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Synthesis of a hemifluorinated amphiphile designed for self-assembly and two-dimensional crystallization of membrane protein

Julien Dauvergne [a], Ange Polidori [a,*], Catherine Vénien-Bryan [b], Bernard Pucci [a,*]

[a] *Université d'Avignon et des pays du vaucluse, laboratoire de chimie bioorganique et des systèmes moléculaires vectoriels, 33 rue Louis Pasteur, F-84000 Avignon, France*
[b] *Laboratory of Molecular Biophysics, University of Oxford, South Park Road, OX1 3QU Oxford, UK*

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This work is dedicated to the memory of our colleague Dr. Charles Mioskowski

Abstract

The work reported herein deals with the synthesis and the preliminary physical–chemical analysis of new hemifluorinated surfactant made up of one fluorinated chain linked to a tricarboxylic acid polar head which is able to complex a Ni atom and should favor the two-dimensional crystallization of membrane proteins. Such a compound forms a Langmuir film which is a fluid at 20 °C and not perturbed by the presence of hydrocarbon detergent in aqueous solution.

Keywords: Hemifluorinated surfactants; Langmuir film; Membrane proteins; Nickel complexation; 2D proteins crystallization

Knowledge of the three-dimensional structure of proteins helps the understanding of biological mechanisms at the molecular level. Atomic resolution structures can currently be determined by NMR of proteins in solution, or by X-ray diffraction of three-dimensional (3D) crystals, or by electron diffraction of two-dimensional (2D) crystals, the latter using electron microscopy. Some proteins may form 2D crystals on a lipid monolayer at an air–water interface.[1] This method may be improved by anchoring the protein onto a Langmuir film using specific ligands attached to the lipids or surfactants.[2–5] The polar head of a lipid monolayer can be loaded with Nickel atoms which can bind to a protein expressed with histidines tag. After a complex is formed between the Ni atom and polyhistidine tag, translational and rotational diffusion within the film can result in the protein becoming organized into 2D arrays. The quality and the rate of protein crystallization are strongly dependent on the intrinsic film properties, notably its fluidity.[6–8,3,9,10] However, this technique cannot be applied to membrane proteins, as the surfactants or detergents commonly used to handle these proteins in solution would solubilize the lipid monolayer.[11] To overcome this problem, Mioskowski et al. have designed lipids with a bi-tailed fluorinated hydrophobic segment.[12,11] Perfluorinated compounds do not mix with either hydrocarbons or hydrophilic compounds, as they are lipophobic and hydrophobic. Therefore, fluorinated surfactant monolayers are not destabilized by the presence of hydrocarbon detergents in the aqueous solution.[12–14]

Furthermore, adding a hydrocarbon segment to the end of the fluorinated chains minimizes the association of the fluorinated chains and consequential formation of a pseudo-crystal phase, which is detrimental to the formation of a fluid-phase lipid layer and hence the crystallization of the protein.

Thorough investigations remain to be done in this field since until now only a few hemifluorocarbon surfactants

* Corresponding authors. Tel.: +33 4 90 14 44 45; fax: +33 4 90 14 44 49 (A.P.); tel.: +33 4 90 14 44 42; fax: +33 4 90 14 44 49 (B.P.).
E-mail addresses: ange.polidori@univ-avignon.fr (A. Polidori), bernard.pucci@univ-avignon.fr (B. Pucci).

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have been designed and prepared for specific biological application.[15,16] In this context, we have previously shown that non-ionic perfluorinated surfactants (such as C_6F_{13}SOTHAM) can lead to stable Newton Black Films (NBFs). The possibility of organizing detergent-solubilized membrane proteins in a plane within the core of these NBFs was also demonstrated.[17] From these preliminary results, we report herein the synthesis and some physical–chemical properties of a new hemifluorocarbon surfactant called PhenylHFNTANi made up of one fluorinated chain linked to a tricarboxylic acid polar head which is able to complex a Ni atom. To provide the necessary fluidity of the lipid monolayer made of this fluorinated surfactant, the perfluorinated tail is end-capped with a phenyl propyl group (Fig. 1).

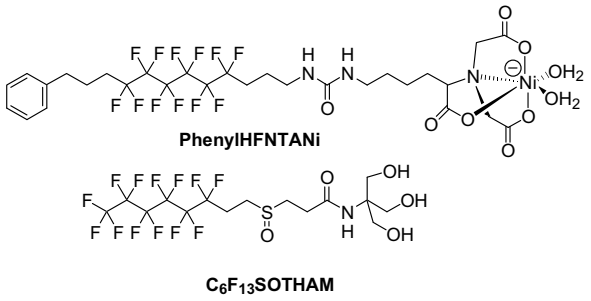


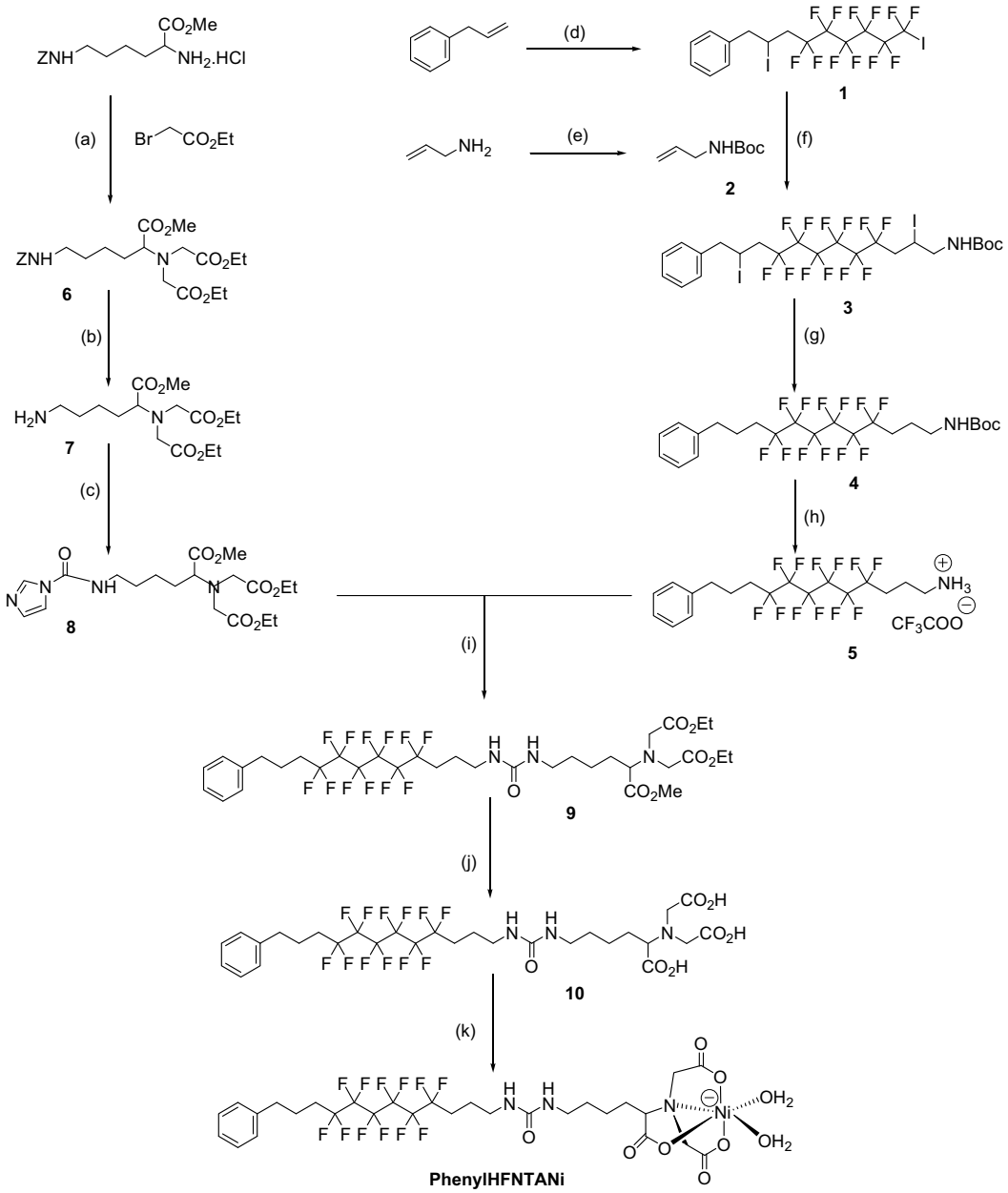
Fig. 1. Structures of the partially fluorinated PhenylHFNTANi and fluorinated sulfoxide surfactant C_6F_{13}SOTHAM.

PhenylHFNTANi surfactant was synthesized following a convergent synthetic pathway through the preparation of the hemifluorinated hydrophobic part and the lysine-derived nickel-chelating nitrilotriacetic group. The hemifluorinated segment was first synthesized from 1,6-diiodoperfluorohexane as starting material (Scheme 1). Phenyl-3-propene was grafted onto the perfluorocarbon chain by using AIBN as the radical initiator. The monofunctionalization of the perfluorinated chain afforded compound **1** in 51% yield. Radical addition of Boc-allylamine **2** was carried out with sodium dithionite as the radical initiator following the method developed by Huang.[18] Compound **3** so obtained was dehalogenated by using tributyltin hydride in the presence of AIBN to give compound **4** in 70% yield. Thus, treatment with TFA in methylene chloride led to the deprotection of amino group to provide compound **5**. Second time around, the tricarboxylic polar head was obtained from N^{ε}-benzyloxycarbonyl L-lysine carboxymethyl ester. Substitution of ethyl bromoacetate by lysine amino group provided triester **6** in 89% yields. Thus, the hydrogenolysis of benzyloxycarbonyl group of lysine derivative led to an amino group on the lateral chain which was coupled with DCI to gave the imidazoyl compound **8**. The latter was coupled to amino group of compound **5** through an urea link to provide compound **9**. The saponification of carboxylate gave the nitrilotriacetic (NTA) compound **10** in 76% yield. The complexation reaction of Ni^{2+} was carried out in a chloroform solution: triacid derivative **10** was stirred with a phosphate buffer solution at pH 8 containing 1 equiv of $NiCl_2{\cdot}6H_2O$. After 1 h we observed a blue coloration of the organic phase showing the complexation of nickel cation by the NTA group. The organic phase was decanted and concentrated under reduced pressure to obtain the nickel-chelating compound PhenylHFNTANi, which was purified by size exclusion chromatography. PhenylHFNTANi, was obtained as a blue-green amorphous solid. This compound is weakly soluble in water. The structure and purity of PhenylHFNTA was assessed and confirmed by proton, ^{19}F and ^{13}C NMR spectroscopy, and mass spectroscopy.[19]

We have investigated some physical properties of PhenylHFNTANi and C_6F_{13}SOTHAM using tensiometry measurements[20] and surface pressure measurements.[21]

The surface tensions of C_6F_{13}SOTHAM and PhenylHFNTANi have been determined when the surface was saturated with surfactant molecules (at a concentration of surfactant above the critical micelle concentration—cmc-value). Three hundred microliters of a solution of C_6F_{13}SOTHAM (50 mM in water) was spread on a 20 ml water solution (final concentration of 0.75 mM, about twice the cmc value, which is 0.37 mM). We measured a value of 21.5 mN m^{-1} for the surface tension. Successive additions of the detergent DDM (*n*-dodecyl-β-D-maltopyranoside from Anatrace, USA) to the subphase leading to final concentrations in the range from 0.09 mM (0.5 × cmc) to 1.08 mM (6 × cmc) did not modify the surface tension value of C_6F_{13}SOTHAM, indicating that the langmuir film was not disturbed by the presence of detergent in solution. The surface tension value of a saturated monolayer of PhenylHFNTANi was 33.8 mN m^{-1} (200 µl of a solution of 18 mM PhenylHFNTANi in chloroform/hexane 1/1 was spread on a 20 ml buffer subphase[21] giving a final concentration of 0.2 mM, about twice the cmc which is 0.1 mM). This value being comparable to the surface tension value of the DDM alone (34.9 mN m^{-1}), we chose another hydrocarbon surfactant (H_{12}-TAC) to perform the test of stability of the PhenylHFNTANi monolayer upon the addition of detergent in the subphase. H_{12}-TAC, a telomer surfactant derived from trishydroxymethylaminomethane with a cmc of 0.5 mM, has been extensively tested for its detergent properties.[22] The surface tension value of H_{12}-TAC is 39.3 mN m^{-1} at a concentration four times higher than its cmc. Successive additions of H_{12}-TAC (leading to final concentrations in the range from 0.25 mM to 1.5 mM) to the subphase of a monolayer of PhenylHFNTANi did not modify its surface tension value, which kept constant at 33.8 mN m^{-1}. This demonstrates that H_{12}-TAC is not miscible with a monolayer of PhenylHFNTANi. These data agree with the results of previous work where a Langmuir film made from $C_{18}F_{17}$NTANi (a comparable compound to Phenyl NTANi) was not perturbed by the presence of detergent in the subphase.[17]

These results show that both surfactants C_6F_{13}SOTHAM and PhenylHFNTANi are not miscible in the



Scheme 1. Synthesis of hemifluorinated PhenylHFNTANi surfactant. Reagents and conditions: (a) MeONa (7 equiv), CH_3CN, Δ, 24 h, 89%; (b) H_2, Pd–C 10%, MeOH, 7 bars, 3 h, 100%; (c) CDI (4 equiv), DMAP cat, CH_2Cl_2, 2 h, 57%; (d) $I(CF_2)_6I$, AIBN cat, CH_3CN, Δ, 72 h, 51%; (e) Boc_2O, Et_3N, CH_2Cl_2, 4 h, 48%; (f) $NaHCO_3$, $Na_2S_2O_4$, CH_3CN/H_2O: 3/1, 5 h, 55%; (g) Bu_3SnH, AIBN, CH_3CN, Δ, 24 h, 70%; (h) CH_2Cl_2/TFA: 4/1, 1 h, 100%; (i) Et_3N, DMAP cat, CH_2Cl_2, 20 h, 69%; (j) KOH 0.5 M, MeOH, 4 days, 76%; (k) $NiCl_2{\cdot}6H_2O$, phosphate buffer pH 8, $CHCl_3$, 48 h, 100%.

monolayer with two detergents, respectively, DDM and H_{12}-TAC which are generally used for the solubilization of membrane proteins. This property makes these (hemi)-fluorinated compounds very good candidates and suitable for the 2D crystallization of membrane proteins at an air–water interface.

The π–A isotherm of a monolayer made from pure PhenylHFNTANi spread on a buffer solution (NaCl 250 mM; Tris 20 mM, pH 8.0) is shown in Figure 2. The PhenylHFNTANi isotherm was stable up to 38 mN m^{-1}. The collapse area was 43 $Å^2$. PhenylHFNTANi did not display a phase transition pressure at 20 °C, indicating that PhenylHFNTANi is fluid at this temperature. Same behavior was observed when the buffer subphase was replaced by water (data not shown). The π–A isotherm of a monolayer made from pure C_6F_{13}SOTHAM spread on a water subphase was performed (data not shown), too. No phase transition pressure or collapse was noticed. The fluidity and high solubility of this compound in water might explain this behavior.

In conclusion, we have shown that a langmuir film made of C_6F_{13}SOTHAM or PhenylHFNTANi is not perturbed by the presence of detergent in the subphase. Moreover, PhenylHFNTANi and C_6F_{13}SOTHAM films are fluid at

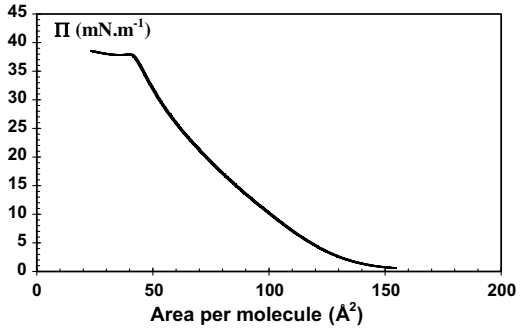


Fig. 2. Surface pressure (π)–area isotherm of PhenylHFNTANi monolayer spread on buffer subphase at 20 °C.

20 °C. Both conditions are key features in the 2D crystallization of membrane proteins on a lipid monolayer experiments. The membrane proteins are usually solubilized in detergent. The monolayer matrix made from these two compounds is not perturbed by the presence of detergent in the subphase and therefore provide a suitable anchor for the solubilized membrane proteins. Then there is a direct correlation between the fluidity of the monolayer and the crystallization process. Once the proteins are adsorbed and concentrated at the lipid or surfactant monolayer, the organization of proteins within a 2D crystal results from the in-plane diffusion of the protein–surfactant complexes both in rotation and in translation. This diffusion process is partly controlled by the fluidity property of the Langmuir film. We have shown that C6F13SOTHAM and PhenylHFNTANi films are fluid at 20 °C. We think that these two newly designed components are good candidates for the crystallization of membrane proteins

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19. *Selected spectra for PhenylHFNTA*: ^{1}H NMR (DMSO-d_6) 7.28 (m, 5H, phenyl); 3.50 (m, 4H, NCH_2COOH); 3.36 (t, 1H, CHLys); 3.08 (t, 2H, CH_2NH); 2.96 (t, 2H, CH_2NH); 2.71 (t, 2H, CH_2Ph); 2.22 (m, 4H, CH_2CF$_2$); 1.85 (m, 2H, C$_{H2}$βPh); 1.61 (m, 4H, CH$_2$βNHCONH); 1.35 (m, 4H, CH$_2$ Lys). ^{19}F NMR (DMSO-d_6) −113.23 (4F, s, CH$_2$CF_2); −121.69 (4F, s, CF_2βCH$_2$); −123.18 (4F, s, (CF$_2$)$_2$). ^{13}C NMR (DMSO-d_6) 174.27 (*C*O$_2$H Lys); 173.5 (2CH$_2$*C*O$_2$H); 158.55 (*C*O urea); 141.37 (C phenyl); 126.6–128.9 (*C*H phenyl); 64.7 (*C*H Lys); 53.7 (*C*H$_2$CO$_2$H); 39.7 (NHCONH*C*H$_2$); 39.1 (*C*H$_2$NHCO); 34.4 (*C*H$_2$Ph); 28.1−30.2 (*C*H$_2$); 21.5–23.6 (*C*H$_2$).
Mass spectrum for PhenylNTANi: (ESI-MS in a presence of ammonium acetate, TOF analyser) *m*/*z*: 836 [M−Ni+2NH$_4^+$]$^+$, *m*/*z*: 822 [M−2H$_2$O+2H]$^+$.
20. Surface tension measurements were made on a Krüss (Hamburg, Germany) K10T maximum pull digital tensiometer with a Pt/Ir ring. Solution of PhenylHFNTANi (18 mM) was prepared in chloroform/hexane (1:1 v/v). C$_6$F$_{13}$SOTHAM (50 mM) was prepared in water.
21. The surface pressure *p*, was measured using an automated Wilhelmy film balance (Nima technology Ltd Coventry, UK). The pressure-measuring systems was equipped with a filter paper (Whatman 541, periphery 4 cm). The trough was made from a 535 cm^2 Teflon-coated brass. Solution of PhenylHFNTANi (18 mM) was prepared in chloroform/hexane (1:1 v/v). C$_6$F$_{13}$SOTHAM (19 mM) was prepared in water. Three microliters of the surfactants were spread on the subphase. The spreading solvent was allowed to evaporate for 15 min prior to compression. The monolayer was compressed at a speed of 50 cm^2/min. The substrate solution was either distilled water (surface tension, 73 mN m^{-1}) or a buffer (NaCl 250 mM, Tris 20 mM, pH 8.0).
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