

BIOCOMPATIBLE NON -IONIC SURFACTANTS FOR BIOMEDICAL UTILIZATION : DOUBLE- TAILED HYDROCARBON AND/OR FLUOROCARBON SYNTHETIC GLYCOLIPIDS

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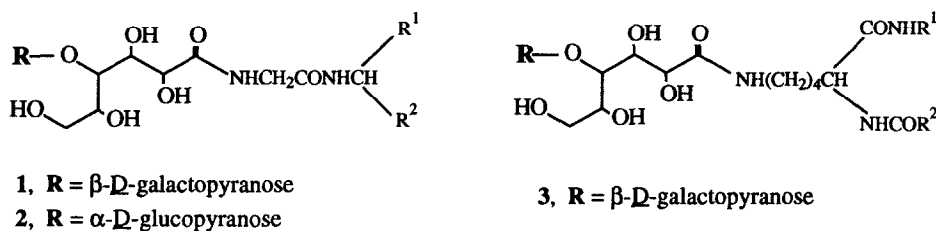
Abstract : Two classes of double-tailed hydrocarbon and/or fluorocarbon glycolipids containing either a gluco- or a galactopyranose residue in their hydrophilic head have been synthesized. The first was obtained upon opening a maltono- or lactonolactone moiety by the amino group of glycine followed by condensation of the glycine carboxylic function with a double-chain primary amine. The second involves L-lysine in place of glycine. Formation of vesicular structures upon conventional treatment was confirmed by preliminary measurements.

In the last decade researches have concentrated on potentialities provided by self-organized structures derived from amphiphilic molecules for *in vivo* carrying and/or delivering drugs and diagnosis agents. Among the numerous systems investigated, liposomes prepared from membrane phospho- and/or glycolipids or analogs have received particular attention¹⁻⁶.

Liposomes prepared from either natural or synthetic glycolipids may additionally be expected to target biomolecules via the specific recognition of the outer carbohydrate layer by cell membrane lectins. Preliminary results reported in this communication are part of a program aimed at assessing the potentialities and limits of synthetic glycolipid-made vesicles for *in vivo* drug delivery and cell targeting.

A series of biantennary hydro- and/or fluorocarbon non-ionic amphiphiles derived from lactose and maltose were synthesized. The structures of compounds **1a-1g**, **2** and **3** are shown in figure 1, together with partial physico-chemical data. These were obtained by treatment of a maltono- or lactonolactone moiety by a primary amine (see scheme 1 and 2) according to a procedure previously described^{7,8}. Reaction proceeds in good yield, provided the nucleophilic amine is unbranched, without protection of the carbohydrate moiety. In addition, the polyol chain resulting from the opening of the lactone ring is expected to : i) improve the packing of the surfactant molecules and facilitate the formation of vesicles, ii) confer to the galactopyranose (compounds **1a-1g**, **3**) and glucopyranose (compound **2**) terminus a highly flexible configuration and consequently a better accessibility to the solvent and/or the lectin receptor, iii) increase the hydrophilicity of the polar head compared to the sole monosaccharide. We decided to interpose an aminoacid spacer (glycine in compounds **1** and **2**, L-Lysine in compound **3**) between the hydrophilic head and the biantennary hydrophobic tail. This spacer plays a triple role : (1) it improves significantly the yield of the condensation step since in all cases the nucleophile is an unbranched primary amine, (2) it allows to further modulate the hydrophilic-lipophilic balance (HLB) while increasing at the same time the possibility of packing through intramolecular

hydrogen bonds, (3) in the case of L-lysine the α -amino and α -carboxy functionalities are readily condensed with respectively fatty acids and amines by conventional peptide-coupling methods. This strategy proved successful and allows the synthesis of novel amphiphilic molecules carrying either two hydrocarbon or two fluorocarbon chains or one of each as in the case of compound 3. Neither glycine, nor lysine should affect the biocompatibility of surfactants.

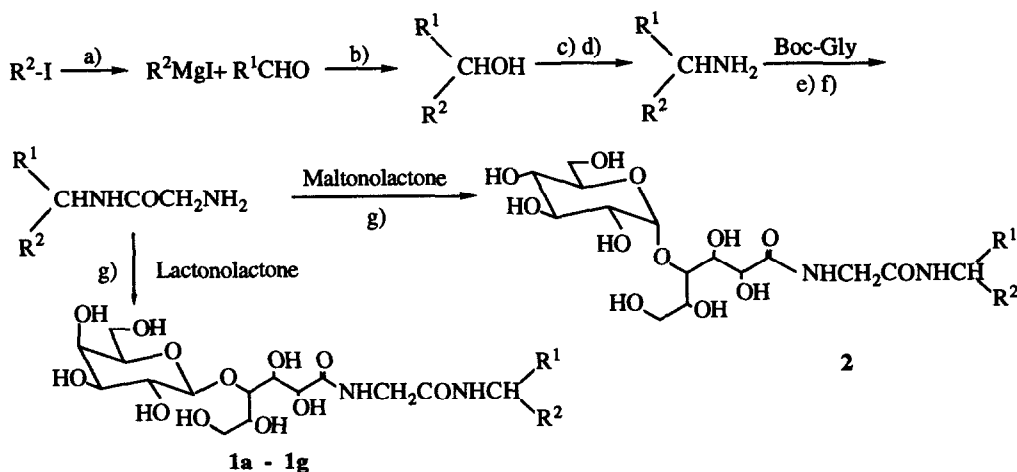


	R ¹	R ²	Yield a)	F ^{b)}	(α) _D ^{c)}
1a	-(CH ₂) ₈ CH=CH ₂	-(CH ₂) ₁₁ CH ₃	53.5	188	+13°
1b	-(CH ₂) ₁₀ CH ₃	-(CH ₂) ₁₁ CH ₃	67	154	+13.2°
1c	-(CH ₂) ₈ CH ₃	-(CH ₂) ₂ C ₆ F ₁₃	35	200	+13.3°
1d	-(CH ₂) ₈ CH=CH ₂	-(CH ₂) ₂ C ₆ F ₁₃	33	179	+15.4°
1e	-(CH ₂) ₈ CH ₃	-(CH ₂) ₂ C ₈ F ₁₇	21	163	+12.9°
1f	-(CH ₂) ₈ CH=CH ₂	-(CH ₂) ₂ C ₈ F ₁₇	32	175	+13°
1g	(CH ₂) ₂ S(CH ₂) ₂ C ₆ F ₁₃ ^{d)}	-(CH ₂) ₂ C ₆ F ₁₃	21	182	+13.3°
2	-(CH ₂) ₈ CH=CH ₂	-(CH ₂) ₁₁ CH ₃	44	162	+59.8°
3	-(CH ₂) ₁₀ CH ₃	-(CH ₂) ₂ C ₈ F ₁₇	16	171	+13°

a) Overall yield ; b) Décomposition ; c) In DMSO (c,1%) ; d) See ref.9

Figure 1. Schematic structure of compounds 1a-1g, 2, 3 and partial physico-chemical data.

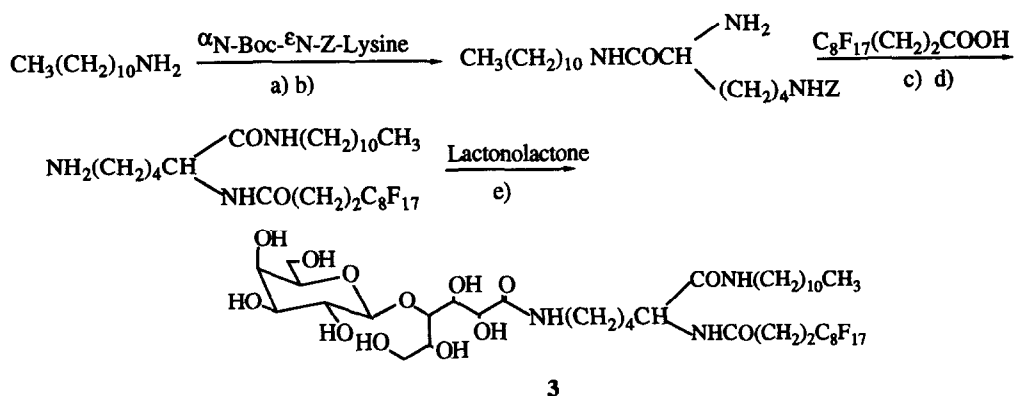
Fluorocarbon surfactants have proved more appropriate than hydrocarbon analogs for the emulsification of fluorodecaline (FDC) or perfluorooctylbromide (PFOB) that are used as oxygen carrier *in vivo*¹⁰⁻¹². In addition, single-chain fluorocarbon amphiphiles used as co-surfactants were shown to facilitate the formation of vesicles while increasing their stability¹³. Double-tailed hybrid surfactants 1c-1g were synthesized with the purpose to substantiate these observations and assess the contribution, if any, of the fluorocarbon branch to the stability of liposomes. Glycine- and lysine- containing surfactants were obtained according to the strategy summarized in scheme 1 and 2 respectively.



a) anhydrous Et₂O, Mg ; b) anhydrous Et₂O, Δ, 2h ; c) (PhO)₂P(O)N₃, EtOC(O)N=NC(O)OEt, Ph₃P, 1h, THF, 20°
 d) LiAlH₄, Et₂O ; e) DCC, HOBT, CH₂Cl₂, 6h, 20°C ; f) CF₃CO₂H, CH₂Cl₂, 2h, 20°C ; g) CH₃OH, Et₃N, Δ, 12h

Scheme 1. Synthetic route to hydrocarbon and fluorocarbon double-tailed surfactants containing a glycine spacer.

Conversion of the hydroxyl into the amino group was achieved in two steps producing the biantennary amine in good yield. It is worth noting that ultrasonication improved significantly the yield of the so-called Mitsunobu reaction¹⁴ (step c) while decreasing considerably the reaction time (5-10 min). Condensation of the amine with lactono- or maltonolactone prepared following the procedure described by Williams *et al*⁸ was performed in boiling methanol and slightly alkaline conditions. Compounds **1a-1g** and **2** were purified by column-chromatography on silica gel (AcOEt-MeOH 8:2, v/v) followed by gel filtration on Sephadex LH 60 (CH₂Cl₂- MeOH, 1:1, v/v) and isolated as amorphous white material.



a) DCC, HOBT, CH₂Cl₂, 20°C, 12h (98%) ; b) CF₃CO₂H, CH₂Cl₂, 1h (98%) ; c) DCC, HOBT, DMF, 20°C, 12h (40%)
 d) H₂, 10% Pd-C, CH₃OH (98%) ; e) CH₃OH, Δ, 12h (40%).

Scheme 2. Synthetic route to double-tailed non-ionic surfactants derived from lactose containing a lysine spacer.

The synthesis of compound **3** starting from α N-Boc- ϵ N-Z-L-Lysine is reported on scheme 2. Most of the steps proceeded in excellent yield. The reaction involving the fluorocarbon derivative (step c) was unsatisfactory whatever the coupling reagent : DCC-HOBT or EEDQ or BOP. This was ascribed to a reduced reactivity of the L-Lysine α -amino group as a consequence of steric hindrance as well as to the low solubility of reactants in DMF. Purification was achieved as previously described to afford compound **3** as a white amorphous powder insoluble in water.

When an aqueous suspension of compounds **1**, **2**, and **3** was sonicated according to conventional procedure¹⁵, clear solution was observed which indicates the formation of vesicular structures. Preliminary measurements by laser light scattering (LLS), transmission electron microscopy (TEM) and cryo-transmission electron microscopy (cryo-TEM) substantiates this behavior. For a given conditions of sonication (power, time and temperature) surfactant dispersion produced different vesicle microstructures which seemed to be correlated with the nature of both the carbohydrate head and the amino-acid spacer. Moreover, ultrasonication of fluorocarbon-tailed surfactants produced large nonuniform particles. Increasing the sonication time at the same power setting resulted in a dramatic reduction of both the polydispersity and size of the vesicles. Vesicle microstructures as well as stability are currently under investigation in our laboratory.

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References and Notes

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- 15 - We have selected compound **1a** as model compound. **1a** (30 mg) was coated on the wall of a glass tube upon removal of the solvent (CHCl₃-MeOH, 1:1, v/v, 4 ml). The tube was then evacuated under reduced pressure (10⁻³ mmHg, 3h) to make sure that there was no solvent left in the system. After highly purified water (3 mL) was added to the tube, the mixture was vortex mixed for about 3 min. The suspension was then sonicated for 15 min at 40°C (Branson B30, 7 mm probe, power 5, mode pulse 50) to give a slightly blue transparent solution which was submitted to spectral measurements.