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Synthesis and surface properties of new semi-fluorinated sulfobetaines potentially usable for 2D-electrophoresis

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Abstract

New semi-fluorinated amidosulfobetaines, homologs of hydrocarbon amidosulfobetaines (ASB) commonly used in two-dimensional gel electrophoresis (2DE), were prepared in three steps from 2-F-alkylethyl iodide or F-alkyl iodide. Their synthesis was described and their air–water interface properties were investigated and compared with their perhydrogenated counterpart properties. The influence of the relative lengths of the perfluorinated and hydocarbonated moieties was discussed. 2DE of a rat testicular membrane fraction was performed comparatively using one of these fluorinated sulfobetaines and its hydrocarbon homolog; these preliminary results showed the great potential of the semi-fluorinated sulfobetaines in proteomic analysis.

Keywords: Sulfobetaine; Semi-fluorinated; Surfactant; Critical micelle concentration; Surface tension; 2D electrophoresis

1. Introduction

When a surfactant, a liquid crystal, a monomer, a polymer and so on, present an activity or a specific property, most of the time, this activity or property is largely modified when a hydrogenated fragment of its structure is replaced by a homologous perfluorinated moiety [1]. This quality of the perfluoroalkyl chains has been used for a long time to enhance the surface properties of surfactants in various domains like paints [2], cleaning [3,4], electronics [5], emulsion [6], microemulsions [7], fire retardant (AFFF) [8,9] or blood substitute [10]. A field in which these exceptional properties have just started to be explored is that of detergents for the extraction and solubilization of membrane proteins. First attempts were made by Shepherd and Holzenburg [11] on various membrane proteins and seemed promising. They demonstrated that the use of a charged detergent, i.e. ammonium perfluorooctanoate, enabled high concentrations in surfactant (40–80 g L^{-1}) to be obtained, in which the membranes (2–5 g proteins L^{-1}) are at least partially solubilized. Perfluorophosphocholines were used for the purification of flavocytochrome B_{558} [12], but the encouraging results obtained were not confirmed by the solubilization of other membrane proteins. Pucci et al. showed that fluorinated amphipols were able to preserve proteins in their native state, which allowed the study of their activities [13,14]. All these results suggest that semi-fluorinated surfactants have a role to play in proteomic analysis. Furthermore, the impact of the perfluorinated segment in membrane extraction and solubilization followed by two-dimensional gel electrophoresis (2DE) has never been demonstrated.

Recently, amidosulfobetaine detergents (see ASB-n in Fig. 1) have been shown to be effective in 2DE [15] with respect to a great number of membrane proteins; efficiency is modulated by the length of the hydrocarbon tail, which can be related directly to the hydrophobic properties. For instance, amidosulfobetaine-14 (ASB-14) was very efficient in solubilizing the membrane

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FASB-p,m ASB-n

Fig. 1. Synthesized amidosulfobetaines FASB-*p*,*m* and their perhydrogenated analogs ASB-n.

H^+ATPase [16] and the highly hydrophobic transmembrane protein B and 3 characterized by 12 transmembrane domains [17,18].

For these reasons, while preserving the properties and advantages brought by the polar head of amidosulfobetaine detergents, we decided to modulate their hydrophobic and lipophilic properties by introducing perfluorinated fragments in the hydrophobic tail. In consequence, we decided to synthesize semi-fluorinated detergents which have within the same structure: a sulfobetaine polar head, an amide connector and a perfluorinated terminal fragment of variable length (C_2F_5, C_4F_9, C_6F_{13}, C_8F_{17}), itself connected to the structure by a short hydrocarbon spacer (C_2H_4) or a longer one ($C_{10}H_{20}$). All the structures synthesized are shown in Fig. 1. They were named FASB-*p*,*m*, where *p* is the number of carbons of the perfluorinated moieties and *m* the number of carbons in the hydrocarbon spacer.

In order to support our hypothesis for the possible use of semi-fluorinated sulfobetaines for 2DE analysis, a preliminary experiment was performed on a membrane fraction of rat testicular tissue by using extraction/solubilization followed by a first dimension separation with these fluorinated surfactants. As we were interested by the influence of the fluorinated chains, we choose to start with the compound having the higher contents in fluorine. That is the reason why FASB-8,2 was taken as a model for this preliminary investigation.

2. Results and discussion

2.1. Synthesis

The semi-fluorinated surfactants FASB-*p*,*m* were obtained from 2F-alkyl-ethyl iodides ($R_FC_2H_4I$) for compounds with $m = 2$, and from perfluoroalkyl iodides (R_FI) for compounds with a longer hydrocarbon spacer ($m = 10$) according to the general reaction pathway summarized in Fig. 2.

2.1.1. Step 1

The iodides were initially transformed into acids **1**. The procedures involved in these syntheses depend on the length of the hydrocarbon linker. The synthesis of the short hydrocarbon segment acids involves the prior formation of the magnesium form; the latter will react with carbon dioxide to form the carboxylate intermediate. Acidic hydrolysis allowed us to obtain the corresponding 3-perfluoroalkyl-propanoic acids.

In order to synthesize the surfactants with long hydrocarbon fragments (ten atoms of carbon in our case), the corresponding acids were prepared using the method published by Brace [19]. It involved the homolytic rupture of the C–I bond in perfluoroalkyl iodides followed by its radical addition to long chain ω-olefins α-functionalized.

The Brace condensation on undecenyl acid, followed by reduction of the iodized synthon allowed us to obtain the desired semi-fluorinated acids. Several methods have been described in the literature for the reduction itself, we decided to use the one involving metal zinc in acid medium [19].

2.1.2. Step 2

All the acids synthesized were then converted into amides **2** via a condensation reaction with 3-*N*,*N*-dimethylaminopropylamine. This conversion has been accomplished through three different ways. Attempts were made first with the commercial acid $C_8F_{17}C_2H_4COOH$. The first two methods involved the use of different coupling agents which are classically used in peptide syntheses, a third one utilises of a non conventional synthesis using microwave irradiation. In general, the formation of amides from amines and carboxylic acids implies the activation of the carboxy group by either the prior conversion to a more reactive acylating agent such as acyl chloride or in situ activation by coupling reagents [20]. The formation of the compound FASB-8,2 via the perfluorooctylpropanoic acid chloride was claimed in a patent describing the synthesis and the use of various fluorinated sulfobetaines as fire extinguishing agents [21]. However, we did not succeed to reproduce this experiment because the product obtained while following the protocol led to the formation of the salt $C_8F_{17}C_2H_4C(O)NH(CH_2)_3N^+H(CH_3)_2{\cdot}Cl^-$ which was difficult to deprotonate [21]. We tested two coupling agents known to give good results in the literature [20]. One of the reactions involved dicyclohexylcarbodiimide (DCC) assisted by hydroxysuccinimide (HOSu) and the other 3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) and is catalysed with 4-dimethylaminopyridine (DMAP). In the DCC/HOSu couple experiment, the DCC reacts with the perfluorooctylpropanoic acid to form a reactive complex. Then, HOSu reacts with the latter to form an even more reactive entity. Once the acid is activated, the amine can condense and form the desired amide with dicyclohexylurea (DCU), which precipitates in the medium and allows elimination of water; HOSu is regenerated.

In this case, the amide $C_8F_{17}C_2H_4C(O)NH(CH_2)_3N(CH_3)_2$ was obtained with 63% yield. With EDC/DMAP, the principle is the same. EDC $(CH_3)_2N(CH_2)_3N{=}C{=}NCH_2CH_3{\cdot}HCl$, is also a carbodiimide which can activate the acid and trap the water formed in the same way as DCC. DMAP catalyses the reaction by forming an even more reactive species. In this case, the treatment is simplified compared to the preceding couple, since EDC and the urea formed are water soluble and can be eliminated by a simple washing. This method allowed us to obtain the amide $C_8F_{17}C_2H_4C(O)NH(CH_2)_3N(CH_3)_2$ with 86% yield.

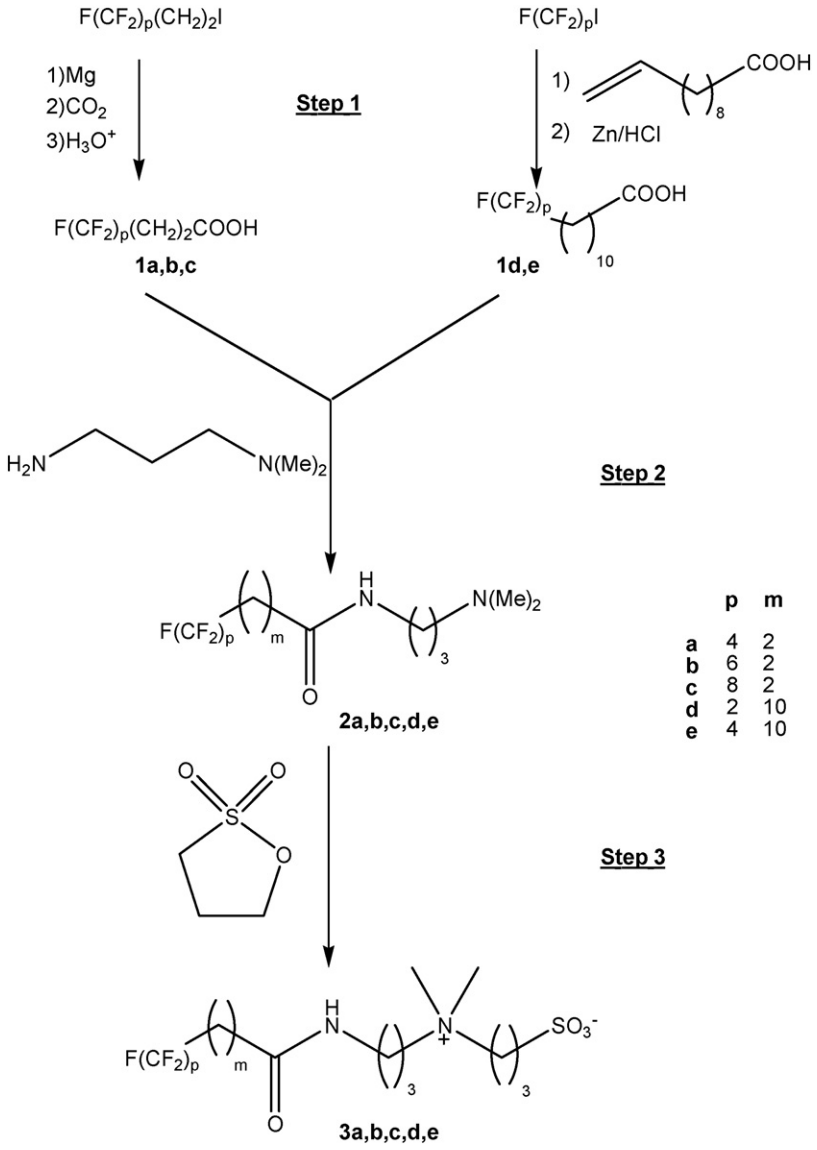

Fig. 2. Reaction pathway for the synthesis of semi-fluorinated amidosulfobetaines FASB-*p*,*m*.

Lastly, we were also interested in a ''non-usual'' technique of activation involving microwave irradiation [22]. It is now well documented that solvent-free methods coupled to microwave (MW) irradiation results in very efficient and clean procedures with noticeable improvements over classical methods [23]. In order to test this protocol, we used a domestic microwave. Different combinations of time and irradiation power were achieved in order to observe the complete conversion of the acid into amide. The best results were obtained after three consecutive irradiations of 1 min with full power (800 W). In these conditions, we observed the complete conversion of the starting acid without formation of side products or any noticeable decomposition. However, the isolated yield (43%) remains relatively weak, in part due to the evaporation of the products, and this reaction would deserve to be optimized in closed vessels with a laboratory microwave oven.

Table 1 summarizes the yields obtained for the synthesis of the amide $C_8F_{17}C_2H_4C(O)NH(CH_2)_3N(CH_3)_2$ according to the various processes used.

Taking into consideration the results obtained with these different procedures, we decided to continue the synthesis of our surfactants by using the method involving the couple EDC/DMAP.

Table 1
Various processes used for the synthesis of the amide $C_8F_{17}C_2H_4C(O)NH(CH_2)_3N(CH_3)_2$

Method	Reaction time (min)	Treatments	Isolated yield (%)
DCC/HOSu[a]	1440	Column chromatography Kügelrohr	63
EDC/DMAP[b]	1440	Water extraction Kügelrohr	86
Microwave 800 W	3 × 1	Kügelrohr	43

[a] DCC: dicyclohexylcarbodiimide; HOSu: hydroxysuccinimide.
[b] EDC: 3-(dimethylamino)propyl]-3-ethylcarbodiimide; DMAP: 4-dimethylaminopyridine.

Table 2
Yields obtained for the different steps of FASB-*p*,*m* synthesis

Surfactants FASB-*p*,*m*	Step 1: isolated yield (%)	Step 2: (EDC/DMAP)[a] isolated yield (%)	Step 3: isolated yield (%)	Global yield (%)
FASB-4,2	50	82	66	27
FASB-6,2	40	83	59	20
FASB-8,2	Commercially available	86	62	53
FASB-2,10	48	71	49	17
FASB-4,10	56	78	54	24

[a] EDC: 3-(dimethylamino)propyl]-3-ethylcarbodiimide; DMAP: 4-dimethylaminopyridine.

2.1.3. Step 3

The third step, which consisted in opening the propane sultone with the different amides synthesized, allowed us to obtain the semi-fluorinated amidosulfobetaines. The reaction proceeded in a Et_2O/CH_3CN 2:1 mixture in which the starting materials are soluble whereas the surfactants formed precipitate. Consequently, the amidosulfobetaines synthesized could be isolated by filtration except for FASB-2,10 which was soluble in the media and required purification by column chromatography over silicagel.

The results obtained are summarized in Table 2.

2.2. Physicochemical study

The critical micelle concentration (CMC) can be determined by various methods (conductimetry, colorimetry, tensiometry, solubilization, etc.). We chose to use the tensiometric method, which is based on the measure of the surface tension (γ_S) versus the surfactant concentration (C) of an aqueous solution. Graphs representing the variation of surface tension (γ_S) against log concentration indicate clearly that γ_S decreases until a particular concentration, the CMC, is reached and it remains constant for concentrations higher than the CMC (Fig. 3). This change in the slope of the curve is characteristic of micellar aggregation and indicates the surfactant properties of the semi-fluorinated amidosulfobetaines synthesized.

Since, the surfactants synthesized were not totally water-soluble, an aqueous solution containing 10% of ethanol was used. In these conditions, the CMC varies very slightly [24]. The values of the γ_S and CMC were obtained at 25 °C and are reported in Table 3.

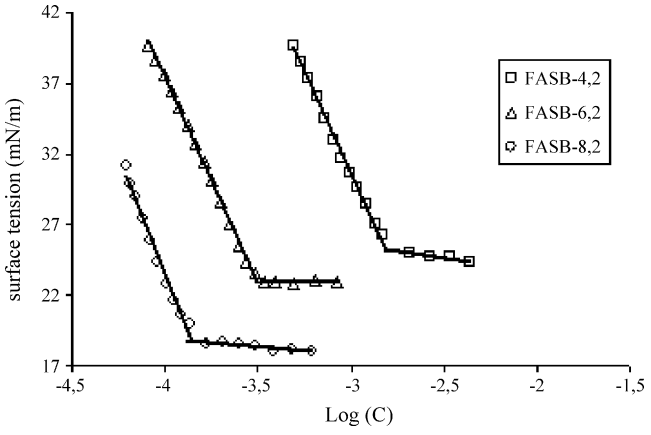

Fig. 3. Surface tension against log (concentration of FASB-*p*,2).

For comparison, we also report here the values measured for ASB-14 which is the hydrocarbon homologous of our semi-fluorinated structures. This surfactant is commercially available and the values of the γ_S and the CMC we obtained in water or in an aqueous solution containing 10% of ethanol were identical.

From these data, it is apparent that all of the molecules can self-organize at very low concentrations (CMC values range from 0.018 to 6.03 mmol L^{-1}) and have a high surfactant activity, reducing the surface tension of water from 69 mN m^{-1} (reference sample) to 18.5–34.3 mN m^{-1} at the CMC at 25 °C. As predicted by the literature, the introduction of a semi-fluorinated chain into the surfactant structure involves a dramatic reduction of the CMC (0.018 mmol L^{-1} for the best value obtained with FASB-4,10 against 6.03 mmol L^{-1} for the totally hydrogenated counterpart), along with a reduction of the surface tension γ_S (from 18.5 to 25.2 for the semi-fluorinated compounds against 34.3 for the perhydrogenated ASB-14).

As expected within the semi-fluorinated series, we observed that for a predetermined hydrocarbon spacer, the surface tension γ_S decreases when the length of the perfluorinated chain increases. The same trend was observed for the CMC values which vary from 1.58 mmol L^{-1} for FASB-4,2 to 0.16 mmol L^{-1} for FASB-8,2. The CMC measured for the surfactants with a long hydrocarbon spacer ($C_{10}H_{20}$) are remarkably much lower than the one measured for the surfactant with a shorter spacer. This is not surprising and can be explained by the fact that micelle formation is favoured when stabilizing hydrophobic interactions are accentuated [25].

2.3. 2D gel electrophoresis attempt

In order to test the potential of these semi-fluorinated surfactants for 2DE analysis, some preliminary experiments were performed on a rat testicular membrane by using FASB-8,2 that presents an intermediate CMC value among the series

Table 3
Surface properties of amphiphilic compounds

Surfactants	γ_s (mN m^{-1})[a]	CMC (mmol L^{-1})
ASB-14	34.3	6.03
FASB-4,2	25.2	1.58
FASB-6,2	22.6	0.31
FASB-8,2	18.5	0.16
FASB-2,10	22.1	0.19
FASB-4,10	20.5	0.018

[a] Value at the critical micelle concentration (CMC).

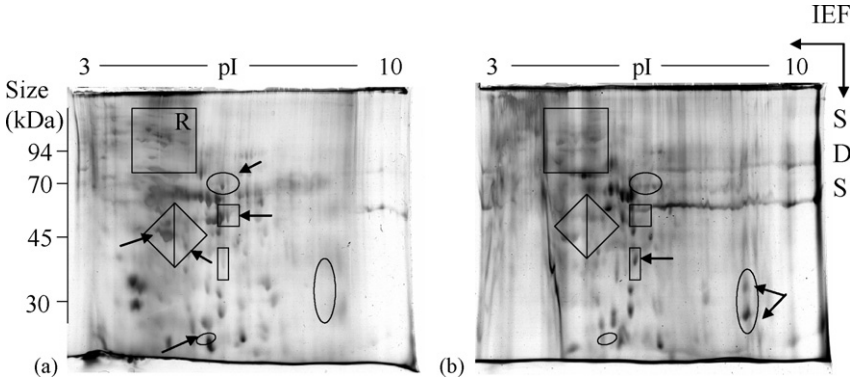

Fig. 4. Differential 2DE analysis after silver staining of a rat testicular membrane fraction extracted and solubilized with: (a) the classical detergent ASB-14; (b) a semi-fluorinated surfactant FASB-8,2. Presence of a spot in a gel compared to the other gel is indicated by an arrow (→); better spot resolution area (R) in (a); uncleared separation (*) in a narrow acidic zone in (b).

synthesized and it is the synthesized surfactant having the most fluorinated tail.

In the 2DE technology, proteins are separated according to their isoelectric point by isoelectric focusing (IEF) in the first dimension and according to their molecular weight by sodium dodecylsulfate (SDS) electrophoresis in the second dimension [26,27]. Thus, 2DE patterns of a rat testicular membrane fraction were compared after that extraction/solubilization and IEF were performed in a 3–10 linear IPG pH gradient, respectively with ASB-14 (Fig. 4a) and FASB-8,2 (Fig. 4b). Second dimensional gels were obtained with 3% SDS at the equilibration step. The amount of protein loaded is highly dependent on the separation distance in IEF, and in this experiment the pH gradient is only a 7 cm IPG strip. The comparison of the 2DE patterns obtained with ASB-14 and FASB-8,2 (Fig. 4) displayed differences in the number of spots thus showing the specificity of these surfactants; five spots or train of spots appeared only when ASB-14 was used and three spots appeared when FASB-8,2 was used. Some spots were less resolved and some smears were present in the acidic end of the 2DE pattern resulting from FASB-8,2 extraction and separation. From these promising preliminary results it appears that further investigation will be necessary to optimize the fluorinated surfactant concentration as well as the SDS concentration in the second dimension. Use of different separation conditions will let us to enhance the sensitivity and the resolution of the 2DE map.

3. Conclusion

The diversity of the biological samples analyzed in 2DE leads to the development of protocols with numerous steps of extraction and solubilization requiring the use of one or a combination of surfactants. In this perspective, simple and efficient synthesis of a new series of semi-fluorinated amidosulfobetaine amphiphiles homolog to hydrocarbon amidosulfobetaines ASB-n largely used in proteomic analysis is described. These fluorinated compounds self-organize in water at low concentrations and present interesting surfactant properties. The preliminary results obtained in rat testicular membrane extraction and solubilization followed by 2DE showed that the synthesized semi-fluorinated surfactants were able to extract different membrane proteins than those extracted by their hydrocarbon homologous. The specificity observed with these new semi-fluorinated amidosulfobetaines let us to foresee their use in serial extraction of membrane proteins for 2DE. Further investigation is necessary to optimize the patterns in order to enhance this specificity. Work on red blood cell membrane will be reported elsewhere [28].

4. Experimental

4.1. General experimental procedures

^{1}H NMR and ^{19}F NMR spectra were obtained using a Bruker AC-200 spectrometer, in $CDCl_3$ for 2-perfluoroalkylethanoic acid, ^{1}H NMR, 2D ^{1}H NMR, ^{19}F NMR and ^{13}C NMR spectra were obtained in MeOD for all the other compounds. Chemical shifts δ are given in ppm, using TMS as internal standard for ^{1}H NMR, 2D ^{1}H NMR and ^{13}C NMR, and $CFCl_3$ for ^{19}F NMR. Mass spectra were obtained from a Finnigan MAT. (Thermo Corp.) LCQ with an ESI API 2 source and a ITD (ion trap) analyzer. High resolution mass spectra for the final amidosulfobetaines were performed on a Waters QStar Elite (Applied Biosystems SCIEX) spectrometer with an ESI API source and a TOF (time of flight) analyzer.

Fusion points were obtained from a Büchi 510 apparatus. Critical micelle concentration (CMC) and superficial tensions were obtained using a tensiometer K100 from Kruss.

1-Iodo-2-perfluoroalkylethanes, 1-iodoperfluoroalkanes, azobis-(dimethylvaleronitrile) (AIVN) were supplied by DuPont de Nemours. Undecenyl acid, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP), propanesultone, zinc granular 20 mesh and 3-[N,*N*-dimethyl(3-myristoylaminopropyl)ammonio]propanesulfonate (ASB-14) were purchased from Sigma–Aldrich, and silica gel 60 from Fluka.

4.2. Synthesis of 3-perfluoroalkylpropanoic acid (*1a–c*)

Magnesium (2.4 g, 0.1 mol) was added to 25 mL of anhydrous diethyl ether under nitrogen atmosphere, followed

by a drop wise addition of 1-iodo-2-perfluoroalkylethane (0.1 mol) dissolved in anhydrous diethyl ether for 2 h. The reaction was stirred for 2 h at room temperature, and then the mixture was cooled in an ice water bath. Ten grams of dry ice were added and the mixture was stirred at room temperature for 15 min. The reaction was cooled and a 10% aqueous solution of sulfuric acid was added. The aqueous and organic layers were separated. The organic layer was dried and evaporated under vacuum. The acids $F(CF_2)_n(CH_2)_2COOH$ were obtained as white powders.

1a—$R_F = C_4F_9$; $m = 2$; m.p. = 43–44 °C; ^{1}H NMR ($CDCl_3$) δ: 2.3–2.8 (4H, m, $CF_2\boldsymbol{CH_2}\boldsymbol{CH_2}COOH$). ^{19}F NMR ($CDCl_3$) δ: −82.5(3F, $\boldsymbol{CF_3}$); −116 (2F, $\boldsymbol{CF_2}CH_2$); −125 (2F, $CF_3CF_2\boldsymbol{CF_2}$); −127.5 (2F, $CF_3\boldsymbol{CF_2}$).

1b—$R_F = C_6F_{13}$; $m = 2$; m.p. = 63–64 °C; ^{1}H NMR ($CDCl_3$) δ: 2.3–2.8 (4H, m, $CF_2\boldsymbol{CH_2}\boldsymbol{CH_2}COOH$). ^{19}F NMR ($CDCl_3$) δ: −82.5 (3F, $\boldsymbol{CF_3}$); −116 (2F, $\boldsymbol{CF_2}CH_2$); −123 (2F, $\boldsymbol{CF_2}CF_2CH_2$); −124 (2F, $\boldsymbol{CF_2}CF_2CF_2CH_2$); −125 (2F, $CF_3CF_2\boldsymbol{CF_2}$); −127.5 (2F, $CF_3\boldsymbol{CF_2}$).

1c—$R_F = C_8F_{17}$; $m = 2$; m.p. = 91–92 °C; commercial; ^{1}H NMR ($CDCl_3$) δ: 2.3–2.8 (4H, m, $CF_2\boldsymbol{CH_2}\boldsymbol{CH_2}COOH$). ^{19}F NMR ($CDCl_3$) δ: −81.2 (3F, $\boldsymbol{CF_3}$); −113.9 (2F, $\boldsymbol{CF_2}CH_2$); −122.2 (6F, $(\boldsymbol{CF_2})_3CF_2CH_2$); −123.3 (2F, $C\boldsymbol{F_2}(CF_2)_3CF_2CH_2$); −123.9 (2F, $CF_3CF_2\boldsymbol{CF_2}$); −126.6 (2F, $CF_3\boldsymbol{CF_2}$).

4.3. Synthesis of 11-perfluoroethylundecanoic acid (*1d*)

1-Iodoperfluoroethane (18.4 g, 75 mmol), 2,2′-azobis(2,4-dimethyl)valeronitrile (AIVN) (248 mg, 1 mmol) and 10-undecenoic acid (9.2 g, 50 mmol) were added in a flask to be sealed, cooled in a water iced bath. The flask was then cooled in liquid nitrogen and sealed. The reaction was stirred for 24 h at room temperature. The sealed flask was then opened and the 10-iodo-11-perfluoroethylundecanoic acid was not isolated, but directly reduced.

The reaction was cooled at room temperature, 0.22 mol of an aqueous solution of hydrochloric acid 37% was added and the mixture was heated at 70 °C. Metallic zinc (1.4 g, 22 mmol) in powder was added and the reaction was stirred at 70 °C for 12 h.

The reaction mixture was cooled at room temperature and filtered, rinsed with cold water. The product was purified by crystallization in hexane. The 11-perfluoroethylundecanoic acid was obtained in 48% yield as white powder.

1d—$R_F = C_2F_5$; m = 10; m.p. = 37–38 °C; ^{1}H NMR (MeOD) δ: 1.25 (12H, m,$(\boldsymbol{CH_2})_6CH_2C_2H_4CO_2H$); 1.5 (4H, m, $R_FCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}$); 1.95 (2H, m, $R_F\boldsymbol{CH_2}(CH_2)_8CH_2CO_2H$); 2.25 (2H, m, $\boldsymbol{CH_2}COOH$). ^{19}F NMR (MeOD) δ: −88.5 (3F, $\boldsymbol{CF_3}$); −121 (2F, $\boldsymbol{CF_2}CH_2$).

4.4. Synthesis of 11-perfluorobutylundecanoic acid (*1e*)

1-Iodoperfluorobutane (25.9 g, 75 mmol) and 2,2′-azobis(2,4-dimethyl)valeronitrile (AIVN) (248 mg, 1 mmol) were placed under a nitrogen atmosphere. The reaction was heated at 65 °C and 10-undecenoic acid (9.2 g, 50 mmol) was added. The mixture was stirred for 12 h at 65 °C, 10-iodo-11-perfluorobutylundecanoic acid was not isolated, but directly reduced.

The procedure followed for the reduction was the same as the one previously described for the reduction of 10-iodo-11-perfluoroethylundecanoic acid.

The 11-perfluorobutylundecanoic acid was obtained in 56% yield as a white powder.

1e—$R_F = C_4F_9$; $m = 10$; m.p. = 48–49 °C; ^{1}H NMR (MeOD) δ: 1.25 (12H, m,$(\boldsymbol{CH_2})_6CH_2C_2H_4CO_2H$); 1.5 (4H, m, $R_FCH_2\boldsymbol{CH_2}(CH_2)_6\boldsymbol{CH_2}$); 1.95 (2H, m, $R_F\boldsymbol{CH_2}(CH_2)_8CH_2CO_2H$); 2.25 (2H, m, $\boldsymbol{CH_2}COOH$). ^{19}F NMR (MeOD) δ: −84.5 (3F, $\boldsymbol{CF_3}$); −118 (2F, $\boldsymbol{CF_2}CH_2$); −128.5 (2F, $\boldsymbol{CF_2}CF_2CH_2$); −129.5 (2F, $CF_3\boldsymbol{CF_2}$).

4.5. Synthesis of amides (*2a–e*)

4.5.1. By coupling agents (DCC/HOSu)

3-Perfluorooctylpropionic acid (807 mg, 1.64 mmol), diclyclohexylcarbodiimide (339 mg, 1.64 mmol) and *N*-hydrosuccinimide (189 mg, 1.64 mmol) were added to 10 mL of dichloromethane. The mixture was stirred for 1 h at room temperature and 3-*N*,*N*-dimethylaminopropylamine (184 mg, 1.8 mmol) was added. After stirring for 24 h at room temperature, the solvent was evaporated under reduced pressure. The residue was purified by column chromatography over silica gel using methanol as the eluant. The product was obtained in 63% yields as colourless liquid.

4.5.2. By coupling agents (EDC/DMAP)

Acid (1.64 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (314 mg, 1.64 mmol) and 4-dimethylaminopyridine (20 mg, 0.164 mmol) were added to 10 mL of dichloromethane. The mixture was stirred for 1 h at room temperature and 3-*N*,*N*-dimethylaminopropylamine (184 mg, 1.8 mmol) was added. After stirring for 24 h at room temperature, the reaction was washed three times with water. The organic layer was dried and evaporated under reduced pressure.

The amides were obtained in 71–86% yields as colourless liquids, excepted for FASB-4,10 which was obtained as white powder.

4.5.3. By microwave irradiation

In order to test this protocol, a domestic microwave was used.

3-Perfluorooctylpropionic acid (807 mg, 1.64 mmol), and 3-*N*,*N*-dimethylaminopropylamine (500 mg, 4.9 mmol) were introduced in a 50 mL flask. Different combinations of time and irradiation power were achieved in order to observe the complete conversion of the acid into amide. The best results were obtained after three consecutive irradiations of 1 min with full power (800 W). In these conditions, we observed the complete conversion of the starting acid without formation of side products or any noticeable decomposition. The pure product was obtained after evaporation of the excess of amine using a Kugelrohr apparatus. Isolated yield: 43%.

2a—$R_F = C_4F_9$; $m = 2$; bp (°C)/mmHg = 121/7.6 × 10^{-3}; ^{1}H NMR (MeOD) δ: 1.65 (2H, m, $NHCH_2\boldsymbol{CH_2}$); 2.2 (6H, s,

N(CH_3)$_2$); 2.35 (2H, m, CH_2 N(CH_3)$_2$); 2.4–2.6 (4H, m, $CF_2CH_2CH_2$); 3.15 (2H, t, J = 7 Hz, NHCH_2). ^{19}F NMR (MeOD) δ: −82.5 (3F, CF_3); −116 (2F, CF_2CH_2); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2).

2b—$R_F = C_6F_{13}$; m = 2; bp (°C)/mmHg = 128/7.6 × 10^{-3}; ^{1}H NMR (MeOD) δ: 1.65 (2H, m, NHCH$_2CH_2$); 2.2 (6H, s, N(CH_3)$_2$); 2.35 (2H, m, CH_2N(CH_3)$_2$); 2.4–2.6 (4H, m, $CF_2CH_2CH_2$); 3.15 (2H, t, J = 7 Hz, NHCH_2). ^{19}F NMR (MeOD) δ: −82.5(3F, CF_3); −116 (2F, CF_2CH_2); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2).

2c—$R_F = C_8F_{17}$; m = 2; bp (°C)/mmHg = 140/7.6 × 10^{-3}; ^{1}H NMR (MeOD) δ: 1.65 (2H, m, NHCH$_2CH_2$); 2.2 (6H, s, N(CH_3)$_2$); 2.35 (2H, m, CH_2 N(CH_3)$_2$); 2.4–2.6 (4H, m, $CF_2CH_2CH_2$); 3.15 (2H, t, J = 7 Hz, NHCH_2). ^{19}F NMR (MeOD) δ: −82.5 (3F, CF_3); −116 (2F, CF_2CH_2); −123 (2F, $(CF_2)_3$CF2CH2); −124 (2F, $CF_2CF_2CF_2CH_2$); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2).

2d—$R_F = C_2F_5$; m = 10; bp (°C)/mmHg = 112/7.6 × 10^{-3}; ^{1}H NMR (MeOD) δ: 1.3 (12H, m, $(CH_2)_6CH_2C_2H_4CO$); 1.5 (6H, m, $R_FCH_2CH_2(CH_2)_6CH_2$, NHCH$_2CH_2$); 2.2 (12H, m, CF_2CH_2,CH_2CO,$CH_2N(CH_3)_2$); 3.2 (2H, m, NHCH_2). ^{19}F NMR (MeOD) δ: −88.5 (3F, CF_3); −121 (2F, CF_2CH_2)

2e—$R_F = C_4F_9$; m = 10; m.p. = 40–41 °C; ^{1}H NMR (MeOD) δ: 1.3 (12H, m, $(CH_2)_6CH_2C_2H_4CO$); 1.5 (6H, m, $R_FCH_2CH_2(CH_2)_6CH_2$, NHCH$_2CH_2$); 2.2 (12H, m, CF_2CH_2, CH_2CO,$CH_2N(CH_3)_2$); 3.2 (2H, m, NHCH_2). ^{19}F NMR (MeOD) δ: −84.5 (3F, CF_3); −118 (2F, CF_2CH_2); −128.5 (2F, $CF_2CF_2CH_2$); −129.5 (2F, CF_3CF_2).

*4.6. Synthesis of surfactants (**3a–e**)*

A mixture of 2 × 10^{-3} mole of the amide previously prepared and 5 × 10^{-3} mole of propanesultone was added to 20 mL of a mixture of diethyl ether/acetonitrile (2/1). The reaction was stirred and heated at 50 °C for 24 h.

White precipitates were filtered and washed with acetone several times. FASB-2.10 was also purified by column chromatography over silica gel using methanol as the eluant.

The surfactants were obtained in 49–66% yield as oils, excepted for FASB-4,2 which was obtained as white powder and FASB-2,10 as colourless liquid.

3a—$R_F = C_4F_9$; m = 2; m.p. = 108–109 °C; ^{1}H NMR (MeOD) δ: 1.9 (2H, m, NHCH$_2CH_2$); 2.2 (2H, m, $CH_2CH_2SO_3^-$); 2.5 (4H, m, $CF_2CH_2CH_2$); 2.85 (2H, m, $CH_2SO_3^-$); 3.05 (6H, s, $N^+(CH_3)_2$); 3.3–3.6 (6H, m, NHCH_2,$CH_2N^+(CH_3)_2CH_2$); ^{19}F NMR (MeOD) δ: −82.5 (3F, CF_3); −116 (2F, CF_2CH_2); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2). ^{13}C NMR (MeOD) δ: 18.5(1C, s, $CH_2CH_2SO_3^-$); 22(1C, s, NHCH$_2CH_2$); 26.5 (2C, m, $CF_2CH_2CH_2$); 36 (1C, s, NHCH_2); 47(1C, s, $CH_2SO_3^-$); 50.5 (2C, s, $N^+(CH_3)_2$); 62 (2C, m, $CH_2N^+(CH_3)_2CH_2$). This structure was also confirmed by the 2D ^{1}H NMR COSY spectrum. Mass spectrum (ESI+)—*m/z*: 499.3 [M + H]$^+$; 521.3 [M + Na]$^+$. HRMS: (*m/z*) calcd for $C_{15}H_{23}N_2O_4SF_9$ [M + H]$^+$, 499.1307; found 499.1308.

3b—$R_F = C_6F_{13}$; m = 2; ^{1}H NMR (MeOD) δ: 1.95 (2H, m, NHCH$_2CH_2$); 2.2 (2H, m, $CH_2CH_2SO_3^-$); 2.5 (4H, m, $CF_2CH_2CH_2$); 2.85 (2H, m, $CH_2SO_3^-$); 3.05 (6H, s, $N^+(CH_3)_2$); 3.3–3.6 (6H, m, NH$CH_2CH_2N^+(CH_3)_2CH_2$). ^{19}F NMR (MeOD) δ: −82.5 (3F, CF_3); −116 (2F, CF_2CH_2); −123 (2F, $CF_2CF_2CH_2$); −124 (2F, $CF_2CF_2CF_2CH_2$); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2). ^{13}C NMR (MeOD) δ: 18.5(1C, s, $CH_2CH_2SO_3^-$); 22(1C, s, NHCH$_2CH_2$); (1C, s, $CH_2SO_3^-$); 26.5 (2C, m, $CF_2CH_2CH_2$); 36 (1C, s, NHCH_2); 47(1C, s, $CH_2SO_3^-$); 50.5 (2C, s, $N^+(CH_3)_2$); 62 (2C, m, $CH_2N^+(CH_3)_2CH_2$). This structure was also confirmed by the 2D ^{1}H NMR COSY spectrum. Mass spectrum (ESI+)—*m/z*: 599.3 [M + H]$^+$; 621 [M + Na]$^+$; 1219.5 [2M + Na]$^+$. HRMS: (*m/z*) calcd for $C_{17}H_{23}N_2O_4SF_{13}$ [M + H]$^+$, 599.1242; found 599.1244.

3c—$R_F = C_8F_{17}$; m = 2, refractive index = 1.4196; ^{1}H NMR (MeOD) δ: 1.95 (2H, m, NHCH$_2CH_2$); 2.2 (2H, m, $CH_2CH_2SO_3^-$); 2.5 (4H, m, $CF_2CH_2CH_2$); 2.85 (2H, m, $CH_2SO_3^-$); 3.05 (6H, s, $N^+(CH_3)_2$); 3.3–3.6 (6H, m, NHCH_2, $CH_2N^+(CH_3)_2CH_2$). ^{19}F NMR (MeOD) δ: −82.5 (3F, CF_3); −116 (2F, CF_2CH_2); −123 (2F, $(CF_2)_3CF_2CH_2$); −124 (2F, $CF_2CF_2CF_2CH_2$); −125 (2F, $CF_3CF_2CF_2$); −127.5 (2F, CF_3CF_2). ^{13}C NMR (MeOD) δ: 18.5(1C, s, $CH_2CH_2SO_3^-$); 22(1C, s, NHCH$_2CH_2$); 26.5 (2C, m, $CF_2CH_2CH_2$); 36 (1C, s, NHCH_2); 47(1C, s, $CH_2SO_3^-$); 50.5 (2C, s, $N^+(CH_3)_2$); 62 (2C, m, $CH_2N^+(CH_3)_2CH_2$). This structure was also confirmed by the 2D ^{1}H NMR COSY spectrum. Mass spectrum (ESI+)—*m/z*: 699.3 [M + H]$^+$. HRMS: (*m/z*) calcd for $C_{19}H_{23}N_2O_4SF_{17}$ [M + H]$^+$, 699.1179; found 699.1181.

3d—$R_F = C_2F_5$; m = 10; bp (°C)/mmHg = 160/7.6 × 10^{-3}; refractive index = 1.4280; ^{1}H NMR (MeOD) δ: 1.3 (12H, m,$(CH_2)_6CH_2C_2H_4CO$); 1.6 (4H, m, $R_FCH_2CH_2(CH_2)_6CH_2$); 1.95 (2H, m, NHCH$_2CH_2$); 2.2 (6H, m, CF_2CH_2,CH_2CO,$CH_2CH_2SO_3^-$); 2.85 (2H, m, $CH_2SO_3^-$); 3.05 (6H, s, $N^+(CH_3)_2$); 3.3–3.5 (6H, m, NHCH_2,$CH_2N^+(CH_3)_2CH_2$). ^{19}F NMR (MeOD) δ: −88.5 (3F, CF_3); −121 (2F, CF_2CH_2); ^{13}C NMR (MeOD) δ: 18.5 (1C, s, $CH_2CH_2SO_3^-$); 20 (1C, s, $CF_2CH_2CH_2$); 22(1C, s, NHCH$_2CH_2$); 25 (1C, s, CH_2CH_2CO); 28 (6C, s, $CH_2(CH_2)_6CH_2$); 29,5 (1C, m, CF_2CH_2); 36 (2C, s, $CH_2CONHCH_2$); 47 (1C, s, $CH_2SO_3^-$); 50.5 (2C, s, $N^+(CH_3)_2$); 62 (2C, m, $CH_2N^+(CH_3)_2CH_2$). This structure was also confirmed by the 2D ^{1}H NMR COSY spectrum. Mass spectrum (ESI+)—*m/z*: 511.4 [M + H]$^+$; 533.4 [M + Na]$^+$; 1043.7 [2M + Na]$^+$; HRMS: (*m/z*) calcd for $C_{21}H_{39}N_2O_4SF_5$ [M + H]$^+$, 511,2623; found 511,2624.

3e—$R_F = C_4F_9$; m = 10; refractive index = 1.4430; ^{1}H NMR (MeOD) δ: 1.3 (12H, m,$(CH_2)_6CH_2C_2H_4CO$); 1.6 (4H, m, $R_FCH_2CH_2(CH_2)_6CH_2$); 1.95 (2H, m, NHCH$_2CH_2$); 2.2 (6H, m, CF_2CH_2,CH_2CO,$CH_2CH_2SO_3^-$); 2.85 (2H, m, $CH_2SO_3^-$); 3.05 (6H, s, $N^+(CH_3)_2$); 3.3-3.5 (6H, m, NHCH_2, $CH_2N^+(CH_3)_2CH_2$; ^{19}F NMR (MeOD) δ: −84.5 (3F, CF_3,3F); −118 (2F, CF_2CH_2); −128.5 (2F, $CF_2CF_2CH_2$); −129.5 (2F, CF_3CF_2); ^{13}C NMR (MeOD) δ: 18.5 (1C, s, $CH_2CH_2SO_3^-$); 20 (1C, s, $CF_2CH_2CH_2$); 22(1C, s, NHCH$_2$ CH_2); 25 (1C, s, CH_2CH_2CO); 28 (6C, s, $CH_2(CH_2)_6CH_2$); 29,5 (1C, m, CF_2CH_2); 36 (2C, s, $CH_2CONHCH_2$); 47 (1C, s, $CH_2SO_3^-$); 50.5 (2C, s, $N^+(CH_3)_2$); 62 (2C, m, $CH_2N^+(CH_3)_2$ CH_2). This structure was also confirmed by the 2D ^{1}H NMR COSY spectrum. Mass spectrum (ESI+)—*m/z*: 611.4 [M + H]$^+$;

633.4 $[M + Na]^+$; 1243.7 $[2M + Na]^+$; HRMS: (m/z) calcd for $C_{23}H_{39}N_2O_4SF_9$ $[M + H]^+$, 611.2559; found 611.2555.

4.7. 2D gel electrophoresis experiments

Rat testis (600 mg) was homogeneized in a Thomas potter with 6 mL of 50 mmol L^{-1} Tris–HCl buffer pH 7.5 containing a protease inhibitor cocktail Complete™ (1 mmol L^{-1} EDTA) from Roche Applied Science (Roche, Mannheim, Germany) as well as 10 mmol L^{-1} β-glycerophosphate and phosphatases inhibitors: 1 mmol L^{-1} sodium orthovanadate and 50 mmol L^{-1} sodium fluoride. The homogenate was centrifuged for 15 min at 8000 × g, then for 40 min at 100,000 × g. The pellet was called testicular membrane fraction. Proteins of this pellet (100 μg) were placed in 135 μL of the extraction/solubilization buffer containing 2 M thiourea, 7 M urea purified by Amberlite MB-150 mixed bed ion exchange resin (10 mg mL^{-1}) obtained from ICN Pharmaceuticals (Costa Mesa, CA, USA), 46 mmol L^{-1} ASB-14 (Sigma–Aldrich, St. Louis, MO, USA) or 23 mmol L^{-1} FASB-8,2, 65 mmol L^{-1} dithiothreitol (DTT), 0.8% (w/v) Pharmalyte 3–10 (Amersham Biosciences, Orsay, France), 10% isopropanol (v/v) and the inhibitors cited above. After 60 min, the sample was centrifuged at 20,000 × g in an Eppendorff centrifuge at 16 °C during 60 min. The supernatant completed to 155 μL by the solubilization buffer is introduced during reswelling of the immobilized pH gradient (IPG) strip pH range 3–10 overnight [29,30] and then separated by IEF on a Multiphor II apparatus (Amersham Biosciences). The run was performed with a preliminary voltage gradient to 3500 V, then at 3500 V to reach a total of 7500 Vh. IPG strips were then equilibrated for the second dimension in a 50 mmol L^{-1} Tris–HCl pH 8.5 buffer of high viscosity as previously described [31]. SDS concentration was raised to 3% at the equilibration step and 0.1 mmol L^{-1} EDTA was added. The first step of equilibration used 65 mmol L^{-1} DTT and the second used 260 mmol L^{-1} iodoacetamide [32]. Both steps lasted 15 min. Vertical second dimension was performed vertically in a 10% T 2.6% C acrylamide/bis-acrylamide gel containing 0.1% SDS and in a standard procedure [31]. Polypeptides spots resulting of 2DE were detected in the gel by a silver staining detection [33].

References

[1] E. Kissa, in: Marcel Dekker (Ed.), Fluorinated Surfactants and Repellents (Surfactant Science Series), Marcel Dekker, New York, 2001.
[2] B. Muller, Tenside Surf. Deterg. 37 (2000) 241–244.
[3] J. Block, US Patent 4,144,185 (1979).
[4] P. Bakos, R.E. Darrow, J.P. Funari, R.M. Poliak, Eur. Patent 32,179 (1981).
[5] S. Masuda, M. Izumi, US Patent 3,839,174 (1974).
[6] A.F. Benning, J.D. Park, US Patent 2,396,076 (1946).
[7] M. Visca, D. Lenti, Eur. Patent 292,427 (1989).
[8] R.D. Howard, W.I. Spurr, Ger. Offen. DE 2829594 (1979).
[9] R.A. Falk, US Patent 4,042,522 (1977).
[10] J.G. Riess, M.P. Krafft, Biomaterials 19 (1998) 1529–1539.
[11] F.H. Shepherd, A. Holzenburg, Anal. Biochem. 224 (1995) 21–27.
[12] C. Der Mardirossian, F. Lederer, Biochem. Soc. Trans. 24 (1996) 32S.
[13] J.L. Popot, E.A. Berry, D. Charvolin, C. Creuzenet, C. Ebel, D.M. Engelman, M. Floetenmyer, F. Giusti, Y. Gohon, P. Herve, Q. Hong, J.H. Lakey, K. Leonard, H.A. Shuman, P. Timmins, D.E. Warschawski, F. Zito, M. Zoonens, B. Pucci, C. Tribet, Cell. Mol. Life Sci. 60 (2003) 1559–1574.
[14] C. Breyton, E. Chabaud, Y. Chaudier, B. Pucci, J.L. Popot, FEBS Lett. 564 (2004) 312–318.
[15] M. Chevallet, V. Santoni, A. Poinas, D. Rouquié, A. Fuchs, S. Kieffer, M. Rossignol, J. Lunardi, J. Garin, T. Rabilloud, Electrophoresis 19 (1998) 1901–1909.
[16] V. Santoni, S. Kieffer, D. Desclaux, F. Masson, T. Rabilloud, Electrophoresis 21 (2000) 3329–3344.
[17] S. Luche, V. Santoni, T. Rabilloud, Proteomics 3 (2003) 249–253.
[18] C. Tastet, S. Charmont, M. Chevallet, S. Luche, T. Rabilloud, Proteomics 3 (2003) 111–121.
[19] N.O. Brace, J. Org. Chem. 27 (1962) 4491–4498.
[20] S.Y. Han, Y.A. Kim, Tetrahedron 60 (2004) 2447–2467.
[21] R. Bertocchio, L. Foulletier, A. Lantz, Eur. Patent Appl. EP 17568 (1980).
[22] A. Loupy, Microwaves in Organic Synthesis, Wiley-VCH, Weinheim, 2006
[23] A. Loupy, Comptes Rendus Chim. 7 (2) (2004) 103–112.
[24] K. Esumi, S. Ogiri, Colloid Surf. 94 (1995) 107–110.
[25] M.J. Rosen, Surfactants and Interfacial Phenomena, J. Wiley & Sons, New York, 1989, and references therein.
[26] J. Klose, Hum. Genet. 26 (1975) 231–243.
[27] P.H. O'Farrell, J. Biol. Chem. 250 (1975) 4007–4021.
[28] M. Starita-Geribaldi, P. Thebault, E. Taffin de Givenchy, F. Guittard, S. Geribaldi, Electrophoresis (2007) accepted for publication.
[29] T. Rabilloud, C. Valette, J.J. Lawrence, Electrophoresis 15 (1994) 1552–1558.
[30] J.C. Sanchez, V. Rouge, M. Pisteur, F. Ravier, L. Tonella, M. Moosmayer, M.R. Wilkins, D.F. Hochstrasser, Electrophoresis 18 (1997) 324–327.
[31] A. Görg, G. Boguth, C. Obermaier, A. Posch, W. Weiss, Electrophoresis 16 (1995) 1079–1086.
[32] A. Görg, W. Postel, J. Weser, S. Günther, J.R. Strahler, S.M. Hanash, L. Somerlot, Electrophoresis 8 (1987) 122–124.
[33] H. Blum, H. Beier, H.J. Gross, Electrophoresis 8 (1987) 93–99.