mixture was stirred for 4 h at 80°C. Diethyl 1-iodo-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonate (**3**) was not isolated, but immediately used in the next reaction. Replacement of iodine with hydrogen was performed by tri-*n*-butyltin hydride. AIBN (1.5 mmol) was added to crude, cold diethyl 1-iodo-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonate, followed by drop wise addition of tri-n-butyltin hydride (17.5 g, 60 mmol). The reaction mixture was stirred in argon atmosphere for 12 h at 80°C. After reaction, the lower phase was separated and distilled at reduced pressure.

diethyl 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonate (**4**): colourless liquid, b.p. 86–89°C at p = 20 Pa, yield = 3.23 g (13%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.21–4.05 (m, 4H), 2.50–2.28 (m, 2H), 2.08–1.92 (m, 2H), 1.35 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125–105 (m, 6 × C), 62.2, 25.2 (t, *J* = 23.6 Hz), 17.2 (d, *J* = 146.7 Hz), 16.4. ^{19}F NMR (282 MHz, $CDCl_3$): δ = -80.73 (t, *J* = 9.6 Hz, 3F), 115.32 (m, 2F), 121.88 (m, 2F), 122.81 (m, 2F), 123.30 (m, 2F), 126.12 (m, 2F). ^{31}P NMR (125.5 MHz, $CDCl_3$): δ = 28.9.

4.1.5. Preparation of 1-bromo-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-ol (20 g, 55 mmol) and triphenylphosphine (15.7 g, 60 mmol) were dissolved in 110 mL of anhydrous acetonitrile and deoxygenated with argon. The mixture was heated at 60°C and bromine (9.6 g, 60 mmol) was added drop wise. The mixture was stirred for 5 h at this temperature. After cooling down to room temperature, the reaction mixture was extracted with diethyl ether and the ether layers were washed with brine. After drying with Na_2SO_4, the ether was evaporated and the crude product was dissolved in 100 mL of dichloromethane. This solution was stirred with 50 g of silica gel for 2 h and then filtered and evaporated. The crude product was distilled at reduced pressure.

1-bromo-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane (**6**): colourless liquid, b.p. 93–94°C at 12 kPa, n_D^{20} = 1,3312, yield = 16.4 g (70%). FT-IR (liquid film): υ_{max} = 1453, 1361, 1236, 1196, 1144, 1123, 1079, 951, 845, 812, 745, 734, 702, 629 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 3.51 (t, *J* = 8.2 Hz, 2H), 2.80–2.60 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125–105 (m, 6 × C), 35.0 (t, *J* = 21.8 Hz), 20.2. ^{19}F NMR (282 MHz, $CDCl_3$): δ = -81.27 (t, *J* = 10.0 Hz, 3F), -114.72 (m, 2F), -122.39 (m, 2F), -123.35 (m, 2F), -123.97 (m, 2F), -126.63 (m, 2F).

4.1.6. General procedure for the preparation of phosphonic acid derivatives by Michaelis-Arbuzov reaction

Alkylbromide (4.27 g of 1-bromo-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane or 1.93 g of 1-bromooctane, 10 mmol) was heated with triethyl phosphite (1.83 g, 11 mmol) for 4h at 150°C. After cooling, conc. HBr (12 mL) was added and heated for 4 h at 120°C. The excess HBr and bromoethane was then distilled off and the crude products were crystallised. The same procedure of hydrolysis of diester **4** obtained by first synthetic approach was also used.

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonic acid (**5**): crystallised from a mixture of acetone : $CHCl_3$ 1 : 2, white powder, m.p. = 166–168°C, yield = 1.87 g (66%) for 1st synthetic approach (1 step), yield = 1.24 g (29%) for 2nd synthetic approach (2 steps). FT-IR (KBr): υ_{max} = 1445, 1367, 1302, 1229, 1212, 1186, 1141, 1074, 1012, 954, 778, 741, 697, 653 cm^{-1}. ^1H NMR (300 MHz, CD_3COCD_3): δ = 4.33 (s, 2H), 2.60–2.38 (m, 2H), 2.10–2.02 (m, 2H). ^{13}C NMR (75 MHz, CD_3COCD_3): δ = 125–105 (m, 6 × C), 26.0 (t, *J* = 22.9 Hz), 18.9 (d, *J* = 144.3 Hz). ^{19}F NMR (282 MHz, CD_3COCD_3): δ = -80.62 (t, *J* = 10.4 Hz, 3F), -114.56 (m, 2F), -121.40 (m, 2F), -122.37 (m, 2F), -122.83 (m, 2F), -125.70 (m, 2F). ^{31}P NMR (125.5 MHz, CD_3COCD_3): δ = 28.7. HRMS calcd. for $C_8H_5F_{13}PO_3$ = 426.9763; found *m/z*: 426.9762 $[M-H]^-$

Octylphosphonic acid (**8**): crystallised from hexane, white powder, m.p. = 99–101°C, yield = 1.44 g (74%). FT-IR (KBr): υ_{max} = 2958, 2929, 2853, 1468, 1260, 1230, 1173, 1107, 1006, 995, 947, 931, 780, 716 cm^{-1}. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 5.36 (s, 2H), 1.58–1.40 (m, 4H), 1.40 – 1.18 (m, 10H), 0.86 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CD_3SOCD_3): δ = 31.3, 30.1 (d, *J* = 16.2 Hz), 28.6, 28.5, 27.5 (d, *J* = 137.0 Hz), 22.6 (d, *J* = 4.8 Hz), 22.1, 13.9. ^{31}P NMR (125.5 MHz, CD_3SOCD_3): δ = 28.4. HRMS calcd. for $C_8H_{18}PO_3$ = 193.0990; found *m/z*: 193.0995 $[M-H]^-$

4.1.7. General procedure for preparation of alkyl tosylates

Alcohol (8.74 g of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctanol or 3.13 g of octanol, 24 mmol) and tertiary amine (30 mmol, triethylamine (4.2 mL) in the case of fluorinated alcohol and pyridine (2.4 mL) in the case of octanol) were dissolved in 50 mL of dichloromethane and *p*-toluenesulfonyl chloride (4.77 g, 25 mmol) was added in small portions. The reaction

mixture was stirred overnight. The dichloromethane solution was then washed with water, dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was crystallised from methanol in the case of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl tosylate. Crude octyl tosylate was used in the next step without further purification.

Octyl tosylate (**9**): colourless liquid, yield = 76%. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 4.01 (t, *J* = 6.5 Hz, 2H), 2.45 (s, 3H), 1.68–1.57 (m, 2H), 1.36–1.16 (m, 10H), 0,88 (t, *J* = 6.7 Hz, 3H).

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl tosylate (**10**): colourless crystals, m.p. = 54.5–55°C, yield = 11.4 g (92%). FT-IR (KBr): υ_{max} = 1597, 1495, 1424, 1399, 1363, 1318, 1250, 1202, 1173, 1137, 1079, 987, 972, 933, 892, 815, 778, 697, 663, 646 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 4.30 (t, *J* = 6.7 Hz, 2H), 2.60–2.43 (m, 2H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 145.4, 132.3, 130.1, 128.0, 125–105 (m, 6 × C), 61.6, 31.0 (t, *J* = 21.7 Hz), 21.7. ^{19}F NMR (282 MHz, $CDCl_3$): δ = -81.29 (t, *J* = 10.0 Hz, 3F), -114.01 (m, 2F), -122.38 (m, 2F), -123.36 (m, 2F), -124.08 (m, 2F), -126.65 (m, 2F).

4.1.8. General procedure for the preparation of choline tosylates

2-(*N*,*N*-Dimethylamino)ethanol (1.07 g, 12 mmol) and an alkyl tosylate (5.18 g of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl tosylate or 2.84 g of octyl tosylate, 10 mmol) were dissolved in 25 mL of acetonitrile and refluxed for 24 h. After cooling down the acetonitrile was evaporated in vacuum. The resulting mixture was crystallised from acetone.

***N*-(2-hydroxyethyl)-*N*,*N*-dimethyloctane-1-ammonium tosylate** (**11**): white powder, m.p. = 169–170°C, yield = 2.54 g (68%). FT-IR (KBr): υ_{max} = 2956, 2923, 2856, 1492, 1467, 1219, 1184, 1171, 1120, 1033, 1010, 819, 681 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 7.9 Hz, 2H), 7.15 (d, *J* = 7.9 Hz, 2H), 5.43 (t, *J* = 5.3 Hz, 1H), 4.10–4.00 (m, 2H), 3.64–3.55 (m, 2H), 3.39–3.28 (m, 2H), 3.20 (s, 6H), 2.34 (s, 3H), 1.64–1.54 (m, 2H), 1.37–1.12 (m, 10H), 0.88 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 143.3, 139.8, 128.9, 125.8, 65.8, 65.6, 56.3, 51.6, 31.8, 29.2, 26.3, 22.8, 22.7, 21.4, 14.1. HRMS calcd. for $C_{12}H_{28}ON$ = 202.2165; found *m/z*: 202.2165 $[M\text{-}Ts]^+$

***N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-ammonium tosylate** (**12**): white powder, m.p. = 219–221°C, yield = 1.64 g (27%). FT-IR (KBr): υ_{max} = 1495, 1362, 1320, 1296, 1231, 1203, 1189, 1144, 1121, 1108, 1009, 820, 723, 683, 653 cm^{-1}. ^{1}H NMR (300 MHz, CD_3SOCD_3): δ = 7.48 (d, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.37 (t, *J* = 4.7 Hz, 1H), 3.95–3.68 (m, 4H), 3.58–3.48 (m, 2H), 3.17 (s, 6H), 3.06–2.82 (m, 2H), 2.29 (s, 3H). ^{13}C NMR (75 MHz, CD_3SOCD_3): δ = 145.7, 137.6, 128.0, 125.4, 125–105 (m, 6 × C), 64.9, 55.7, 54.9, 50.9, 24.4 (t, *J* = 21.0 Hz), 20.7. ^{19}F NMR (282 MHz, CD_3SOCD_3): δ = -79.8 (t, *J* = 9.0 Hz, 3F), -111.97 (m, 2F), -121.21 (m, 2F), -122.15 (m, 4F), -125.32 (m, 2F). HRMS calcd. for $C_{12}H_{15}ONF_{13}$ = 436.0941; found *m/z*: 436.0938 $[M\text{-}Ts]^+$

4.1.9. General procedure for the preparation of dialkylphosphonatocholines

Pyridinium salts of alkyl dihydrogen phosphonates were prepared by the treatment of alkylphosphonic acids (0.428 g of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonic acid or 0.194 g of octylphosphonic acid, 1 mmol) with pyridine (8 mL) for 2 h at 50°C. The excess pyridine was evaporated and the rest was dissolved in 5 mL of anhydrous pyridine. The

appropriate choline (0.911 g of *N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-ammonium tosylate or 0.374 g of *N*-(2-hydroxyethyl)-*N*,*N*-dimethyloctane-1-ammonium tosylate, 1.5 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (0.45 g, 2 mmol) in pyridine (15 mL) were added to the solution of pyridinium alkylphosphonate. The solution was stirred at 40 °C overnight. After cooling, the mixture was hydrolyzed by addition of H_2O (1.5 mL) and stirred for 1 h at room temperature. The solvents were evaporated in vacuum, and the resulting crude solid was dissolved in a mixture of tetrahydrofuran and water (5:1, V/V). An exchange resin, Amberlite MB3, was added sequentially to the stirred solution until the colour of the resin ceased to change. Then the resin was filtered off and the solvents were evaporated in vacuum. The remaining mixture was coevaporated with propan-2-ol several times. The residue was dissolved in chloroform and precipitated with acetone. Dialkylphosphonatocholines were filtered off and crystallised from a mixture of chloroform and acetone. Products were dried in a vacuum dessicator over P_4O_{10}.

2-[dimetyl(octyl)ammonio]ethyl octylphosphonate (**HH**): white powder, m.p. = 227–227.5°C, yield = 0.131 g (33%). FT-IR (KBr): υ_{max} = 3297, 2918, 2851, 1467, 1195, 1091, 1057, 962, 936, 781, 717, 704 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ 500 µL + CD_3OD 100 µL): δ = 4.24–4.16 (m, 2H), 3.55–3.45 (m, 2H), 3.37–3.26 (m, 2H), 3.14 (s, 6H), 1.80–1.66 (m, 2H), 1.65–1.47 (m, 4H), 1.45–1.19 (m, 20H), 0.95–0.82 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 66.5, 64.9, 56.7, 51.6, 32.0, 31.8, 31.4 (d, *J* = 17.1 Hz), 29.4(3), 29.3(5), 29.2, 29.1, 27.6 (d, *J* = 136.0 Hz), 26.4, 23.8, 23.7, 22.8, 22.7, 14.1(2), 14.0(6). ^{31}P NMR (125.5 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 28.0. HRMS calcd. for $C_{20}H_{44}O_3NPNa$ = 400.2951; found *m/z*: 400.2950 $[M+Na]^+$

2-[dimetyl(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)ammonio]ethyl octylphosphonate (**HF**): white powder, m.p. = 188–189°C, yield = 0.136 g (22%). FT-IR (KBr): υ_{max} = 3274, 2933, 2860, 1240, 1222, 1139, 1097, 1060, 964, 920, 780, 738, 710 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ 500 µL + CD_3OD 100 µL): δ = 4.26–4.17 (m, 2H), 3.82–3.70 (m, 2H), 3.65–3.57 (m, 2H), 3.24 (s, 6H), 2.89–2.69 (m 2H), 1.67–1.46 (m, 4H), 1.41–1.18 (m, 10H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 125–115 (m, 6 × C), 64.9, 58.2, 56.7, 51.8, 32.0, 31.3 (d, *J* = 17.3 Hz), 29.4, 29.3, 27.5 (d, *J* = 136.3 Hz), 25.1 (t, *J* = 21.6 Hz), 23.6, 22.8, 14.1. ^{19}F NMR (282 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = -81.20 (t, *J* = 10.0 Hz, 3F), -113.94 (m, 2F), -122.25 (m, 2F), -123.27 (m, 4F), -126.54 (m, 2F). ^{31}P NMR (125.5 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 29.0. HRMS calcd. for $C_{20}H_{31}O_3NF_{13}PNa$ = 634.1726; found *m/z*: 634.1725 $[M+Na]^+$

2-[dimetyl(octyl)ammonio]ethyl 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctylphosphonate (**FH**): white powder, m.p. = 214–216°C, yield = 0.312 g (50%). FT-IR (KBr): υ_{max} = 3365, 2932, 2857, 1212, 1143, 1061, 972, 928, 822, 775, 745, 732 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ 500 µL + CD_3OD 100 µL): δ = 4.29–4.18 (m, 2H), 3.62–3.49 (m, 2H), 3.42–3.28 (m, 2H), 3.14 (s, 6H), 2.50–2.26 (m, 2H), 1.91–1.58 (m, 4H), 1.45–1.20 (m, 10H), 0.89 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 125–115 (m, 6 × C), 66.5, 64.9, 57.1, 51.6, 31.8, 29.2, 26.4, 26.1 (t, *J* = 22.1 Hz), 22.8, 22.7, 18.1 (d, *J* = 138.8 Hz), 14.0. ^{19}F NMR (282 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = -81.27 (t, *J* = 10.0 Hz, 3F), -115.74 (m, 2F), -122.38 (m, 2F), -123.31 (m, 2F), -123.84 (m, 2F), -126.60 (m, 2F). ^{31}P NMR (125.5 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 23.5. HRMS calcd. for $C_{20}H_{31}O_3NF_{13}PNa$ = 634.1726; found *m/z*: 634.1727 $[M+Na]^+$

2-[dimetyl(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)ammonio]ethyl 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl-phosphonate (**FF**): white powder, m.p. = 205.5–207°C, yield = 0.211 g (25%). FT-IR (KBr): υ_{max} = 3409, 1230, 1185, 1139, 1058, 963, 776, 710 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$ 500 µL + CD_3OD 100 µL): δ = 4.32–4.16 (m, 2H), 3.84–3.70 (m, 2H), 3.68–3.58 (m, 2H), 3.23 (s, 6H), 2.90–2.68 (m, 2H), 2.50–2.24 (m, 2H), 1.90–1.72 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 125–115 (m, 12 × C), 65.0, 58.0, 56.9, 51.8, 26.1 (t, *J* = 22.4 Hz), 25.1 (t, *J* = 21.8 Hz), 18.1 (d, *J* = 139.3 Hz). ^{19}F NMR (282 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = -81.33 (m, 6F), -114.03 (m, 2F), -115.92 (m, 2F), -122.37 (m, 4F), -123.32 (m, 6F), -123.91 (m, 2F), -126.62 (m, 4F). ^{31}P NMR (125.5 MHz, $CDCl_3$ 500 µl + CD_3OD 100 µl): δ = 24.0. HRMS calcd. for $C_{20}H_{18}O_3NF_{26}PNa$ = 868.0501; found *m/z*: 868.0500 $[M+Na]^+$

4.2. Equilibrium surface tension measurements

The critical micelle concentration values of the surfactants were determined from the surface tension isotherm. The solvent surface tension values were measured by the Wilhelmy plate technique using a Krüss 100 MK2 tensiometer. Deionised water was used in the preparation of all samples. The temperature of the measurements was kept at 25 ± 0.1°C. Measurements of equilibrium surface tension were taken repeatedly (every 6 min.) until the change in surface tension was less than 0.05 $mN.m^{-1}$. The critical micelle concentrations (CMC) and surface tensions at the CMC (γ_{CMC}) were determined from the intersection of two lines – one premicellar and second one postmicellar – of the surface tension vs. log c curve. The values of surfactant concentrations when premicellar aggregates start to form (c_1) were calculated in a same way. From the surface tension data, the adsorbed amount of the surfactant γ_{CMC} is calculated utilising the Gibbs adsorption isotherm

$\Gamma_{CMC} = -[d\gamma/d \log c]_T/(2.303iRT)$ (1)

where γ is the surface tension ($mN.m^{-1}$), c is the surfactant concentration, *i* is the prefactor, T is the absolute temperature and R the gas constant. Surface excess may be determined from the slope below the CMC in the surface tension vs. log c plots. Surface area at the surface saturation per head group (A_{CMC}) is obtained from the equation

$A_{CMC} = 10^{20}/N_A\Gamma_{CMC}$ (2)

were N_A is the Avogadro constant.

4.3. Solubilization of griseofulvin

Saturated griseofulvin solutions were prepared in glass vessels by mixing an excess powdered drug (5 mg) with 2.5 mL of deionised water or surfactant solution with an appropriate concentration (c_{surf}) and stirring (250 rpm) at a constant temperature t = 25 ± 1 °C for 72 hours before filtering (Millipore, 0.22 μm) to remove any unsolubilized material. The extent of dissolution was determined by UV spectroscopy. The filtered solution (1 mL) was diluted quantitatively with methanol in a 25 mL volumetric flask. Absorbance was measured at the optimum wavelength, λ = 292 nm, which was then compared with the appropriate Beer's law plot for the drug in methanol. Water content in the measured solution was low enough to allow the calibration with methanol solutions to be used without correction. MSR was calculated according to the equation:

513 $MSR = (c - c_w)/(c_{surf} - CMC)$

514

515 where c is the concentration of griseofulvin in a solution of surfactant and c_w is concentration
516 of griseofulvin in deionised water.

517

518 **4.4. *In vitro* amoebicidal activity assay**

519 The cytotoxic activity of dialkyphosphonatocholines was tested on two clinical isolates of
520 free-living amoebae, i.e., *Acanthamoeba lugdunensis* AcaVNAK02 and *Acanthamoeba quina*
521 AcaVNAK03, isolated from the corneas of two patients with amoebic keratitis.
522 Briefly, from the 2-day monoxenic cultures on agar plates, the trophozoites were axenised by
523 inoculation into the Bacto-Casitone/Serum medium (BCS) with penicillin and ampicillin.
524 After 72 h**,** the active trophozoites were transferred into peptone-yeast extract-glucose
525 medium (PYG) with penicillin and ampicillin. After 5 passages, the trophozoites were
526 transferred into a PYG medium without antibiotics and subsequently cultivated in this
527 medium. Cytotoxicity measurements were carried out in sterile 96-well microtitre plates. Each
528 well was seeded with 100 µL (2×10^5 cells mL^{-1}) of a trophozoite suspension. Then, 100 µL
529 of a freshly prepared medium containing a tested compound at 10 concentrations was added
530 to all wells except untreated control wells that received 100 µL of pure medium. Each
531 compound was tested at final concentrations of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9,
532 1.95, and 0.98 µM. The reduction of trophozoites was recorded after 24 h by counting the
533 surviving cells in a Bürker-Türk hemocytometer. Viability of trophozoites was determined by
534 trypan blue exclusion; 100% eradication was confirmed by transferring 50 µL of the
535 suspension to a PYG medium, then recording the amoeba growth for 14 days. The lowest
536 concentration of tested compounds leading to 100% eradication of the trophozoites was
537 defined as the minimal trophocidal concentration (MTC). The experiments were repeated 8

538 times for each concentration. The cultivations and the cytotoxicity measurements were carried
539 out at 37°C.
540
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550 **References**
551
552 [1] F.M. Menger, A.V. Peresypkin, K.L. Caran, A.L. Apkarian, Langmuir 16 (2000) 9113–
553 9116.
554 [2] F.M. Menger, A.V. Peresypkin, J. Am. Chem. Soc. 123 (2001) 5614–5615.
555 [3] C. Menez, P. Legrand, V. Rosilio, S. Lesieur, G. Barratt, G. Mol. Pharmacol. 4 (2007)
556 281–288.
557 [4] M. Lukáč, I. Prokipčák, I. Lacko, F. Devínsky, Eur. J. Pharm. Sci. 44 (2011) 194–199.
558 [5] S. Drescher, S. Sonnenberger, A. Meister, A. Blume, B. Dobner, Monatsh. Chem. 143
559 (2013) 1533–1543.
560 [6] W.J. van Blitterswijk, M. Verhej, Biochim. Biophys. Acta 1831 (2013) 663–674.
561 [7] S.L. Croft, K. Seifert, M. Duchêne, Molecular Biochem. Parasitol. 126 (2003) 165–172.
562 [8] M. Pisárčik, M. Polakovičová, M. Lukáč, F. Devínsky, M. Pupák, Colloids Surf. A -
563 Physicochem. Eng. Asp. 407 (2012) 169–176.

564 [9] A. Zaggia, B. Ameduri, Curr. Opin. Colloid Interface Sci. 17 (2012) 188–195.
565 [10] T. Asakawa, K. Ishikawa, S. Miyagishi, J. Colloid Interface Sci. 240 (2001) 365–367.
566 [11] P. Quagliotto, C. Barolo, N. Barbero, E. Barni, C. Compari, E. Fisicaro, G. Viscardi, Eur.
567 J. Org. Chem. (2009) 3167–3177.
568 [12] M. Uomar, E.T. de Givenchy, S.Y. Dieng, S. Amigoni, F. Guittard, Langmuir 27 (2011)
569 1668–1674.
570 [13] N. Bongartz, S.R. Patil, C. Stubenrauch, D. Blunk, Colloids Surf. A - Physicochem. Eng.
571 Asp. 414 (2012) 320–326.
572 [14] T. Schuster, S. Schellenberger, R. Friedrich, M. Klapper, K. Müllen, J. Fluorine Chem.
573 154 (2013) 30–36.
574 [15] A. Peresypkin, C. Clavel, F.M. Menger, Mendeleev Commun. 17 (2007) 82–84.
575 [16] P. Nitoń, A. Żywociński, J. Paczesny, M. Fiałkowski, R. Hołyst, B. Glettner, R. Kieffer,
576 C. Tschierske, D. Pociecha, E. Górecka, Chem. Eur. J. 17 (2011) 5861–5873.
577 [17] M.Dupuy, A. Pasc, E. Mayot, S. Cosgun, C. Gérardin-Charbonier, J. Fluorine Chem. 134
578 (2012) 115–121.
579 [18] J.G. Ries, M.P. Krafft, Biomaterials 19 (1998) 1529–1539.
580 [19] R. Kaplánek, O. Paleta, I. Ferjentsiková, M. Kodíček, J. Fluorine Chem. 130 (2009) 308–
581 316.
582 [20] L. Massi, F. Guittard, S. Géribaldi, R. Levy, Y. Duccini, Int. J. Antimicrob. Agents 21
583 (2003) 20–26.
584 [21] L. Massi, F. Guittard, R. Levy, S. Géribaldi, Eur. J. Med. Chem. 44 (2009) 1615–1622.
585 [22] T. Platen, T. Schüler, W. Tremel, A. Hoffmann-Röder, Eur. J. Org. Chem. (2011) 3878–
586 3887.
587 [23] E. Klein, C. Leborgne, M. Ciobanu, J. Klein, B. Frisch, F. Pons, G. Zuber, D. Scherman,
588 A. Kichler, L. Lebeau, Biomaterials 31 (2010) 4781–4788.

589 [24] N.R. Cichowicz, P. Nagorny, Org. Lett. 14 (2012) 1058–1061.
590 [25] P. Fourgeaud, C. Midrier, J.P. Vors, J.N. Volle, J.L. Pirat, D. Virieux, Tetrahedron 66
591 (2010) 758–764.
592 [26] P. Jansa, A. Holý, M. Dračínsky, O. Baszczyňski, M. Česnek, Z. Janeba, Green Chem. 13
593 (2011) 882–888.
594 [27] H. Shao, L. Jiang, W.D. Meng, F.L. Qing, J. Fluorine Chem. 124 (2003) 89–91.
595 [28] J.A. Bennett, E.G. Hope, K. Singh, A.M. Stuart, J. Fluorine Chem. 130 (2009) 615–620.
596 [29] X.T. Huang, Q.Y. Chen, J. Org. Chem. 66 (2001) 4651–4656.
597 [30] C. Naud, P. Calas, H. Blancou, A. Commeyras, J. Fluorine Chem. 104 (2000) 173–178.
598 [31] S. Elshani, E. Kobzar, R.A. Bartsch, Tetrahedron 56 (2000) 3291–3301.
599 [32] T. Calogeropoulou, P. Angelou, A. Detsi, I. Fragiadaki, E. Scoulika, J. Med. Chem. 51
600 (2008) 897–908.
601 [33] I. Papanastasiou, K.C. Prousis, K. Georgikopoulou, T. Pavlidis, E. Coulica, N.
602 Kolocouris, T. Calogeropoulou, Biorg. Med. Chem. Lett. 20 (2010) 5484–5487.
603 [34] M. Lukáč, M. Mrva, E. Fischer-Fodor, I. Lacko, M. Bukovský, N. Miklášová, F.
604 Ondriska, F. Devínsky, Bioorg. Med. Chem. Lett. 19 (2009) 6346–6349.
605 [35] A.V. Peresypkin, F.M. Menger, Org. Lett. 1 (1999) 1347–1350.
606 [36] L.D. Song, M.J. Rosen, Langmuir 12 (1996) 1149–1153.
607 [37] C. Xue, H. Zhu, T. Zhang, D. Cao, Z. Hu, Z. Colloid Surf. A - Physicochem. Eng. Asp.
608 375 (2011) 141–146.
609 [38] D. Arabadzhieva, B. Soklev, E. Mileva, E. Colloids Surf. A - Physicochem. Eng. Asp.
610 419 (2013) 194–200.
611 [39] T.H.V. Ngo, C. Damas, R. Naejus, R. Coudert, J. Colloid Interface Sci. 359 (2013) 157–
612 163.

[40] M. Crothers, Z. Zhou, N.M.P.S. Ricardo, Z. Yang, P. Taboada, C. Chaibundit, D. Attwood, C. Booth, Int. J. Pharm. 293 (2005) 91–100.

[41] K. Matsuoka, E. Takeuchi, M. Takahashi, S. Kitsugi, C. Honda, K. Endo, J. Colloid Interface Sci. 333 (2009) 641–645.

[42] H. Xing, P. Yan, J.X. Xiao, Soft Matter 9 (2013) 1164–1171.

[43] M. Mrva, M. Garajová, M. Lukáč, F. Ondriska, J. Parasitol. 97 (2011) 538–540.

[44] J. Walochnik, M. Duchêne, K. Seifert, A. Obwaller, T. Hottkowitz, G. Wiedremann, H. Eibl, H. Aspöck, Antimicrob. Agents Chemother. 46 (2002) 695–701.

[45] M. Lukáč, M. Mrva, M. Garajová, G. Mojžišová, L. Varínska, J. Mojžiš, M. Sabol, J. Kubincová, H. Haragová, F. Ondriska, F. Devínsky, Eur. J. Pharm. Sci. 66 (2013) 46-55.

[46] M. Lukáč, M. Garajová, M. Mrva, M. Bukovský, F. Ondriska, E. Máriássy, F. Devínsky, I. Lacko, Int. J. Pharm. 423 (2012) 247–256.

[47] P. Balgavý, F. Devínsky, Adv. Colloid Interface Sci. 66 (1996) 23–63.

626 **Figure and table captions**

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628 Scheme 1. Preparation of alkylphosphonic acids

629 Scheme 2. Preparation of choline derivatives

630 Scheme 3. Preparation of dialkylphosphonatocholines

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632 Figure 1. Plots of surface tension *vs.* log concentration curves of dialkylphosphonatocholines

633 Figure 2. Plots of solubility of griseofulvin *vs.* concentration of dialkylphosphonatocholines

634

635 Table 1. Chemical shifts of the signal of some groups of dialkylphosphonatocholines in ^{1}H,

636 ^{13}C, ^{19}F, ^{31}P NMR

637 Table 2. Physicochemical properties of dialkylphosphonatocholines

638 Table 3. Minimal trophocidal concentration of dialkylphosphonatocholines after 24 hours of

639 incubation