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Synthesis of Polymerizable Double-Chain Glycolipids derived from Tris. Polymerization in aqueous Media. Preliminary Investigation of their Colloidal Behavior.

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Abstract : A series of mono and double tailed hydro and perfluorocarbon surfactant derived from Tris(hydroxymethyl)aminomethane and bearing an acryloyl moiety on the hydrophilic head have been prepared. The bicodal surfactants dispersed in water by ultrasonication formed vesicles which precipitated after UV irradiation. We describe a new method to stabilize these vesicular dispersions during the polymerization by addition of monocodal amphiphile. © 1997 Published by Elsevier Science Ltd.

Vesicles are being studied extensively for their usefulness as models for biological membranes or for their use as novel drug carriers[1,3]. In that respect, we reported recently the synthesis of new double-tailed glycolipidic amphiphiles[4]. These compounds bear an aminoacid or peptidic spacer interposed between the hydrophilic and the hydrophobic moities and an acryloyl group grafted at one of the hydrocarbon chain termini. The investigation of the supramolecular arrangement produced in aqueous medium showed the morphology of these assemblies to depend especially on the volumetric ratio of hydrophilic head and hydrophobic tail and on the nature of aminoacid spacer. The introduction of a chiral aminoacid induced the formation of tubules and helices[5], this phenomenon was already observed with many surfactants[6,7]. However, we observed that polymerization in water by UV irradiation of such glycolipids at a temperature above their Tc led to the formation of solely slightly heterogenous unilamellar vesicles[8]. No fibers nor tubules were observed after few weeks storage.

In the course of our project dealing with the understanding and modulation of different parameters involved in the stabilization of vesicular systems, these last results allow us to synthesize and investigate the colloidal behavior of new glycolipidic polymerizable amphiphiles derived from galactosylated Tris(hydroxymethyl) aminomethane (THAM), as depicted in formula **A**.

With these amphiphiles, the polymerizable group (acrylamide function) was introduced not in the hydrophobic core (as previously described[8]) but on the hydrophilic part using the amino group of THAM. The fluorocarbon chain was expected to allow the modulation of the vesicle membrane's permeability since fluorinated bilayers have been show to be less permeable to ions and small molecules than those made of hydrocarbon analogs[9,10].

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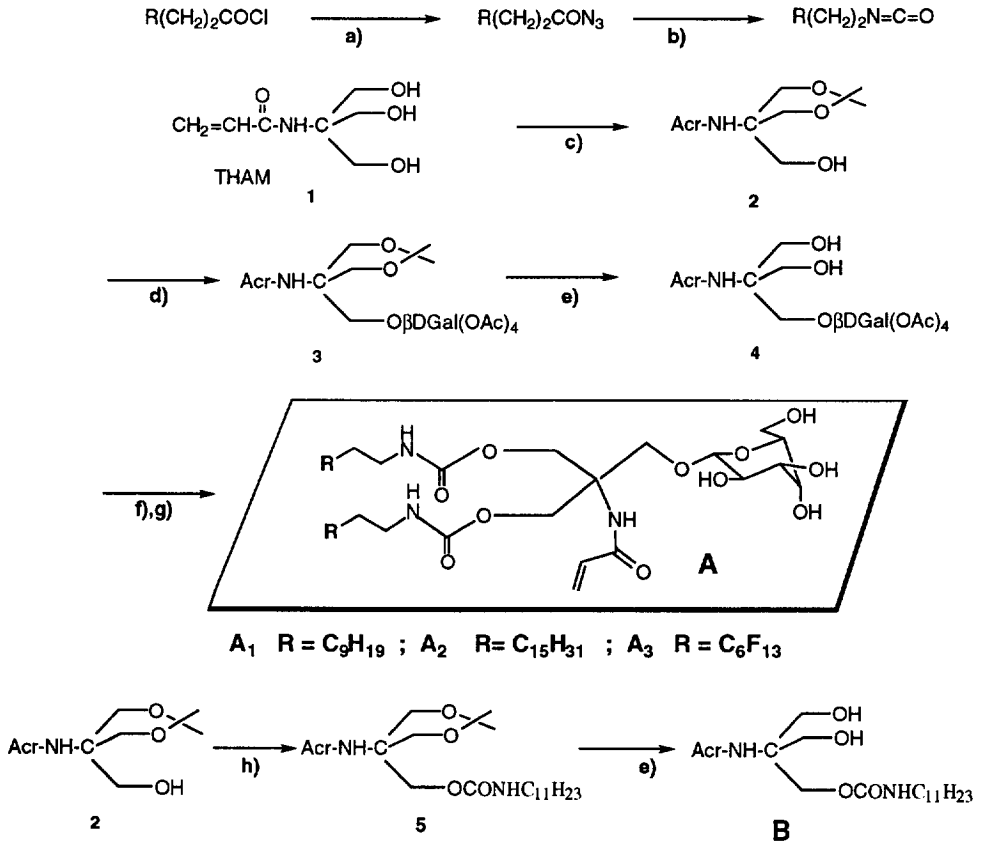
A synthetic route to compounds A is presented in scheme 1. This preparation calls several remarks :

-Glycosylation of THAM was effected according to Helferich procedure. After purification through column chromatography on silica gel, only β-anomers were obtained with 50% yield[11].

-After acid hydrolysis of ketal group, the hydrophobic chains were introduced on galactosylated THAM. For the sake of convenience, we chose to use high reactivity of isocyanate function. The condensation reaction of undecanoyl, heptadecanoyl or 2H,2H,3H,3H perfluorooctanoyl isocyanate on monogalactosylated THAM were carried out with good yield in hot toluene (50-60°C) under a nitrogen atmosphere. Pure diadducts were isolated after column chromatography on silica gel (AcOEt/Hexane).

-After acetyl group removing, glycolipids A_1-A_3 were isolated as amorphous solid. All compounds gave satisfactory elemental analysis and were fully characterized by NMR spectroscopy (^1H, ^{13}C, ^{19}F) and mass spectrometry[12].



A_1 R = C_9H_{19} ; A_2 R= $C_{15}H_{31}$; A_3 R = C_6F_{13}

a) : NaN_3, acetone /water (1/1) ; **b)** cyclohexane,Δ ; **c)** apts, dimethoxypropane, CH_2Cl_2, 12h, 80% ;
d) acetobromogalactose, $Hg(CN)_2$, CH_3CN, 20°, 24h, 50% ; **e)** CH_3CO_2H,H_2O (7/3), 60°,6h,70% :
f) $RCH_2CH_2N=C=O$, 2 eq., toluene, 50-60° ; **g)** MeONa, MeOH, 20°, 98% ;**h)** $C_{11}H_{23}N=C=O$, Toluene, 50°

Scheme 1 : Synthesis of mono and bicodal glycolipids derived from THAM.

Aqueous dispersion of hydrocarbon glycolipids A_1 or A_3 led after ultrasonication to a bluish translucid solution, whereas the higher glycolipid A_2 give a much more turbid solution. In each cases, one can observed formation of a vesicular system by using Transmission Electron Microscopy (TEM) after negative staining with

phosphotungstic acid. These vesicles are homogeneous for A_1 and A_3 derivatives. The C16 hydrocarbon compound A_2 coalesces.

After a week, dispersion of A_2 precipitates at room temperature, while solution of A_1 forms tubular systems (photo 1, observed by using TEM method) and A_3 sample slowly coalesce.

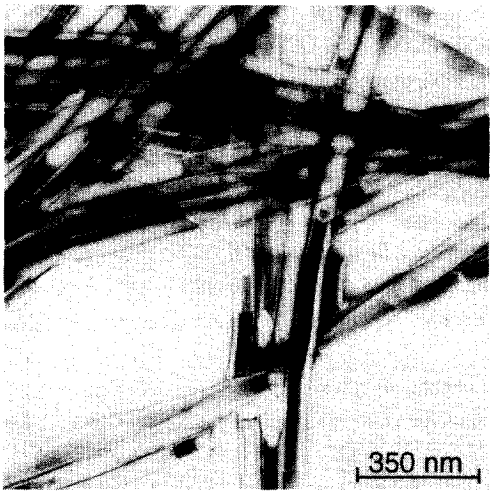


Photo 1 : Aqueous dispersion of A_1 after a week storage.

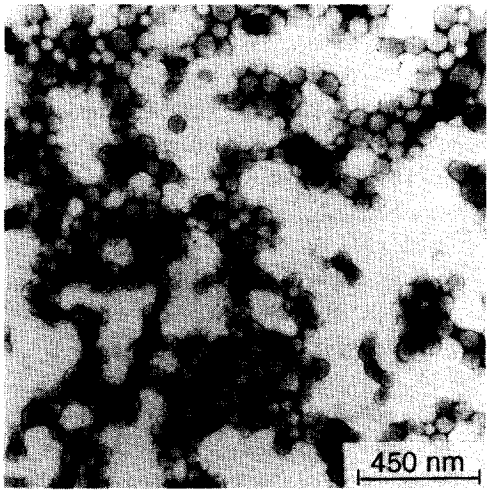


Photo 2 : Aqueous dispersion of a mixture A_1 and B (30% mole) after polymerisation

(TEM negative staining with phosphotungstic acid).

In order to stabilize these systems, we polymerized them in aqueous media after ultrasonication. Oxygen, which is a radical inhibitor, was removed from solution by bubbling nitrogen. The mixtures were heated at 80° C under nitrogen. The polymerization was unduced by UV irradiation (λ = 240-400 nm) until no remaining monomer was observed (5mn).

In one hand the polymerizations of hydrocarbon surfactants A_1 and A_2 were not achieved properly. After 5 minutes irradiation we observed a precipitate. In fact, it seems that the reticulation of polar heads induces steric hindrance which disorganizes the bilayer supramolecular structures. The polymer built in such conditions would not show a vesicular or bilayer structures anymore and would become hydrophobic.

On the other hand, with the perfluorocarbon compound A_3, the polymerization reaction was realized after 10 minutes irradiation. Then, we obtain a very stable translucent bluish solution which reveals homogeneous vesicles with a 40-50 nm average size. According to Israelachvili[13], in aqueous solution the molecular geometry of surfactant must follow precise constraints to give vesicular structures. Notably, it is necessary that the volume of bicodal surfactants occupies the shape of a truncated cone, the polar head forming the larger cone section. In this case, we can assume that the polymerization brings together the polar moieties, and therefore decreases their solvation volume. These effects modify the volume ratio between the polar head and the hydrophobic moiety which avoid the formation vesicular system. Concerning the fluorocarbon surfactant A_3, it seems reasonable to suggest that the rigidity and strong hydrophobic interactions between fluorinated chains balance the lateral tensions brought by reticulating the polar heads. Indeed, it is well known that aggregates formed by fluorinated surfactants are more organized and more stable than those formed by their direct hydrocarbon analogues[9].

According to the previous hypothesis, a decrease of lateral tensions between hydrocarbon tails should induce the formation of polymerized vesicular system.

Keeping the above remark in mind, the monomers A_1 and A_2 were dispersed in the presence of a monocodal cosurfactant with an analogous structure **B** in which the hydrophilic / hydrophobic volume ratio is higher. Such compounds should increase the bilayer curvature in water.

Compound **B** was prepared by grafting nonyl isocyanate with THAM isopropylidene derivative followed by an acid hydrolysis. Acrylamide and remaining hydroxyle functions constitute the polar moiety of this surfactant. This sample is insoluble in water even after sonication. However, its dispersion in the presence of the bicodal surfactants A_1 and A_2 gives homogeneous low diameter vesicles (40 to 100 nm).

These solutions were polymerized as previously described. No remaining monomers were observed after 5 minutes irradiation. A resulting translucent bluish solution without any precipitate was observed. Polymerization does not induce morphologic alteration, in each cases homogeneous vesicles (40 to 100 nm) were visible (photo 2). The dispersions were very stable on standing , we have noted no alteration (checked by TEM) after a 3 months period.

The first results obtained in this work allow us to formulate the following remarks.

-Polymerization of hydrophilic moities may induce steric hindrance. This phenomenon seems disorganize vesicle whereas the hydrocarbon tail reticulation via acrylamide functions (i.e polymerization inside the hydrophobic bilayers) does not induce disorder[8].

-The use in the mixture of monocodal polymerizable surfactants allow to i) modulate the hydrophilic / hydrophobic volume ratio, ii) minimize steric hindrance and lateral tensions, iii) stabilize the bilayers and produce stable polymerized vesicles.

The polymerization of these vesicules should not affect their recognition as a guest by galactose specific lectins. Such systems could be exploited as in vivo drug targeting and encapsulating agent.

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12/ Main physico chemical data of glycolipids. for example compound A_2 : $[\alpha]^{D}_{20}$ (c, 1, MeOH) = + 1.4 ; Mp = 169-170°C; NMR ^{1}H (CD_3OD) δ : 6.35-6.13 (2H, 2dd, Acryl). ; 5.66-5.64 (1H, dd, Acryl) 4.46-3.48 (CH Gal) ; 3.07 (4H, t, CH_2NH) ; 1.47-1.29 [$(CH_2)_n$] ; 0.90 (6H, t, CH_3). NMR ^{13}C (CD_3OD) δ : 170.36, 158.56,(C=O) ; 132.54, 126.79 (Acryl) ; 105.44 (C anomère) ; 76.78-62.45 (C sugar, C Tris) ; 41.89 (CH_2NH_2) ; 33.09-23.75 [(CH_2)n] ; 14.48 (CH_3). Anal. Calc. For $C_{49}H_{93}O_{11}N_3$: C 65.40, H 10.34, N 4.67 ; found : C 65.27, H 10.28, N 4.72.
Compound **B** : Mp = 60-61°C ; NMR ^{1}H ($CDCl_3$) δ : 6.71 (1H, s, NH) ; 6.34-6.10 (2H, 2dd, Acryl) ; 5.74-5.69 (1H, dd, Acryl) ; 4.31 (2H, s, CH_2OCO) ; 3.70-3.51 (4H, dd, CH_2OH) 3.20-3.12 (2H, m, CH_2NH) ; 1.49-1.35 [$(CH_2)_9$] ; 0.88 (3H, t, CH_3) ; NMR ^{13}C (CDCl3) δ : 166.90, 157.58 (C=O) ; 130.67,127.60 (CH_2=CH) ; 62.44, 62.27 (C tris) ; 41.32 (CH_2NH) ; 31.93-22.69 [$(CH_2)_9$] ; 14.11 (CH_3). Anal. Calc. For $C_{19}H_{36}O_5N_2$: C 61.29, H 9.60, N 7.50 ; found : C 61.03, H 9.75, N 7.60.

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