

Synthesis and Preliminary Assessments of Ethyl-Terminated Perfluoroalkyl Nonionic Surfactants Derived from Tris(hydroxymethyl)acrylamidomethane

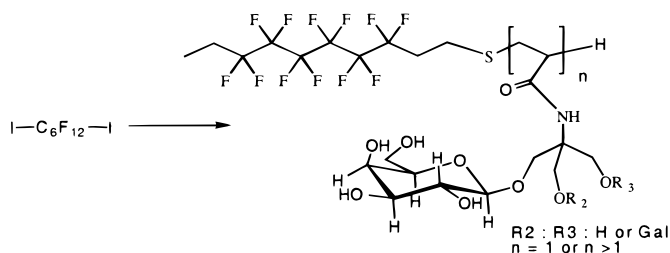
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ABSTRACT



We describe the synthesis and preliminary physicochemical and biological assessments of a new class of nonionic hybrid hydrofluoro amphiphiles derived from tris(hydroxymethyl)aminomethane (THAM). The synthesis of the hydrophobic tail of these amphiphiles is based on the preparation of an asymmetrical hydrofluorocarbon derivative containing an ethyl segment, a fluorocarbon core, and an ethyl thiol moiety. This molecule led to either THAM galactosylated monoadducts or telomers. These amphiphiles exhibit neither detergency toward cell membranes nor membrane protein denaturation.

Stabilizing membrane proteins in aqueous solution under their native state is a difficult task. The lipids and proteins that make up biological membranes classically can be dispersed using detergents. The hydrophobic moiety of detergents competes with lipids for the transmembrane, hydrophobic surface of the proteins and thereby renders them water-soluble. Substituting detergent for lipids however frequently inactivates the protein. While the mechanism of this inactivation generally is not known, it is likely to involve either the removal of critical lipids and/or a direct perturba-

tion by the detergent of the protein's structure. To alleviate this problem, we are exploring the use of less dissociating molecules, namely fluorinated surfactants.^{1,2} While highly tensioactive, perfluorinated surfactants are not detergents and do not solubilize biological membranes.¹ Some of them, nevertheless, have proven able to keep water-soluble the membrane proteins that had been extracted using a classical hydrogenated detergent.² In the course of this previous study, we noted however that perfluorinated molecules that exhib-

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ited this interesting property were rare and that, somewhat predictably, they had difficulty preventing the proteins from aggregating.² In the present work, we describe the synthesis and preliminary characterization of hydrofluorinated surfactants. These molecules feature a largely fluorinated alkyl chain terminated by an ethyl group. The rationale behind their design is that the ethyl group would improve the affinity of the tip of the tail for the hydrogenated surface of the protein, while doing little to restore detergency.

More than three decades ago, Brace reported that long-chain alkanolic acids bearing perfluoroalkyl terminal segments had unique surface active and wettability properties.³ Since then, numerous works have described the potentialities of hydrofluorocarbon compounds. Hybrid anionic surfactants containing fluorocarbon and hydrocarbon chains have already been prepared and studied.⁴ Such hybrid compounds tend to form supramolecular assemblies,⁵ some of which have potential biological applications.⁶ Recently, hybrid bola-amphiphiles have been synthesized to study the “flip-flop” behavior of spin label in vesicles.⁷ Few articles however have described the synthesis of perfluoro compounds with an alkyl terminal part.⁸ Among them, one can point out the work achieved by Rondestvedt who prepared methyl-terminated perfluoroalkyl iodides (from telomerization reactions). Such compounds can provide very useful starting materials.⁹ So far, only a handful of hydrofluoro surfactants have been prepared.¹⁰

The work reported here deals with the synthesis and preliminary physicochemical assessment of new ethylfluorocarbon surface-active molecules derived from galactosylated tris(hydroxymethyl)acrylamidomethane.

Results and Discussion. 1. Synthesis. The key synthetic task was to obtain a nonsymmetrical fluorocarbon derivative containing a distal alkyl segment, a fluorocarbon core, and a proximal ethyl thiol moiety. This molecule will be the hydrophobic tail of the surfactant. Such a building block is not commercially available. To open a new route toward mixed nonionic amphiphiles, symmetrical α,ω -diiodoperfluorohexyl was chosen as a starting material.

As previously described,¹¹ the bis-monoethylenation of diiodoperfluorohexane leads to the expected starting material 1,10-diiodo-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluorodecane **1** (Figure 1).

The second step involves the selective reduction of one iodine only achieved by tri-*n*-butyltin hydride. The outcoming radical is trapped by tin hydride providing a chain reaction

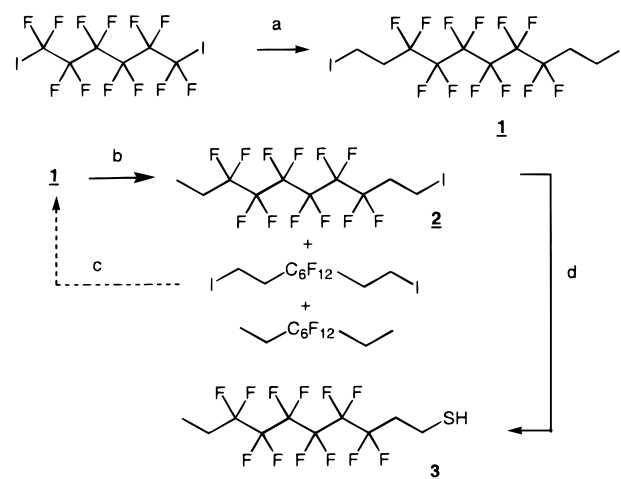


Figure 1. Synthesis of the hydrofluorocarbon thiol **3**. Conditions: (a) C_2H_4 , CuI , $165\text{ }^\circ\text{C}$, 15 h, yield 80%; (b) AIBN, tri-*n*-butyltin hydride, THF, Δ , 6 h; (c) the residual diiodo is reused; the yield rises to 60%; (d) thiourea, THF/ H_2O 20/1 Δ , followed by NH_4OH at room temperature, yield 95%.

initiated with azobis(isobutyronitrile) (AIBN). This step affords monoiodo, diiodo, and a very small amount of bis-hydrogenated compounds. Diiodo compound **1** can be easily recovered by rapid chromatography on silica gel (eluent: hexane) and reused to complete the reaction. 1-Iodo-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluorodecane **2** reacts with the thiourea to give, after hydrolysis, thiol derivative **3** (Figure 1).

Since hydrofluorocarbon thiol **3** is now available, the preparation of novel nonionic ethyl-terminated fluorocarbon surfactants becomes feasible. Basically, the thiol derivative opens an access to a new class of nonionic surfactants, which can be either monoadducts or amphiphile telomers.

Several telomers derived from the THAM (tris(hydroxymethyl)acrylamidomethane) bearing hydrocarbon or fluorocarbon tails have already been prepared; however, none of them carry a mixed hydrofluoro chain.¹²

The telomerization reaction was performed in the presence of AIBN and thiol **3** as chain-transfer reagent (Figure 2). Telomers were isolated by precipitation in methanol–ether mixture (10/90, v/v). The byproducts, mainly the monoadduct, were soluble in this solvent.

Unlike the usual fluorocarbon telomers, the average degree of polymerization (DP_n) of these compounds can be deter-

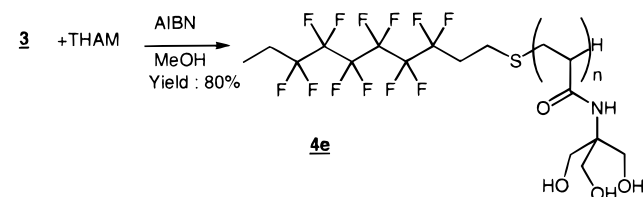


Figure 2. Hydrofluorocarbon telomer ($\text{DP}_n = 10$).

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mined more easily by ^1H NMR spectroscopy. Indeed, since the integration of the terminal methyl protons resonance is measured accurately, the DP_n value becomes reliable. It was worthwhile to prepare such a hydrofluorocarbon telomer by using similar ratio thiol/THAM as perfluorocarbon compounds to eventually verify and confirm the DP_n previously measured. (One can assume that the transfer constant is quite equivalent for both telogen agents.)

According to the thiol/THAM ratio chosen, the THAM monoadduct can be obtained and isolated after silica gel chromatography (eluent: MeOH/AcOEt, 10/90, v/v). However, the compound bearing a trishydroxyl head shows a poor solubility in water. Obviously, such a property is required to get efficient amphiphiles. Thus, sugar moieties were grafted on the hydroxyl groups to increase the water solubility of the whole molecule. The THAM galactosylated monoadducts were then prepared under free-radical conditions following the procedure previously described¹³ (Figure 3). For example, main physicochemical data of galactosylated (tetraacetyl) surfactant **4a** are reported in full note 20.

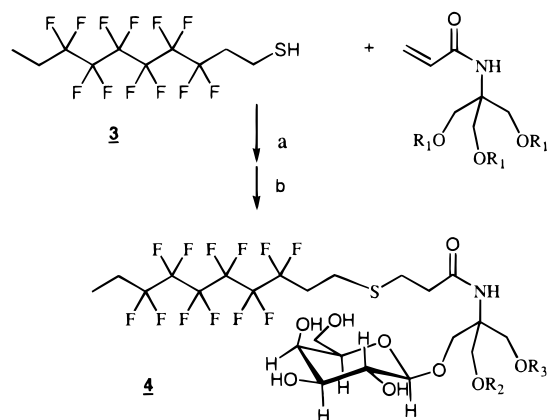


Figure 3. Synthesis of sugar derivatives. Substituents: R_1 , β -D-Gal(OAc)₄ or H; R_2 , R_3 , β -D-Gal or H. Conditions: (a) AIBN, MeOH, 65 °C; (b) MeONa (catalytic), MeOH, 20 °C. **4a** = R_2 , R_3 : β -D-galactose; **4b** = R_2 : H; R_3 : β -D-galactose; **4c** = R_2 , R_3 : H; **4d** = R_2 , R_3 : β -D-galactose, diadduct.

2. Physicochemical Properties. Surfactants containing a fluorocarbon chain as hydrophobic group display high surface activity and low CMC (critical micelle concentration). Recently, it was shown that the hydrophobicity is higher in the case of perfluorinated ionic surfactants than their hydrogenated analogues, suggesting that the mechanism of the micellization process is similar.¹⁴ Hybrid surfactants bearing hydro- and fluorocarbon tails are quite unknown in terms of tensioactive properties.

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The aim of this study is to examine the physicochemical data obtained with our ethyl perfluorocarbon nonionic surfactants. The results are compared to those measured with hydrogenated and/or fluorinated homologues previously described.

The CMC data of ethyl-terminated perfluoro nonionic surfactants were determined following the usual procedure by measuring their surface tension properties. The CMC's and limit tension results obtained are listed in Table 1.

Table 1. Physicochemical Data of Surfactants Bearing Ethylfluorocarbon, Hydrofluorocarbon, and Perfluorocarbon Tails

P	product no.	CMC, mM ^d	γ limit, mN/m
$\text{C}_2\text{H}_5(\text{C}_6\text{F}_{12})\text{CH}_2\text{CH}_2$ -STHAM TriGal	4a	0.5	25/26
$\text{C}_2\text{H}_5(\text{C}_6\text{F}_{12})\text{CH}_2\text{CH}_2$ -STHAM DiGal	4b	0.35	26
$\text{C}_2\text{H}_5(\text{C}_6\text{F}_{12})\text{CH}_2\text{CH}_2$ -STHAM MonoGal	4c	0.3	25/26
$\text{C}_2\text{H}_5(\text{C}_6\text{F}_{12})\text{CH}_2\text{CH}_2$ -S(THAM) ₂ TriGal	4d	0.35	25/26
$\text{C}_2\text{H}_5(\text{C}_6\text{F}_{12})\text{CH}_2\text{CH}_2$ -STeloTHAM, $\text{DP}_n \approx 10$	4e	0.45	32
$\text{C}_{10}\text{H}_{21}$ -Telo, $\text{DP}_n = 5, 7$	5^c	0.6	
$\text{C}_{12}\text{H}_{25}$ -Telo, $\text{DP}_n = 4$	6^b	0.15	31.3/32
$\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2$ -Telo, $\text{DP}_n = 6$	7^b	0.03	25/27
$\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2$ STHAM TriGal	8^b	0.038	27
$\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2$ STHAM DiGal	9^b	0.046	25
$\text{C}_6\text{F}_{13}\text{CH}_2\text{CH}_2$ STHAM TriGal	10^b	0.28	34
$\text{C}_{10}\text{H}_{21}$ STHAM TriGal	11^b	1	
$\text{C}_{12}\text{H}_{25}$ STHAM DiGal	12^b	0.28	35
$\text{C}_{12}\text{H}_{25}$ STHAM TriGal	13^b	0.23	45

^a Telo: telomer. ^b See ref 13. ^c See ref 1. ^d CMC were measured with a tensiometer Kruss 2000.

As observed previously for perfluoro- or hydrocarbon THAM derivatives (telomers, monoadducts; see Table 1), the CMC of hydrofluorocarbon surfactants depends essentially on the hydrophobic part of the molecule. Indeed, no drastic changes of CMC values are observed when the hydrophilic head volume increases (CMC_{telo} **4e**, 0.45 mM; CMC_{trigal} **4a**, 0.5 mM; CMC_{monogal} **4c**, 0.3 mM). Since the CMC depends essentially on the nature and the length of the tail, it was reasonable to expect, in the case of ethylfluorocarbon compounds, a CMC close to that of perfluoro analogues **8** or **9**, which bear a $\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2$ tail (CMC $\approx 0.04\text{mM}$). Actually, the CMC measured is 10 times higher even though it remains lower than that of the corresponding hydrogenated compounds (**11**: tail, $\text{C}_{10}\text{H}_{21}$; CMC, 1mM).

Free energies of micellization can be evaluated from the CMC.¹⁵ They show that micelles form more favorably in the case of ethylfluorocarbon compounds ($\text{C}_2\text{H}_5\text{C}_6\text{F}_{12}\text{C}_2\text{H}_4$ -, $\Delta G \approx -19$ kJ) than for hydrocarbon surfactants ($\text{C}_{10}\text{H}_{21}$ -,

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$\Delta G \approx -16.7$ kJ) and less easily than for their hydrogenated analogues¹⁶ ($\text{C}_8\text{F}_{17}\text{C}_2\text{H}_4-$, $\Delta G \approx -25$ kJ).

It was found previously that for a given length of the fluorinated chain (in a nonionic surfactant series), increasing the length of the hydrogenated spacer (hydrocarbon moiety located between the polar head and the fluorinated tail) did not lower the CMC values as much as could have been expected.¹⁷ This may indicate that the length of the fluorinated chain controls the micellization phenomenon, the hydrocarbon part (as a spacer) playing only a minor role. In the present work, the grafting of a hydrocarbon moiety at the end of the fluorocarbon tail seems to destabilize the micelle organization, i.e., the packaging of fluorocarbon chain in the micelle core, and thus increases their CMC. This would suggest that interactions between hydrocarbon and fluorinated parts in the core of the micelles are unfavorable.

3. Biochemical Properties. Biochemical tests conducted as described in ref 2 showed that neither of our hybrid surfactants exhibited any detergency. Specifically, none of them extracted integral membrane proteins from thylakoid membranes from the unicellular alga *Chlamydomonas reinhardtii*. These observations suggest that, as expected, hybrid hydrophobic tails show too low a partition coefficient into lipid bilayers for a disruptive concentration to be reached. Cytochrome $b_6 f$ complex, the plastoquinol-plastocyanin oxidoreductase from oxygenic photosynthesis,¹⁸ was used as a convenient model system with which to

examine the ability of hybrid surfactants to keep membrane proteins in solution under their native state. Cytochrome $b_6 f$ is highly sensitive to denaturation by classical detergents when those are used in the absence of protective lipids.¹⁹ Following transfer of purified cytochrome $b_6 f$ to lipid-free solutions of either compound **4a** or **4e** according to the sucrose gradient procedure described in ref 2, the complex remained water-soluble, monodisperse, intact (with regard to its subunit and pigment composition), and enzymatically active. These observations bode well for the usefulness of hydrofluorocarbon surfactants in membrane protein biochemistry.

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(20) **HFTHAMtri(GalOAc)₄ 4a**: NMR ^1H (CDCl_3) δ (in ppm) 1.07 (t, 3H, $^3J_{\text{HH}} = 7.5$ Hz, CH_3), 2.1 (m, 4s, 14H, CH_3CH_2 , 3(CH_2CO)₄), 2.4 (m, 4H, CH_2CO , CF_2CH_2), 2.8 (m, 4H, CH_2SCH_2), 3.7 (m, 2H, H_6), 4.16 (m, 9H, H_5 , 3(CH_2O), 4.5 (d, 3H, H_1), 5.05 (dd, 3H, H_2), 5.15 (dd, 3H, H_3), 5.5 (m, 3H, H_4), 6.1 (s, 1H, NH); NMR ^{13}C (CDCl_3) δ (in ppm) 4.8 (t, $^3J_{\text{CF}} = 5$ Hz, CH_3), 20.5, 20.7 (CH_3CO), 23, 5 (CH_2S), 25.6 (t, $^2J_{\text{CH}} = 24.5$ Hz, $\text{CH}_3\text{CH}_2\text{CF}_2$), 25, 8 (CH_2S), 33.1 (t, $^2J_{\text{CH}} = 24.4$ Hz, CH_2CF_2), 36.9 (CH_2CO), 61.2 ($\text{C}(\text{CH}_2)_3$), 63.0 (C_6), 67.1 (C_4), 68.5 (CH_2Ogal), 69.2 (C_2), 70.5 (C_5 or C_3), 70.9 (C_5 or C_3), 101.5 ($\text{C1 } \beta$ anomer), 170.1 (COCH_3), 172.7 (CO); NMR ^{19}F (CDCl_3) δ (in ppm) -114, 8 (2F, CF_2CH_2), -116, 8 (2F, CF_2CH_2), -122, 2 (4F, $\text{CH}_2\text{CF}_2\text{CF}_2$), -124.0 (4F, $\text{CF}_2\text{CF}_2\text{CF}_2$).