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NEW FLUORINATED BIOCOMPATIBLE NON-IONIC TELOMERIC AMPHIPHILES BEARING TRISHYDROXYMETHYL GROUPS[1]

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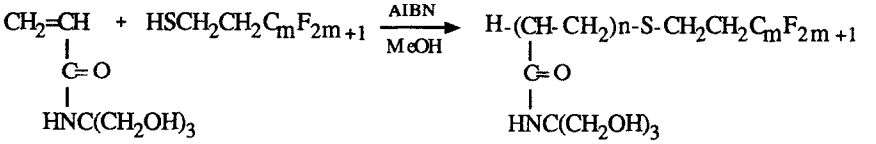
Abstract : New perfluoroalkylated telomeric surfactants derived from tris(hydroxymethyl)aminomethane were synthesized in two steps and 50% overall yield. These surfactants display significantly better surface activity and enhanced capacity to emulsify fluorocarbons compared to Pluronic F-68®. Preliminary *in vitro* and *in vivo* evaluation (cell cultures, hemolysis, LD50 *i.v.* in mice) indicates that this type of compounds is remarkably non-toxic, and could find applications in preparations for biomedical uses including injectable fluorocarbon emulsions.

Considerable efforts are presently being devoted to the development of injectable fluorocarbon emulsions to be used as oxygen carriers, contrast agents and drug delivery systems[2,3]. The surfactant plays a key role in the formulation of such emulsions as it largely determines the stability, *in vivo* acceptance and other crucial properties of such preparations[4,5]. The surfactants used in the presently approved emulsion - Pluronic F-68 and egg-yolk lecithin - are not particularly well suited for the emulsification of fluorocarbons, they allow little room for controlling the characteristics of the emulsions, and present various insufficiencies[6]. Future progress in the field will closely depend on the availability of more appropriate and especially more fluorophilic surfactants.

We report here the synthesis of a new class of fluorinated non-ionic surfactants with a telomeric structures and tris(hydroxymethyl)amidomethane hydrophilic groups, and preliminary data on their emulsifying properties and biocompatibility.

We have previously shown[7-9] that radical telomerization of a polymerizable hydrophilic moiety such as tris(hydroxymethyl)acrylamidomethane, in the presence of an alkylthiol as a chain transfer reagent, leads to the formation of amphiphilic molecules whose properties can be modulated by an appropriate choice of the length of the hydrophobic thioalkyl chain and of the number of repeating hydrophilic units. This method has now been adapted to the synthesis of fluorinated surfactants by substituting the thioalkylchain-transfer

reagent by the thioperfluoroalkyl analog, $HSCH_2CH_2C_mF_{2m+1}$. The telomerization reaction occurs in boiling methanol under a nitrogen atmosphere in the presence of α,α'-azobisisobutyronitrile as a radical initiator. It is pursued until the monomer has totally disappeared. The telomers are isolated by precipitation in anhydrous ether. The transfer constant Ct (the ratio between the rate constant of chain transfer and the rate constant of propagation of the monomer) of the tris(hydroxymethyl)acrylamidomethane unit to thioalkyl telogen used is close to 1 ; consequently Ro, the ratio between the initial concentration of the transfer reagent and that of the monomer allows an efficient control of the DP_n (average degree of polymerization in number) of the telomer[10]. The DP_n is determined both by elemental analysis (percentage of fluorine and nitrogen contained in the macromolecule), and by ^{1}H-NMR analysis in DMSO-d6 (by comparing the areas under the signals ascribed to the methylene α to sulfur in the alcanoyl chain (δ = 2.75ppm) to that corresponding to the amide protons (δ = 7.4ppm)). The telomers are isolated as amorphous hygroscopic white solids. They are highly soluble in water (solubility > 200 g/l, lathering profusely) and stable for 6 hours when heated in the standard, FDA-recommended conditions (121°C, ^{1}H NMR monitoring). The synthesis and the main physicochemical data of compounds obtained with various fluorinated thioalkyl-type telogenic agents are reported in Scheme I and Table I respectively.



Scheme I

Table I : Physicochemical data for telomers obeying the generic structure of scheme I.

m	Ro	Telomer yield	DPn	CMC* (mM/l)	m.p.
6	0.5	62	1		100°
6	5	80	6.9	0.35	180° (dec)
6	4	73	5.4	0.33	-
6	6	81	10	0.33	-
8	0.5	65	1	-	120°
8	4	71	5.75	0.033	205° (dec)
8	5	77.5	5.8	0.03	-
8	6	82	12.2	0.035	-
10	5	80	6.8	0.005	-

* The CMC were determined by the Menger and Portnoy method[11]

These results call for two important observations :

1) The monoadducts, which are purified by chromatography over silica gel (eluent AcOEt), are insoluble in water ; significant solubility appears for compound with DPn>2.
2) The CMC depends on the nature of the fluorocarbon chain and appears to be essentially independent of the DPn ; it is approximatively 10 times lower than that found for the hydrocarbon analogs[9].

The toxicity of these surfactants was evaluated by *in vitro* tests on cell cultures[12] and human red blood cells[13], and their *in vivo* toxicity was determined by intravenous injection in mice. The incubation of a suspension of a Namalva cell culture (37°C, 4 days) with an equal volume of a solution of the surfactant to be tested in physiologic water (Table II) showed no effect on the growth and viability of the culture until a rather high, 5 g/l, concentration was reached. These surfactants display no detectable hemolytic activity when incubated with a 1% suspension of red blood cells in isotonic NaCl/water even at a 200 g/l initial concentration. These results are remarkable in view of the high surface activity of these compounds. Their intravenous injection in mice also establishes an unusually high tolerance for such strongly efficient surfactants, with an LD_{50} as high as 2.4g/kg body weight.

Table II. Toxicity data for the telomeric surfactants.

m	DP_n	Cell cultures[12]			i.v. injection in mice			
		g/l	mM/l	% growth /% viability	g/l	mM/l	mg/kgbw	survival (/10animals)
6	5.4	5	3.77	46/96	100	75.5	2400	6*
					75	56.6	1850	10
8	5.75	5	3.33	77/97	100	66.7	2400	4*
					75	50	1850	10

* ex. LD_{50}

The new telomeric surfactants display significantly higher surface activity than Pluronic F-68®, the main surfactant used in the presently approved injectable fluorocarbon emulsion. Thus, for example, a 0.1 g/l solution of the telomer with m = 8 displays a surface tension of 32.2 mNm^{-1} and an interfacial tension between water and perfluorodecalin of 12.9 mNm^{-1}, compared to respectively 48.7mNm^{-1} and 29.6 mNm^{-1} for a 0.1 g/l solution of Pluronic F-68.

Emulsions prepared with such surfactants require less energy during preparation and display enhanced stability compared to those prepared with Pluronic F-68® as the sole surfactant (Table III).

Table III. Particle size evolution in F-decalin emulsions prepared with the perfluoroalkylated telomeric surfactants.

Formulation % w/v			Mean diameter at initial and after 3 months			
F-decalin	Pluronic F-68	m = 10	Initial, after sterilization	4 °	25 °	40 °C
50	3	-	0.32	0.81	0.85	1.10
50	-	3	0.31	0.28	0.38	0.47

These preliminary biocompatibility and emulsification data indicate that the new surfactants described here could be used in injectable preparations including fluorocarbon emulsions. The preparation of more concentrated emulsions (90% w/v) and additional biological assessment (exchange perfusion in rats) are in progress.

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10. **Example of synthesis of a perfluoroalkylated telomer** : Tris(hydroxymethyl)acrylamidomethane (8.75g, 0.05 mol) was dissolved in 100ml of methanol at 40-50°C under nitrogen. Perfluoro-1H, 1H, 2H, 2H octanethiol (3.8g, 0.01 mol, Ro = 5)and AIBN (0.087g) were then added. The mixture was refluxed and the reaction monitored by CCM. After the trisacryl was consumed, the solution was concentrated and poured dropwise in anhydrous ether (200ml) under vigourous stirring. The precipitate was filtered then washed with anhydrous ether, and the telomer was dried under reduced pressure (10g, 80% with respect to the trisacryl). The ^1H NMR analysis shows that the DPn is equal to 6.6. Elemental analysis -%C 42.18 ; %H 5.81 ; %N 6.34 ; %F 16.77-give DPn = 6.24 ; Calc (for Dpn = 6.24) : %C, 42.13 ; %H, 5.85 ; %N, 5.94 ; %F, 16.78.
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