ELSEVIER

Chemistry and Physics of Lipids
72 (1994) 153–173

Chemistry and Physics
of
LIPIDS

Vesicles and other supramolecular systems from biocompatible synthetic glycolipids with hydrocarbon and/or fluorocarbon chains

C. Guedj[a], B. Pucci[a], L. Zarif[b], C. Coulomb[c], J.G. Riess[b], A.A. Pavia*[a]

[a]*Laboratoire de Chimie Bioorganique,* [c]*Laboratoire de Biologie Cellulaire, Université d'Avignon, 33 Rue Louis Pasteur, 84000 Avignon, France*

[b]*Unité de Chimie Moléculaire associée au CNRS, Université de Nice Sophia-Antipolis, Parc Valrose, 06108 Nice Cedex, France*

Received 24 March 1994; revision received 15 June 1994; accepted 16 June 1994

Abstract

A series of double-tailed hydrocarbon and/or fluorocarbon glycolipids derived from galactose and glucose have been prepared. These compounds were obtained upon opening a lactono- and maltonolactone moiety by the amino group of either a glycine, glycylglycine or lysine residue. The carboxyl terminus of the glycyl and glycylglycine conjugates was further reacted with the appropriate double-tailed amine. In the case of lysine, the lactonamide conjugate was functionalized with a hydrocarbon and/or fluorocarbon fatty amine and acid, respectively. The ability of such glycolipids to disperse in water, the morphology of self-assemblies formed and the stability of the supramolecular structure obtained were shown to depend on the presence or absence and on the nature of the aminoacid spacer. Most of the compounds described were shown by conventionnal techniques (TEM, Cryo-TEM, LLS, etc.) to produce stable vesicular systems.

Keywords: Synthetic glycolipids; Aminoacids; Surfactants; Vesicles; Fibers; Electron microscopy

1. Introduction

First described in 1964 [1], classical vesicles or liposomes possessing closed hydrocarbon shells separating well-defined interior and exterior aqueous phases are prepared by dispersing lamellar liquid crystals of phospholipids in water. Since then, many other amphiphiles were shown to exhibit a phase behavior similar to that of lecithin and to form vesicles. In recent years, much research has concentrated on the potential provided by such self-organized structures for in vivo carriage and/or delivery of drugs, antigens, adjuvants or diagnosis agents. Among the numerous systems investigated, liposomes prepared from membrane phospho- and/or glycolipid analogs have received particular attention [2–7].

Even if, *a priori,* vesicles prepared from either natural or synthetic amphiphiles represent a promising approach, in vivo utilization of such systems suffers various limitations, especially that of rapid clearance from the bloodstream and accum-

* Corresponding author.

0009-3084/94/$07.00
SSDI 0009-3084(94)02351-5

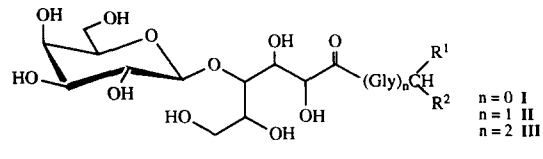
ulation in the reticuloendothelial system (RES). Indeed, one of the earliest and best-known observations in the field of liposome research is that parenterally injected liposomes are rapidly ingested by macrophages, particularly in the liver and spleen, where they are gradually degraded in lysosomal vacuoles [8–10]. Several modifications have been envisaged in order to minimize such drawbacks [11–13]; these include the utilization of polyethyleneglycol [14–16] or carbohydrate derivatives [17–19] as surfactants or cosurfactants of natural phospholipids.

Improving the vesicle-shell stability and drug encapsulation ability constitute other key objectives. Incorporation of a fluorinated film within the vesicle's membrane by using surfactants shows promise in this respect (Refs. [20–22], and F. Frezard, C. Santaella, P. Vierling and J.G. Riess, submitted for publication).

Liposomes prepared from either natural or synthetic glycolipids may additionally be expected to target biological molecules via the specific recognition of the outer carbohydrate layer by cell membrane lectins [3].

The goal of the research developed in our laboratories is to assess the potential and limits of synthetic glycolipid-made vesicles for in vivo drug delivery and cell targeting via specific membrane lectins. This paper deals with the synthesis and lyotropic behavior of a series of biantennary hydro- and/or fluorocarbon amphiphiles with a hydrophilic moiety derived from lactose or maltose. In order to modify the hydrophilic-lipophilic balance (HLB) of such amphiphiles, we decided to interpose an aminoacid (glycine or lysine) or a peptide (glycylglycine) spacer between the hydrophilic head and the double-chain. The general structures of such surfactants are shown in Fig. 1.

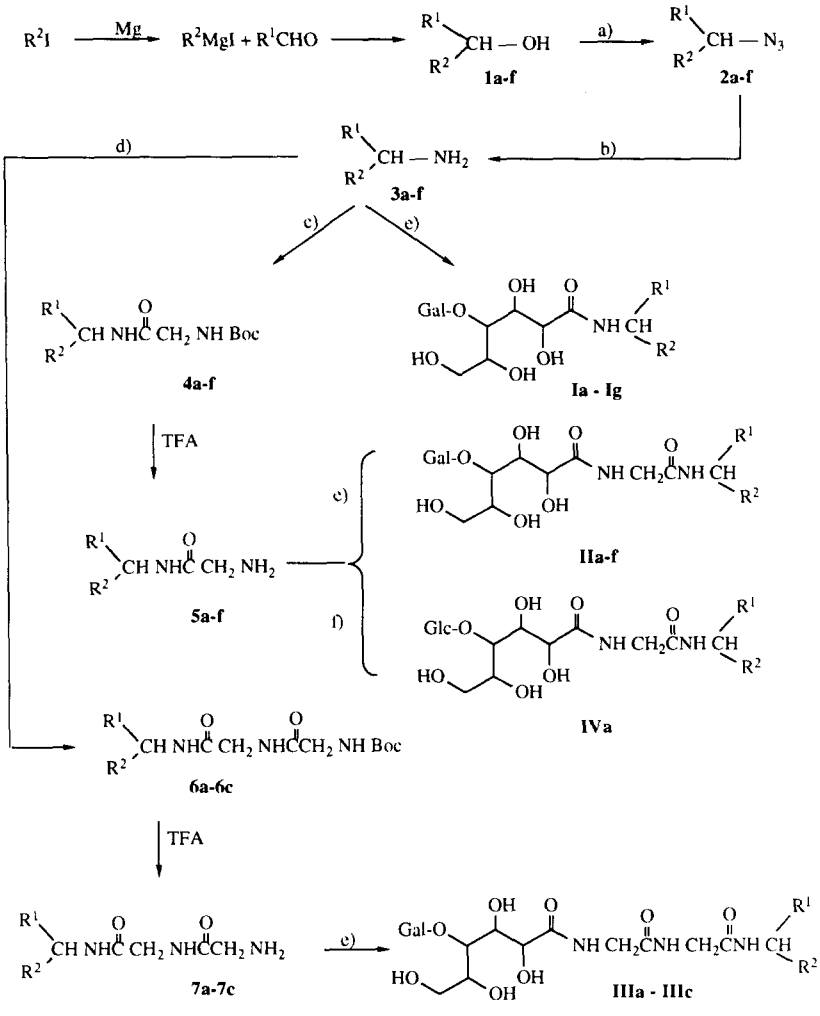
The formation of vesicular structures upon conventional treatment was assessed by laser light scattering (LLS) transmission electron microscopy (TEM) and cryo-TEM. The nature (glycine or lysine) and number (one or two) of aminoacids are determinant for both the dispersibility of amphiphilic molecules and their ability to produce vesicles or lamellar systems.



IV

V

Fig. 1. General structures of glycolipid surfactants; for the definitions of R^1 and R^2 see Table 1.



Scheme 1: Synthetic route to double-tailed hydrocarbon and fluorocarbon glycolipids containing either a glycyl or a glycylglycine spacer.

2. Results and discussion

The amphiphiles I to V were obtained by treatment of a malto- or a lactonolactone moiety by a primary amine according to a procedure previously described [23,24]. The method reported by Williams et al. [23] has several advantages: the reaction proceeds in good yield, 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 possibly facilitate the formation of vesicles, (ii) confer on the galacto- or glucopyranose terminus a more flexible configura-

tion and consequently a better accessibility to the solvent and/or the lectin receptor and (iii) increase the hydrophilicity of the polar head compared with the monosaccharide alone.

We first synthesized the glycolipid **I** (scheme 1) bearing a hydrophobic tail directly linked to the glycosidic residue by an amide bond. Such products not being dispersible in water upon sonication, we decided to interpose an aminoacid spacer (glycine in compounds **II–IV**, lysine in compound **V**) between the hydrophobic tail and the hydrophilic head. The spacer plays a triple role: (i) it significantly improves the yield of the condensation step, since in all cases the nucleophile is an unbranched primary amine, (ii) it allows further modulation of the HLB while at the same time increasing the possibility of packing through additional intermolecular hydrogen bonds and (iii) in the case of L-lysine, the α-amino and α-carboxy functionalities are readily condensed with fatty acids and amines, respectively, by conventional peptide-coupling methods to afford double-chain amphiphiles. In addition, neither glycine nor lysine should affect the biocompatibility of surfactants. Double-tailed hybrid surfactants (R^1: hydrocarbon, R^2: fluorocarbon chain) were designed with the purpose of introducing a bilayer rigidifying effect. These modifications were expected to increase vesicle stability and possibly lifetime in the bloodstream [12,25] and confer some impermeability on the vesicular membrane.

Glycine- and lysine-containing surfactants (Table 1) were obtained according to the strategy summarized in schemes 1 and 2, respectively.

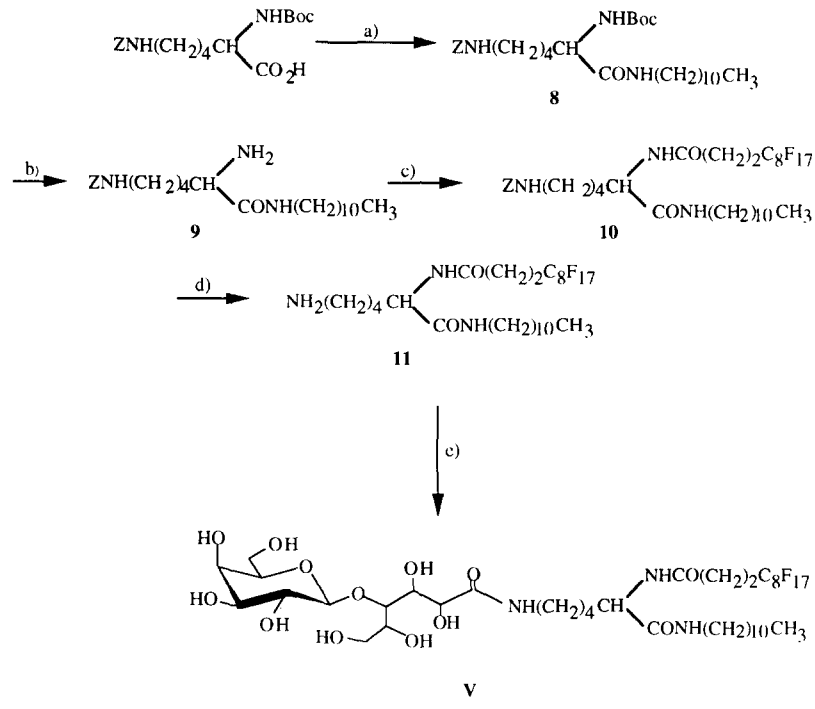
Grignard addition of alkylmagnesium bromide to aldehydes affords secondary alcohols in excellent yield; with a perfluoroalkyl reagent, we observed the formation of Wurtz by-products, which slightly reduce reaction yields. Conversion of the hydroxyl into the amino group was achieved in two steps, producing the biantennary amine in good yield. It is worth noting that ultrasonication improved significantly the yield of the so-called Mitsunobu reaction [26], while considerably decreasing the reaction time (5–15 min).

Condensation of the amine with *tert*-butyloxycarbonyl glycine (BocGlyOH) was achieved with dicyclohexyl carbodiimide (DCC) as coupling reagent. Final addition of amine **3**, **5** or **7** to lacto- or maltonolactone prepared following the procedure described by Williams et al. [23] was performed in boiling methanol and slightly alkaline conditions. Compounds **I** to **IV** were obtained as amorphous white material after purification by column chromatography on silica gel followed by gel filtration on Sephadex LH60.

Table 1
Partial physicochemical data for compounds **I–V**

	R^1	R^2	Yield (%)[a]	F (°C)[b]	$[\alpha]_D^{20}$[c]
Ia	$(CH_2)_8CH{=}CH_2$	$(CH_2)_{11}CH_3$	51	188	+18
Ig	$(CH_2)_9CH_3$	$(CH_2)_{10}CH_3$	33	182	+15.3
IIa	$(CH_2)_8CH{=}CH_2$	$(CH_2)_{11}CH_3$	53.5	188	+13
IIb	$(CH_2)_{10}CH_3$	$(CH_2)_{11}CH_3$	67	154	+13.2
IIc	$(CH_2)_8CH_3$	$(CH_2)_2C_6F_{13}$	35	200	+13.3
IId	$(CH_2)_8CH{=}CH_2$	$(CH_2)_2C_6F_{13}$	33	179	+15.4
IIe	$(CH_2)_8CH_3$	$(CH_2)_2C_8F_{17}$	21	163	+13.2
IIf	$(CH_2)_8CH{=}CH_2$	$(CH_2)_2C_8F_{17}$	32	175	+13
IIIa	$(CH_2)_8CH{=}CH_2$	$(CH_2)_{11}CH_3$	68	175	+19.2
IIIc	$(CH_2)_8CH_3$	$(CH_2)_2C_6F_{13}$	17	181	+15.2
IVa	$(CH_2)_8CH{=}CH_2$	$(CH_2)_{11}CH_3$	44	168	+59.8
V	$(CH_2)_{10}CH_3$	$(CH_2)_2C_8F_{17}$	16	171	+13

[a]Overall yield.
[b]Decomposition.
[c]In DMSO (c, 1).



a) $CH_3(CH_2)_{10}NH_2$, DCC, HOBT ; b) TFA, CH_2Cl_2 ; c) $C_8F_{17}(CH_2)_2CO_2H$, DCC, HOBT
d) H_2, Pd/C ; e) Lactonolactone, MeOH, Δ

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

For the synthesis of compound **V** (scheme 2), starting from N^{α}Boc-N^{ϵ}-Z-L-lysine, most of the steps proceeded with good yield. However, the reaction involving the fluorocarbon derivative was unsatisfactory whatever the coupling agent (DCC-HOBT, EEDQ, 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 the reactants, even in DMF. Purification was achieved as previously described, to afford compound **V** as a white amorphous powder, insoluble in water.

We thus obtained a series of double-tailed hydrocarbon or mixed fluorocarbon/hydrocarbon amphiphiles. In some cases (**IIa**, **IId**, **IIf**, **IIIa** and **IVa**) the hydrophobic chain bears a double bond in order to allow a local polymerisation with the purpose of further reinforcing the cohesion of the hydrophobic chains in the membrane's core [3].

Dispersion in water of each of the preceding products could be achieved by the classic sonication procedure. Supramolecular structures were assessed by two complementary techniques: negative-staining transmission electron microscopy and transmission electron microscopy after freeze-fracture. Particle size measurements were established by photon correlation spectroscopy, and the phase transition temperatures were determined whenever possible.

The effect of several structural parameters, especially the nature of the hydrophilic tail and of the aminoacid spacer, on the morphology and stability of the resulting organized systems was investigated. The impact of the addition of a cosurfactant on both the dispersibility and the organization of the new glycolipid amphiphiles was determined.

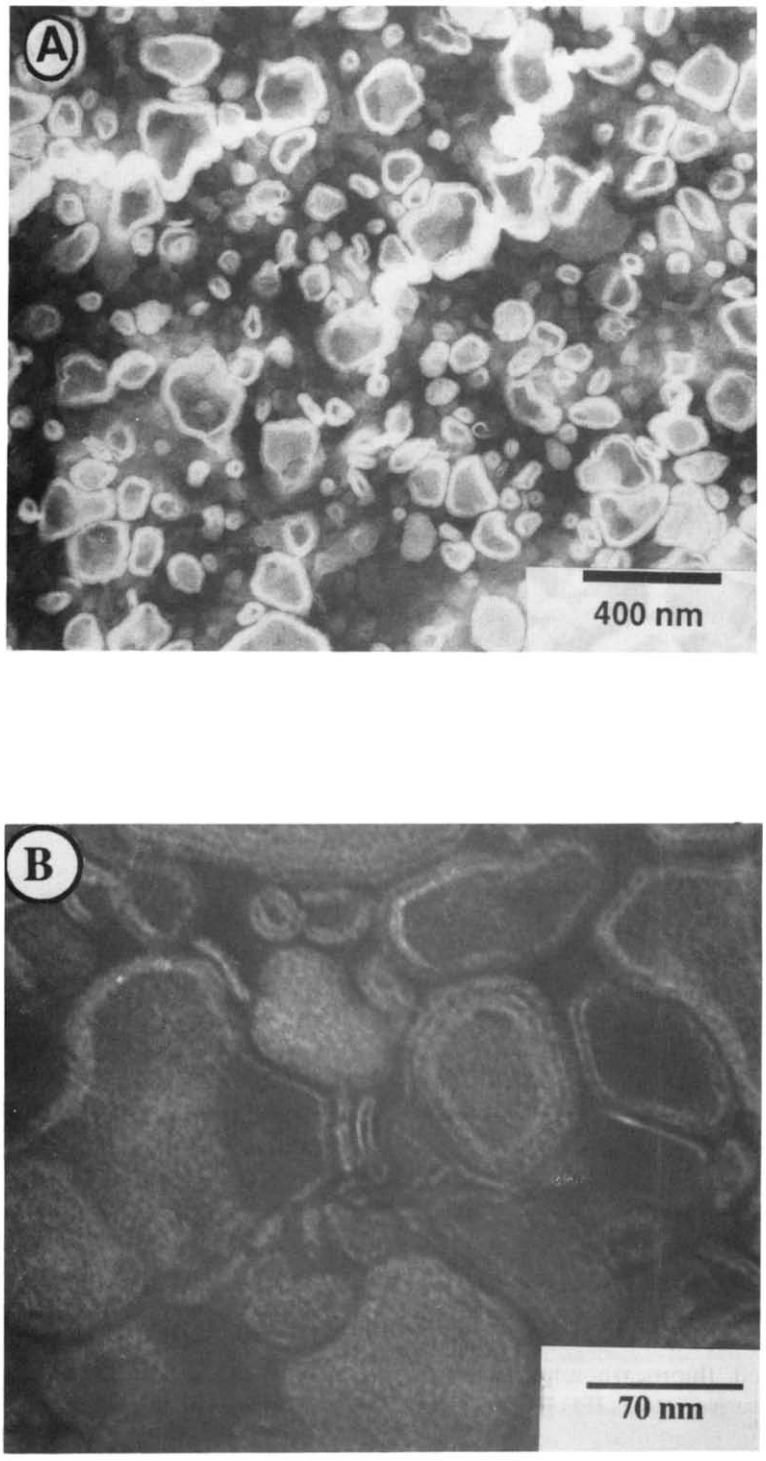


Fig. 2. Electron micrographs of compounds **IV** (A) and **IIa** (B) after negative staining by phosphotungtic acid

2.1. Effect of the nature of the hydrophilic head

Glycolipids derived from lactose and maltose showed different dispersibility behaviour. Compound **IVa**, with a glucose terminus, was easily dispersible (sonication 5 min, power setting 7, 7-mm probe, room temperature), while compound **IIa**, bearing a galactose terminus, needed more energy (sonication 15 min, power setting 7, 13-mm probe, room temperature). This difference in behavior can be related to the differences in the anomeric (α or β) configurations as well as in that of the hydroxyl group in position 4, which is equatorial and axial in glucose and galactose, respectively. In the galactose derivative hydrogen bonding can occur between position 4 and 6, thus diminishing the hydration possibility of the sugar ring; this situation does not exist for glucose (maltose derivative), and better hydration of the hydrophilic head is expected. The hydration process is known to be determinant for the preparation of vesicular structures. Both compounds, however, produced well-organized structures, mainly single-walled and multilayered vesicles for compounds **IVa** and **IIa**, respectively, as demonstrated by negative staining TEM (Fig. 2).

2.2. Effect of the nature of the hydrophobic tail

The mixed fluorocarbon/hydrocarbon glycolipids form dispersions that are more complex than those obtained from double-tailed hydrocarbon amphiphiles (**IIa–IIf**, Table 1). For example, a homogenous population of vesicles was obtained with compound **IIa** after sterilization (Fig. 3A), while in the same conditions **IId** formed two distinct populations (Fig. 3B).

2.3. Effect of the nature of the aminoacid spacer

Unusual morphology was obtained, depending on the nature of the aminoacid spacer that links the hydrophobic tail to the hydrophilic sugar head. Compounds **IIa–IIf** and **IVa**, containing a glycine spacer, readily formed multilayered vesicles (Fig. 2B), while compounds **IIIa** and **IIIc**, with a glycylglycine spacer, readily produced clear blue dispersions by gentle shaking, without sonication. The latter dispersions were shown to contain single-walled vesicles (Fig. 4 and Table 2).

Compound **V**, which is equipped with a lysine spacer, mainly yielded stacked disk-like assemblies as well as tubules and helically twisted structures

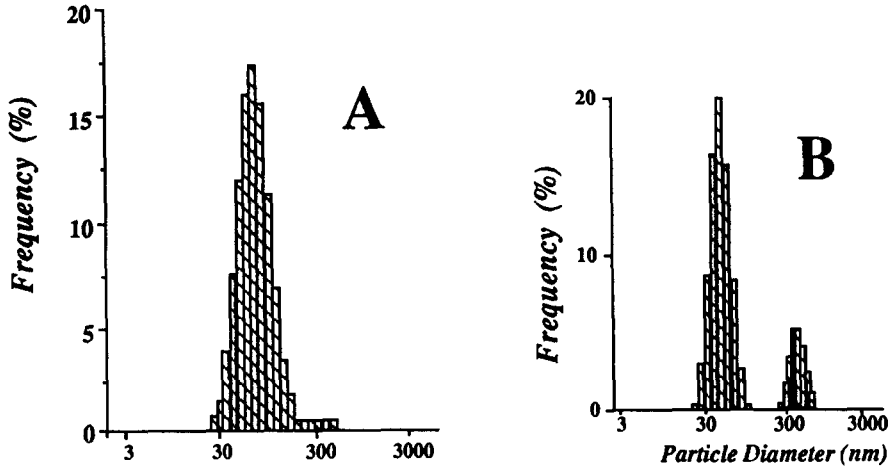


Fig. 3. Particle size distribution measured by LLS of (A) a 10^{-2} M aqueous sterilized dispersion of compound **IIa** and (B) a 10^{-2} M aqueous sterilized dispersion of compound **IId**.

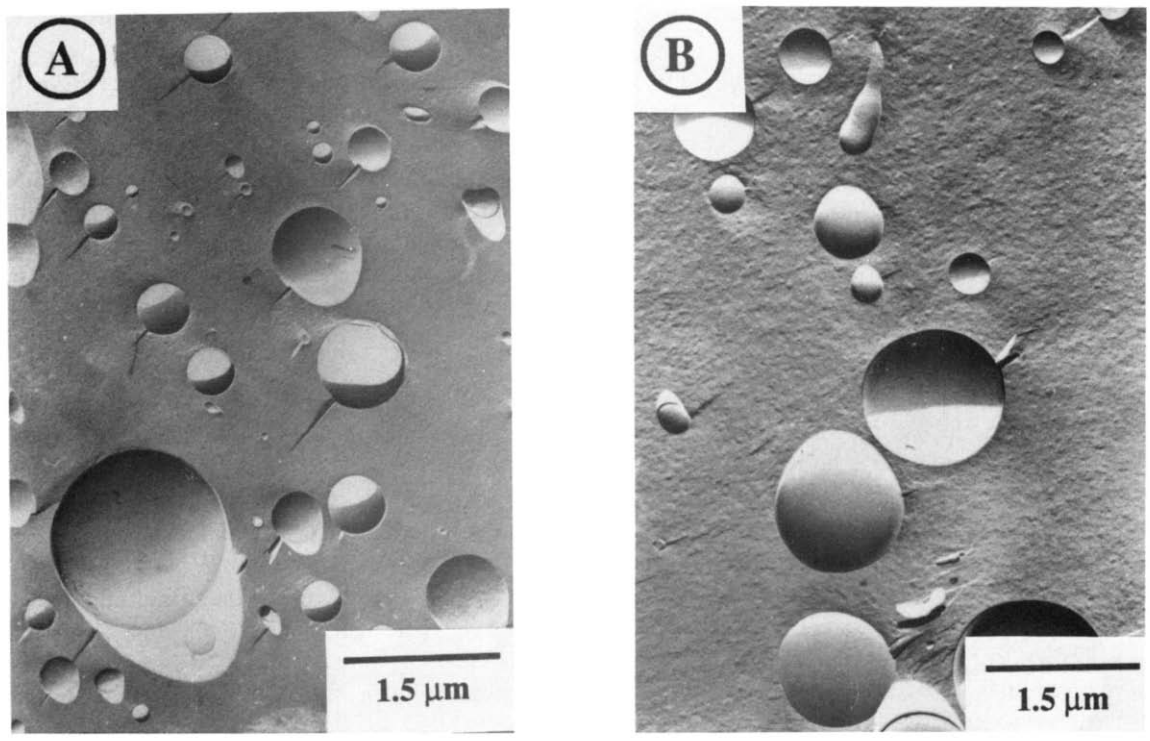


Fig. 4. Freeze-fracture electron micrographs of sonicated solutions of compounds **IIIa** (A) and **IIIc** (B).

(L = 30 nm), along with rare vesicles (Fig. 5A). Prolonged sonication favored the formation of helical superstructures (Fig. 5B).

Similar helical aggregates, already observed by Pfannemüller [27] with gluconamide-derived amphiphiles, are, according to this author, a consequence of the presence of hydroxyl groups and amide bonds in the hydrophilic head. In water,

Table 2
Impact of the nature of the aminoacid spacer on the dispersibility and morphology of glycolipid assemblies

Compounds	Concentration ($\times 10^2$ M)	Preparation condition[a]	Mean diameter (nm) (SD)	Observations
IIf	1	Vortex 2 min, Soni 13 mm, P_5 5 min, 40°C	1620 (780)	Multilayered vesicles
IIIa	6	Vortex 2 min, Soni 3 mm, P_5, RT, 5 min	40	Unilamellar vesicles
IIa	0.5	Vortex 2 min, Soni 13 mm, P_5 50%, 15 min, 30°C	160 (520)	Multilayered vesicles and membranes Polydisperse
IIIc	6	Vortex 2 min, Soni 3 mm, P_5, RT, 5 min	35	Unilamellar vesicles
IIc	1	Vortex 2 min, Soni 13 mm, P_5 50%, 20 min, 30°C	318 (110)	Multilayered vesicles and membranes Polydisperse

[a]Soni, sonicator probe; P_5, power 5; RT, room temperature.

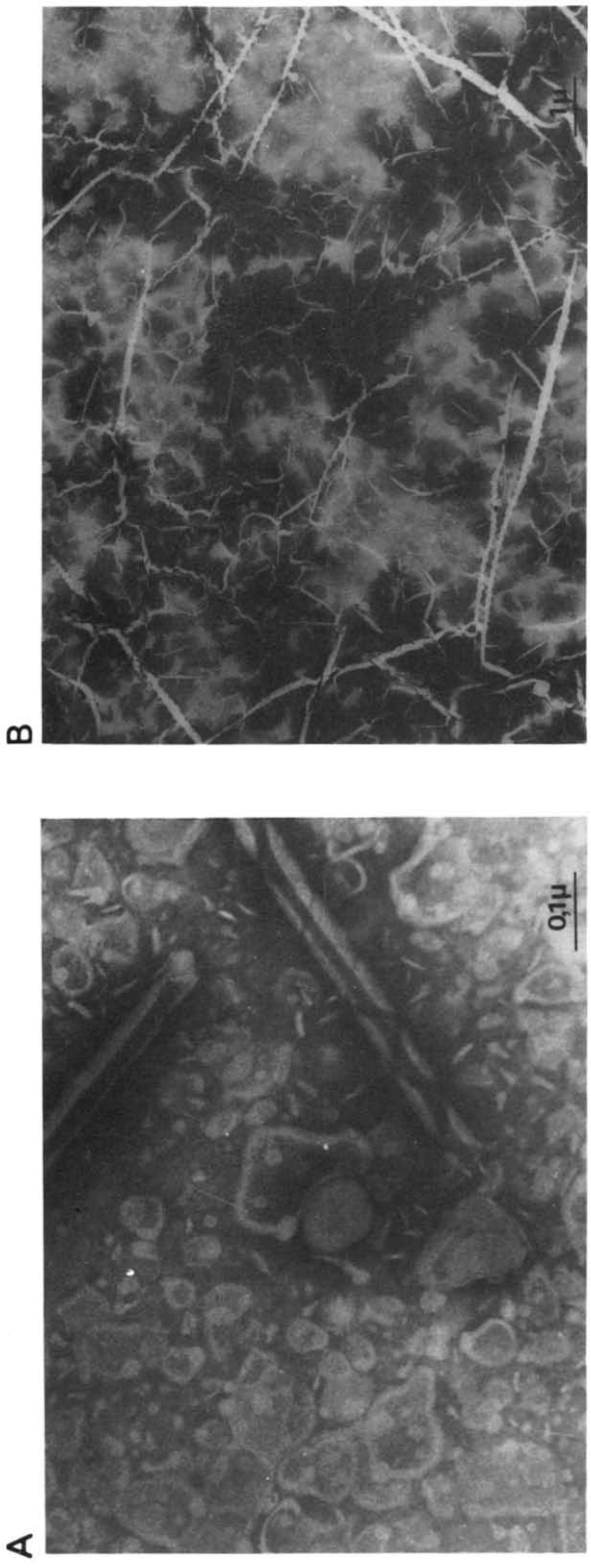
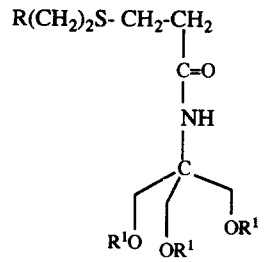


Fig. 5. Electron micrographs of compound V (after negative staining by phosphotungtic acid) before (A) and after (B) sonication.



R^1 = β-D-Gal

VI: R = C_8F_{17}

VII: R = $C_{10}H_{21}$

Fig. 6. Structure of tris-galactosylated surfactants **VI** and **VII**.

both features seem to promote intermolecular hydrogen bonds, resulting in the formation of gels. Quasi-crystalline aggregation of molecules is observed, producing fibrils [28]. Idaka [29] postulated that clockwise and/or anticlockwise helicity is related to the chirality of the aminoacid spacer. Hence L-*N*-(2-hydroxy)-dodecylvaline gives fibrous aggregates with right-handed helicity, whereas the D-enantiomer produces left-handed helices.

Kunitake [30] has also reported the evolution of a vesicular suspension obtained from double-chain ionic amphiphiles into helical structures. As noted by the author, this transformation is reversible: vesicles are recovered upon heating helix-containing systems above the phase-transition temperature.

2.4. Effect of the addition of a cosurfactant to the glycolipid dispersions

The main potential application of the glycolipid amphiphiles is for drug encapsulation and delivery; it has been shown that maximum efficacy in that respect is obtained with small unilamellar vesicles (SUV) [31].

The glycolipids **IIa–IIf**, with one glycine spacer, were shown to give mainly multilayered vesicles after brief sonification; more energy (40 min of sonication, 13 mm-probe, 40°C) must be applied to the resulting dispersions in order to obtain single-walled vesicles.

To overcome the risk of chemical degradation that may occur when more energy is applied, and in order to obtain a better dispersibility, we decided to add a cosurfactant to the glycolipid to be dispersed. Therefore a single-chain fluorocarbon surfactant derived from *O*-galactosyl-tris(hydroxymethyl)aminomethane [32] **VI** and the hydrocarbon analogs **VII** (Fig. 6) were added to the hybrid fluorocarbon/hydrocarbon glycolipid **IIc**

Table 3
Effect of the addition of a surfactant to glycolipid dispersions

Compounds	Concentration (× 10^2 M)	Preparation conditions[a]	Mean diameter (nm) (SD)	Observations
IIa	0.5	Vortex 2 min, Soni 13 mm P_5 50%, 15 min, 30°C	160 (520)	Multilayered vesicles and membranes, Polydisperse
IIa + VII	0.6 59% w/w of **VII**	Vortex 2 min, Soni 13 mm P_8 50%, 15 min, 30°C	60 (39)	Unilamellar vesicles
IIc	1	Vortex 2 min, Soni 13 mm, P_5 50%, 20 min, 30°C	318 (110)	Multilayered vesicles and membranes, Polydisperse
IIc + VI	0.6 60% w/w of **VI**	Vortex 2 min, Soni 13 mm P_5 50%, 15 min, 30°C	127 (63)	Unilamellar vesicles

[a]Soni, sonicator probe; P_5, power 5.

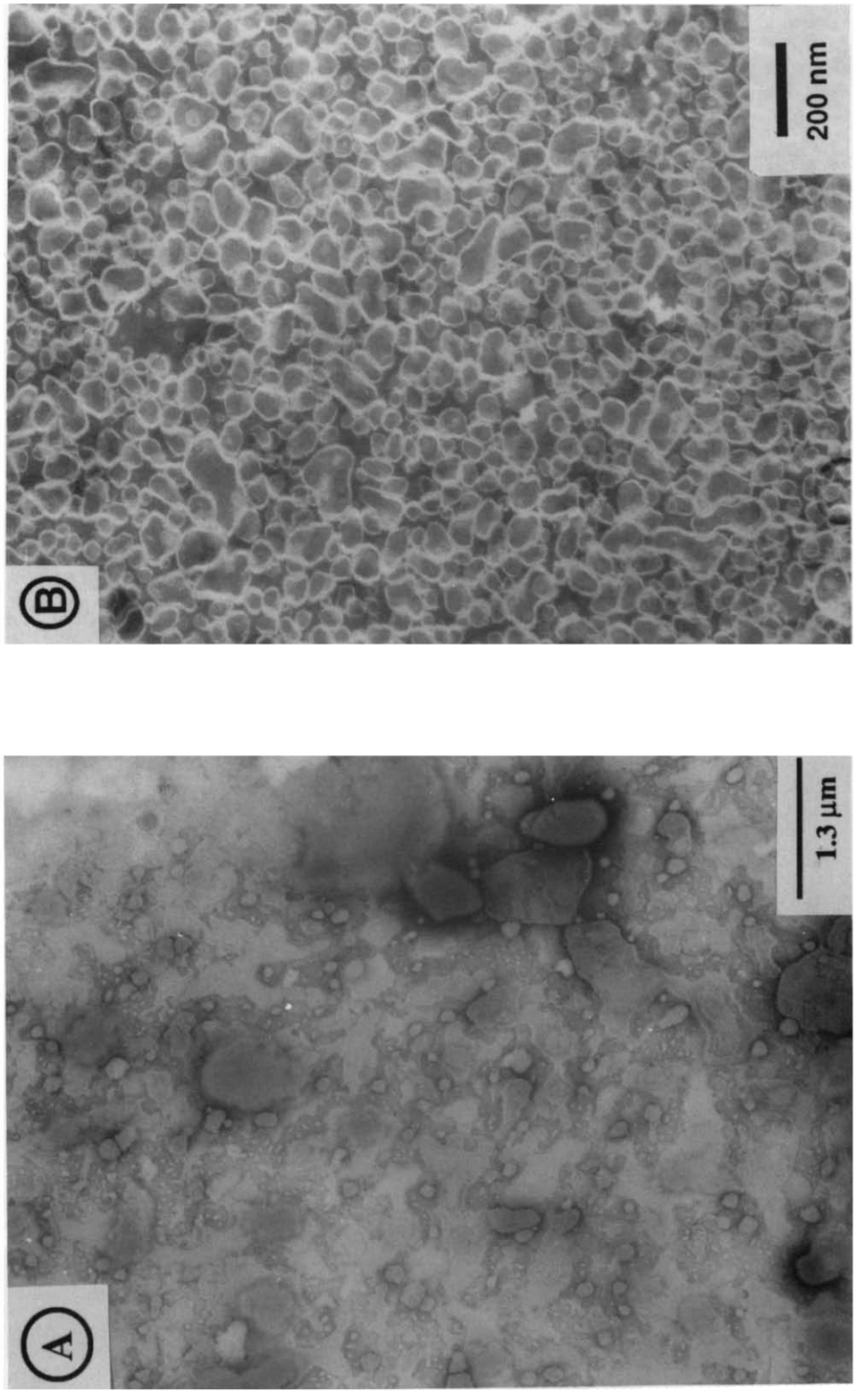


Fig. 7. Effect of the addition of a hydrocarbon cosurfactant. This diagram represents the electron micrographs (negative staining EM) of a dispersion of compound **IIa** (A) without cosurfactant and (B) with 60% (w/w) of the tris-derived cosurfactant **VII**.

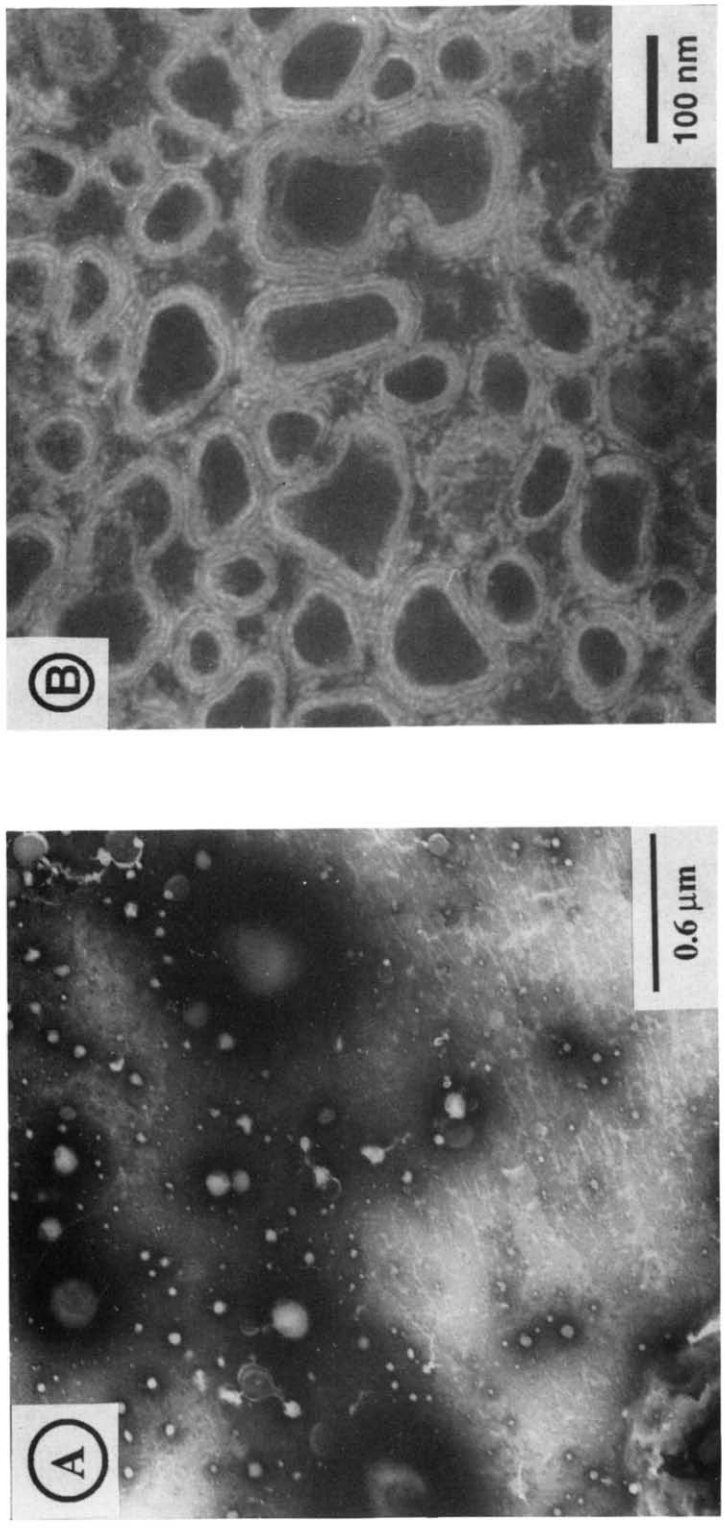


Fig. 8. Effect of the addition of a fluorocarbon cosurfactant. This diagram represents the electron micrographs (negative staining EM) of a dispersion of compound IIc (A) without cosurfactant and (B) with 59% of the tris-galactosylated derivative VI.

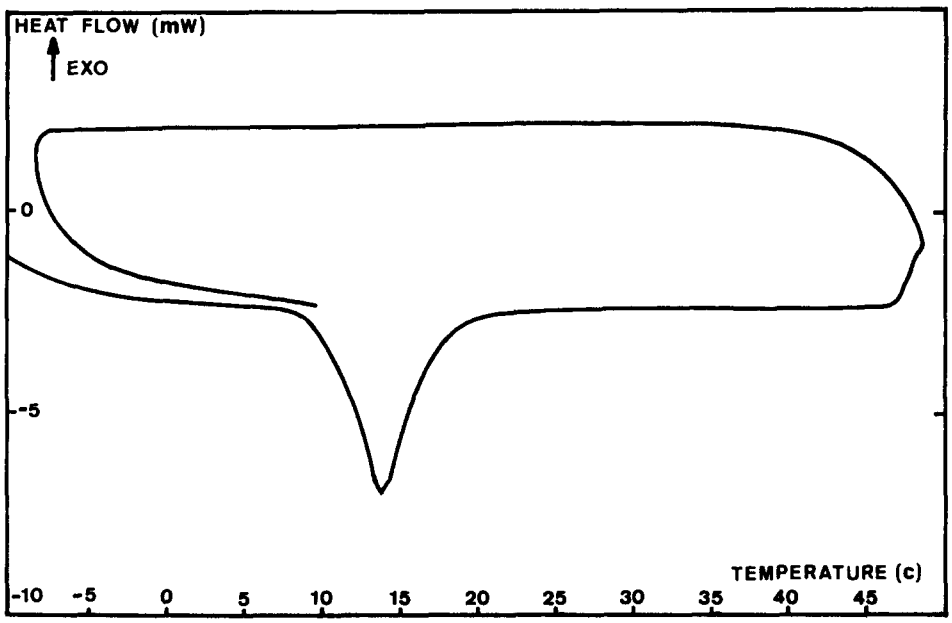


Fig. 9. Differential scanning calorimetry curves of bilayers of compounds **IIa** and **IIb** in a water/ethyleneglycol 60/40 mixture.

and to the hydrocarbon/hydrocarbon analog **IIa**, respectively. The hypothesis was that the addition of such a 'cosurfactant' would increase the curvature of the bilayers [4] and thus favor their arrangement into vesicles.

Table 3 shows how the aggregation behaviour was altered by the addition of the cosurfactant. Whereas compound **IIa** shows (TEM negative staining) the presence of multilayered vesicles of high polydipersibility along with non-closed membranes as demonstrated by photon correlation spectroscopy (ϕ = 160 ± 520 nm), in the same conditions the addition of ~ 60% w/w of the hydrocarbon cosurfactant **VII** to the glycolipid results in the formation of unilamellar vesicles with a small average diameter (ϕ = 60 ± 39 nm) (Table 3; Fig. 7).

The same phenomenon was observed with the fluorocarbon/hydrocarbon mixed amphiphile **IIc** upon addition of the fluorinated cosurfactant **VI**; the formation of SUV's is favored (Table 3; Fig. 8)

Table 4
Results of the biological tests performed on the glycolipid dispersions on mice and cell cultures

Compounds	Mice			Cell culture	
	Concentration (g/l)	Concentration (mg/kg body weight)	Survival ratio	Concentration (g/l)	Result (% v. controls) growth/viability
IIa	20	500	10/10	0.1	86/86
IId	20	500	10/10	0.1	72/100
IIf	20	500	10/10	0.1	—

2.5. Thermotropic behavior

The DSC thermograms of compounds **IIa** and **IIb** show that the phase transition temperature (T_c) of the hydrocarbon bilayers is detected at 22°C ($\Delta H = -11.6$ mcal/mg) and 14°C ($\Delta H = -10.29$ mcal/mg), respectively (Fig. 9).

No phase transition temperature could be determined for the fluorocarbon/hydrocarbon hybrid amphiphiles, even for compounds **IIc**, **IId** and **IIf**, which were shown to give multilayered vesicles. This behaviour should be ascribed to the small enthalpy change that occurs in the case of mixed bilayers when the membrane structure changes from the gel state to the fluid state and vice versa, upon heating and cooling, respectively.

2.6. Stability of the liposome systems

An important requirement for material destined for drug delivery is its capability to withstand heat (sterilization). Another important requirement is the stability of this drug delivery material upon storage.

The glycolipid-based amphiphiles reported here proved to withstand standard heat-sterilization conditions (121°C, 15 min, 15 psi). Photon correlation spectroscopy measurements were used to assess this stability. The dispersions remained stable in terms of particle sizes and particle size dispersion for at least 1 month at room temperature after sterilization.

2.7. Biological tolerance

Preliminary biological tests performed on these new glycolipid dispersions are promising. Compounds **IIa**, **IId** and **IIf** were well tolerated (100% of survival) when injected intraperitoneally in mice at a dose of 500 mg/kg body weight ($n = 10$). The glycolipid derivatives were innocuous on cell cultures at concentrations of 0.1 g/l (Table 4).

3. Experimental procedures

The progress of the reactions and the homogeneity of the compounds were monitored by thin-layer chromatography (TLC, Merck 254). Compound detection was achieved by iodine absorption or exposure to UV light (254 nm), by spraying a 50% sulfuric acid methanolic solution or 5% ninhydrin ethanolic solution (to detect the amine-containing compounds) and heating at 150°C. Purifications were performed by column chromatography over silica gel (Merck 60) or on permeation gel Sephadex LH 60 (Pharmacia LKB). Melting points were measured on a Tottoli apparatus and are reported uncorrected. The ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded at 250 MHz on a Bruker AC 250 apparatus. Chemical shifts are given in ppm relative to tetramethylsilane using the deuterium signal of the solvent as a heteronuclear reference for ^{1}H and ^{13}C and relative to trichlorofluoromethane as an internal reference for ^{19}F. Elemental analyses were performed by the Service Central de Microanalyses of the CNRS at Montpellier. Mass spectra were recorded on a DX 300 Jeol apparatus.

Sonication was performed by pulse method power 6 with a titanium probe of 13 mm diameter on a Branson B-30 cell disruptor working at a frequency of 20 kHz with a maximum power of 350 W.

3.1. Synthesis

Example: 11-(*N*-1-lactobionocarbonylglycinamido) trieicos 1-ene **IIa**

3.1.1. Trieicos 1-en 11-ol **1a**

Undec 10-enal (7.57 g; 0.045 mol) diluted in 50 ml of anhydrous ether was added dropwise under inert atmosphere to 45 ml of a molar solution of dodecyl magnesium bromide. The reaction mixture was maintained for 1.5 h at boiling point, then the solution was cooled and 100 ml of a saturated solution of ammonium chloride was added. After decantation, the organic phase was washed with water, dried over sodium sulfate and concentrated under reduced pressure. After chromatography over a silica gel column (eluent: ether/hexane, 1/1), an amorphous white solid (14 g) was isolated.

Yield 92%; m.p. = 65°C. ^{1}H-NMR ($CDCl_3$): 5.8 (1H, m, CH=); 4.9 (2H, m, CH_2=); 3.6 (1H, m; >CHOH); 2 (2H, td, =CH—CH_2); 1.3 (36H, m, CH_2); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$):

139.21 ($CH{=}CH_2$); 114.12 ($CH{=}CH_2$); 72.03 ($>CHOH$); 14.11 (CH_3). MS: 429 (M-H$^+$ glycerol)$^+$; 337 (M-H)$^+$; 321 (M-OH)$^+$.

3.1.2. 11-Azido trieicos 1-ene **2a**

To a mixture of diethyl azodicarboxylate (DEAD) (191.4 mg; 1.1 mmol), alcohol **1a** (338 mg; 1 mmol) and triphenyl phosphine (288 mg; 1.1 mmol) in anhydrous tetrahydrofurane (THF), stirred for a few minutes at room temperature, was added diphenyl phosphorazide (302.5 mg; 1.1 mmol). This solution was submitted to ultrasound for 15 min (mode pulse 1/1, power 7). After the complete disappearance of alcohol **1a** (CCM), THF was evaporated under reduced pressure and the residue chromatographed on a silica-gel column (hexane). The azide **2a** (350 mg; 96%) was recovered as an oil.

^{1}H-NMR ($CDCl_3$): 5.8 (1H, m, CH=); 4.9 (2H, m, CH_2=); 3.2 (1H, m, $>CHN_3$); 2 (2H, td, $=CHCH_2$); 1.4 (36 H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 139.18 ($CH{=}CH_2$); 114.14 ($CH{=}CH_2$); 63.17 ($>CHN3$); 14,1 (CH_3).

3.1.3. 11-Amino trieicos 1-ene **3a**

The azide **2a** (3 g; 8.28 mmol) was dissolved in anhydrous diethyl ether (150 ml). To the solution cooled to 0°C was added a 1-M solution (10 mmol) of lithium aluminium hydride ($LiAlH_4$) in ether (10 ml). After 15 min the reduction of the azide was completed. The excess of $LiAlH_4$ was destroyed by the addition of water. After filtration of the aluminium hydroxide, the organic phase was dried over sodium sulfate and concentrated under reduced pressure. Amine **3a** (2.72 g; 98%) was isolated as an oil.

^{1}H-NMR ($CDCl_3$): 5.8 (1H, m, CH=); 4.9 (2H, m, CH_2=); 2.7 (1H, m, $>CHNH_2$); 2 (2H, td, $=CHCH_2$); 1.3 (36H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 139.22 ($CH{=}CH_2$); 114.12 ($CH{=}CH_2$); 51.29 ($>CHNH_2$), 14.11 (CH_3).

3.1.4. 11-(N-tert-butyloxycarbonyl glycinamido) trieicos 1-ene **4a**

To anhydrous methylene chloride (20 ml) was added the amine **3a** (0.919 g; 2.72 mmol), dicyclohexylcarbodiimide (DCC) (0.674 g; 3.27 mmol), *N-tert*-butyloxycarbonylglycine (Boc-GlyOH) (0.572 g; 3.27 mmol) and 100 mg of hydroxybenzotriazole (HOBT). After 15 min stirring at room temperature the reaction was completed. The dicyclohexylurea (DCU) formed was filtered off, the solvent evaporated under reduced pressure and the residue chromatographed on silica-gel column (ether/hexane 1/1). Product **4a** (1.3 g; 97%) was isolated as a wax.

^{1}H-NMR ($CDCl_3$): 6.14 (1H, d, NH); 5.8 (1H, m, CH=); 5.43 (1H, m, NH); 4.9 (2H, m, CH_2=); 3.9 (1H, m, >CH); 3.7 (2H, d, $CH_2C(O)$); 2 (2H, td, $=CH{-}CH_2$); 1.5 (9H, s, $(CH_3)_3$); 1.3 (36 H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 168.92 (C(O)NH); 155.02 (—O—C(O)NH); 139.17 ($CH{=}CH_2$); 114.13 ($CH{=}CH_2$); 78 ($C(CH_3)_3$); 49.44 (CH_2-gly-NH); 44.11 (>CHNH); 14.11 (CH_3). MS: 493 M-H$^+$; 395 $NH_3CH_2C(O)NHCHR_1R_2$]$^+$; 336 $NHCHR_1R_2$]$^+$.

3.1.5. 1-Perfluorohexyl 3-dodecanol **1c**

$C_6F_{13}CH_2CH_2I$ (9.48 g; 20 mmol) dissolved in 40 ml of anhydrous diethyl ether was added under inert atmosphere to 1 g (41.6 mmol) of magnesium in anhydrous diethyl ether. When the reaction had started the solution of alkyl iodide was added dropwise so as to maintain refluxing. At the end of the addition refluxing was continued for 1 h; decanal (3.7 g; $2.4 \cdot 10^{-2}$ mol) was added dropwise. The solution was then treated as above. Pure alcohol **1c** (6.25 g, 62%, m.p. = 70–72°C) was isolated. ^{19}F-NMR ($CDCl_3$): −81.31 (3F, CF_3); −114.82 (2F, $CF_2\alpha CH_2$); −122.36 (2F, $CF_2\beta CH_2$); −123.16 (2F, $CF_2\gamma CH_2$); −123.88 (2F, $CF_2\delta CH_2$); 126.82 (2F, $CF_2\epsilon CH_2$). ^{1}H-NMR ($CDCl_3$): 3.65 (1H, s, $>CHOH$); 2.4–2 (2H, m, CH_2CF_2); 1.8–1.6 (2H, m, $CH_2CH_2CF_2$); 1.47–1.27 (16H, m, $(CH_2)_8$); 0.9 (3H, t; CH_3). ^{13}C-NMR ($CDCl_3$): 70.92 (>CH—OH); 14.1 (CH_3). MS: 503 M-H$^+$.

Products **1** to **6** were prepared according to the procedure described above. ^{1}H, ^{13}C and ^{19}F spectra were in accordance with the expected structures.

3.1.6. 12-Tetraeicosanol **1b**

From 8.28 g (0.045 mol) of dodecanal and 45 ml of a molar solution of dodecyl magnesium bromide, 15.3 g of alcohol **1b** was obtained. Yield 96%, m.p. = 70°C.

3.1.7. 12-Azidotetraeicosane **2b**

Procedure identical to that followed for **2a**. An oil was obtained in 92% yield.
^{1}H-NMR ($CDCl_3$): 3.21 (1H, m; CHN_3); 1.5–1.2 (42 H, m, $(CH_2)_{21}$); 0.9 (6H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 63.19 (CHN_3); 14.13 ($2CH_3$).

3.1.8. 12-(N-tert-butyloxycarbonylglycinamido) tetraeicosane **4b**

The procedure was identical to that followed for **4a**. A wax was obtained, in 98% yield, from **2b**.
^{1}H-NMR ($CDCl_3$): 5.85 (1H, d, NH); 5.2 (1H, m, NH); 3.9 (1H, m, >CH); 3.76 (2H, d, $CH_2\alpha$); 1.45–1.11 (51 H, m, $(CH_2)_{21}$ + $(CH_3)_3$); 0.9 (6H, t, 2 CH_3). ^{13}C-NMR ($CDCl_3$): 167.36 (C(O)NH); 155.05 (—C(O)O); 78.05 (C ^{t}Bu); 47.99; 43.89; 12.67.

3.1.9. 3-(N-tert-butyloxycarbonylglycinamido) 1-perfluorohexyldodecane **4c**

Procedure identical to that followed for **4a**. **4c** (96%) was isolated as a wax.
^{1}H-NMR ($CDCl_3$): 6.14 (1H, d, NH); 5.26 (1H, t, NH); 4 (1H, m, >CH); 3.8 (2H, d, $CH_2\alpha$); 2.3–2 (2H, m, CH_2CF_2); 1.9–1.5 (2H, m, $CH_2CH_2CF_2$); 1.4–1.3 (25H, m, $(CH_2)_8$ + $(CH_3)_3$); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 167.57; 156; 80; 49.7; 45; 14.09. ^{19}F-NMR ($CDCl_3$): −81.28; −114.79; −122.4; −123.22; −123.70; −126.03. MS: 661 M + H^{1+}.

3.1.10. 3-(N-tert-butyloxycarbonylglycinamido) 1-perfluorohexyltridec 12-ene **4d**

Procedure identical to that followed for **4a**. From **3d**, **4d** was isolated as wax (yield 90%).
^{1}H-NMR ($CDCl_3$): 6.1 (1H, d, NH); 5.8 (1H, m, CH=); 5.2 (1H, t, NH); 4.9 (2H, m, CH_2=); 3.96 (1H, m, >CH); 3.77 (2H, d; $CH_2\alpha$); 2.19–2 (4H, m, CH_2CF_2 + =CHCH_2); 1.86–1.6 (2H, m, $CH_2CH_2CF_2$); 1.58–1.39 (9H, s $(CH_3)_3C$); 1.27–1.16 (14H, m, $(CH_2)_7$). ^{13}C-NMR ($CDCl_3$): 169.58; 155; 139.16 (CH=CH_2); 114.18 (CH_2=CH); 80.1; 48.71; 45. ^{19}F-NMR ($CDCl_3$): −81.26; −114.7; −122.38; −123.18; −123.66; −126.6.

3.1.11. 3-(N-tert-butyloxycarbonylglycinamido) 1-perfluorooctyldodecane **4e**

Procedure identical to that used for the synthesis of **4a**. From **3e**, **4e** was isolated as a wax (yield 92%).
^{1}H-NMR ($CDCl_3$): 6.02 (1H, d, NH); 5.16 (1H, t, NH); 3.96 (1H, m, CH); 3.75 (2H, d, $CH_2\alpha$); 2.15–2 (2H, m, CH_2CF_2); 1.86–1.81 (2H, m, $CH_2CH_2CF_2$); 1.63–1.45 (25H, m $(CH_3)_3$ + $(CH_2)_8$); 0.9 (3H, t; CH_3). ^{13}C-NMR ($CDCl_3$): 169.5; 153.3; 77.8; 48.68; 44.94; 14.04. ^{19}F-NMR ($CDCl_3$): −80.6 (3F, CF_3); −114.4 (2F, $CF_2\alpha$); −122.38 (6F, $CF_2\beta$, γ et δ CH_2); −122.66 (2F, $CF_2\gamma CF_3$); −123.6 (2F, $CF_2\beta CF_3$); −126.6 (2F, $CF2\alpha CF_3$). MS: 761 M + H^{1+}; 661 NH_3CH_2-(CO)NHCHR^{1}R$^{2]+}$; 602 NHCHR^{1}R$^{2]+}$; 476 NHCHR^{1}R$^{2]+}$.

3.1.12. 3-(N-tert-butyloxycarbonylglycinamido) 1-perfluorooctyltridec 12-ene **4f**

Procedure identical to that used for the synthesis of **4a**. **4f** was isolated as a wax (yield 71%).
^{1}H-NMR ($CDCl_3$): 5.97 (1H, d, NH); 5.8 (1H, m, CH=); 5.11 (1H, m, NH); 4.95 (2H, m, CH_2=); 3.96 (1H, m, CHNH); 3.76 (2H, d, $CH_2\alpha$); 2.14 (4H, m, CH_2CF_2 + CH_2CH=); 1.85 (2H, m, $CH_2CH_2CF_2$); 1.57 (9H, s, $(CH_3)_3$); 1.45 (14H, m, $(CH_2)_7$). ^{13}C-NMR ($CDCl_3$): 169; 155.2; 139.18; 114.15; 80.1; 48.69; 43.15. ^{19}F-NMR ($CDCl_3$): −81.2; −114.7; −122.6; −123.18; −123.66; −126.6.

3.1.13. 11-(N-1-tert-butyloxycarbonylglycylglycinamido) trieicos 1-ene **6a**

To CH_2Cl_2 (50 ml) were added BocGlyGlyOH (1.13 g; 4.89 mmol), 11-amino 1-trieicosen **3a** (1.5 g; 4.45 mmol), DCC (1 g; 4.89 mmol) and finally HOBT (1 g). The reaction was completed after 1 h stirring at room temperature. The DCU formed was filtered off, the solvent evaporated and the residue chromatographed on a silica gel column (hexane/Et_2O 1/1). Product **6a** (2.207 g; 90%) was isolated as a wax.
^{1}H-NMR ($CDCl_3$): 7.47 (1H, t,NH); 6.7 (1H, d, NH); 5.74 (2H, m, CH= and NH); 4.94 (2H, t, CH_2=); 3.9 (4H, system AB, CH_2C(O)—); 2.04 (2H, m, CH_2—CH=CH_2); 1.44 (9H, m, $(CH_3)_3C$);

1.3 (36H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR ($CDCl_3$): 168.74; 166.75; 154.61; 137.44; 112.47; 78.34; 48.09; 42.59; 41.53; 12.44.

3.1.14. 3-(N-tert-butyloxycarbonylglycinamido) 1-perfluorohexyldodecane **6c**

Procedure identical to that used for the synthesis of **6a**. Compound **6c** was isolated as a wax in 97% yield.

^{1}H-NMR ($CDCl_3$): 7.07 (1H, s, NH); 6.61 (1H, d, NH); 5.34 (1H, t, NH); 3.96 (4H, System AB, $CH_2\alpha$Gly); 3.80 (1H, δ, CHNH); 2.07 (2H, m, CH_2CF_2); 1.93–1.67 (2H, m, $CH_2CH_2CF_2$); 1.45 (9H, s $(CH_3)_3$); 1.25 (16H, m $(CH_2)_8$); 0.9 (3H, s, CH_3). ^{13}C-NMR ($CDCl_3$): 168.58; 167.14; 155.5; 79.27; 47.76; 43.1; 41.83; 12.6. ^{19}F-NMR ($CDCl_3$): −79.81; −112.79; −121.31; −122.38; −122.6; −125.34.

3.2. Synthesis of glycolipids

3.2.1. 11-(N-1-lactobionocarbonylglycinamido)trieicos 1-ene **IIa**

Lactobionic acid (0.907 g; 2.64 mmol), methoxyethanol (30 ml), toluene (30 ml) and 1 drop of trifluoroacetic acid (TFA) were stirred 1 h at room temperature, then concentrated at 50°C under reduced pressure. The ammonium trifluoroacetate **5a** precipitated upon addition of anhydrous ether.

Condensation between 1,5-lactobionolactone and the amine **5a** was achieved at reflux of methanol in the presence of triethylamine (pH = 8). After 12 h the solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column (AcOEt/MeOH/H_2O, 80/18/2). A white amorphous solid (970 mg; 63.5%) was isolated; m.p. = 188°C (decomposition). $[a]_D^{20}$ = +19° (c 1, $CHCl_3$).

^{1}H-NMR (DMSO): 7.9 (1H, t, NH); 7.3 (1H, d, NH); 5.8 (1H, m, CH=); 5.4 (1H, d); 5.2 (1H, d); 5 (1H, t, CH_2=); 4.8 (2H, t); 4.65 (1H, t); 4.55 (1H, t); 4.45 (1H, d); 4.22 (1H, d); 4.15 (2H, m); 4 (1H, m); 3.73–3.24 (13H, m, H_6, H_6', >CHNH, CH_2gly); 2(2H, td, CH_2—CH=); 1.3 (36 H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172.37 (C(O)NH); 167.66 (C(O)NH); 137 (*C*H=CH_2); 114.44 (CH=*C*H_2); 104.51 (Clb); 83.31; 75.5; 73.1; 71.8; 71.3; 71.04; 70.96; 67.9; 62; 60.28; 48.6 ($CH_2\alpha$Gly); 41.78 (>CHNH); 13.8 (CH_3). MS: 757 M + Na^+; 395 $NH_3CH_2C(O)NHCHR^1R^{2+}$; 336 NH_2—CHR^1R^{2+}.

3.2.2. N-1-lactobionocarbonyltrieicos 1-en 11-amine **Ia**

Procedure identical to that used for the preparation of **IIa**. Product **Ia** was isolated from **3a** (60%). m.p. = 188°C. $[\alpha]_D^{20}$ = +18° (c 1, MeOH).

^{1}H-NMR (DMSO d_6): 7.2 (1H, d, C(O)NH); 5.8 (1H, m, CH=); 5.4 (1H, s); 5.2 (1H, s); 5(2H, t, CH_2=); 4.7 (2H, s); 4.58 (1H, s); 4.55 (1H, s); 4.5 (1H, s); 4.3 (1H, d); 4.26 (1H, d); 4.1 1H, m; 3.8–3.4 (11H, m); 2(2H, td, CH_2—CH=); 1.3 (36H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172 (*C*(O)); 139 (*C*H=CH_2); 114 (CH=*C*H_2); 104 (Cβ); 84; 78; 74; 68; 63; 61; 48; 41.89; 35; 14. MS: 700 M + Na^+.

3.2.3. N-1-lactobionocarbonyldoeicos 11-amine **Ig**

Procedure identical to that followed for the synthesis of **IIa**. Starting from **3g** (1.7 g), **Ig** was obtained in 71% yield (2 g). m.p. = 182°C. $[\alpha]_D^{20}$ = +15.3° (c 1, DMSO).

^{1}H-NMR (DMSO d_6): 7.12 (1H, C(O)NH); 5.4 (1H, m); 5.16 (1H, m); 4.8 (2H, s); 4,66 (1H, s); 4.48 (2H, d); 4.28 (1H, s); 4.13 (1H, s); 3.98 (2H, s); 3.7–3.3 (11H, m); 1.24 (38H, m, $(CH_2)_{19}$); 0.9 (6H, t, CH_3). ^{13}C-NMR (DMSO): 171.56; 106.66; 87.17; 75.67; 73.25; 71.9; 71.5; 71.1; 70.52; 68.14; 62.39; 60.55; 48.57; 41.69;13.9.

3.2.4. 12-(N-1-lactobionocarbonylglycinamido) tetraeicosane **IIb**

Procedure identical to that used for the preparation of **IIa**. From **4b** (2.1 g), **IIb** (2.17 g) was obtained in 80% yield. m.p. = 154°C (dec). $[\alpha]_D^{20}$ = +13.2° (c 1, DMSO).

^{1}H-NMR (DMSO d_6): 7.9 (1H, t, NH); 7.35 (1H, d, NH); 5.44 (1H, d); 5.18 (1H, d); 4.83 (2H, m); 4.61 (1H, t); 4.53 (1H, t); 4.49 (1H, d); 4.26 (1H, d); 4.18 (2H, m); 3.99 (1H, s); 3.72–3.34 (13H, m); 1.23 (42H, m, $(CH_2)_{21}$); 0.87 (6H, t, $2CH_3$). ^{13}C-NMR (DMSO d_6): 172.16; 167.78; 104.8; 83.43; 75.61; 73.22; 72; 71.41; 71.1; 70.94; 68; 62.24; 60.4; 48.07; 41.9; 13.7. MS: 773 M + Na^+; 411 $NH_3CH_2C(O)NHR^1R^{2+}$.

3.2.5. 3-(N-1-lactobionocarbonylglycinamido) 1-perfluorohexyldodecane **IIc**

Procedure identical to that used for the preparation of **IIa**. **4c** (2.4 g) produced **IIc** (1.7 g; 52%). m.p. = 181°C (dec). $[\alpha]_D^{20}$ = 13.3°(c 1, DMSO). ^{1}H-NMR (DMSO d_6): 7.98 (1H, d, NH); 7.4 (1H, dd, NH); 5.43 (1H, dd); 5.2 (1H, d); 4.81 (2H, m); 4.62 (1H, t); 4.5 (2H, m); 4.28 (1H, d); 4.26 (1H, d); 4.03 (1H, m); 3.75–3.35 (13H, m); 2.2 (2H, m, CH_2CF_2); 1.58 (2H, m, $CH_2CH_2CF_2$); 1.41 (2H, m, CH_2); 1.24 (14H, m $(CH_2)_7$); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172.8; 168.4; 104.7; 83.3; 75.3; 73.2; 72.0; 71.51 71.24; 71.14; 68.16; 62.29; 47.72; 41.94; 13.86. ^{19}F-NMR (DMSO d_6): −80; −112.9; −118.9; −121.34; −122.16; −125.46. MS: 923 M + Na$^+$; 602 $NH_3CHR^1R^{2+}$.

3.2.6. 3-(N-1-lactobionocarbonylglycinamido) 1-perfluorohexyltridec 12-ene **IId**

Procedure identical to that used for the preparation of **IIa**. From **4d** (2.3 g), **IId** was obtained (2.46 g; 79%). m.p. = 179°C (decomposition). $[\alpha]_D^{20}$ = 15.4°(c 1, DMSO).

^{1}H-NMR (DMSO d_6): 8 (1H, d, NH); 7.5 (1H, t, NH); 5.83 (1H, m, CH=); 5.47 (1H, m); 5.22 (1H, d); 4.9 (2H, m, CH_2=); 4.8 (2H, d); 4.6 (1H, t); 4.54–4.5 (2H, d); 4.3 (2H, d); 4.15 (1H, d); 4.15 (1H, d); 4 (1H, m); 3.74–3.27 (13H, m); 2.3–2.15 (2H, m, CH_2CF_2); 2 (2H, td, CH_2—CH=); 1.68–1.4 (2H, m, $CH_2CH_2CF_2$); 1.2 (14H, m $(CH_2)_7$). ^{13}C-NMR (DMSO d_6): 172.7; 168.5; 138.81; 114.51; 104.8; 83.3; 75.7; 73.3; 72.1; 71.55; 71.24; 71.15; 68.15; 62.3; 60.46; 47.73; 42.06; 28.5 (t, CH_2CF_2). ^{19}F-NMR (DMSO d_6): −80.02; −113.07; −121.04; −122.31; −122.51; −125.64. MS: 935 M + Na$^+$; 573 $NH_3CH_2C(O)NHR^1R^{2+}$; 514 $NH_3CHR^1R^{2+}$.

3.2.7. 3-(N-1-lactobionocarbonylglycinamido) 1-perfluorooctyldodecane **IIe**

Procedure identical to that used for the preparation of **IIa**. From **4e** (1 g) **IIe** (0.763 g) was isolated in 68% yield. m.p. = 164°C (decomposition). $[\alpha]_D^{20}$ = +13.2°(c 1, DMSO).

^{1}H-NMR (DMSO d_6): 8 (1H, d, NH); 7.46 (1H, t, NH); 5.48 (1H, m); 5.2 (1H, d); 4.8 (2H, m); 4.56 (1H, m); 4.51 (2H, m); 4.3 (2H, m); 4.18 (1H, m); 3.99 (1H, s); 3.8–3.2 (13H, m); 2.23 (2H, m, CH_2CF_2); 1.4–1.3 (18H, m $(CH_2)_8$ + CH_2CH_2-CF_2); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172.72; 169.04; 104.76; 83.30; 76.66; 73.22; 72.02; 71.44; 71.21; 71.07; 68.04; 62.23; 60.49; 47.66; 42.02; 34.19; 13.9. ^{19}F-NMR (DMSO d_6): −78.8; −113.4; −121.3; −122; −122.8; −125.38. MS: 1023 M + Na$^+$; 821 M + H-Gal$^+$; 661 NH_3glyCHR$^1R^{2+}$; 602 $NHCHR^1R^{2+}$.

3.2.8. 3-(N-1-lactobionocarbonylglycinamido) 1-perfluorooctyltridec 12-ene **IIf**

Procedure identical to that used for the preparation of **IIa**. From 2.1 g of **4f**, **IIf** was isolated in 64% yield (1.26 g). m.p. = 175°C (dec). $[\alpha]_D^{20}$ = +13°(c 1, DMSO).

^{1}H-NMR (DMSO d_6): 7.97 (1H, d, NH); 7.46 (1H, NH); 5.75 (1H, m, CH=); 5.44 (1H, t); 5.23 (1H, d); 5.01–4.85 (3H, m); 4.67 (1H, t); 4.57 (1H, t); 4.51 (1H, d); 4.35 (2H, d); 4.15 (1H, m); 3.98 (1H, m); 3.7–3.1 (13H, m); 2.15 (2H, m, CH_2CF_2); 2 (2H, td, CH_2CH=); 1.5 (2H, m, $CH_2CH_2CF_2$); 1.3 (14H, m $(CH_2)_7$). ^{13}C-NMR (DMSO d_6): 172.8; 168.5; 139.66; 114.57; 104.6; 83.35; 75.72; 73.3; 72.1; 71.52; 71.16; 68.17; 62.3; 60.56; 48.61; 42.06; 28.02 (t). ^{19}F-NMR (DMSO d_6): −80.21; −113; −121.48; −122.32; −122.51; −125.64. MS: 1035 M + Na$^+$; 673 NH_3CH_2C-(O)NHCHR$^1R^{2+}$; 614 $NH_3CHR^1R^{2+}$.

3.2.9. 11-(N-1-lactobionocarbonylglycylglycinamido) trieicos 1-ene **IIIa**

Procedure identical to that used for the preparation of **IIa**. From 2 g of **6a**, **IIIa** was isolated (2.27 g; 80%). m.p. = 175–182°C (dec). $[\alpha]_D^{20}$ = +19.2°(c, 1, DMSO).

^{1}H-NMR (DMSO d_6): 8 (2H, m, NH); 7.42 (1H, d, NH); 5.8 (1H, m, CH=); 5.44 (1H, d); 5.2 (1H, d); 5.0 (2H, m, CH_2=); 4.8 (2H, m); 4.66 (1H, t); 4.57 (1H, t); 4.52 (1H, d); 4.3 (2H, m); 4.19 (1H, d); 4.03 (1H, m); 3.8–3.35 (15H, m); 2 (2H, td, CH_2CH=); 1.25 (36H, m, $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172.9; 168.9; 167.85; 138.8; 114.5; 104.76; 83.4; 75.73; 73.2; 72.04; 71.38; 71.1; 70.96; 68.2; 62.24; 60.62; 48.14; 41.94; 41.54; 13.91. MS: 814 M + Na$^+$; 652 M + Na-gal$^+$; 452 NH_3glyglyCHR$^1R^{2+}$; 395 NH_3glyCHR$^1R^{2+}$; 336 $NHCHR_1R_2^+$.

3.2.10. 3-(N-1-lactobionocarbonylglycylglycinamido) 1-perfluorohexyldodecane **IIIc**

Procedure identical to that used for the synthesis of **IIa**. **6c** (2.1 g) afforded **IIIc** (1.79 g; 64%). m.p. = 181°C (dec). $[\alpha]_D^{20}$ = +15.2°(c, 1, DMSO). ^{1}H-NMR (DMSO d_6): 8.05 (2H, m, NH); 7.55 (1H, m, NH); 5.4 (1H, d); 5.2 (1H, d); 4.79 (2H, t); 4.62 (1H, t); 4.55 (1H, t); 4.5 (2H, m); 4.28 (1H, d); 4.26 (1H, m, OH); 4.17 (1H, m); 4.03 (1H, m); 3.73–3.49 (15H, m); 2.17 (2H, m, CH_2CF_2); 1.6 (2H, m, $CH_2CH_2CF_2$); 1.41 (2H, m); 1.24 (14H, m $(CH_2)_7$); 0.86 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 173.11; 169; 168.43; 104.8; 83.3; 75.7; 73.2; 72; 71.35; 71.07; 70.88; 68.2; 62.2; 60.6; 47.55; 42.05; 28.7 (t); 13.9. ^{19}F-NMR (DMSO d_6): −79.81; −112.79; −121.31; −122.38; −122.6; −125.34.

3.2.11. 11-(N-1-maltobionocarbonylglycinamido) trieicos 1-ene **IVa**

The maltobionolactone was obtained according to Kobayashi's procedure [33]. Condensation between the amine **5a** and maltobionolactone was achieved at pH = 8 in boiling methanol under inert atmosphere. After refluxing for 48 h, the solvent was evaporated under reduced pressure and the residue was chromatographed on a silica-gel column (AcOEt/MeOH/H_2O 80/18/2), followed by filtration on a column of sephadex LH60 (MeOH/CH_2Cl_2 1/1). From amine **5a** (2 g), compound **IVa** (2.22 g; 76%) was recovered as a white powder. m.p. = 155–162°C. $[\alpha]_D^{20}$ = +59.8° (c 1, DMSO).

^{1}H-NMR (DMSO d_6): 7.97 (1H, t, NH); 7.35 (1H, d, NH); 5.8 (1H, m, CH=); 5.68 (2H, m); 5 (6H, m, CH_2 = + 2H); 4.53 (3H, m); 4,1 (2H, m); 3.8–3.4 (13H, m); 2 (2H, td, CH_2CH=); 1.3 (36H, m $(CH_2)_{18}$); 0.9 (3H, t, CH_3). ^{13}C-NMR (DMSO d_6): 172.9; 168.25; 139.2; 114.94; 101.31; 83.52; 73.74; 73.55; 72.66; 72.44; 72.31; 70.31; 62.94; 61.04; 60.4; 48.5; 44.2; 14.3. MS: 757 M + $Na]^+$; 555 M-gal$]^+$; 395 $NH_3CH_2CONHCHR^1R^2]^+$; 336 $NHCHR^1R^2]^+$.

3.2.12. N^{α}-1-(3-perfluorooctylpropionocarbonyl) N^{ϵ}-1-(lactobionocarbonyl) undecyllysinamide **V**

To undecanamine (2.29 g; 13.4 mmol) in dichloromethane (30 ml) were added BocLys(Z)OH (5 g; 13,4 mmol), DCC (2.75 g; 13.4 mmol) and HOBT (200 mg). The reaction mixture was stirred at room temperature for 24 h in an inert atmosphere. The DCU was then filtered off and the reaction mixture concentrated under reduced pressure. The crude product **8** (7.2 g) was used without purification.

Compound **8** was dissolved in dichloromethane (30 ml) to which TFA (10 ml) was added. After 1 h stirring at room temperature the solvent was evaporated under reduced pressure. The residue was diluted with diethyl ether (100 ml), washed with a saturated solution of $NaHCO_3$, then with water, dried over sodium sulfate and concentrated under reduced pressure.

The amine **9** was then dissolved in DMF (30 ml) to which 2H,2H,3H,3H perfluoroundecylic acid (6.6 g; 13.4 mmol), DCC (2.7 g; 13.4 mmol) and HOBT (100 mg) were added. The reaction was monitored by CCM, and proved to be completed after 24 h.

Compound **10** was obtained (contaminated by traces of DCU) after column-chromatography on silica-gel and/or filtration on Sephadex LH-60 and/or crystallisation. Compound **10** (2 g) was dissolved in anhydrous methanol (50 ml) containing 20% palladium on charcoal (100 mg). Hydrogenation was achieved at room temperature (10 atm; 2 h). The solution was filtered on Celite and the solvent evaporated under reduced pressure to give **11** (0.82 g).

Compound **10** (0.622 g; 0.8 mmol) and lactobiono-1,5 lactone (0.285 g; 0.8 mmol) were dissolved in MeOH (40 ml). The pH of the solution was set up to 8 by the addition of TEA and brought to reflux under a nitrogen atmosphere. The reaction was monitored by CCM (AcOEt/MeOH/H_2O 80/18/2). After 24 h the reaction was shown to be completed. The solvent was evaporated under reduced pressure and the residue chromatographed on a silica-gel column (AcOEt. MeOH.H_2O 80/18/2) then on Sephadex LH 60 (MeOH/CH_2Cl_2 1/1). Product **V** (620 mg; 16%) was isolated. m.p. = 171°C. $[\alpha]_D^{20}$ = +13° (c 1, DMSO).

^{1}H-NMR (DMSO-d_6): 8.6 (1H, NHC(O)); 7.83 (1H, C(O)NH); 7.59 (1H, t, C(O)NH); 5.3–3.2 (24H, m); 3.1 (4H, m); 2.45 (4H, m, CH_2F_2); 1.1 (22H, m, $(CH_2)_{10}$); 0.85 (3H, t; CH_3). ^{13}C-NMR

(DMSO d_6): 172.07; 171.26; 169.31; 104.61; 82.94; 75.74; 73.27; 72.07; 71.15; 70.47; 68.26; 62.38; 60.70; 52.9; 13.88. ^{19}F-NMR (DMSO d_6): −81.25; −114.45; −122.37; −123.19; −124; −126.6. MS: 1114 M + H^+; 1136 M + Na^+.

3.3. Identification of liposomes by transmission electron microscopy (TEM)

The formation of liposomes was observed by transmission electron microscopy according to the negative staining method. The dispersion was applied on a grid covered with a FORMVAR membrane, using the drop method: a drop of the lipid dispersion was placed on the grid for 1 min, and the excess was then removed with filter paper. The sample was colored by depositing a drop of phosphotungstic acid (2%, pH adjusted to 7) for 1 min, the excess being removed using a filter paper. The grid was then dried in an oven at 60°C. The sample was examined using a Philips microscope (CM/2 model) at 80 kV.

3.4. Observation of the liposomes by transmission electron microscopy after freeze-fracture

One drop of lipidic dispersion (~0.5 ml) was placed on a copper support. Freezing was obtained by rapid immersion in liquid propane. The fractures were obtained using a Balzers BAF 301 at −120°C in vacuo (10^{-6} Torr) with a liquid nitrogen cooled knife. The replication was performed with unidirectional Pt-C shadowing; the mean thickness of the metal deposit was ~1.5 nm. The replicas were then washed with ethanol and water and examined with a Philips EM 301 electron microscope.

3.5. Differential scanning calorimetry

The phase transition temperatures and the transition enthalpies were determined after hydration by a known quantity of water, using a Setaram DSC-92 by heating then cooling at different speeds.

Product **IIa** (10.8 mg) was hydrated with a mixture of water/ethyleneglycol 60/40 (hydration rate 37%). Heating and cooling between −20 and 40°C at a speed of 1°C/min showed that the T_c was 22°C and the transition enthalpy −11.6 mcal/mg.

Product **IIb** (16 mg) was hydrated with 10.2 ml of a mixture of water/ethyleneglycol 60:40 (hydratation percentage 28%). Heating and cooling between −10°C and 45°C at a speed of 2°C/min showed that the T_c was 14°C and that the transmission enthalpy was −10.3 mcal/mg.

3.6. Biological tests

The toxicity on Namalva cell cultures was determined as follows. A series of five flasks was filled with 2 ml of cell-culture medium (RPMI containing 10% of fetal calf serum) at 37°C under 7% of CO_2 containing ~3.10^5 cells/ml and 2 ml of the dispersion of the compound to be tested. A reference series was realized under the same conditions. After 4 days of incubation the cells were counted and the viability was determined by the Trypan blue dye exclusion method. The results are presented in Table 4 and expressed as growth rate/viability ratio with respect to control. In vivo toxicity was assessed by injecting 500 μl of an isotonic dispersion of the amphiphile to be tested intraperitoneally into 10 OF1 male mice of 20–25 g. The growth of the animals, compared with controls, was recorded over a 1-month observation period.

4. Conclusions

These results represent an essential step in our research on the vectorization of drugs using biocompatible organized systems. The synthetic glycolipids reported here, which are equipped with a peptide link, form liposomes presenting a good thermal and storage stability. The choice of the hydrophobic or hydrophilic peptide spacer largely determines the morphology of the organized systems. Research is under way in two complementary directions: the study of the encapsulation and kinetics of the release of drugs, and the recognition of the vesicles by specific membrane lectins in vitro.

Acknowledgments

We thank Dr. T. Gulik-Krzywicki (Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette) for his interest and help in the morphological

characterization of some of our dispersions by freeze-fracture electron microscopy, and ATTA (Camille Guedj, Leila Zarif) for financial support.

References

[1] A.D. Bangham and R.W. Horne (1964) J. Mol. Biol. 8, 660.
[2] G. Gregoriadis and A.C. Allison (1980) in: Liposomes in Biological Systems, Wiley, Chichester, p. 166.
[3] H. Ringsdorf, B. Schlarb and J. Venzmer (1988) Angew. Chem. Int. Ed. Engl. 27, 113.
[4] D. Lasic (1992) Am. Sci. 80, 20.
[5] R.A. Schwendener (1992) Chimia 46, 69.
[6] Y. Okahata, G.I. Nakamura and H. Noguchi (1987) J. Chem. Soc. Perkin Trans. 22, 1317.
[7] G.R. Alving, E.A. Steck, W.L. Chapman, V.B. Warls Jr, L.D. Hendrik, G.M. Swartz and W.L. Harson (1978) Proc. Natl. Acad. Sci. 75, 2959.
[8] A.W. Segal, E.J. Wills, J.E. Richmond, G. Slavin, C.D.V. Black and G. Gregoriadis (1974) Br. J. Exp. Pathol. 55, 320.
[9] J. Senior, J.C.W. Crawley and G. Gregoriadis (1985) Biochim. Biophys. Acta 839, 1.
[10] J. Ogihara, S. Kojima and M. Jay (1986) Eur. J. Nucl. Med. 11, 40.
[11] J.M. Allen and A. Chonn (1987) FEBS Lett. 223, 42.
[12] Y.J. Kao and R.L. Juliano (1981) Biochim. Biophys. Acta 677, 453.
[13] R.L. Souhami, H.M. Patel and B.E. Ryman (1981) Biochim. Biophys. Acta 674, 354.
[14] G. Blume and G. Cevc (1990) Biochim. Biophys. Acta 1029, 91.
[15] T.M. Allen, C. Hansen, F. Martin, C. Redermann and A. Yauyoung (1991) Biochim. Biophys. Acta 1066, 29.
[16] A. Mori, A.L. Klibanov, V.P. Torchilin and L. Huang (1991) FEBS Lett. 284, 263.
[17] D. Papahadjopoulos, T.M. Allen, A. Gabizon, F. Mayhew, K. Matthay, S.K. Huang, K.D. Lee, M.C. Woodle, D. Lasic, C. Redermann and F.J. Martin (1991) Proc. Natl. Acad. Sci. USA 88, 11460.
[18] Y. Daicho, S. Okada and R. Goto (1992) Biochim. Biophys. Acta 1107, 61.
[19] K. Maruyama, A. Mori, S. Bhadra, M.T. Ravi Subbiah and L. Huang (1979) Biochim. Biophys. Acta 1070, 246.
[20] J.G. Riess (1994) Colloids and Surfaces, in press.
[21] C. Santaella, P. Vierling and J.G. Riess (1991) Angew. Chem. Int. Engl. Ed. 30, 567.
[22] C. Santaella, P. Vierling, J.G. Riess, T. Gulik-Krzywicki, A. Gulik and B. Mouassa (1994) Biochim. Biophys. Acta, in press.
[23] T.J. Williams, N.R. Plessas and J.J. Goldstein (1979) Arch. Biochim. Biophys. 195, 145.
[24] B. Boyer, S. Durand, G. Lamaty, J.M. Moussamou-Missima, A.A. Pavia, B. Pucci, J.P. Roque and J. Rouvière (1991) J. Chem. Soc. Perkin Trans. 22, 1391.
[25] C. Santaella, F. Frezard, P. Vierling and J.G. Riess (1994) FEBS Lett., in press.
[26] O. Mitsunobu (1981) Synthesis 1, 1.
[27] B. Pfannemüller and W. Welte (1985) Chem. Phys. Lipids 37, 227.
[28] J.H. Fuhrhop and J. Mathieu (1984) Ang. Chem. Int. Engl. Ed. 23, 100.
[29] H. Idaka (1991) Colloids and Surfaces 58, 1.
[30] T. Kunitake (1992) Angew. Chem. Int. Ed. Engl. 31, 709.
[31] D.A. Tyrell, T.D. Heath, C.M. Colley and B.E. Ryman (1976) Biochim. Biophys. Acta 457, 259.
[32] A. Polidori, B. Pucci, J.C. Maurizis and A.A. Pavia (1994) New J. Chem., in press.
[33] K. Kobayashi, H. Sumimoto and Y. Ina (1985) Polymer J. 17, 567.