

Perfluoroalkylated amphiphiles with a morpholinophosphate or a dimorpholinophosphate polar head group

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Some previously synthesized (perfluoroalkyl)alkyldimorpholinophosphates, $C_nF_{2n+1}C_mH_{2m}OP(O)[N(CH_2CH_2)_2O]_2$, were found remarkably to stabilize heat sterilizable water-in-fluorocarbon reverse emulsions and to have a strong proclivity to self-aggregate into microtubular assemblies when dispersed in water. This series has now been extended in order to allow structure–property relationships to be established and product optimization to be achieved. A new series of even more fluorophilic compounds consisting in bis[(perfluoroalkyl)alkyl]monomorpholinophosphates, $(C_nF_{2n+1}C_mH_{2m}O)_2P(O)N(CH_2CH_2)_2O$, was also synthesized. Preliminary surfactant activity and biocompatibility data are presented and compared to data obtained with non-fluorinated analogues.

There is a need for novel, highly effective and biocompatible surfactants to serve as components of drug preparation and delivery systems. A large variety of well-defined pure perfluoroalkylated amphiphiles have been synthesized in recent years with this objective in mind^{1–4} and were investigated as emulsion stabilizers, vesicle-forming components and for other uses.^{5,6} Among the most fluorophilic neutral compounds synthesized were some (perfluoroalkyl)alkyldimorpholinophosphates, $C_nF_{2n+1}C_mH_{2m}OP(O)[N(CH_2CH_2)_2O]_2$, (FnCmDMPs).⁷

Investigation of this initial series of compounds revealed several unexpectedly interesting features, including: (i) a remarkable capacity to allow the preparation and stabilization of water-in-fluorocarbon reverse emulsions; these emulsions, which were stable enough to withstand heat sterilization, could be loaded with a variety of drugs and other agents, and have potential in drug delivery through the pulmonary route;⁸ (ii) a remarkable tendency to self-aggregate when dispersed in water and to yield sturdy, regularly shaped, long microtubules in which the surfactant molecules appear to be highly ordered;^{9,10} when heated, these tubules reversibly convert into giant vesicles. When hydrocarbon analogues were dispersed in water no aggregates larger than micelles were seen, in line with the usual behaviour of single-chain amphiphiles; (iii) low acute toxicities, whether when administered intraperitoneally or intravascularly, as well as the absence of hemolytic activity in spite of their high surface activity. By contrast, the hydrogen analogues are highly hemolytic.

A more extensive series of dimorpholinophosphates was needed in order to optimize and establish these results on a firmer basis and to determine structure–property relationships. Compounds with higher and lower Fn : Cm ratios

were synthesized as well as compounds with intermediate hydrocarbon spacer lengths, since such intermediate spacer lengths were shown to have an influence on the ordering of the surfactant films.¹¹ In addition, the synthesis of even more fluorophilic compounds, with two perfluoroalkyl chains, was decided upon. These compounds consist in the bis(perfluoroalkyl)alkylmonomorpholinophosphates, $(C_nF_{2n+1}C_mH_{2m}O)_2P(O)N(CH_2CH_2)_2O$, code-named D(FnCm)MMP. Some hydrogenated analogues were also prepared in order to determine the impact of the fluorinated chains on the amphiphiles' behaviour.

This paper describes the synthesis of the new F-alkylated mono- and dimorpholinophosphates, their characterization and a preliminary assessment of their surface activity and biocompatibility.

Experimental

Materials and methods

The perfluoroalkylated alcohols and iodides utilized were a gift from Atochem (Pierre-Bénite, France) or were synthesized as described below. They were recrystallised from chloroform or distilled under reduced pressure prior to use. The alcohols with a longer hydrocarbon spacer were synthesized according to Brace by radical addition of $C_nF_{2n+1}I$ to terminally unsaturated alcohols $CH_2=CH(CH_2)_{m-2}OH$, followed by reduction with HCl–Zn in anhydrous ethanol.¹² (F-Heptyl)methanol, $C_7F_{15}CH_2OH$, was prepared from the methyl perfluorooctanoate ester $C_7F_{15}COOCH_3$ by $NaBH_4$ reduction in isopropanol.¹³ Synthesis of the (F-alkyl)propanols is described elsewhere.¹⁴ Phosphorus oxytrichloride, triethylamine and morpholine (Aldrich) were distilled prior to use. Perfluorodecalin (FDC) was a gift from Air Products (USA).

TLC analyses were performed on RP-18 silica gel F-254 plates (Merck). Silica gel (70–230 mesh, Merck) was used for

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column chromatography. Melting points (uncorrected) were determined with a Reichert apparatus. IR spectra were recorded on a Bruker IFS 45 and NMR spectra on a Bruker AC 200 spectrometer (the FT-IR and NMR spectra of F6C3DMP, F7C1DMP, F8C3DMP and F10C3DMP were recorded on Bruker IFS 55 and Bruker 500 MHz spectrometers by Dr. A. Csámpai, Hungary). The elemental analyses were performed by the Service Central de Microanalyses of the Centre National de la Recherche Scientifique (Lyon, France). Surface tensions, γ_s , and interfacial tensions, γ_i , were measured with an automatic Lauda tensiometer (platinum ring, Lecomte du Noüy method): aqueous solutions of the amphiphiles were prepared using deionized water (Elgastat UHQ system, $\gamma_s = 72 \text{ mN m}^{-1}$, and γ_i water/FDC = 56 mN m^{-1}). Tension values were obtained after careful equilibration of the interface. Critical micellar concentrations (cmc) were estimated from the well-marked breaking points of the γ_s -log C curves.

Biological tests

In a typical cell culture test,¹⁵ ten flasks were filled with 2 mL of a suspension of N_1 Namalva lymphoblastoid cells in a nutrient medium; 2 mL of a 0.9% NaCl solution of the perfluoroalkylated surfactant to be tested, or 2 mL of physiological water for the control series, was then added. After three days of incubation, the number of cells were counted (N_2) and their viability determined by the Trypan Blue dye exclusion method: results were expressed as the ratio of living (non-colored) cells to the total number of cells.

The hemolytic activity was evaluated on human red blood cells according to ref. 16: 2 mL of a 1% suspension of human red blood cells were incubated for 1 h at 37 °C with 2 mL of a 0.9% NaCl solution of the surfactant to be tested. Detoxified water and physiological water were submitted to the same procedure and taken as a positive reference and a blank, respectively. After centrifugation, the optical density (OD) of the supernatant was measured at 540 nm and the percentage of hemolysis relative to detoxified water was calculated according to the relationship: $100(\text{OD}_{\text{test}} - \text{OD}_{\text{NaCl}})/(\text{OD}_{\text{H}_2\text{O}} - \text{OD}_{\text{NaCl}})$.

The *in vivo* test consisted in injecting 0.5 mL of an isotonic solution of the surfactant to be tested into the tail vein (intravenous administration, i.v.) of 10 Dawley mice, weighing 20–25 g. Intraperitoneal administration (i.p) was achieved with a solution of surfactant in detoxified perfluorooctyl bromide. Growth, behaviour and any symptoms of intoxication of the animals were monitored over a period of one month and compared to those of a reference population of mice injected with the same volume of saline.

Synthesis of the (perfluoroalkyl)alkyldimorpholinophosphates, F n C m DMPs

[2-(*F*-Butyl)ethyl]dimorpholinophosphate, F4C2DMP. 2-(*F*-Butyl)ethanol (10.14 g, 38.4 mmol) and 14 mL of triethylamine (2.5 equiv) were dissolved in 50 mL of dry ether and added dropwise, at 0 °C and under anhydrous nitrogen, to 5.9 g (38.5 mmol) of phosphorus oxytrichloride in 70 mL of ether. The mixture was stirred at 0 °C for 1 h. [2-(*F*-Butyl)ethoxy]phosphoryl dichloride was identified as the major product (>90%) of the reaction: ¹H NMR (δ , CDCl₃): 2.57 (tt, 2H, ³J_{HH} = 5.5 Hz, ³J_{HF} = 18 Hz, R_FCH₂), 4.3 (dt, 2H, ³J_{HH} = 5.5 Hz, ³J_{HP} = 7 Hz, CH₂OP); ³¹P NMR (δ , CDCl₃): -6.1, signal from by-product bis[2-(*F*-Butyl)ethoxy]phosphoryl monochloride: $\delta = -10$, less than 10% of the total signal area.

A solution of 6.68 g (76.7 mmol) of morpholine and 28 mL (2.5 equiv) of triethylamine in 50 mL of ether was then added dropwise to the stirred reaction mixture, which was maintained free from oxygen and cooled with an ice-bath. After stirring for 18 h, the mixture was allowed to warm up to room temperature, triethylamine hydrochloride was filtered off, and

the solvent and excess of amine were removed under vacuum. The clear oily residue was dried under reduced pressure and purified by chromatography (ethyl acetate-methanol 9 : 1), leading to 12.65 g of the final product (69% with respect to the alcohol). Purity was controlled by TLC (R_f value = 0.7, ethyl acetate-methanol 9 : 1, 1% fluorescein in ethanol solution spray); GC: 99.7%. MW = 482 g, liquid at 25 °C. ¹H NMR (δ , TMS ref., CDCl₃): 2.5 (tt, 2H, ³J_{HH} = 5.6 Hz, ³J_{HF} = 20.2 Hz, R_FCH₂), 3.1 (td, 8H, ³J_{HH} = 5.0 Hz, ³J_{HP} = 3.7 Hz, NCH₂), 3.6 (t, 8H, ³J_{HH} = 5.1 Hz, CH₂OCH₂), 4.2 (td, 2H, ³J_{HH} = 6.0 Hz, ³J_{HP} = 7.6 Hz, CH₂OP); ¹⁹F NMR (δ , CFCl₃ ref., CDCl₃): -81.5 (CF₃), -114.3 (CF₂CH₂), -125.0 (CF₃CF₂CF₂), -126.5 (CF₃CF₂); ¹³C NMR (δ , TMS ref., CDCl₃): 32.1 (td, ²J_{CF} = 21.5 Hz, ³J_{CP} = 7.1 Hz, R_FCH₂), 44.6 (s, NCH₂), 57.2 (d, ²J_{PC} = 4.2 Hz, CH₂OP), 67.1 (d, ³J_{CP} = 6.5 Hz, CH₂OCH₂); ³¹P NMR (δ , H₃PO₄ ref., CDCl₃): 14.1; IR (ν/cm^{-1}): 1250–1180 (P=O, CF), 971 (PN). Anal. calcd (%) for C₁₄H₂₀F₉N₂O₄P: C 34.85; H 4.15; N 5.81, P 6.43; found C 34.64; H 4.13; N 5.73; P 6.29.

The same procedure was applied for the synthesis of all dimorpholinophosphates using the quantities mentioned below.

[3-(*F*-Hexyl)propyl]dimorpholinophosphate, F6C3DMP. 3-(*F*-Hexyl)propanol (21.2 mmol), 21.2 mmol of OPOCl₃ and 53 mmol of morpholine. Yield = 69% after chromatography (ether-chloroform-methanol 4.5 : 5 : 0.5) and recrystallisation in isooctane. MW = 596 g, m.p. = 40 °C. ¹H NMR (δ , CDCl₃): 1.9 (m, 2H, R_FCH₂), 2.1 (m, 2H, R_FCH₂CH₂), 3.1 (td, 8H, ³J_{HH} = 6.6 Hz, ³J_{HP} = 6.7 Hz, NCH₂), 3.6 (t, 8H, ³J_{HH} = 6.3 Hz, CH₂OCH₂), 4.0 (td, 2H, ³J_{HH} = 7.8 Hz, ³J_{HP} = 9.8 Hz, CH₂OP). ¹³C NMR (δ , CDCl₃): 21.9 (s, R_FCH₂), 27.7 (t, R_FCH₂CH₂), 44.8 (s, NCH₂), 63.9 (d, ²J_{CP} = 5.0 Hz, CH₂OP), 67.2 (d, ³J_{CP} = 5.5 Hz, CH₂OCH₂). ³¹P NMR (δ , CDCl₃): 14.8.

[1-(*F*-Heptyl)methyl]dimorpholinophosphate, F7C1DMP. 1-(*F*-Heptyl)methanol (10.0 mmol), 10.0 mmol of POCl₃ and 25 mmol of morpholine. Yield = 60%. MW = 618 g, m.p. = 49 °C. ¹H NMR (δ , CDCl₃): 3.1 (td, 8H, ³J_{HH} = 5.0 Hz, ³J_{HP} = 5.8 Hz, NCH₂), 3.7 (t, 8H, ³J_{HH} = 4.5 Hz, CH₂OCH₂), 4.4 (td, 2H, ³J_{HH} = 6.7 Hz, ³J_{HP} = 13.3 Hz, CH₂OP). ¹³C NMR (δ , CDCl₃): 44.7 (s, NCH₂), 61.7 (t, CH₂OP), 67.1 (s, CH₂OCH₂). ³¹P NMR (δ , CDCl₃): 15.5. IR (ν/cm^{-1}): 1240–1210 (CF), 2890–2850 (CH), 974 (PN).

[3-(*F*-Octyl)propyl]dimorpholinophosphate, F8C3DMP. 3-(*F*-Octyl)propanol (12.5 mmol), 12.5 mmol of OPOCl₃ and 31.4 mmol of morpholine. Yield = 64% after chromatography (ether-chloroform-methanol 4.5 : 5 : 0.5) and recrystallisation in isooctane. MW = 696 g, m.p. = 59 °C. ¹H NMR (δ , CDCl₃): 2.0 (m, 2H, R_FCH₂), 2.2 (m, 2H, R_FCH₂CH₂), 3.1 (td, 8H, ³J_{HH} = 6.6 Hz, ³J_{HP} = 6.7 Hz, NCH₂), 3.6 (t, 8H, ³J_{HH} = 6.3 Hz, CH₂OCH₂), 4.0 (td, 2H, ³J_{HH} = 7.8 Hz, ³J_{HP} = 9.8 Hz, CH₂OP). ¹³C NMR (δ , CDCl₃): 21.9 (s, R_FCH₂), 27.7 (t, R_FCH₂CH₂), 44.8 (s, NCH₂), 63.9 (d, ²J_{CP} = 5.0 Hz, CH₂OP), 67.2 (d, ³J_{CP} = 5.5 Hz, CH₂OCH₂). ³¹P NMR (δ , CDCl₃): 14.8.

[5-(*F*-Octyl)pentyl]dimorpholinophosphate, F8C5DMP. 5-(*F*-Octyl)pentanol (32.6 mmol), 32.6 mmol of OPOCl₃ and 70.4 mmol of morpholine. Yield = 73%. MW = 724 g; m.p. = 78 °C. ¹H NMR (δ , CDCl₃): 1.5 (s, 2H), 1.7 (m, 4H), 2.1 (tt, 2H, ³J_{HH} = 6.2 Hz, ³J_{HF} = 19.1 Hz, R_FCH₂), 3.1 (td, 8H,

$^3J_{\text{HH}} = 6.1$ Hz, $^3J_{\text{HP}} = 4.5$ Hz, NCH_2), 3.6 (t, 8H, $^3J_{\text{HH}} = 6.1$ Hz, CH_2OCH_2), 3.9 (td, 2H, $^3J_{\text{HH}} = 6.2$ Hz, $^3J_{\text{HP}} = 6.9$ Hz, CH_2OP). ^{19}F NMR (δ , CDCl_3): -81.2 (CF_3), -114.8 (CF_2CH_2), -122.3 (6F), -123.2 (2F), -123.9 (2F), -126.6 (CF_3CF_2). ^{13}C NMR (δ , CDCl_3): 20.1 (t, $^3J_{\text{CF}} = 5.5$ Hz, $\text{R}_\text{F}\text{CH}_2\text{CH}_2$), 25.5, 30.3 (s, 2 CH_2), 31.1 (t, $^2J_{\text{CF}} = 21.5$ Hz, $\text{R}_\text{F}\text{CH}_2$), 44.9 (s, NCH_2), 65.2 (d, $^2J_{\text{CP}} = 3.8$ Hz, CH_2OP), 67.3 (d, $^3J_{\text{CP}} = 5.5$ Hz, CH_2OCH_2). ^{31}P NMR (δ , CDCl_3): 13.7. IR (ν/cm^{-1}): 1250–1150 (P=O, CF), 974 (PN), 2930–2850 (CH). Anal. calcd (%) for $\text{C}_{21}\text{H}_{26}\text{F}_{17}\text{N}_2\text{O}_4\text{P}$: C 34.81; H 3.59; N 3.87; P 4.28; found: C 34.64; H 3.74; N 3.89; P 4.35.

[1-(*F*-Nonyl)methyl]dimorpholinophosphate, F9C1DMP. 1-(*F*-Nonyl)methanol (8.8 mmol), 8.9 mmol of OPCl_3 and 17.7 mmol of morpholine. Yield = 71%. MW = 718 g, m.p. = 67 °C. ^1H NMR (δ , CDCl_3): 3.1 (td, 8H, $^3J_{\text{HH}} = 6.1$ Hz, $^3J_{\text{HP}} = 3.1$ Hz, NCH_2), 3.6 (t, 8H, $^3J_{\text{HH}} = 6$ Hz, CH_2OCH_2), 4.4 (td, 2H, $^3J_{\text{HF}} = 22.7$ Hz, $^3J_{\text{HP}} = 7.5$ Hz, CH_2OP). ^{19}F NMR (δ , CDCl_3): -81.2 (CF_3), -120.7 (CF_2CH_2), -122.3 (4F), -123.1 (2F), -123.5 (6F), -126.6 (CF_3CF_2). ^{13}C NMR (δ , CDCl_3): 44.8 (s, NCH_2), 61.8 (dt, $^2J_{\text{CF}} = 20.0$ Hz, $^2J_{\text{CP}} = 4.0$ Hz, CH_2OP), 67.1 (d, $^3J_{\text{CP}} = 5.5$ Hz, CH_2OCH_2). ^{31}P NMR (δ , CDCl_3): 14.7. IR (ν/cm^{-1}): 1250–1150 (P=O, CF), 976 (PN). Anal. calcd (%) for $\text{C}_{18}\text{H}_{18}\text{F}_{19}\text{N}_2\text{O}_4\text{P}$: C 30.08; H 2.52; N 3.9; P 4.32; found: C 30.21, H 2.34; N 3.85; P 4.38.

[2-(*F*-Decyl)ethyl]dimorpholinophosphate, F10C2DMP. 2-(*F*-Decyl)ethanol (18.4 mmol), 18.4 mmol of OPCl_3 and 36.9 mmol of morpholine. Yield = 65%. GC: 99.7%. MW = 782 g, m.p. = 91 °C. ^1H NMR (δ , CDCl_3): 2.5 (tt, 2H, $^3J_{\text{HH}} = 7.8$ Hz, $^3J_{\text{HF}} = 19.6$ Hz, $\text{R}_\text{F}\text{CH}_2$), 3.1 (td, 8H, $^3J_{\text{HH}} = 6.6$ Hz, $^3J_{\text{HP}} = 6.7$ Hz, NCH_2), 3.6 (t, 8H, $^3J_{\text{HH}} = 6.3$ Hz, CH_2OCH_2), 4.3 (td, 2H, $^3J_{\text{HH}} = 7.8$ Hz, $^3J_{\text{HP}} = 9.8$ Hz, CH_2OP). ^{19}F NMR (δ , CDCl_3): -81.3 (CF_3), -114.1 (CF_2CH_2), -122.3 (10F), -123.2 (2F), -124.1 (2F), -126.7 (CF_3CF_2). ^{13}C NMR (δ , CDCl_3): 32.3 (td, $^2J_{\text{CF}} = 21.3$ Hz, $^3J_{\text{CP}} = 5.7$ Hz, $\text{R}_\text{F}\text{CH}_2$), 44.8 (s, NCH_2), 57.4 (d, $^2J_{\text{CP}} = 5.0$ Hz, CH_2OP), 67.1 (d, $^3J_{\text{CP}} = 5.5$ Hz, CH_2OCH_2). ^{31}P NMR (δ , CDCl_3): 14.2. IR (ν/cm^{-1}): 1250–1150 (P=O, CF), 978 (PN). Anal. calcd (%) for $\text{C}_{20}\text{H}_{20}\text{F}_{21}\text{N}_2\text{O}_4\text{P}$: C 30.69; H 2.56; N 1.79; P 3.96; found: C 30.74; H 2.46; N 1.82; P 3.85.

[3-(*F*-Decyl)propyl]dimorpholinophosphate, F10C3DMP. 3-(*F*-Decyl)propanol (10.4 mmol), 10.4 mmol of OPCl_3 and 26 mmol of morpholine. Yield = 52% after chromatography (ether–chloroform–methanol 4.5 : 5 : 0.5) and recrystallisation in isoctane. MW = 796 g, m.p. = 89 °C. ^1H NMR (δ , CDCl_3): 1.9 (m, 2H, $\text{R}_\text{F}\text{CH}_2$), 2.2 (m, 2H, $\text{R}_\text{F}\text{CH}_2\text{CH}_2$), 3.1 (td, 8H, $^3J_{\text{HH}} = 6.6$ Hz, $^3J_{\text{HP}} = 6.7$ Hz, NCH_2), 3.6 (t, 8H, $^3J_{\text{HH}} = 6.3$ Hz, CH_2OCH_2), 4.1 (td, 2H, $^3J_{\text{HH}} = 7.8$ Hz, $^3J_{\text{HP}} = 9.8$ Hz, CH_2OP). ^{13}C NMR (δ , CDCl_3): 21.9 (s, $\text{R}_\text{F}\text{CH}_2$), 27.7 (t, $\text{R}_\text{F}\text{CH}_2\text{CH}_2$), 44.8 (s, NCH_2), 63.9 (d, $^2J_{\text{CP}} = 5.0$ Hz, CH_2OP), 67.2 (d, $^3J_{\text{CP}} = 5.5$ Hz, CH_2OCH_2). ^{31}P NMR (δ , CDCl_3): 14.8.

Pentadecyldimorpholinophosphate, C15DMP. Pentadecanol (25.5 mmol), 25.6 mmol of OPCl_3 and 51.1 mmol of morpholine. Yield = 65%. MW = 446 g, m.p. = 45 °C. ^1H NMR (δ , CDCl_3): 0.8 (t, CH_3), 1.2 (m, 24 H), 1.6 (m, $\text{CH}_2\text{CH}_2\text{OP}$), 3.1 (td, 8H, $^3J_{\text{HH}} = 6.2$ Hz, $^3J_{\text{HP}} = 2.7$ Hz, NCH_2), 3.6 (t, 8H, $^3J_{\text{HH}} = 6.2$ Hz, CH_2OCH_2), 3.9 (dt, 2H, $^3J_{\text{HH}} = 6.6$ Hz, $^3J_{\text{HP}} = 6.9$ Hz, CH_2OP). ^{13}C NMR (δ , CDCl_3): 14.2 (s, CH_3),

22.8, 25.8, 29.3, 29.4, 29.6, 29.7, 32.0 (all s, 12 CH_2), 30.6 (d, $^3J_{\text{CP}} = 6$ Hz, $\text{CH}_2\text{CH}_2\text{O}$), 44.8 (s, NCH_2), 65.7 (d, $^2J_{\text{CP}} = 5.5$ Hz, CH_2OP), 67.3 (d, $^3J_{\text{CP}} = 5.7$ Hz, CH_2OCH_2). ^{31}P NMR (δ , CDCl_3): 13.7. IR (ν/cm^{-1}): 1260–1230 (P=O), 962 (PN), 2930–2850 (CH). Anal. calcd (%) for $\text{C}_{23}\text{H}_{47}\text{N}_2\text{O}_4\text{P}$: C 31.88; H 10.54; N 6.28; P 6.95; found: C 31.95; H 10.68; N 6.35; P 7.02.

Synthesis of the bis(perfluoroalkyl) monomorpholinophosphates, D(*F**n**C**m*)MMPs

Bis[2-(*F*-hexyl)ethyl]monomorpholinophosphate, D(F6C2)-MMP. OPCl_3 (8.38 g, 54.6 mmol) was dissolved in 100 ml of dry ether under dry nitrogen. 2-(*F*-Hexyl)ethanol (39.9 g, 109.6 mmol) and 10.2 g (14 mL, 112 mmol) of NEt_3 in 50 mL of Et_2O were added dropwise to this solution at 50 °C. The mixture was stirred for 12 h at room temperature, filtered and the solvent and the excess of amine were removed under vacuum. Morpholine (10.1 g, 116 mmol) in 50 mL Et_2O was then added dropwise at 0 °C. The reaction mixture was refluxed and stirred for 12 h. The precipitate was then filtered, washed with Et_2O and the solvent evaporated. The crude product was purified by column chromatography on silica (gradient from 10% ethyl acetate in hexane to 100% ethyl acetate). Recrystallisation from hexane yielded 30.4 g (65% with respect to the fluorinated alcohol) of the final product. MW = 859 g, m.p. = 48 °C. ^1H NMR (δ , CDCl_3): 2.45 (m, 4H, $\text{R}_\text{F}\text{CH}_2$), 3.09 (m, 4H, CH_2N), 3.6 (m, 4H, CH_2OCH_2), 4.25 [m, 4H, $(\text{CH}_2\text{O})_2\text{PO}$]. ^{19}F NMR (δ , CDCl_3): -81.3 (CF_3), -114.0 (CF_2CH_2), -122.4 (2F), -123.4 (2F), -124.1 (2F), -126.6 (CF_3CF_2). ^{31}P NMR (δ , CDCl_3): 8.34. IR (ν/cm^{-1}): 1250–1150 (P=O, CF), 970 (PN). Anal. calcd (%) for $\text{C}_{20}\text{H}_{16}\text{F}_{26}\text{NO}_4\text{P}$: C 27.95; H 1.88; N 1.63; P 1.64; found: C 27.91; H 1.82; N 1.59; P 1.60.

Bis[2-(*F*-octyl)ethyl]monomorpholinophosphate, D(F8C2)-MMP. The same procedure was applied to 6.91 g (45.0 mmol) of OPCl_3 , 41.82 g (90.2 mmol) of 2-(*F*-octyl)ethanol and 11.3 mL (8.51 g, 90.3 mmol) of NEt_3 . The reaction mixture, after having been stirred for 12 h at room temperature, was refluxed for 4 h. Morpholine (7.93 g, 91.1 mmol) was then added at room temperature and the mixture was refluxed for 12 h and filtered. The precipitate was extracted with a CHCl_3 – H_2O mixture. The ether and chloroform solutions were removed and the combined residues were chromatographed on silica with the same gradient as above. Recrystallisation from hexane yielded 24.3 g (55%) of a white material. MW = 1059 g, m.p. = 79 °C. ^1H NMR (δ , CDCl_3): 2.55 (m, 4H, $\text{R}_\text{F}\text{CH}_2$), 3.19 (m, 4H, CH_2N), 3.7 (m, 4H, CH_2OCH_2), 4.33 [m, 4H, $(\text{CH}_2\text{O})_2\text{PO}$]. ^{19}F NMR (δ , CDCl_3): -81.3 (CF_3), -114.2 (CF_2CH_2), -122.5 (4F), -123.4 (4F), -124.1 (2F), -126.6 (CF_3CF_2). ^{31}P NMR (δ , CDCl_3): 8.29. IR (ν/cm^{-1}): 1250–1150 (P=O, CF), 970 (PN). Anal. calcd (%) for $\text{C}_{24}\text{H}_{16}\text{F}_{34}\text{NO}_4\text{P}$: C 27.20; H 1.52; N 1.32; P 2.92; found: C 27.22; H 1.50; N 1.36; P 2.90.

Bis[1-(*F*-nonyl)methyl]monomorpholinophosphate, D(F9C1)-MMP. The same procedure as for D(F8C2)MMP, when applied to 1.93 g (45.0 mmol) of OPCl_3 , 41.84 g (90.2 mmol) of 1-(*F*-nonyl)methanol, 11.3 mL (8.51 g, 90.3 mmol) of NEt_3 and, subsequently, 7.93 g (91.1 mmol) of morpholine, yielded 5.42 g (37%) of D(F9C1)MMP. MW = 1131 g, m.p. = 82 °C. ^1H NMR (δ , CDCl_3): 3.2 (m, 4H, CH_2N), 3.65 (m, 4H, CH_2OCH_2), 4.2 [m, 4H, $(\text{CH}_2\text{O})_2\text{PO}$]. ^{19}F NMR (δ , CDCl_3):

–81.5 (CF₃), –114.1 (CF₂CH₂), –122.5 (4F), –123.4 (6F), –124.2 (2F), –126.5 (CF₃CF₂); ³¹P NMR (δ, CDCl₃): 8.35. IR (ν/cm⁻¹): 1250–1150 (P=O, CF), 970 (PN). Anal. calcd (%) for C₂₄H₁₆F₃₄NO₄P: C 25.48; H 1.07; N 1.24; P 2.90; found: C 25.12; H 0.99; N 1.25; P 2.60.

Results and Discussion

Synthesis

The series of (perfluoroalkyl)alkyldimorpholinophosphate surfactants has been extended; the yields were in the 52–73% range. The procedure consists, in its first step, in the phosphorylation of the fluorinated alcohol by phosphorus oxychloride as shown in Scheme 1. The formation of di- and triesters and the chlorination of the alcohol can be avoided by using dry ether and an excess of triethylamine, which result in the precipitation of triethylammonium chloride.¹⁷ The (perfluoroalkyl)alkyldimorpholinophosphates were subsequently obtained by reaction with morpholine. *Ca.* 5% of the crude material obtained consisted of a by-product, which was identified by NMR to be the diester D(FnCm)MMP. No pyrophosphate was detected. The stability of the FnCmDMPs was assessed by heating aqueous solutions of these compounds (10

g L⁻¹) for 18 h. After evaporation of the water, the residue was dissolved in CDCl₃ and analysed by ¹H and ³¹P NMR. No degradation of the products was detected.

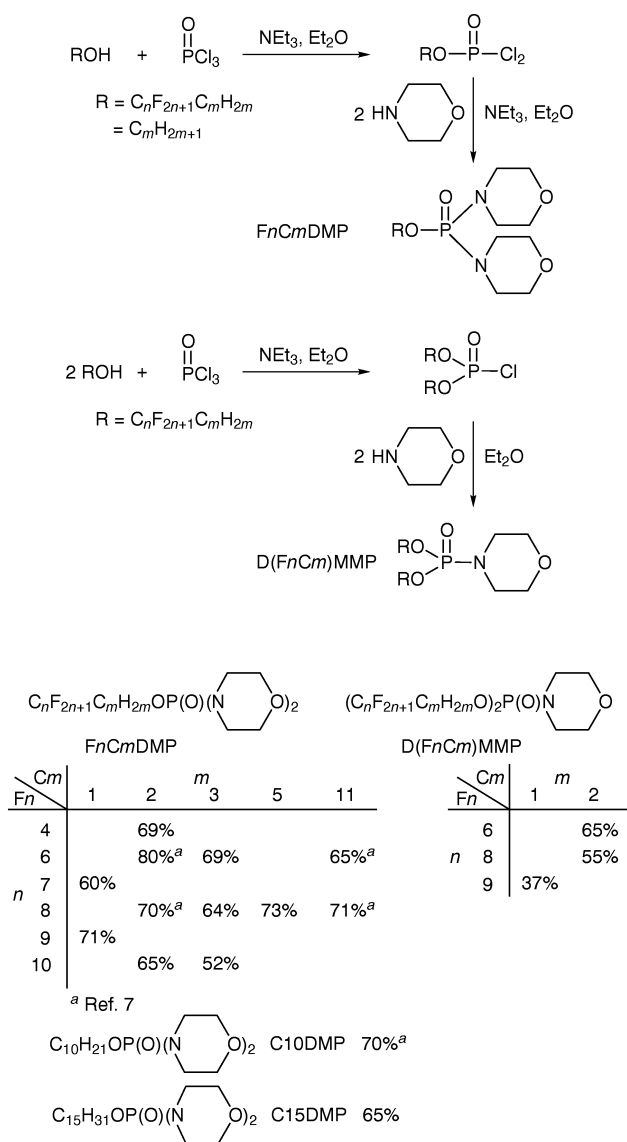
The synthesis of bis[(F-alkyl)alkyl]monomorpholinophosphates also involve the direct phosphorylation of the F-alkylated alcohols by OPCl₃, followed by reaction with morpholine. However, the duration of the first step was increased because of steric crowding due to the presence of two bulky and rigid perfluoroalkylated chains. In the case of D(F8C2)MMP and D(F9C1)MMP, heating at reflux was even necessary to achieve phosphorylation of the alcohols. Furthermore, two equivalents of morpholine without triethylamine were needed: it was impossible to obtain the D(FnCm)MMPs with only one equivalent of morpholine in the presence of an excess of triethylamine as in the case of the dimorpholinophosphates. This can be explained by a competition between the two bases for the remaining, crowded and less reactive P–Cl bond. With the procedure reported here, the D(FnCm)MMPs could be obtained in 37–65% yield.

Surface and interfacial tension measurements

It is well-known that fluorinated surfactants are much more surface active at the air/water interface than standard hydrogenated surfactants.^{1–4,18,19} We have assessed the surface activity of the perfluoroalkylated mono- and dimorpholinophosphates by measuring the surface tension, γ_s, of their aqueous solutions and the interfacial tension between these solutions and perfluorodecalin, γ_i(FDC). Long equilibration times (several hours) were necessary, especially for the surfactants with the highest Fn : Cm ratio, in order to reach equilibrium.²⁰ The results are collected in Table 1. It can be seen that the FnCmDMPs can reduce the surface tension of water from 72 to 21.2–24.5 mN m⁻¹, even when they are very short, as in the case of F4C2DMP. In comparison, the hydrogenated analogues, C10DMP and C15DMP, are much less effective and reduce the γ_s of water to 30–32.5 mN m⁻¹ only, even when higher concentrations of the surfactant (*e.g.* 10⁻⁴ mol L⁻¹) are used. The length of the hydrophobic chain has only a small impact on the surface tension value at the critical micellar concentration (cmc), in line with other results on nonionic surfactants.²¹ The cmc of the fluorinated products are very low and decrease, as expected, when the degree of fluorination and/or the hydrocarbon spacer length increase. It is well-known that fluorinated surfactants have a higher tendency to form micelles than hydrogenated analogues.^{18,19} The cmc value of F8C2DMP is *ca.* two orders of magnitude lower than that of C10DMP. On the other hand, it was found that for a given length of the fluorinated chain, increasing the length of the hydrogenated spacer did not lower the cmc value as much as would have been expected; see for example the cmc of F6C2DMP and F6C11DMP (97.3 × 10⁻⁶ and 25.4 × 10⁻⁶ mol L⁻¹, respectively). This observation may indicate that it is the length of the fluorinated chain that actually controls micellization and that the spacer only plays a minor role.²²

The fluorinated FnCmDMPs were also found to reduce the interfacial tension between water and perfluorodecalin very effectively, *i.e.* from 56 to *ca.* 1 mN m⁻¹, while their hydrogenated analogues reduce γ_i only to 10 mN m⁻¹.

The D(FnCm)MMPs were found to be less-surface active than the FnCmDMPs. D(F6C2)MMP reduces the surface tension of water to 23.5 mN m⁻¹ but only at concentrations higher than 1.5 × 10⁻⁵ mol L⁻¹. Likewise, high concentrations were needed to reduce the interfacial tension between water and perfluorodecalin (FDC) significantly (γ_i = 12.5 mN m⁻¹ at 10⁻² mol L⁻¹). D(F6C2)MMP being soluble in FDC, a significant amount of the surfactant remains in the form of monomers, and is therefore not available to form the monolayer. The high interfacial tension values obtained with D(F8C2)MMP and D(F9C1)MMP (γ_i = 15.5 mN m⁻¹ and



Scheme 1 Synthesis of (F-alkyl)alkyldimorpholinophosphates and bis[(F-alkyl)alkyl]monomorpholinophosphates

Table 1 Surface and interfacial tensions of aqueous solutions of perfluoroalkylated dimorpholinophosphates and monomorpholinophosphates at 25 °C and biological data

Compound	cmc/ mol L ⁻¹	γ_s / mN m ⁻¹	c/ mol L ⁻¹	γ_i (FDC)/ mN m ⁻¹	Acute toxicity in mice LD ₅₀			
					i.v./ mg (kg body weight) ⁻¹	Survival	i.p./ g (kg body weight) ⁻¹	Survival
F4C2DMP	3.2 × 10 ⁻³	24.5	8.3 × 10 ⁻³	3.7	> 500	10/10	—	—
F6C2DMP	1.0 × 10 ⁻⁴	22.0	3.2 × 10 ⁻³	2.3	> 250	10/10	—	—
F6C11DMP	2.6 × 10 ⁻⁵	23.5	2.8 × 10 ⁻⁴	0.9	> 25	10/10	—	—
F7C1DMP	1.5 × 10 ⁻⁵	21.2	2.0 × 10 ⁻³	3.7	—	—	—	—
F8C2DMP	2.0 × 10 ⁻⁶	22.5	1.5 × 10 ⁻⁴	0.9	> 25	10/10	> 2	10/10
F8C5DMP	8.9 × 10 ⁻⁷	22.5	6.9 × 10 ⁻⁵	3.8	—	—	≈ 2	5/10
F8C11DMP ^a	1.0 × 10 ⁻⁶	23.0	—	—	> 2000	10/10	≈ 4	5/10
F9C1DMP	7.7 × 10 ⁻⁷	23.0	2.5 × 10 ⁻³	0.9	—	—	—	—
F10C2DMP ^b	1.0 × 10 ⁻⁶	23.5	—	—	—	—	—	—
C10DMP	2.5 × 10 ⁻⁴	30.0	—	—	> 140	10/10	—	—
C15DMP	1.6 × 10 ⁻⁶	32.5	5.4 × 10 ⁻⁵	9.4	—	—	—	—
D(F6C2)MMP	1.5 × 10 ⁻⁵	23.5	1.7 × 10 ⁻²	12.7	—	—	> 4	8/10
D(F8C2)MMP ^b	2.1 × 10 ⁻⁶	23.5	1.0 × 10 ⁻²	15.5	—	—	—	—
D(F9C1)MMP ^b	1.0 × 10 ⁻⁵	25.3	9.9 × 10 ⁻³	14.5	—	—	> 2	9/10

^a Calculated. ^b Measured at 60 °C.

$\gamma_i = 14.5 \text{ mN m}^{-1}$ at $10^{-2} \text{ mol L}^{-1}$), which are very soluble in the fluorocarbon phase, confirm this view. The quasi-insolubility of the monomorpholinophosphates in water explains that D(F8C2)MMP and D(F9C1)MMP reduce the surface tension of water to 23.5 and 25.3 mN m⁻¹, respectively, but only at 60 °C.

Acute toxicity and hemolysis data

The preliminary biological tests performed on the new perfluoroalkylated amphiphiles are collected in Table 1. Dispersions of F6C2DMP, F6C11DMP, F8C2DMP and F8C11DMP (0.05 to 0.5 g L⁻¹ in 0.9% of NaCl) did not perturb the growth and viability of Namalva strain lymphoblastoid cell cultures, while a solution of the non-fluorinated analogue C10DMP (0.05 g L⁻¹) totally inhibited cell growth and viability.⁷ Likewise, solutions of the fluorinated surfactants did not show any hemolytic activity on a suspension of human red blood cells: in contrast, a solution of C10DMP (0.5 g L⁻¹) was strongly hemolytic.

Aqueous dispersions of the above fluorinated surfactants were well-tolerated when administered intravenously (tail vein) in mice; the i.v. LD₅₀ values were higher than 200–250 mg (kg body weight)⁻¹ (10/10 survivals) for F6C2DMP and F8C2DMP (limit of protocol/solubility). To increase the amount of surfactant that could be administered, we prepared a dispersion of the poorly water-soluble F8C11DMP in 0.9% NaCl using egg yolk phospholipids. The toxicity tests indicated a LD₅₀ value higher than 2 g (kg body weight)⁻¹ (limit of protocol, 10/10 survivals).

Intraperitoneal administration in mice of FnCmDMPs in perfluorooctyl bromide (PFOB) gave LD₅₀ values higher than 2 g (kg body weight)⁻¹ for F8C2DMP (10/10 survivals) and around 4 g (kg body weight)⁻¹ for F8C11DMP (5/10 survivals). Very encouraging results have also been obtained for the bis-perfluoroalkylated monomorpholinophosphates with i.p. LD₅₀ values larger than 4 and 2 g (kg body weight)⁻¹ for D(F6C2)MMP (8/10 survivals) and D(F9C1)MMP (9/10 survivals), respectively.

All these results confirm that in spite of their high surface activity, fluorinated morpholinophosphates are well tolerated by cell cultures, are non-hemolytic, and present low to very low acute toxicity, even when administered intravascularly. They confirm our opinion that perfluoroalkylated morpholinophosphates have potential as components of colloidal systems intended for biomedical applications.

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