Perfluoroalkylated amphiphiles with a morpholinophosphate or a dimorpholinophosphate polar head group

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Some previously synthesized (perfluoroalkyl)alkylmorpholinophosphates, CnFm+1CH2nOP(O)-[N(CH2CH2)mO]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, CnFm+1CH2nO)(CH2CH2)mO₄, 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 mind1–4 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)alkylmorpholinophosphates, CnFm+1CH2nOP(O)[N(CH2CH2)mO]2, (FrCmMMP).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 microtubes in which the surfactant molecules appear to be highly ordered;9,10 when heated, these tubes 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 inpraperitoneally 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 Fr: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, (CnFm+1CH2nO)(CH2CH2)mO₄, code-named D(FrCmMMP). 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 to terminally unsaturated alcohols CH₃=CH(CH2)m-OH, followed by reduction with HCl-Zn in anhydrous ethanol.12 (F-Heptylmethanol, C₇F₁₅CH₂OH, was prepared from the methyl perfluorooctanate ester C₇F₁₅COOC₂H₅ by NaBH₄ reduction in isopropanol.13 Synthesis of the (F-alkyl)propanols is described elsewhere.14 Phosphorus oxychloride, triethylamine and morpholine (Aldrich) were distilled prior to use. Perfluordecaclin (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_{lq}, 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, γ_w = 72 mN m^{-1}, and γ_l 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, 10 ten flasks were filled with 2 mL of a suspension of N_{2} Namalva lymphoblastoid cells in a nutrient medium; 2 mL of a 0.9% NaCl solution of the perfluorooalkylated 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 was counted (N_t) 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 the relationship: 100(OD_{red} – OD_{control})/(OD_{control} – OD_{no sucked})

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., CDCl_3): 2.5 (t, 2H, J_{HH} = 5.6 Hz, J_{HF} = 20.2 Hz, R_C6H_4Cl_3), 3.1 (td, 8H, J_{HH} = 5.0 Hz, J_{HF} = 3.7 Hz, NCH_3), 3.6 (t, 8H, J_{HH} = 5.1 Hz, CH_2CH_3OH), 4.2 (td, 2H, J_{HF} = 6.0 Hz, J_{HH} = 7.6 Hz, CH_2OP); 13C NMR (δ, CFC1, ref., CDCl_3): –81.5 (CF_3), –114.3 (CF_2CH_3), –125.0 (CF_2CF_2CF_3), –126.5 (CF_5CF_3); 13C NMR (δ, TMS ref., CDCl_3): 32.1 (td, J_{HF} = 21.5 Hz, 3J_{HF} = 7.1 Hz, R_C6H_4Cl_3), 44.6 (s, NCH_3), 57.2 (d, J_{HF} = 4.2 Hz, CH_3OP), 67.1 (d, J_{HF} = 6.5 Hz, CH_3CH_2OH). 13C NMR (δ, CDCl_3): 41.4; IR (v/cm^{-1}): 1250–1180 (P=O, CF), 971 (PN). Anal. calc’d (%) for C_{34}H_{48}F_8N_2O_4P: 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.

**Synthesis of the (perfluoroalkyl)alkyldimorpholinophosphates, F_{n}C_{m}DMPs**

**[2-(F-Butyl)ethyl]dimorpholinophosphates, F4C2DMP.** 2-(F-Butyl)ethyl(10.14 g, 38.44 mmol) and 14 mL of triethylene (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 oxychioride in 70 mL of ether. The mixture was stirred at 0 °C for 1 h. 2-(F-Butyl)ethylphosphoryl dichloride was identified as the major product (>90%) of the reaction: 1H NMR (δ, CDCl_3): 2.57 (tt, 2H, J_{HH} = 5.5 Hz, 3J_{HF} = 18 Hz, R_C6H_4Cl_3), 4.3 (dt, 2H, J_{HF} = 5.5 Hz, J_{HH} = 7 Hz, CH_2OP); 31P NMR (δ, CDCl_3): –6.1, signal from by-product bis[2-(F-Butyl)ethyl]phosphoryl monochloride: δ = –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 triethylene 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, triethylene hydrochloride was filtered off, and
[3] J₃₀ = 6.1 Hz, J₂₁ = 4.5 Hz, NCHO). 3.6 (t, 8H, J₁₂ = 6.1 Hz, H₃(CH₂OH)). 3.9 (td, 2H, J₁₂ = 6.2 Hz, J₁₂ = 6.9 Hz, CH₂OPO). 19F NMR (δ, CDCI₃): -81.2 (CF₃), -114.8 (CF₂CH₂). -122.3 (6F), -123.2 (2F), -123.9 (2F), -126.6 (CF₂CH₂). ¹³C NMR (δ, CDCI₃): 20.1 (t, J₁₂ = 5.5 Hz, R₃(CH₂CH₂O)), 25.5, 30.3 (s, 2 C), 31.1 (t, J₂₁ = 21.5 Hz, R₃(CH₂CH₂O)). 44.9 (s, NCH₃), 65.2 (d, J₂₁ = 3.8 Hz, CH₂OP), 67.3 (d, J₁₂ = 5.5 Hz, CH₂OCH₃). ³¹P NMR (δ, CDCI₃): 13.7. IR (v/cm⁻¹): 1250-1150 (P=O, CF), 974 (PN), 2930-2850 (CH). Anal. calcld (%) for C₃₆H₇₉F₅N₇O₈P: C 34.81; H 3.59; N 3.26; P 14.8.

Synthesis of the bis(perfluorooalkyl) monomorpholinophosphates, D(F6C2)MMP

[2-(F-hexyl)ethyl] monomorpholinophosphates, D(F6C2)-MMP. OPC1 (83.8 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 NEt₃ in 50 ml of Et₂O 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 Et₂O was then added dropwise at 0 °C. The reaction mixture was refluxed and stirred for 12 h. The precipitate was then filtered, washed with Et₂O 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 fluororinated alcohol) of the final product.

MW = 859 g, m.p. = 48 °C. ¹¹B NMR (δ, CDCI₃): 245.4 (m, 4H, R₃(CH₂CH₂O)), 3.09 (m, 4H, CH₂N), 3.6 (m, 4H, CH₂OCH₃). 4.18 (m, 4H, CH₂N), 4.25 [m, 4H, (CH₃O)₂PO]. 4.23 [m, 4H, (CH₃O)₂PO]. 81.3 (CF₃), -114.1 (CF₂CH₂), -122.3 (10F), -123.2 (2F), -124.1 (2F), -126.7 (CF₂CH₂). ¹³C NMR (δ, CDCI₃): 27.3 (s, R₃(CH₂CH₂O)). 124.2 (s, R₃(CH₂CH₂O)). 126.6 (s, R₃(CH₂CH₂O)). 31P NMR (δ, CDCI₃): 14.2. IR (v/cm⁻¹): 1250-1150 (P=O, CF), 970 (PN). Anal. calcld (%) for C₃₂H₅₂F₃N₅O₇P: C 72.95; H 1.88; N 1.63; P 1.64; found: C 72.91; H 1.82; N 1.59; P 1.60.

[3]-[F-decyl]dimorpholinophosphate, F10C3DMP

[3]-[F-decyl]propanol (10.4 mmol), 10.4 mmol of OPC1 and 26 mmol of morpholine. Yield = 52% after chromatography (ether–chloroform–methanol 4:5:0.5) and recrystallisation in isooctane. MW = 496 g, m.p. = 89 °C. ¹¹B NMR (δ, CDCI₃): 1.9 (m, 2H, R₃(CH₂CH₂O)), 2.2 (m, 2H, R₃(CH₂CH₂O)), 3.1 (td, 8H, J₁₂ = 6.6 Hz, J₁₂ = 6.6 Hz, NCH₃), 3.6 (t, 8H, J₁₂ = 6.3 Hz, CH₂OCH₃). 4.1 (td, 2H, J₁₂ = 7.8 Hz, J₁₂ = 9.8 Hz, CH₂OCH₃). 1.9 (s, 6H, NCH₃), 27.7 (t, R₃(CH₂CH₂O)), 44.8 (s, NCH₃), 63.9 (d, J₁₂ = 5.0 Hz, CH₂OCH₃). 67.2 (d, J₁₂ = 5.5 Hz, CH₂OCH₃). ³¹P NMR (δ, CDCI₃): 14.8.

Pentadecyl morpholinophosphate, C15DMP. Pentadecanol (25.5 mmol), 25.6 mmol of OPC1 and 51.1 mmol of morpholine. Yield = 65%. MW = 446 g, m.p. = 45 °C. ¹¹B NMR (δ, CDCI₃): 0.8 (t, CH₃), 1.2 (m, 24 H), 1.6 (m, CH₂CH₂O), 3.1 (td, 8H, J₁₂ = 6.2 Hz, J₁₂ = 2.7 Hz, NCH₃), 3.6 (t, 8H, J₁₂ = 6.2 Hz, CH₂OCH₃), 3.9 (dt, 2H, J₁₂ = 6.6 Hz, J₁₂ = 6.9 Hz, CH₂OCH₃). ¹³C NMR (δ, CDCI₃): 14.2 (s, CH₃), 148.2 (m, 4H, CH₂N), 297.1 (m, 4H, CH₂OCH₃). 4.2 (m, 4H, (CH₂O)₂PO). 19F NMR (δ, CDCI₃): -81.3 (CF₃), -114.2 (CF₂CH₂), -122.5 (4F), -123.4 (2F), -126.6 (CF₂CH₂). ³¹P NMR (δ, CDCI₃): 8.29. IR (v/cm⁻¹): 1250-1150 (P=O, CF), 970 (PN). Anal. calcld (%) for C₃₆H₇₄F₅N₅O₇P: C 72.27; H 1.52; N 1.32; P 2.92; found: C 72.22; H 1.50; N 1.36; P 2.90.
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 results in the precipitation of triethylammonium chloride. 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(F9C11)MMP. No pyrophosphate was detected. The stability of the F

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, γs, of their aqueous solutions and the interfacial tension between these solutions and perfluorodecalin, γi(FDC). Long equilibration times (several hours) were necessary, especially for the surfactants with the highest Fm:Cm ratio, in order to reach equilibrium.20 The results are collected in Table 1. It can be seen that the FmCmMMPs 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 γs 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 FmCmMMPs 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 γi only to 10 mN m−1.

The D(FmCm)MMPs were found to be less-surface active than the FmCmMMPs. 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 (γi = 12.5 mN m−1 at 10−3 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 (γi = 15.5 mN m−1 and

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

$$-81.5 \text{ (CF}_3)_3, -114.1 \text{ (CF}_3\text{CH}_2), -122.5 \text{ (4F), -123.4 (6F),}$$
$$-124.2 \text{ (2F), -126.5 (CF}_3\text{CF}_2); 3^\text{1}P \text{ NMR (d, CDCl}_3): 8.35.$$
analogue C10DMP (0.05 g L$^{-1}$) perturb the growth and viability of Namalva strain lymphoids of F6C2DMP, F6C11DMP, F8C2DMP and fluoroalkyl amphiphiles are collected in Table 1. Dispersions of the poorly water-soluble F8C11DMP in 0.9% NaCl using egg yolk phospholipids. The toxicity tests indicated a value higher than 2 g (kg body weight)$^{-1}$ for F8C11DMP (8/10 survivals) and F9C11DMP (9/10 survivals), respectively. Likewise, solutions of the fluorinated surfactants around 4 g (kg body weight)$^{-1}$ for D(F6C2)MMP (8/10 survivals) 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 presented in Table 1. Dispersions of F6C2DMP, F6C11DMP, F8C2DMP and F8C11DMP (0.05 to 0.5 g L$^{-1}$ in 0.9% 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. 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. LD$_{50}$ 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 LD$_{50}$ value higher than 2 g (kg body weight)$^{-1}$ (limit of protocol, 10/10 survivals).

Intraperitoneal administration in mice of FmCmDMPs in perfluorooctyl bromide (PFOB) gave LD$_{50}$ 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. LD$_{50}$ 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 intravenously. They confirm our opinion that perfluoroalkylated morpholinophosphates have potential as components of colloidal systems intended for biomedical applications.

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References


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