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# Synthesis and Preliminary Biochemical Assessment of Ethyl-Terminated Perfluoroalkylamine Oxide Surfactants

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**Abstract**—The synthesis and usefulness in membrane biochemistry of a new class of surfactants have been investigated. 1-Ethyl-2-dimethylamine oxide polar heads were grafted onto a hydrocarbon, a fluorocarbon or an ethyl-capped fluorocarbon hydrophobic tail. The ability of the resulting surfactants to extract and/or to stabilize in aqueous solution a test membrane protein, cytochrome $b_6f$, was evaluated. While it is not a detergent, the hemifluorinated derivative efficiently kept purified cytochrome $b_6f$ soluble, native and functional. The data suggest that alkyl-capped fluorocarbon surfactants provide an interesting alternative to classical detergents for handling membrane proteins in aqueous solutions under non-dissociating conditions. 

Detergents are widely used for handling membrane proteins in aqueous solutions.[1] Their dissociative character, however, is responsible for many problems encountered in membrane protein biochemistry.[1,2] Surfactants with fluorocarbon hydrophobic tails, even though they are highly tensioactive, are both hydrophobic *and* lipophobic: because they do not interact favorably with the alkyl chains of natural lipids, their partition coefficient into biological membranes is low, which renders them non-cytolytic.[3,4] This property makes them precious in many applications such as temporary blood substitutes, drug delivery systems or contrast agents.[5] Because they are not detergents, fluorinated surfactants have received little attention from membrane biochemists. In a previous series of experiments, we examined the behavior of membrane proteins transferred to solutions of fluorinated surfactants following their extraction by a classical, hydrogenated detergent.[6] Some of these molecules, obtained by radical telomerization of Tris(hydroxymethyl)-aminomethane (THAM) in the presence of ω-perfluoroalkane thiol,[7] proved able to prevent massive protein precipitation provided they were used at sufficiently high concentration.[6] In order to try and improve interactions between the protein transmembrane surface and the hydrophobic chain of the surfactant without making the latter lipophilic, we then added a hydrocarbon tip to the fluorocarbon tail of a couple of THAM-derived surfactants.[8] In tests conducted using as a model a membrane protein purified from photosynthetic membranes, cytochrome $b_6f$,[2,9] hybrid surfactants proved able to keep this fragile complex soluble in aqueous solutions, native, and highly stable.[8,10] While the data appeared to support the usefulness of ethyl-capped fluorocarbon chains, these first molecules presented practical drawbacks: on the one hand, the polydispersity of THAM-derived telomeric surfactants **1** (Scheme 1) is not a desirable feature in membrane biochemistry; on the other, monodisperse trigalactoside THAM derivatives **2** were laborious to synthesize, and they proved to be insufficiently stable in aqueous solutions for routine biochemical use. Zwitterionic detergents derived from amine oxide, such as dodecyldimethyl-*N*-amine oxide or lauroamido-*N,N*-dimethyl-3-*n*-propylamine oxide have proven useful in membrane biochemistry.[1,11] Perfluorinated amine

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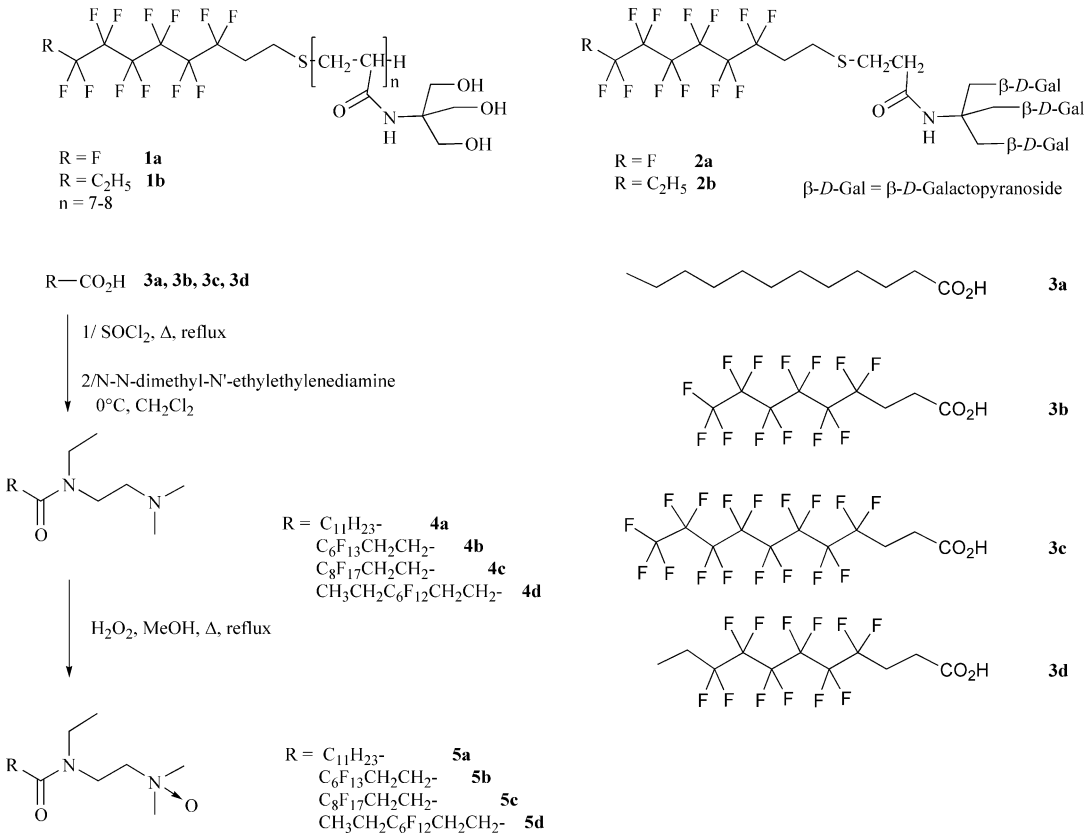


**Scheme 1.** Chemical structure of compounds **1**–**2** and synthesis of amine oxide surfactants **5**.

oxides have been previously synthesized for various applications (fire extinguishing agents,[12–17] blood substitutes or drug delivery systems[5,18–20]). In the present work, we report on the synthesis and preliminary biochemical assessment of fluorinated and hemifluorinated surfactants bearing a 1-ethyl-2-dimethylamine oxide polar head.

## Results and Discussion

### Synthesis

We initially considered grafting the amine oxide to the fluorocarbon tail via an ethyl spacer. However, given the acidity of the methylene group linked to the perfluoroalkyl moiety, the introduction of an amine oxide group in β position could favor a Cope elimination reaction. The amine oxide synthesis recently developed by Gellman et al.[21] was therefore preferred. The amine oxide compounds were obtained in two steps, namely (i) condensation of *N*,*N*-dimethyl-*N*′-ethylethylene diamine on the chosen carboxylic acid, followed by (ii) oxidation of the tertiary amine by either hydrogen peroxide or metachloroperbenzoic acid. This strategy was applied to the different hydro-, fluoro- or hemifluoroacids **3**. Dodecanoic and ω-perfluoroalkyl carboxylic acids are commercially available. The hybrid ethyl-terminated perfluoroalkyl chain was synthesized in the laboratory[22] with 42% overall yield. The amine oxide hydrophilic head was added according to Scheme 1. Amines were obtained in two steps. The first reaction involved an activation of carboxylic acids by thionyl chloride. Crude acyl chlorides were then reacted in cold methylene chloride with two equivalents of diamine to provide the corresponding tertiary amides **4a**–**d** in high yield. Because an excess of amine was used, all coupling reactions were achieved without any additional base. Finally, compounds **4a**–**d** were oxidized into their amine oxide analogues **5a**–**d**. Oxidation was quantitatively performed by an excess of hydrogen peroxide in hot methanol. The amine oxide surfactants were isolated with an overall yield ranging from 74 to 97%. They were fully characterized by NMR spectroscopy and mass spectrometry.[23]

Unlike previous non-ionic fluorinated surfactants, which bore either polyhydroxyl, sulfoxyde or glycosidic polar heads, and whose solubility in water was found to be very sensitive to the nature and length of the perfluorocarbon chain,[6,8,22] all amine oxide surfactants are highly hygroscopic and exhibit a good water solubility, in excess of 5 g $L^{-1}$ ($\sim$10 mM). Preliminary experiments with a Kruss K12 tensiometer using the Wilhelmy plate method yielded the estimates of cmc values (in the Tris buffer solutions used in biochemical assays) shown in Table 1.

Regarding tensioactive properties, it is well-known that surfactants containing a fluorocarbon chain as their hydrophobic moiety display higher surface activity and a lower cmc (critical micellar concentration) than their

**Table 1.** Physico-chemical properties of amine oxide surfactants

| Compd | cmc (mM) | $\gamma_{min}$ (mN m$^{-1}$) |
|---|---|---|
| **5a** | 0.14 | 28 |
| **5b** | 0.16 | 15 |
| **5c** | 0.019 | 16 |
| **5d** | 0.09 | 20 |

Measurements were carried out in 20 mM Tris–HCl buffer solution, pH 8.0. Experimental uncertainties are estimated to be ca. ±0.02 mM on cmc (ca. ±0.005 mM for compound **5c**) and ±1 mN m$^{-1}$ on $\gamma_{min}$ values.

hydrocarbon counterparts of the same length. The physico-chemical data obtained with compounds **5a–d** are in good agreement with this rule (Table 1). Two points are worth underlining: (i) the cmc values obtained are very low and close to but slightly lower than those previously measured with compounds such as **2**,[9] and (ii) once again, one notes that the cmc of the hybrid, hemifluorinated compound $C_2H_5C_6F_{12}CH_2CH_2$–AO **5d** (0.09 mM) is abnormally high as compared to that of $C_8F_{17}CH_2CH_2$–AO **5c** (0.019 mM). This is probably due to unfavorable intermolecular interactions lowering the free energy of transfer of hybrid hydrophobic chains from water to the core of the micelles.

## Biochemical assays

Homologous hydrogenated, fluorinated and hemifluorinated surfactants **5a–d** were tested for their ability (i) to solubilize integral membrane proteins from chloroplast thylakoid membranes and (ii) to maintain a purified membrane protein complex, cytochrome $b_6f$, soluble in aqueous solutions while preserving its integrity, enzymatic activity and monodispersity.

As previously observed for other fluorinated and hemifluorinated surfactants,[6,8,10] none of compounds **5b–d** was able to solubilize thylakoid membranes to any significant degree even at high concentration (24 mM). Their hydrogenated homologue, $C_{11}H_{23}$–AO **5a**, is, on the contrary, an efficient detergent, achieving significant solubilization at 12 mM (corresponding to a ~1:1.2:2 weight ratio of surfactant/lipid/protein).

Surfactants **5a–d** were then tested (at 5 mM) for their ability to keep in a soluble state purified cytochrome $b_6f$ that had been transferred from a dodecylmaltoside ($C_{12}M$) solution. The hydrogenated **5a**, short-chain perfluorinated **5b** and hemifluorinated **5d** compounds successfully passed this test, while the protein precipitated when transferred into $C_8F_{17}C_2H_4$–AO **5c** solutions.

The structural and functional state of cytochrome $b_6f$ complex transferred into surfactants **5a**, **5b**, or **5d** was then evaluated by determining its sedimentation coefficient, monodispersity, integrity and enzymatic activity following ultracentrifugation on sucrose gradients containing 5 mM of the molecule to be tested. Gradient fractions were analyzed by SDS-Page, UV–visible spectrophotometry and electron transfer measurements.[2,6,9] Exposure to $C_{11}H_{23}$–AO **5a** proved to be, under our experimental conditions, detrimental to the stability of cytochrome $b_6f$: the complex released its associated chlorophyll molecule, dissociated into a low-molecular weight form (most likely a monomer), and lost its enzymatic electron transfer activity. These observations are typical of delipidation-induced inactivation.[2] The behavior of the $b_6f$ complex in solutions of $C_6F_{13}C_2H_4$–AO **5b** was similar to that observed previously with some other fluorinated surfactants:[6] a high-molecular weight (presumably dimeric), enzymatically active form of the complex was predominant; however, a significant tendency to aggregation indicated a relatively poor efficiency of the surfactant to prevent protein–protein hydrophobic interactions. With the hemifluorinated compound, $C_2H_5C_6F_{12}C_2H_4$–AO **5d**, no aggregation was observed, most of the complex migrating as a single, heavy species, most probably a dimer. This fraction comprised all of the high molecular weight $b_6f$ subunits, including the Rieske Fe–S protein, as well as the $b_6f$-associated chlorophyll. The latter two components are known to be the first to be lost upon detergent-induced dissociation of the complex.[2] The electron transfer activity of the complex was similar to that of control preparations.

## Conclusion

The results of biochemical assays on compounds **5a–d** can be summarized as follows.

1. $C_{11}H_{23}$–AO **5a** is an efficient detergent. Under our experimental conditions, it inactivated our test membrane protein, cytochrome $b_6f$. This, however, does not rule out its possible usefulness in biochemistry. The $b_6f$ complex is a particularly fragile protein (which is one reason for our using it in these experiments). At the high surfactant concentrations chosen for our tests, dodecylmaltoside ($C_{12}M$), one of the most widely used detergents in membrane biochemistry, has the same inactivating effect.[2]
2. $C_6F_{13}C_2H_4$–AO **5b** and $C_8F_{17}C_2H_4$–AO **5c** behave in much the same way as some other surfactants with perfluorinated alkyl chains that we characterized previously:[6] neither of them solubilized thylakoid membranes; $C_6F_{13}C_2H_4$–AO **5b** was only partially successful at keeping purified cytochrome $b_6f$ soluble when the latter was transferred into it from a $C_{12}M$ solution, while $C_8F_{17}C_2H_4$–AO **5c** was inefficient. The usefulness of these two molecules as tools for handling membrane proteins in aqueous solutions is probably limited.
3. $C_2H_5C_6F_{12}C_2H_4$–AO **5d**, on the contrary, exhibited interesting properties. While it did not solubilize thylakoids either, it efficiently kept purified cytochrome $b_6f$ soluble. The complex appeared structurally and functionally intact, and did not show any tendency to aggregation. In this respect, the properties of this compound are similar to those of two other hemifluorinated surfactants tested previously, $C_2H_5C_6F_{12}C_2H_4$–SteloTHAM **1b** and $C_2H_5C_6F_{12}C_2H_4$–STHAMTriGal **2b**.[8,10] The generality of this observation seems to vindicate the

predicament that led us to devise this family of molecules, namely that they should be simultaneously less aggressive than detergents towards membrane proteins (because of the physical properties of fluoroalkyl tails), while interacting more favorably with them than surfactants with a perfluorinated alkyl chain (because of the ethyl tip).[8,10] The present observations clearly establish the stabilizing effect of the hemifluorinated chain, since the homologous hydrocarbon surfactant, used under the same conditions, totally inactivated our test protein. This discrepancy was not as striking with the two compounds studied earlier, which, probably thanks to their large polar heads, were mild detergents in their hydrocarbon version[8,10] and inactivated cytochrome $b_6f$ only after protracted incubation.[10] By analogy with earlier experiments,[24] we expect that following fractionation on sucrose gradients containing $C_2H_5C_6F_{12}C_2H_4$–AO, most of $C_{12}M$ will have desorbed from the protein. On the other hand, bound lipids ought to have been largely retained. These points will be the object of direct investigations.

From a practical point of view, $C_2H_5C_6F_{12}C_2H_4$–AO **5d** presents significant advantages over the molecules studied thus far : it is monodisperse and its synthesis is simple once the hemifluorinated acid has been prepared. One of the two earlier molecules, $C_2H_5C_6F_{12}C_2H_4$–STHAMTriGal **2b**, was also monodisperse, but its synthesis was particularly difficult and its stability in aqueous solutions proved insufficient for biochemical purposes. The second, $C_2H_5C_6F_{12}C_2H_4$–STeloTHAM **1b**, was appropriately stable, easier if not simple to produce, but featured a polydisperse, oligomeric polar head. While detergents with heterogeneous polar heads, such as Triton X-100, are still widely used in biochemistry, their polydispersity is a source of difficulties, including problematic batch-to-batch reproducibility. $C_2H_5C_6F_{12}C_2H_4$–AO **5d** does not suffer from any of these drawbacks. A full assessment of its potential usefulness in biochemistry will require further studies.

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## References and Notes

1. Le Maire, M.; Champeil, P.; Møller, J. V. *Biochim. Biophys. Acta* **2000**, *1508*, 86.
2. Breyton, C.; Tribet, C.; Olive, J.; Dubacq, J.-P.; Popot, J.-L. *J. Biol. Chem.* **1997**, *272*, 21892.
3. Pucci, B.; Maurizis, J. C.; Pavia, A. A. *Eur. Polym. J.* **1991**, *27*, 1101.
4. Barthélémy, P.; Tomao, V.; Selb, J.; Chaudier, Y.; Pucci, B. *Langmuir*. **2002**, *18*, 2557.
5. Kraft, M.-P. *Adv. Drug Deliv. Rev*. **2001**, *47*, 200.
6. Chabaud, E.; Barthélémy, P.; Mora, N.; Popot, J.-L.; Pucci, B. *Biochimie* **1998**, *80*, 515.
7. Pavia, A. A.; Pucci, B.; Riess, J. G.; Zarif, L. *Makromol. Chem*. **1992**, *193*, 2505.
8. Barthélémy, P.; Améduri, B.; Chabaud, E.; Popot, J.-L.; Pucci, B. *Org. Lett*. **1999**, *1*, 1689.
9. Pierre, Y.; Breyton, C.; Kramer, D.; Popot, J.-L. *J. Biol. Chem*. **1995**, *270*, 29342.
10. Chabaud E. Thèse de Doctorat de l'Université Paris-VI, 2001.
11. Sardet, C.; Tardieu, A.; Luzzati, V. *J. Mol. Biol*. **1976**, *105*, 383.
12. Barry, J. M. (Procter & Gamble), US Patent 3194840, 1965.
13. Stern, R. M.; Fan, W. Q.; Blagev, L. P. PCT Int. Appl., 1997.
14. Szönyi, S.; Vandamme, R.; Cambon, A. *J. Fluor. Chem*. **1985**, *30*, 37.
15. Wirtz, P.; Broell, R. (Hoechst), Ger. Offen. DE 2829594, 1979.
16. Clark, L. C., Jr.; Clark, E. W.; Moore, R. E.; Kinnett, D. G.; Inscho, E. I., Jr. *Prog. Clin. Biol. Res*. **1983**, *122*, 169.
17. Corkill, J. M.; Goodman, J. F.; Ogden, C. P. *Trans. Faraday Soc*. **1965**, *61*, 583.
18. Nivet, J. B.; Le Blanc, M.; Riess, J. G. *J. Dispersion, Sci. Technol*. **1992**, *13*, 627.
19. Riess, J. G. *New J. Chem*. **1995**, *19*, 891.
20. Riess, J. G.; Krafft, M.-P. *Biomaterials* **1998**, *19*, 1529.
21. Mc Quade, D. T.; Quinn, M. A.; Yu, S. M.; Polans, A. S.; Krebs, M. P.; Gellman, S. H. *Angew. Chem., Int. Ed*. **2000**, *39*, 758.
22. Chaudier, Y.; Barthélémy, P.; Pucci, B. *Tet. Lett*. **2001**, *42*, 3583.
23. $^1$H, $^{13}$C and $^{19}$F nuclear magnetic resonance (NMR) spectra were recorded at 250 MHz on a Bruker AC250 spectrometer. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane ($^1$H and $^{13}$C spectra) or $CFCl_3$ ($^{19}$F spectra). Mass spectra were recorded on a DX300 JEOL apparatus in Fab+. Example : compound **5d**. $^1$H NMR (DMSO-$d_6$) $\delta = 1.09$ (m, 6H, $NCH_2CH_3$ isomers *cis–trans* + $CH_3CH_2$); 2.21 (m, 2H $CH_3CH_2CF_2$ $^3J_{HH} = 7.3$ Hz, $^3J_{HF} = 17$ Hz, ), 2.65 (m 2H, $CF_2CH_2$); 3.16–3.48 (m, 12H, $CF_2CH_2CH_2 + N(CH_2CH_3)CH_2CH_2 + NO(CH_3)_2$); 3.71 and 3.81 (t, 2H, $CH_2NO(CH_3)_2$, $^3J = 8$ Hz isomers *cis* + *trans*). $^{19}$F NMR ($CDCl_3$) $\delta = -114.63$ ($CF_2CH_2CH_2CO$); $-116.65$ ($CH_3CH_2CF_2$); $-122.16$ ($(CF_2)_2CF_2CH_2CH_2CO$); $-123.98$ ($CH_3CH_2CF_2(CF_2)_2$). $^{13}$C NMR ($CDCl_3$) $\delta = 5.14$ ($CH_3$); 13.2 ($NCH_2CH_3$); 23.27 ($CF_2CH_2CH_2$); 25.3 ($CH_3CH_2$); 25.73 ($CF_2CH_2CH_2$); 40.43 ($NO(CH_3)_2 + NCH_2CH_3$); 42.18 ($N(Et)CH_2CH_2$); 58.14 ($N(Et)CH_2CH_2$); 106.35–122.56 ($CF_3 + CF_2$); 169.54 (CO) FAB$^+$ MS: $m/z = 517$ $(M+H)^+$. HR MS $m/z = 517.1717$ $(M+H)^+$, calculated for $C_{17}H_{25}O_2N_2F_{12}$: 516.1646.
24. Tribet, C.; Audebert, R.; Popot, J.-L. *Langmuir* **1997**, *13*, 5570.