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Chemistry and Physics of Lipids
77 (1995) 225–251

Chemistry and Physics of LIPIDS

Vesicles and other supramolecular systems made from double-tailed synthetic glycolipids derived from galactosylated tris(hydroxymethyl)aminomethane

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Received 20 April 1995; revision received 1 June 1995; accepted 7 June 1995

Abstract

A series of double-tailed hydrocarbon glycolipids derived from tris(hydroxymethyl)aminomethane (Tris) have been prepared. These amphiphiles consist of three parts: a hydrophilic head derived from mono- or polygalactosylated Tris, a linking arm of peptidic nature and a hydrophobic double tail composed of 12 or 18 carbon atom chains. The ability of the new glycolipids to disperse in water, the morphology of the self-assemblies they form and the stability of the latter were shown to depend largely on the volumetric ratio between hydrophilic head and hydrophobic tails. Comparative studies of such substrates allowed a better understanding of the relative role of the various parameters that govern the formation of tubular systems relative to vesicular organizations. In all cases, the introduction of oligomeric galactosylated heads favoured stable vesicular systems over tubules.

Keywords: Synthetic glycolipids; Amino acids; Amphiphiles; Telomers; Vesicles; Fibers; Tubules; Electron microscopy

1. Introduction

Among the various supramolecular systems investigated in the last 20 years as drug delivery systems (proteins, antibodies microspheres, nanoparticles, polymers, etc.) liposomes have gained special attention [1–3]. They display multiple potential advantages, including their capacity to encapsulate both hydrophilic and hydrophobic drugs, their biocompatibility, as well as their potential as cell targeting devices. However their intravascular use is limited by their rather rapid clearance as a consequence of their uptake by the reticuloendothelial system [4–6]. To overcome this problem several approaches have recently been developed. One consists of including polyethylene glycol or glycolipid-grafted surfactants or cosurfactants into the liposome membrane [7–9] in order to minimize their

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SSDI 0009-3084(95)02475-X

opsonisation and to provide them with 'stealth' character. The second is designed to insure cellular specific targeting via the functionalization of their outer surface with various ligands such as antibodies, hormones or carbohydrates [2,10–12]. Still another approach consists in grafting fluorinated termini onto the hydrophobic tails, which was found to have significant impact on particle recognition [13–15]. In line with the second approach, we have prepared glycolipidic amphiphiles that can organize into liposomes and may achieve targeting via specific recognition of surface carbohydrates by membrane lectins [3,16–18].

We recently reported the synthesis of a variety of double-tailed perhydrogenated and fluorinated glycolipids derived from lactose and maltose containing an amino acid spacer interposed between the hydrophilic and the hydrophobic moieties [19]. The analysis of the supramolecular assemblies they form in aqueous medium showed that the morphology of these assemblies depends largely on the nature of the amino acid and/or peptide spacer [20]. While glycine or glycyl-glycine led to the formation of vesicles, the introduction of a chiral amino acid such as L-lysine induced the formation of tubules and helices. Such structures have already been observed with other ionic or non-ionic mono- and double-tailed surfactants [21–23]. Hydrogen bonds between the hydrophilic heads were considered to be responsible for the formation of tubules from single-chain amphiphiles [22,24]. Tubules were, however, also obtained from non-hydrogen bond-forming fluorinated amphiphiles [25]. The situation was different with double-tailed amphiphiles, for which the morphology of self-assemblies observed in water [21,23] appears to depend on more numerous parameters including the nature and the charge of the hydrophilic head, the chirality of the molecule, the presence of an unsaturation within or at the extremity of the hydrophobic chain.

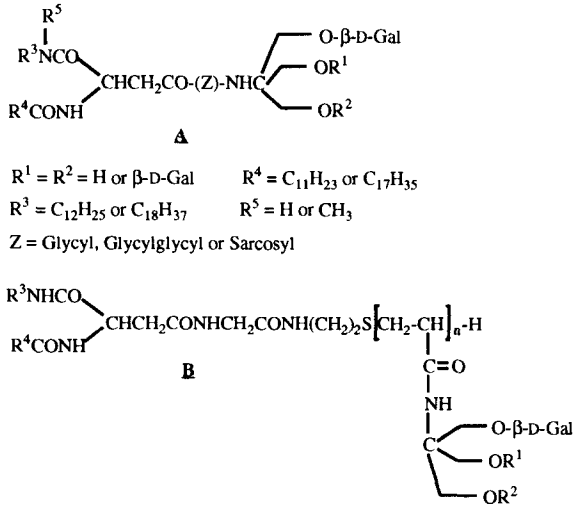
In order to appreciate the influence of such parameters on the aggregation behavior of glycolipids, we have prepared a series of mono- or polyglycosylated double-tailed amphiphiles derived from tris(hydroxymethyl)aminomethane (Tris) with structure **A** and **B** (Scheme 1).

Such materials with their modular structure, should contribute providing new information about the relative role of the various parameters that govern the formation of tubular systems to the detriment of vesicular-type organizations as well as about the type of molecule that may be best adapted to produce drug delivery devices.

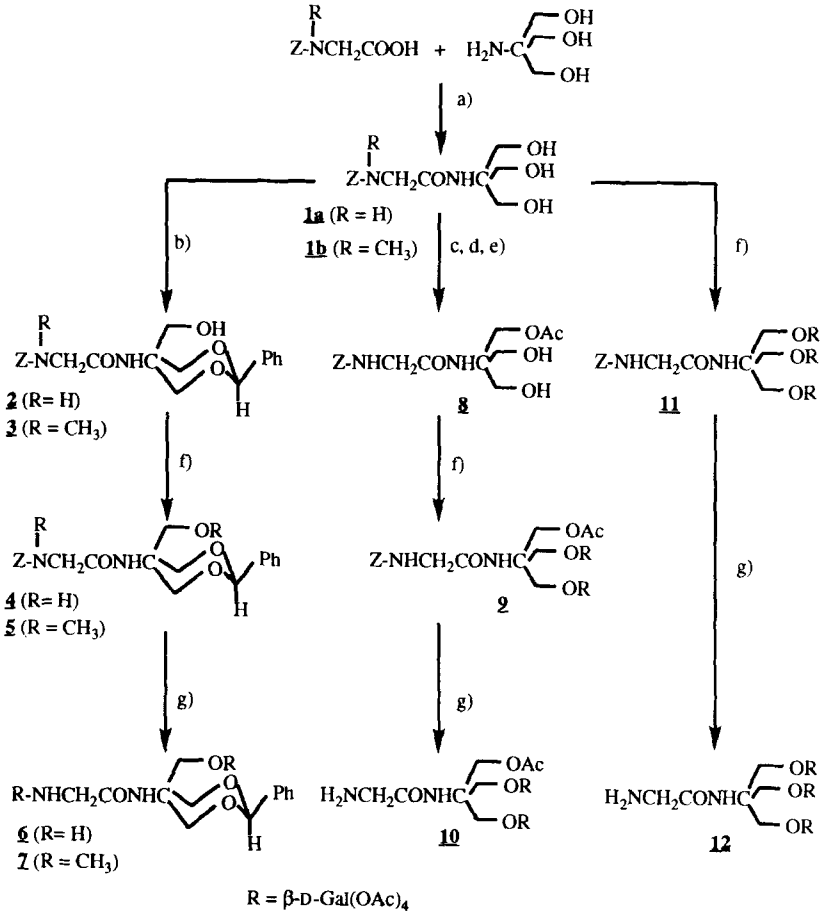
2. Results and discussion

Glycolipid amphiphiles **A** and **B** consist of three parts: a hydrophilic head derived from mono- or polygalactosylated Tris, a linking arm of peptidic nature and a hydrophobic double tail composed of 12 or 18 carbon atom chains.

It is now widely accepted that the volumetric ratio of the hydrophilic to the hydrophobic parts of a surfactant plays a major role in the morphology of the self-assemblies they form in aqueous medium [26,27]. In this respect, the plurifunctionality of Tris is of interest, since it allows to modulate the volume of the hydrophilic head by changing the number of glycosidic groups (structure **A**). Similarly, the technique of telomerization which we have already applied to the preparation of neutral [28–31] or ionic [32] amphiphiles, makes it possible to control the geometry and hydrophilic-lipophilic balance (HLB) of the amphiphiles, as well as its hydrogen bonds-forming



Scheme 1. General structure of Tris-derived amphiphiles.



a) EEDQ, EtOH, Δ ; b) PhCHO, CH_2Cl_2, TsOH ; c) acetone, CH_2Cl_2, TsOH ; d) $(CH_3CO)_2O$, Pyr, 0°C; e) CH_3COOH, H_2O, 70°C ; f) tetracetylgalactopyrannosyl bromide, $Hg(CN)_2$, CH_3CN,))) ; g) H_2, Pd-C, MeOH

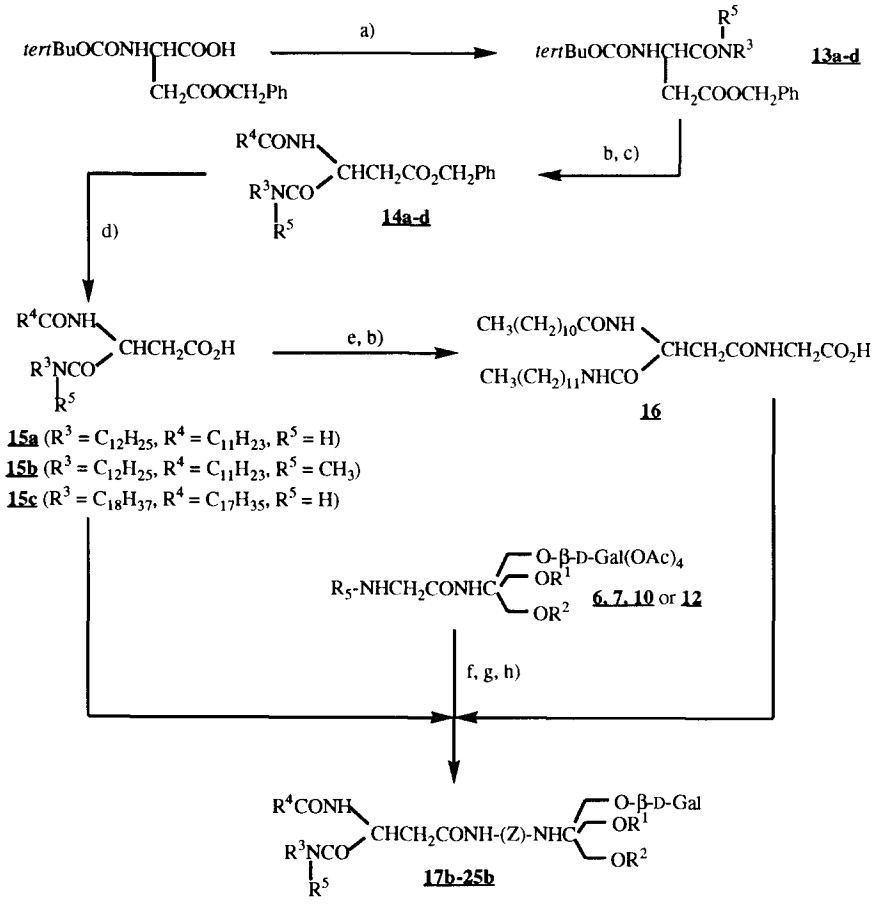
Scheme 2. Synthesis of glycosylated polar heads.

capacity. In the present case, it is possible to significantly increase the volume and, consequently, the steric interactions at the hydrophilic head (structure **B**).

The hydrophobic part of the amphiphiles was obtained by linking a fatty amine and/or acid to the α-carboxy and α-amino function of a L or a (D,L)-aspartic acid moiety. This choice should allow to assess the relative contribution of chirality to the formation of tubular systems, a contribution that was already observed in other circumstances to be important [19–22].

The spacer plays a multiple role: (1) it should confer to the carbohydrate terminus a more flexible configuration, hence possibly a better access to the solvent and/or to the membrane lectins; (2) it significantly increases the yield of the coupling reaction between the bulky hydrophilic and the hydrophobic parts of the molecule; (3) it allows further modulation of the HLB; (4) at the same time it can contribute to the possibility of improving and stabilizing the packing through additional intermolecular hydrogen bonds. Neither glycine nor sarcosine or aspartic acid were expected to affect the biocompatibility of the amphiphiles.

Compounds **A** and **B** were obtained according to the strategy summarized in Schemes 2–4.

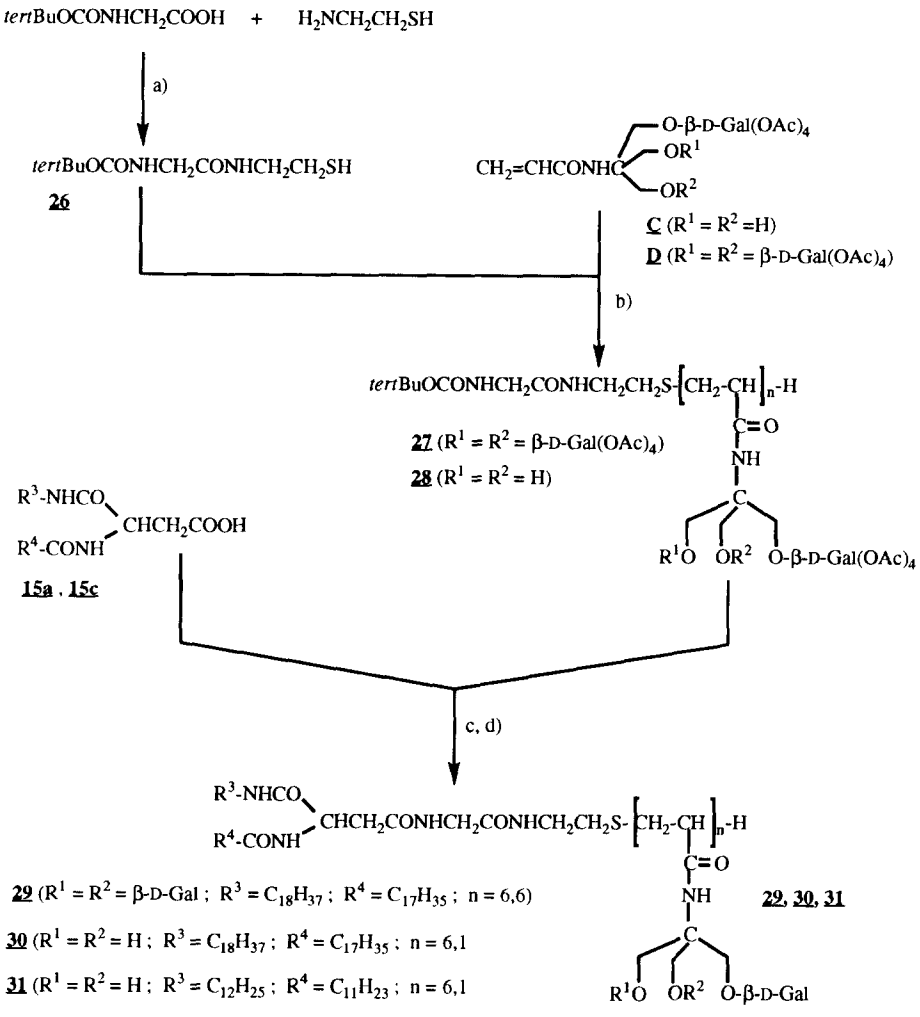


a) R^3NHR^5, DCC, HOBT, CH_2Cl_2 ; b) TFA, CH_2Cl_2 ; c) $CH_3(CH_2)_nCOCl$, CH_2Cl_2, DIEA ; d) H_2, Pd-C, MeOH ; e) $H_2NCH_2CO_2tertBu$, DCC, HOBT, DIEA ; f) **6**, **7**, **10** or **12**, BOP reagent, DMAP, CH_2Cl_2 or DMF ; g) CH_3COOH/H_2O : 7:3, 70°C (for compounds **19** to **22**); h) MeONa, MeOH.

Scheme 3. Synthesis of double-tailed glycolipids.

2.1. Synthesis of the glycolipidic amphiphiles with the monomeric structure **A**

The selective synthesis of mono-, di- and tri-*O*-galactosylated Tris derivatives (scheme 2) has already been described [28]. *N*-benzyloxycarbonylglycine or *N*-benzyloxycarbonylsarcosine was first coupled to Tris in refluxing ethanol in the presence of *N*-ethoxy-1-ethoxy dihydroquinoleine (EEDQ). Replacement of glycine by its *N*-methylated derivative (sarcosine) allows to block one of the sites that can lead to intermolecular hydrogen bonds. Hence, it should be possible to assess the impact of these interactions on the formation of organized molecular systems. After selective protection of one or two of the Tris hydroxyl functions, galactosylation was achieved according to Helferich's method [28] with the aid of ultrasounds. This procedure allows, (1) to obtain diverse galactosylated substrates with yields higher than 60%; (2) an easy



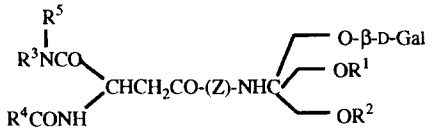
a) DCC, HOBT, CH_2Cl_2 ; b) MeOH, AIBN, Δ ; c) BOP, DMAP, CH_2Cl_2 ; d) MeONa, MeOH

Scheme 4. Synthesis of doubled-tailed glycolipidic telomers.

purification; (3 to prepare the pure β anomer. The cleavage of the protecting benzyloxycarbonyl group was achieved by hydrogenolysis in the presence of Pd-C leading to the Tris galactosylated derivatives **6**, **7**, **10** and **12**.

The double-tailed hydrophobic moiety was built by substituting α-amino and α-carboxy aspartic functions according to scheme 3. The synthesis involved conventional peptide methodology. Condensation of the hydrophobic chains with aspartic acid was achieved in the presence of dicyclohexylcarbodiimide (DCC) or by reaction of an acid chloride with the amino group of aspartic acid. In order to study the effect of the volume of the hydrophobic chain on the formation of vesicular or tubular organized systems in water, hydrocarbon chains of variable length were linked (respectively 12 and 18 carbon atoms for compounds **14a** and **14c**). With *N*-methyl dodecylamine (compound **14b**), it is also possible to block one of the sites that can possibly be involved in intermolecular hydrogen bonding. On the con-

Table 1



Partial physicochemical data of glycolipids **17–25**

Product	-(Z)-	R^1	R^2	R^3	R^4	R^5	Yield (%)*	mp (°C)**	$[\alpha]_D^{20}$**	T_c (°C)	ΔH (J/g)****
17a,b	Gly	Gal	Gal	$C_{12}H_{25}$	$C_{11}H_{23}$	H	16	86	−13	22	−8
18a,b	Gly	Gal	H	$C_{12}H_{25}$	$C_{11}H_{23}$	H	17	78	−9.5	52	−5
19a,b	Gly	H	H	$C_{12}H_{25}$	$C_{11}H_{23}$	H	11	93	−18	57	−3
20a,b	Gly	H	H	$C_{12}H_{25}$	$C_{11}H_{23}$	CH_3	10	—***	—	45	−29
21a,b	Sarcos	H	H	$C_{12}H_{25}$	$C_{11}H_{23}$	H	21	—***	—	44	−4
22a,b	Sarcos	H	H	$C_{12}H_{25}$	$C_{11}H_{23}$	CH_3	19	—***	—	79	−9
23a,b	Gly	Gal	Gal	$C_{18}H_{37}$	$C_{17}H_{35}$	H	17	95	−14	38	−5.5
24a,b	GlyGly	Gal	Gal	$C_{12}H_{25}$	$C_{11}H_{23}$	H	16	88	−18	ND	ND
25a,b	GlyGly	Gal	H	$C_{12}H_{25}$	$C_{11}H_{23}$	H	25	91	−12.5	82	−4.4

*Overall yields (8–10 steps).
Melting points and $[\alpha]_D^{20}$ are measured on *O*-acetylated glycolipids **17a–25a.
***Mixture of two isomers.
****Phase-transition temperature and enthalpy of glycolipids **17b–25b**.
ND, not determined.

trary, the introduction of an extra glycine motif (compound **16**) increases the length of the spacer thus, enhancing the freedom of motion of the hydrophilic head. All the intermediate compounds (products **15a–c**, **16**) were obtained in good yields (> 75% after purification on silica gel column chromatography).

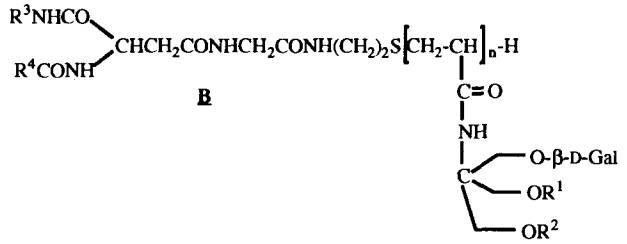
Condensation of the galactosylated hydrophilic moiety on the side-chain aspartic acid was achieved at room temperature in the presence of benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) with a catalytic amount of dimethylaminopyridine (DMAP) (scheme 2). Coupling yields ranged between 35–90%, depending on the respective volumes of the two groups. Generally, they were inversely proportional to the number of carbohydrate residues linked to the Tris motif. Pure **17a–25a** were obtained after chromatography on silica gel followed by gel filtration on Sephadex LH 60.

Compounds **17a–25a** were deacetylated using a catalytic amount of sodium methylate in methanol at room temperature. After dissolution in water and lyophilization, final glycolipids **17b–25b** were obtained as amorphous white solids. Their physicochemical characteristics are shown in Table 1.

2.2. Synthesis of the glycolipidic amphiphiles with the telomeric structures **B**

In a previous work, we emphasized the interest of telomerization [28–30,33]. Telomerization allows easy modulations of the HLB and, consequently, of the hydrosolubility of a surfactant. With respect to cell targeting, telomerization is also of interest: the multiplication of the carbohydrate moieties should favour recognition by membranes lectins as a consequence of a cluster effect. The presence of several galactosylated Tris units on the hydrophilic head results in an increase of the bulkiness of the head, leading to a pronounced curvature of the lipidic bilayers and should, therefore, favour the formation of vesicles to the detriment of tubular or fibrous systems. For these reasons and also in order to compare their behavior in aqueous medium with that of single-chain analogs, we prepared a series of mono- and trigalactosylated glycolipids of telomeric type-**B** structure fitted with 12 or 18 carbon atom hydrophobic chains. The synthesis of compounds **29**, **30** and **31** is reported in scheme 4.

Table 2



B

Analysis by differential scanning calorimetry and overall yields of preparation of the different galactosylated telomers **29–31**

Product	R^1	R^2	R^3	R^4	DPn	Yield (%)*	T_c (°C)**	ΔH (J/g)
29b	Gal	Gal	$C_{18}H_{37}$	$C_{17}H_{35}$	6.6	18	69	−2
30b	H	H	$C_{18}H_{37}$	$C_{17}H_{35}$	6.1	10	38	−0.8
31b	H	H	$C_{12}H_{25}$	$C_{11}H_{23}$	6.1	10	ND	ND

*Overall yields of preparation with respect to monomers **C** or **D**.
**Phase transition temperature.
ND, not determined.

Mono (**C**) and tri-*O*-galactosyl (**D**) tris(hydroxymethyl)acrylamidomethane derivatives were telomerized in the presence of Boc-glycinamidoethanethiol **26** as transfer reagent according to a procedure reported in the literature [28], to afford compounds **27** and **28**, respectively.

Telomerization was achieved in boiling methanol under nitrogen in the presence of α,α'-azobisisobutyronitrile (AIBN) as radical initiator, and the reaction was stopped when the monomer had entirely disappeared. In order to obtain average degrees of polymerization (DPn) ranging between 5 and 7, the initial molar concentration ratio R_0 = (Telogen)/(Monomer) was set at 0.2. After purification by gel filtration on Sephadex LH 60, the DPn of the telomers **27** and **28** was assessed by ^{1}H-NMR by comparing the area of the signal ascribed to the terminal *tert*-butyl group ($\delta = 1.45$ ppm) to that of the signal due to the H_4 protons of the galactopyranose residues ($\delta = 5.4$ ppm). DPn of the trigalactosylated derivative **27** and of the monogalactosylated derivative **28** were 6.6 and 6.1, respectively.

After hydrolysis of the *tert*-butyloxycarbonyl group by treatment with trifluoroacetic acid in dichloromethane (1:1 v/v) at room temperature, the hydrophilic head was linked to the double-tailed hydrophobic moiety **15a** or **15c** in dichloromethane in the presence of the BOP coupling reagent. After deacetylation under the usual conditions followed by lyophilization, compounds **29b**, **30b** and **31b** were obtained as amorphous white solids. The overall yields are reported in Table 2.

Type **A** monomeric glycolipids and type **B** telomers were dispersed in deionized water according to conventional ultrasonication procedures. The supramolecular structures obtained were studied by transmission electron microscopy after negative staining or after freeze-fracture and by optical microscopy (polarization technique). Particle sizes were determined by laser light scattering and phase transition temperatures by differential scanning calorimetry.

2.3. Influence of the volume of the polar head on the self-aggregation behavior

The study of the mono-, di- and trigalactosylated compounds **17b** to **19b** by differential scanning calorimetry indicated that the phase transition temperature (Table 1) increases with decreasing number of galactose residues grafted to the Tris moiety. According to Cvec [7], the variation of T_c can be related to the rate of hydration of the polar heads of the mono- or digalactosylated glycolipids compared to their trigalactosy-

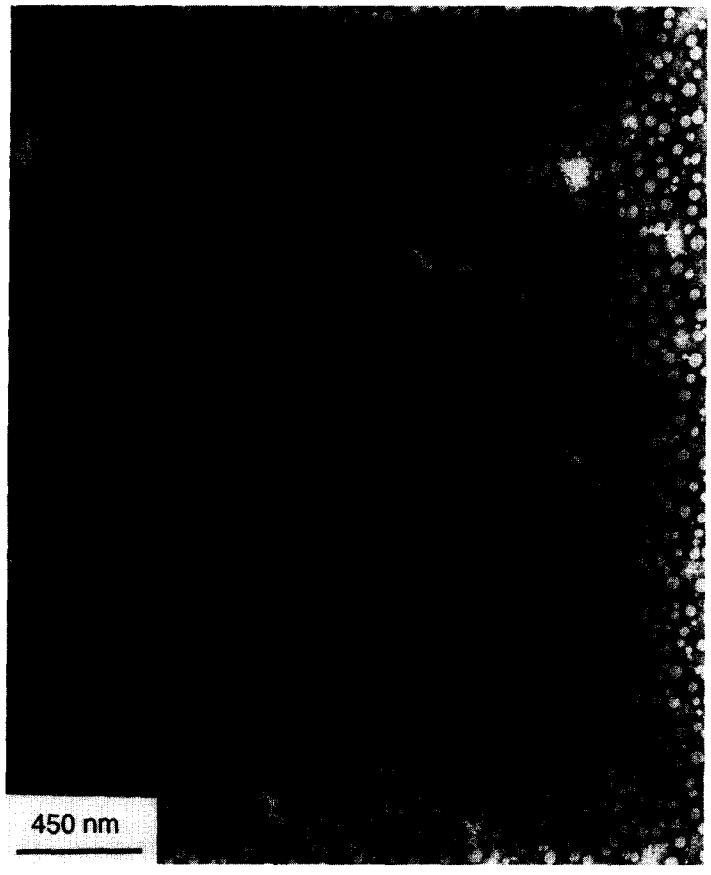


Fig. 1. Electron micrograph of compound **17b** after sonication (negative staining by phosphotungstic acid).

lated analogs. Dispersion of the trigalactosylated compound **17b** in water led after a few minutes to a stable bluish translucid solution, whereas dispersion of glycolipids **18b** and **19b** required prolonged ultrasonication (15 min) above T_c. For **19b** the aqueous dispersions were unstable. After 1 week, their appearance became milky-looking and cloudy and the presence of translucid filaments was observed. A study of dispersions of these three compounds by transmission electron microscopy showed that the trigalactosylated compound **17b** produced vesicles of homogenous size (20 to 40 nm) (Fig. 1), whereas glycolipids **18b** and **19b** gave a mixture of long fibers, tubules and vesicles (Fig. 2). Observation by polarization optical microscopy confirmed the disappearance of the fibers when dispersions of **18b** and **19b** are heated above their phase transition temperature.

A priori, such behavior could result from a balance of two trends:

(1) The formation of elongated structures from mono- and digalactosylated derivatives can reflect the existence of intermolecular hydrogen bonds as a consequence of the presence of the amide function in the linker. The volume and/or the steric constraints of the hydrophilic head could then favour their formation. Pfannemuller [24] has already observed similar behavior with various single-tailed gluconamides.

(2) The formation of stable vesicular aggregates would result from favourable hydrophilic head/hydrophobic tail section ratio [26,27].

2.4. Effect of intermolecular hydrogen bonds

If it is assumed that the formation of tubules is

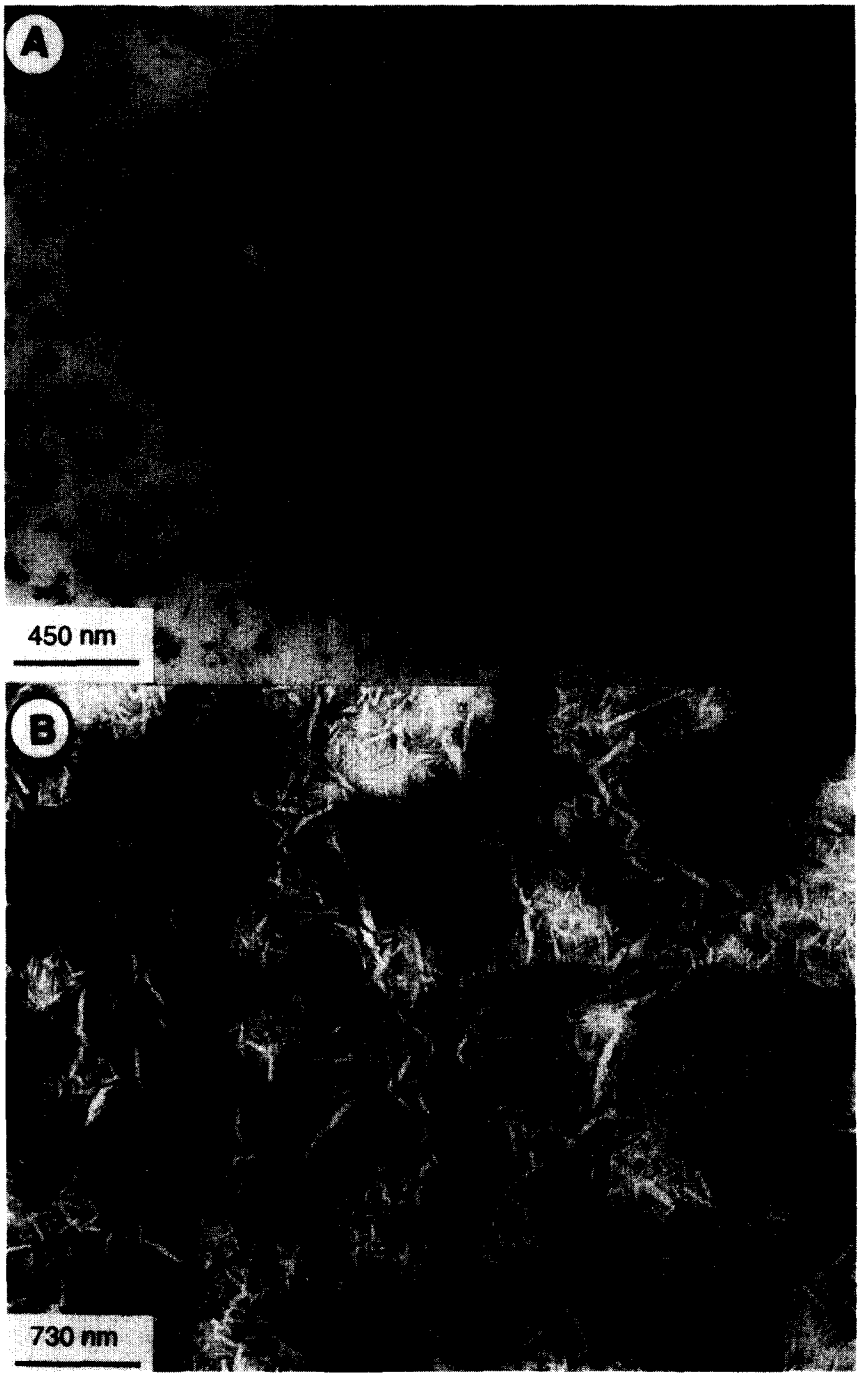


Fig. 2. Electron micrographs of compounds **18b** (A) and **19b** (B) after sonication (negative staining by phosphotungstic acid).

primarily due to the existence of intermolecular hydrogen bonds involving the amide functions of the linking arm, their removal should favour vesicular-type organizations. In order to check

Fig. 3. Electron micrograph of compound **18b** in the presence of urea after sonication (negative staining by phosphotungstic acid).

this hypothesis we prepared dispersions of glycolipids **18b** and **19b** in the presence of a very large excess of urea. It is well known, indeed that urea induces protein denaturation by destroying the hydrogen bonds responsible for their tridimensional structure. After 5 min of ultrasonication in the usual conditions, transmission electron microscopy of the dispersion indicated the presence of a mixture of vesicles and of very elongated fibers (Fig. 3).

N-methylation of the amide bond was then performed. Three substrates were prepared: compound **20b**, which bears a methyl group on the nitrogen atom of the amine linked to the aspartic acid's α-carboxyl group, compound **21b**, in which the nitrogen atom of the glycine had been methylated (sarcosine) and compound **22b**, which carries two *N*-methyl groups (see Table 1). Whereas, the monomethylated compounds **20b** and **21b** were dispersed with great difficulty in water and led to unstable milky dispersions, in the same conditions, their dimethylated analog produced a stable limpid dispersion. In all cases however, transmission electron microscopy revealed the presence of helical fibrous aggregates (Fig. 4). These results show that if hydrogen bonds may influence the dispersibility of these glycolipids and the geometry of the self-assemblies they form in water, additional parameters are definitely also involved. In particular, the overall geometry of the molecule must be taken into account.

2.5. Effect of the geometrical constraints

We have successively studied the behavior in water of glycolipids after modification of either, (1) the length of the hydrophobic chains; (2) the

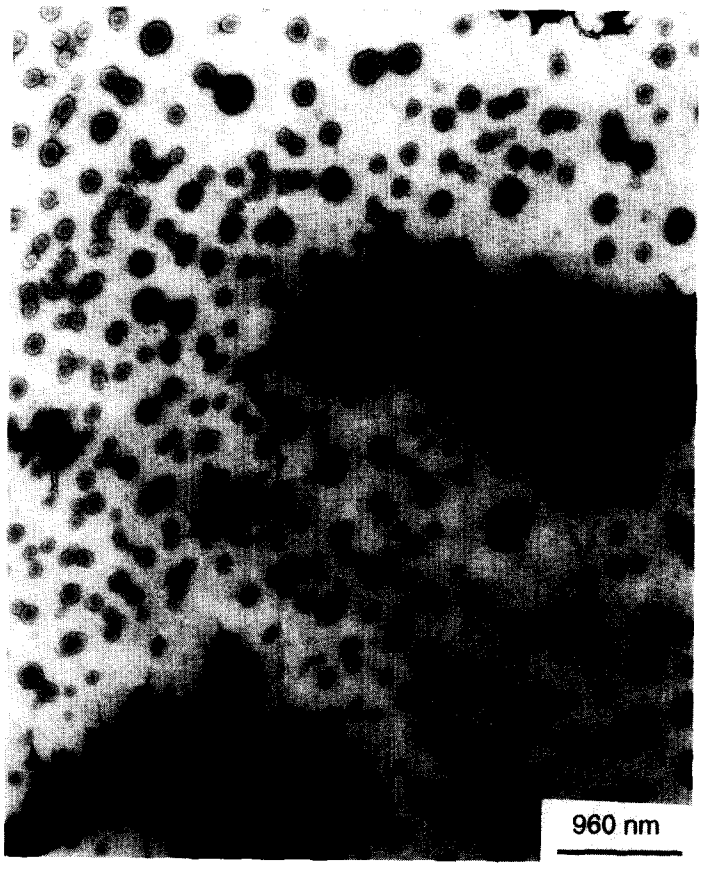


Fig. 4. Electron micrograph of compound **21b** after sonication (negative staining by phosphotungstic acid).

length of the linking arm; or, (3) the volume of the polar carbohydrate head.

Replacement of 12 carbon by 18 carbon atom chains in the trigalactosylated compound **17b** did not affect its dispersibility. Compound **23b** led, after ultrasonication, to stable bluish translucid dispersions (no change of their appearance and constitution was observed after several weeks). Analysis by electron microscopy of dispersions of **23b** revealed the presence of a mixture of fibers and vesicles (Fig. 5).

The length and volume of the peptide spacer is increased when a second glycine residue is added as in compounds **24b** and **25b**. These compounds were easily dispersed by simple mechanical stirring or by ultrasonication. The trigalactosylated compound **24b** displayed a phase transition temperature significantly higher than that of its analog **17b**. This observation tends to show that lengthening the linking arm by a second glycine moiety increases the compacity of the bilayers and possibly decreases the fluidity of the fatty chains. The T_c of the digalactosylated glycolipid **25b** was not detected by DSC; however, the disappearance of the fibrous structures observed by optical microscopy during the heating of the solutions allows its evaluation as 85 $\pm$ 10°C; this temperature is distinctly higher than that of its analog **18b**. The analysis of solutions of **24b** by electron microscopy before and after ultrasonication showed the presence of a very dense network of fibres and liposomes (Fig. 6). Upon sonication or vortexing the digalactosylated glycolipid **25b** produced exclusively fibers (Fig. 7).

These results may indicate that the volumetric hydrophilic head/hydrophobic tail ratio plays a

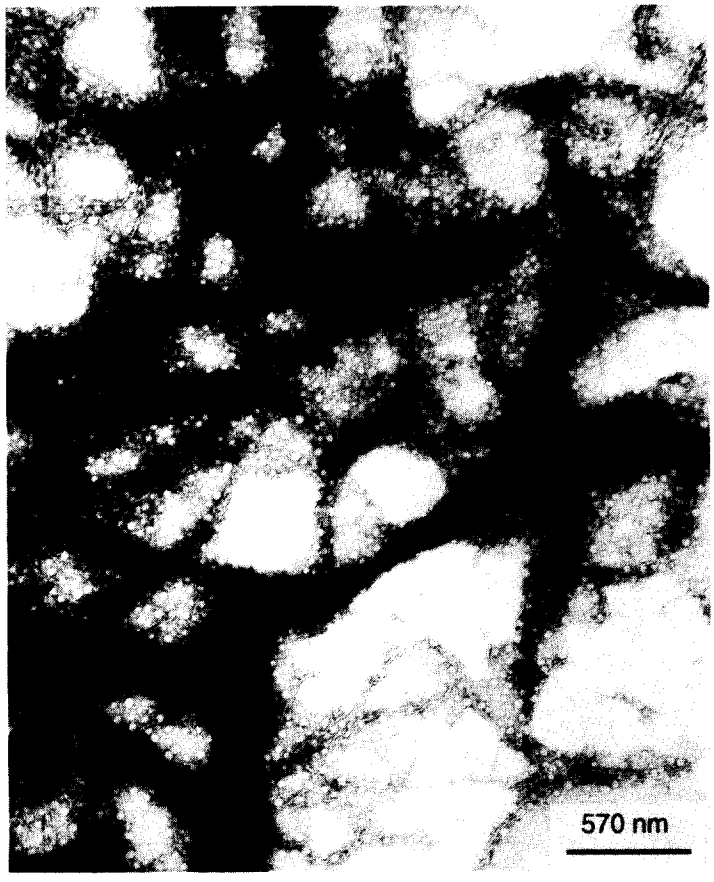


Fig. 5. Electron micrograph of compound **23b** after sonication (negative staining by phosphotungstic acid).

major role in the organization of such systems in water. A bulky hydrophilic head (trigalactosylated compound 17b) should probably restrict the specific orientations of the hydrophobic part [21] and therefore enhance its fluidity. In other words, bulkiness confers to the tail the degree of freedom apparently necessary to the formation of vesicules. Lowering the steric constraints related to the hydrophilic head by elimination of one or two galactopyranose residues (compounds **18b** or 19b), or by lenghtening the spacer (compounds **24b** or **25b**), results in increased steric and configurational constraints in the hydrophobic part, which appears to favour the formation of fibrous and tubular-type lamellar systems. This phenomenon nicely corroborates the observations of Kunitake and co-workers [23,34] with surfactants derived from aspartic acid and bearing separating connectors of various lengths, as well as the interpretation of tubular organization proposed by Schnur and co-workers [35,36].

Thus, it appears that in order to favour the formation of vesicles over tubular structures, it is necessary to increase the volume of the hydrophilic head, which can be readily achieved through telomerization.

2.6. Effect of introducting an oligomeric hydrophilic headgroup

Three types of telomers bearing six monogalactosylated (compounds **30b** and **31b**) or trigalactosylated (compound **29b**) Tris moieties were investigated. These telomers were readily dispersed in deionized water and gave, upon ultra-

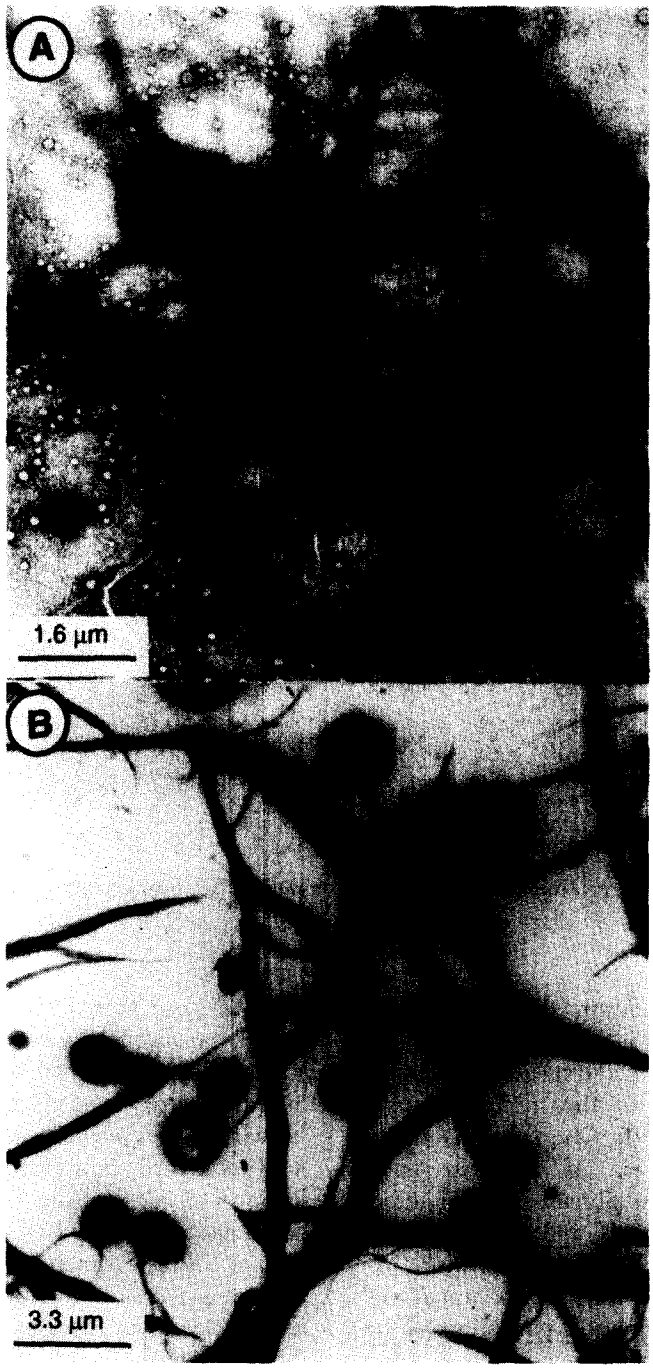


Fig. 6. Electron micrographs of compound **24b** without sonication (negative staining by phosphotungstic acid (A) and uranyl acetate (B)).

sonication stable bluish translucid solutions.

Electron microscopy of aqueous dispersions of telomer **29b** which contain an 18 carbon atom hydrophobic chain, reveals the presence of a ho-

Fig. 7. Electron micrograph of compound **25b** after sonication (negative staining by phosphotungstic acid).

mogeneous vesicle population of average size lower than 50 nm, which is stable over time. No fibers were present in the medium (Fig. 8). Similar results were obtained with the monogalactosylated telomers **30b** and **31b**. One can reasonably assume that the presence of a glycosylated oligomeric chain causes an increase of steric interactions between polar heads and, consequently, confers to the bilayer the necessary curvature for preferential formation of vesicles. The impact of the geometry of the hydrophobic part seems to be lowered. If this hypothesis is founded, one could expect to induce the formation of stable vesicles by the inclusion of a certain amount of telomeric glycolipids in the aqueous suspension of a monomeric surfactant which normally produces fibrous systems.

2.7. Effect of the addition of a telomeric-type co-surfactant on the dispersions of glycolipids

The behavior of an aqueous suspension of a monogalactosylated glycolipid **19b** in the presence of variable proportions of a telomeric monogalactosylated surfactant (**30b**) was studied. Ultrasonication of a mixture of 60% by weight of surfactant **19b** and 40% of telomer **30b** led to a limpid solution which remained homogeneous for several days at room temperature. A large number of vesicles of unequal sizes and few tubules were observed by electron microscopy. An increase in the proportion of telomer (56% by weight, i.e. 25% by mol) led, after this usual treatment, to the complete disappearance of the fibrous systems and to the exclusive formation of vesicles of relatively homogeneous sizes (60–100 nm) (Fig. 9A).

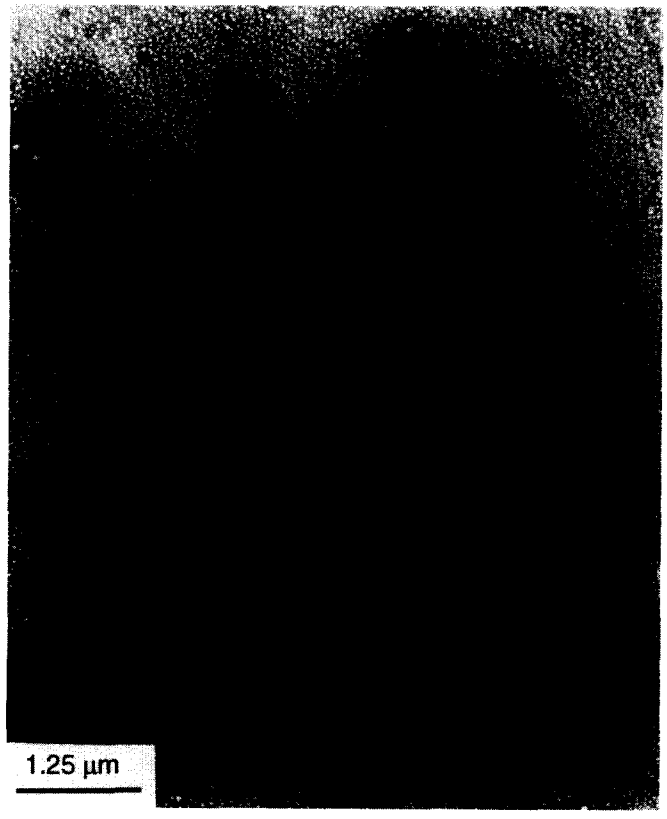


Fig. 8. Electron micrograph of compound **29b** after sonication (negative staining by phosphotungstic acid).

These results corroborate observations previously made with mixtures of the another groups of glycolipids derived from lactose and from single-chain Tris telomer [19]. The increase in steric constraints obtained by introducting oligomeric galactosylated Tris polar heads, probably induces an increase in the asymmetry and/or curvature of the lipid bilayer. In these conditions, the molecular interactions between the hydrophobic chains are not a determinant parameter when the tridimensional organisation of the membranes is concerned.

After several weeks of storage at room temperature, we observed the reappearance of helical fibres in a proportion which grows with time (Fig. 9B). This phenomenon could be explained by a slow demixing of the mixed lipidic bilayers into two homogenous systems constituted, respectively, of monomeric glycolipids **19b**, which give tubular structures and of telomeric amphiphile **30b** which produces vesicular structures.

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- and ^{13}C-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. Elemental

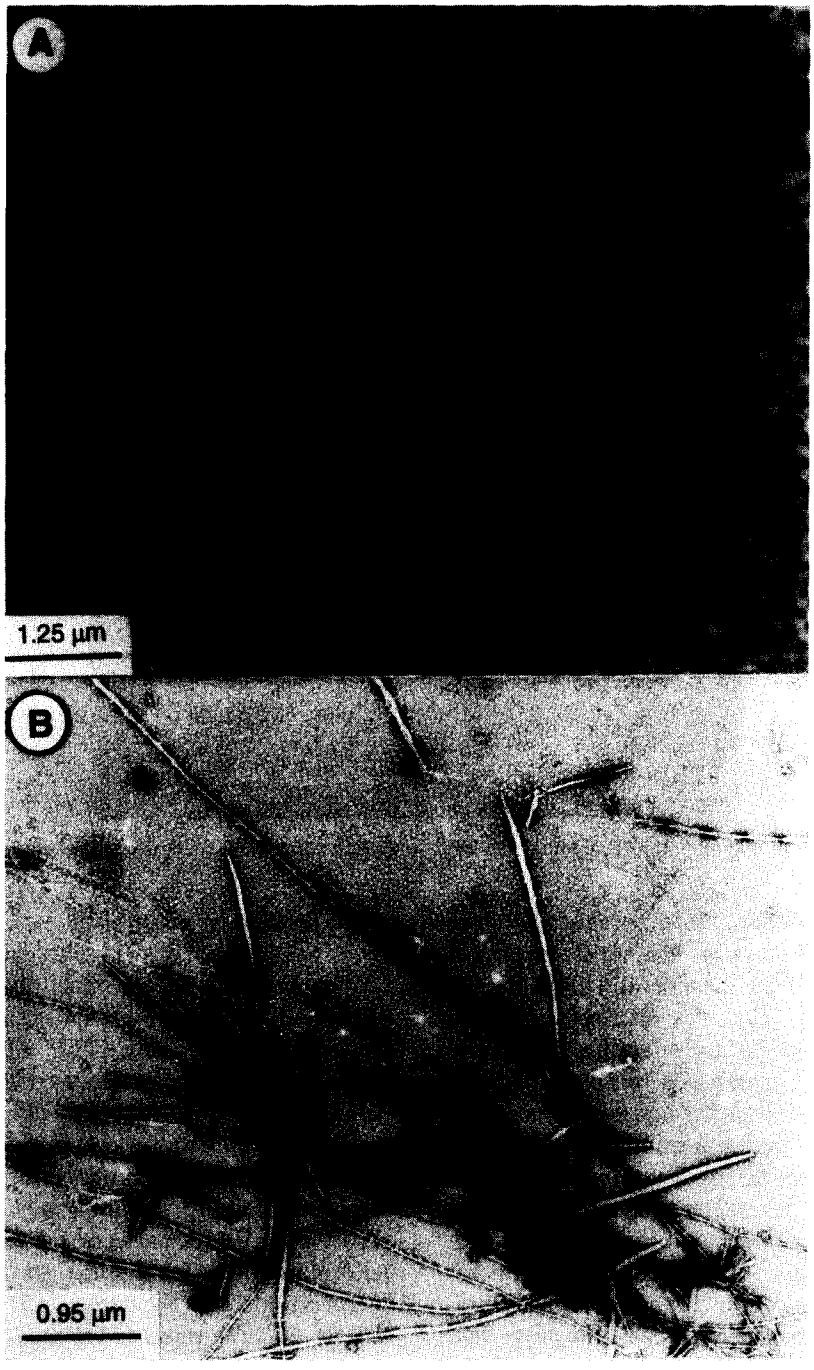


Fig. 9. Effect of the addition of the glycolipid telomer **30b** to the monomer **19b**. These electron micrographs (negative staining by phosphotungstic acid) represents a dispersion of a mixture of compound **19b** (A) with 60% (w/w) of the glycolipid telomer **30b** and, (B) after two months storage.

analysis were performed by the Service Central de Microanalyses of the CNRS at Montpellier. Mass spectra were recorded on a DX 300 Jeol apparatus.

Sonications were 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.

Reactions were performed in anhydrous conditions under dry nitrogen. All the solvents were distilled and dried according to standard procedures. For the telomerization, the solutions were carefully deoxygenated by nitrogen bubbling before use. AIBN was purified by recrystallization from absolute ethanol (twice).

3.1. 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, 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.

3.2. Optical microscopy

The sample were observed using a BHS Olympus microscope. The caracteristic defects of the bilayer, i.e. 'Maltese crosses' which characterise the lamellar faces were visualized by optical polarization microscopy. Fibers were observed by phase contrast optical microscope. Heating over the T_c to visualize transformations of fibers into liposomes were proceeded with regulated platine.

3.3. 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.

Example: compound **17b**

Product **17b** (10.8 mg) was hydrated with a mixture of water/ethylene glycol, 60:40 v/v (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 −8 J/g.

3.4. Synthesis of compounds 4, 5 and 11 N-(benzyloxycarbonyl)glycyl tris(hydroxymethyl)-aminomethane **1a**

Z-glycine (6g, 28.7 mmol) and Tris(hydroxymethyl)aminomethane (3.82 g, 31.5 mmol) were dissolved in absolute ethanol (150 ml). After adding EEDQ (13.3 g, 53.8 mmol), the mixture was refluxed for 6 h. Ethanol was evaporated under vacuum and the resulting oil was triturated after cooling in diethyl ether and the precipitate filtered off. The precipitate was dissolved in methanol (200 ml) and stirred in the presence of H^+-type resin (Amberlite IRC 50). After filtration and evaporation of the solvent, the resulting syrup was purified by recrystallization (methanol/ether) to afford pure compound **1a** as a white powder (5.2 g, 58%). m.p.: 130°C.

^{1}H-NMR (DMSO-d_6) δ: 7.52 (1H, t, $NHCH_2$); 7.35 (5H, m, Ph); 7.18 (1H, s, NHtris); 5.05 (2H, s, CH_2Ph); 4.74 (3H, t, OH); 3.66 (2H, d CH_2NH); 3.55 (6H, d, CH_2OH).

^{13}C-NMR (DMSO-d_6) δ: 170.06 (CONHtris); 156.57 (CO urethane); 137.09, 128.45, 127.89, 127.74 (Ph); 65.61 ($PhCH_2$); 63.50 (CH_2OH); 62.27 (CNH); 60.46 (CH_2NH).

N-(benzyloxycarbonyl)sarcosyl tris(hydroxymethyl)aminomethane **1b**

The procedure was similar to that described above for compound **1a**, starting from *Z*-sarcosine (10g, 44.8 mmol) tris(hydroxymethyl)aminomethane (5.7 g, 47 mmol) and EEDQ (13.3 g, 53.8 mmol). Compound **1b** was isolated after recrystallization (methanol/ether) as a white powder (10 g, 68%). m.p: 104–105°C.

^{1}H-NMR (DMSO-d_6) δ: 7.36–7.28 (5H, m, Ph); 7.24 (1H, 2s, NHtris); 5.09, 5.06 (2H 2s, CH_2Ph); 4.71 (3H, m, OH); 3.92 (2H, s, NCH_2); 3.57, 3.55 (6H, d, CH_2OH); 2.89, 2.87 (3H, 2s, CH_3N).

^{13}C-NMR (DMSO-d_6) δ: 169.32, 169.15 (*C*ONH); 156.08, 155.80 (CO urethane); 137.02, 128.46, 128.37, 127.86, 127.66, 127.48, 127.10 (Ph); 66.38, 66.16 (CH_2Ph); 62.37 (CH_2OH); 60.54 (*C*tris); 51.70, 51.46 (CH_2 CO); 35.88, 35.01 (CH_3N).

5-(N-(benzyloxycarbonyl)glycinamido)-5-hydroxymethyl- 2-phenyl-1,3-dioxane **2**

Compound **1a** (2g, 6.4 mmol) was dissolved in a mixture of benzaldehyde (20 ml) and freshly distilled acetonitrile (40 ml). After adding *p*-toluene sulfonic acid (100 mg), the mixture was stirred at room temperature for 1 day. The solution was neutralized with triethylamine and the solvent removed under reduced pressure. Residual oil was dissolved in dichloromethane (200 ml), washed with a 1 N hydrochloric acid solution and water dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane 2:1 v/v) to afford a mixture of **2a** and **2b** (1.2 g, 47%). Crystallization from dichloromethane/diethyl ether allowed the separation of the major component **2a** in pure form (m.p.: 142-143°C).

Isomer **2a**

^{1}H-NMR ($CDCl_3$) δ: 7.43 and 7.28 (10H, m, Ph); 6.53 (1H, s, NHtris); 5.79 (1H, t, $NHCH_2$); 5.47 (1H, s, CHPh); 5.09 (2H, s, CH_2Ph); 4.27–3.74 (8H, m, CH_2CO, CH_2OH, $2CH_2O$); 2.93 (1H, s, OH).

^{13}C-NMR ($CDCl_3$) δ: 170.70 (CONH); 157.63 (CO urethane); 138.04, 136.64, 129.80, 129.26, 129.00, 128.74 126.77 (Ph); 102.24 (CHPh); 69.40, 68.05 (CH_2OH and CH_2OBzl); 62.67 (CNH); 54.18 (CH_2NH)

Isomer **2b** *(in the mixture* **2a**, **2b***)*

^{1}H-NMR ($CDCl_3$) δ: 7.45 et 7.33 (10H, m, Ph); 7.01 (1H, s, NHtris); 5.67 (1H, t, $CH_2N\mathit{H}$); 5.43 (1H, s, $C\mathit{H}Ph$); 5.05 (2H, s, CH_2Ph); 4.24 to 3.67 (8H, m, CH_2CO, CH_2OH, $2CH_2O$).

^{13}C-NMR ($CDCl_3$) δ: 171.09 (CONH); 157.28 (CO urethane); 137.93, 136.65, 129.84, 129.10, 129.01, 128.91 128.76, 126.63 (Ph); 102.64 (CHPh); 71.98, 67.88 (CH_2OH, CH_2O); 64.06 ($\mathit{C}NH$); 55.99 (CH_2NH).

5-(N-(benzyloxycarbonyl)sarcosinamido)-5-hydroxymethyl- 2-phenyl-1,3-dioxane **3**

The same procedure as used to prepare compound **2** was applied to the synthesis of compound **3** starting from compound **1b** (1.16 g, 4.56 mmol). After purification by column chromatography on silica gel (ethyl acetate/hexane 3:2 v/v) a mixture of several diastereoisomers were obtained as a white powder (1.45 g, 66%).

5-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)hydroxymethyl)-5-(N-(benzyloxycarbonyl)glycinamido)-2-phenyl-1,3-dioxane **4**

Compound **2** (1.2 g, 3.04 mmol) and mercuric cyanide (1.16 g, 4.56 mmol) were dissolved in freshly distilled acetonitrile. After stirring for 15 mn in the presence of drierite, acetobromogalactose (1.9 g, 4.56 mmol) was added and the mixture sonicated under a nitrogen atmosphere for 15 min at room temperature. The precipitate was filtered off and the solvent removed under vacuum. The syrupy residue was dissolved in ethyl acetate and successively washed with saturated sodium hydrogenocarbonate (60 ml), 10% sodium iodide (30 ml), saturated sodium thiosulfate (60 ml) and water (100 ml). The organic layer was dried over sodium sulfate and then concentrated to dryness under reduced pressure. The crude product was purified by column chromatography over silica gel (ethyl acetate/hexane 3:2 v/v) to afford **4** as a white powder (1.4 g, 66%). m.p.: 70–71°C. $[\alpha]_D^{20} = +\ 1.1$ (c, 1, $CHCl_3$). The ^{1}H-NMR and ^{13}C-NMR showed the presence of two diastereoisomers.

^{1}H-NMR (DMSO-d_6) partial data δ: 7.95 7.68 (1H, 2s, NHtris); 7.5 to 7.2 (5H, m, Ph); 5.58, 5.48 (1H 2s, CHPh); 2.2 to 1.8 (12H, m, CH_3CO).

^{13}C-NMR (DMSO-d_6) partial data δ: 156.41 (CO urethane); 100.91, 100.73, 100.60, 100.46 (C_1 anomer β + $\mathit{C}HPh$); 52.57, 51.43 (CH_2gly).

5-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)hydroxymethyl)-5-(N-(benzyloxycarbonyl)sarcosinamido)-2-phenyl-1,3-dioxane **5**

Treatment of mixture **3** (4g, 9.61 mmol) with mercuric cyanide (3.64 g, 14.40 mmol) and acetobromogalactopyranose (5.92 g, 14.4 mmol) gave compound **5** (6.90 g, 95%). m.p.: 61-62°C. $[\alpha]_D^{20} = +\ 4.20$ (c, 1, $CHCl_3$). The ^{1}H-NMR and ^{13}C-NMR showed the presence of several diastereoisomers.

^{1}H-NMR ($CDCl_3$) partial data δ: 5.52, 5.44 (1H, 2s, CHPh); 5.18, 5.08 (2H, 2s, CH_2Ph); 3.04, 2.99 (3H, 2s, CH_3N).
^{13}C-NMR ($CDCl_3$) partial data δ: 101.63 to 101.43 (m, C_1 anomer β + CHPh); 53.29, 52.31 (CH_2gly); 36.38 (m, CH_3N).

Acetoxymethyl bishydroxymethyl-N-(benzyloxycarbonyl)glycyl aminomethane ***8***

Compound **1a** (4.01 g, 12.87 mmol) was dissolved in freshly distilled acetone (150 ml). After adding *p*-toluene sulfonic acid (100 mg), the mixture was stirred at room temperature for 2 h. The solution was neutralized with triethylamine and the solvent removed under reduced pressure. The residual oil was dissolved, at 0°C, in a mixture of acetic anhydride/pyridine 1:1 v/v (70 ml) under nitrogen. The solution was stirred for 16 h at room temperature and then poured into cold water. The aqueous layer was extracted with ethyl acetate (3 × 100 ml). The combined organic phase was washed with 1 N hydrochloric acid and water, before drying over sodium sulfate and concentrated to dryness under reduced pressure. The resulting syrup was dissolved in 100 ml of a mixture of acetic acid/water (7:3 v/v). The solution was stirred at 70°C for 2 h and concentrated to dryness under reduced pressure. The residue was dissolved in toluene (40 ml) and the solvent removed under vacuum. The operation was repeated twice. The resulting oil was subjected to column chromatography on silica gel (ethyl acetate) to afford the pure compound **8** as a colorless oil (3.32 g, 73%).
^{1}H-NMR ($CDCl_3$) δ: 7.31 (5H, m, Ph); 6.92 (1H, s, NHtris); 5.97 (1H, t, $NHCH_2$); 5.09 (CH_2Ph); 4.33 (2H, s, 2OH); 4.23 (2H, s CH_2OAc); 3.81 (2H, d, CH_2NH); 3.77 and 3.63 (4H, 2d, CH_2OH); 2.02 (3H, s, CH_3CO).
^{13}C-NMR ($CDCl_3$) δ: 172.14 (CH_3CO); 171.63 (CONH); 157.61 (CO urethane); 136.50 129.10, 128.82, 128,53 (Ph); 67.79 (CH_2Ph); 63.05 (CH_2OAc); 62.88 (CH_2OH); 61.72 (CNH); 45.36 (CH_2NH); 21.24 (CH_3CO).

Bis-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)hydroxymethyl)acetoxymethyl-N-(benzyloxycarbonyl)glycyl aminomethane ***9***

Treatment of compound **8** (1.2 g, 3.45 mmol) with mercuric cyanide (2.6 g, 10.34 mmol) and acetobromogalactose (4.25 g, 10.34 mmol) gave compound **9**, after purification on silica gel column (ethyl acetate/hexane 7:3 v/v) as a white powder (2.5 g, 75%). m.p.: 71–72°C. $[\alpha]_D^{20} = -3.3$ (c, 1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.38 (5H, m, Ph); 6.35 (1H, s, NHtris); 5.56 (1H, t, $NHCH_2$); 5.38 (2H, m H_4); 5.18–4.98 (6H, m, H_3, H_2), CH_2Ph); 4.42 (2H, d, H_1); 4.30 (2H, q, CH_2OAc); 4.17 (6H, m, CH_2OGal, H_5); 4.09–3.98 (6H, m, CH_2CONH, H_6); 2.05 (27H, 4s CH_3CO).
^{13}C-NMR ($CDCl_3$) δ: 170.89, 170.40 170.36, 170.19, 168.98, 168.34 (CH_3CO CH_2CONH); 156.55 (CO urethane); 136.37, 128.53 128.15, 128.05 (Ph); 101.35, 101.19 (C_1, β-anomer); 70.84, 70.80 (C_5 or C_3); 70.60, 70.52 (C_3 or C_5); 68.98, 68.93 (C_2); 68.13 (CH_2OGal); 67.01 (C_4); 62.97 (CH_2Ph); 61.28, 61.16 (C_6 CH_2OAc); 58.90 (CNH) 44.75 (CH_2NH); 20.76–20.52 (CH_3CO).

Tris-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)hydroxymethyl-N-(benzyloxycarbonyl)glycyl aminomethane ***11***

Treatment of compound **1a** (2g, 6.4 mmol) with mercuric cyanide (7.22 g, 28.8 mmol) and acetobromogalactopyranose (11,86 g, 28.8 mmol) gave after chromatography on silica gel compound **11** (ethyl acetate/hexane 7:3 v/v) as a white powder (5.42 g, 65%). m.p.: 88–90°C. $[\alpha]_D^{20} = +3.3$ (c, 1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.36 (5H, m, Ph); 6.19 (1H, s, NHtris); 5.63 (1H, t, $NHCH_2$); 5.39 (3H, d, H_4); 5.13 (5H, s, dd, H_3), CH_2Ph); 5.03 (3H, dd, H_2); 4.41 (3H, d, H_1); 4.15 (9H, m, H_5 CH_2OGal); 3.93 (3H, t, H_{6a}); 3.80 (5H, m H_{6b}, CH_2NH); 2.05 (36H, 4s, CH_3CO).
^{13}C-NMR ($CDCl_3$) δ: 170.39, 170.18 169.87, 169.49, 169.02 (CONH, CH_3CO); 136.52, 128.52 128.08, 127.91 (Ph); 101.34 (C_1 β anomer); 70.83, 70.57 (C_5, C_3); 69.08 (C_2); 68.19 (CH_2OGal); 67.05, 66.88 (C_4 CH_2Ph); 61.22 (C_6); 59.19 (CNH); 44.10 (CH_2Gly); 20.76, 20.65, 20.58, 20.53 (CH_3CO).

3.5. Synthesis of compounds **6**, **7**, **10** and **12**

Example: compound **12**

Compound **11** (5g, 4 mmol) was dissolved in methanol (50 ml). After cooling the solution, Pd/C (250 mg) was added. The mixture was hydrogenated for 4 h. After filtration on celite the organic phase was concentrated to dryness under reduced pressure. Compound **12** was obtained pure as a white powder (4.82 g, 98%).

The same procedure as that used for the preparation of compound **12** was applied to the synthesis of compounds **6**, **7** and **10**.

3.6. Preparation of hydrophobic moieties **15a**, **15b**, **15c** and **16**

N^{α}-tert-butyloxycarbonyl-β-benzylester dodecyl-L-aspartamide **13a**

To a solution of *N-tert*-butyloxycarbonyl-β-benzylester aspartic acid (5g, 16.2 mmol) and dodecylamine (3.01 g, 16.2 mmol) in cold dichloromethane (100 ml), were added dicyclohexylcarbodiimide (DCC) (3,96 g, 19.2 mmol) and hydroxybenzotriazole (HOBT) (100 mg). The mixture was stirred for 1 h at room temperature. After filtration of the dicyclohexylurea (DCU), the organic phase was washed successively with 1 N hydrochloric acid and saturated sodium hydrogenocarbonate, dried over sodium sulfate and then concentrated to dryness under vacuum. The crude product was purified by chromatography on silica gel (hexane/ethyl acetate 8:2 v/v). After recrystallisation from hexane/diethyl ether, pure compound **13a** was obtained as a white amorphous powder (7.5 g, 97%). m.p.: 66–67°C. $[\alpha]_D^{20}$ = + 6 (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.35 (5H, s, Ph); 6.25 (1H, t, N*H*CH_2); 5.70 (1H, d, NHCH); 5.15 (2H, dd CH_2Ph); 4.48 (1H, m, CH); 3.20 (2H, m CH_2NH); 2.90 (2H, 2dd, CH_2Asp); 1.48 (9H s, Boc); 1.30 (20H, s, $(CH_2)_{10}$); 0.90 (3H t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 171.51 (CONH), 170.56 (COOCH_2Ph); 155.58 (CO urethane); 135.54, 128.54, 128.28 (Ph); 80.17 (C(Me)$_3$); 66.67 (CH_2Ph); 50.79 (CH); 39.63 (CH_2NH); 36.43 (CH_2Asp); 31.91–26.84 ($(CH_2)_{10}$); 22.68 (CH_3 tBu); 14.11 (CH_3).

N^{α}-dodecanoyl-β-benzylester dodecyl-L-aspartamide **14a**

Compound **13a** (4.01 g, 8.42 mmol) was added to a mixture of trifluoroacetic acid/dichloromethane, 1:1 v/v (50 ml) and stirred for 1 h at room temperature. The solution was concentrated to dryness under vacuum. The crude product was dissolved in cold dichloromethane (50 ml), then TEA added until pH = 8. Lauroyl chloride (2.9 ml, 12.6 ml) was added dropwise within 15 min. The pH was adjusted to 8–9 by adding TEA. After stirring for 4 h at room temperature, the mixture was washed with 1 N hydrochloric acid and water, dried over sodium sulfate and concentrated under vacuum. The resulting syrup was purified by recrystallization in ethyl acetate and pure compound **14a** was obtained as white crystals (4.1 g, 90%). m.p.: 100–101°C. $[\alpha]_D^{20}$ = − 18.4 (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, s, Ph); 6.88 (1H, d, NHCH); 6.61 (1H, t, $NHCH_2$); 5.14 (2H, s CH_2Ph); 4.80 (1H, s, CH); 3.18 (2H, q CH_2NH); 2.80 (2H, 2dd, CH_2Asp); 2.19 (2H t, CH_2CO); 1.70–1.20 (38H, m $(CH_2)_{10}$, $(CH_2)_9$); 0.88 (6H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 174.03, 172.04 170.95 (2CONH, COO); 136.06, 129.09, 128.86, 128.74 (Ph); 67.28 (CH_2Ph); 49.84 (CH); 40.24 (CH_2NH); 36.87–26.13 ($(CH_2)_{10}$, $(CH_2)_9$); 14.64 (CH_3).

N^{α}-dodecanoyl dodecyl-L-aspartamide **15a**

Procedure identical to that used for the synthesis of compound **12**. Compound **14a** (4.1 g, 7.16 mmol) afforded **15a** (3.3g, 95%). m.p.: 110–112°C. $[\alpha]_D^{20}$ = − 16.4 (c, 1, DMSO).

^{1}H-NMR ($CDCl_3$) δ: 7.02 (1H, d, NHCH); 6.99 (1H, t, $NHCH_2$); 4.57 (1H, m, CH); 3.30 (2H, m CH_2NH); 2.82 (2H, 2dd, CH_2Asp); 2.30 (2H t, CH_2CO); 1.70–1.20 (38H, m, $(CH_2)_n$); 0.09 (3H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 172.13, 171.69 170.30 (CONH); 49.30 (CH); 40.16 (CH_2NH); 36.16 (CH_2CO); 35.13 (CH_2CO); 33.60–22.04 ($(CH_2)_n$); 13.87 (CH_3).

The same procedure as used for the preparation of compound **15a** was applied to the synthesis of compounds **15b** and **15c**.

N^{α}-tert-butyloxycarbonyl-β-benzylester-N-methyldodecyl-L-aspartamide ***13b***

Procedure identical to that used for the synthesis of compound **13a**. *N-tert*-butyloxycarbonyl-β-benzylester aspartic acid (2g, 6.5 mmol) and *N*-methyl dodecylamine (1.55g, 7.81 mmol) afforded **13b** (3.1g, 97%) as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, m, Ph); 5.36 (1H, d, NHCH); 5.10 (2H, m, CHPh); 4.98 (1H, m, CH); 3.30 (2H m, CH_2N); 3.07 and 2.89 (3H, 2s, CH_3N); 2.86 and 2.59 (2H, 2dd, CH_2Asp); 1.48 (9H, s CH_3tBu); 1.79–1.16 (20H, m $(CH_2)_{10}$); 0.90 (3H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 170.54, 170.47 170.40 (CON or COOBzl); 155.00, 154.76 (CO uréthane); 135.71 135.66, 128.53, 128.37, 128.30, 128.26, 128.24 (Ph); 79.98 ($C(Me)_3$); 66.65, 66.61 (CH_2Ph); 49.97, 48.35 (CH_3N); 47.85, 47.16 (CH); 38.26, 37.82 (CH_2N); 35.21, 33.80 (CH_2CO); 32.44–26.93 ($(CH_2)_n$); 22.68 (CH_3tBu); 14.11 (CH_3).

N^{α}-dodecanoyl-β-benzylester-N- methyl dodecyl-L-aspartamide ***14b***

Procedure identical to that used for the synthesis of compound **14a**. Compound **13b** (2.51 g, 5.15 mmol) afforded **14b** as a white powder (2g, 68%). m.p.: 57–58°C. $[\alpha]_D^{20} = -26.2$ (c, 1 $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, m Ph); 6.51 (1H, d, NH); 5.27 (1H, m, CH); 5.10 (2H, m CH_2Ph); 3.30 (2H, m, CH_2N); 3.07 and 2.89 (3H, 2s, CH_3N); 2.84 and 2.61 (2H, 2dd CH_2Asp); 2.06 (2H, m, CH_2CO); 1.54 and 1.24 (38H, m, $(CH_2)_n$); 0.87 (3H, t CH_3).

^{13}C-NMR ($CDCl_3$) δ: 172.50, 172.29 (CO_2Bzl); 170.45, 170.40, 170.36, 170.23 (CON, CONH); 135.65, 128.54, 128.37, 128.30 (Ph); 66.73 (CH_2Ph); 49.99, 48.38 (CH); 45.79, 45.61 (CH_3N); 37.91, 37.57 (CH_2NH); 36.54, 36.52 (CH_2CO); 35.30–22.69 ($(CH_2)_n$); 14.10 (CH_3).

N^{α}-dodecanoyl-N-methyl dodecyl- L-aspartamide ***15b***

Procedure identical to that used for the synthesis of compound **15a**. Compound **14b** (2 g, 3.49 mmol) afforded **15b** as a white amorphous powder (1.70g, 98%). m.p.: 81–83°C.

^{1}H-NMR (DMSO-d_6) δ: 8.38 (1H, s broadened COOH); 7.20 et 7.17 (1H, d, CON*H*); 5.29 (1H, m, CH); 3.43 and 3.34 (2H, 2t, CH_2N); 3.16 and 2.93 (3H, 2s CH_3N); 2.68 (2H, m, CH_2Asp); 2.21 (2H, t, CH_2CO); 1.60–1.25 (38H, m, $(CH_2)_9$, $(CH_2)_{10}$); 0.88 (6H, t, CH_3).

^{13}C-NMR (DMSO-d_6) δ: 173.51, 173.41 173.20, 171.04, 170.98 (CONH, COOH); 50.22 and 48.72 (CH); 46.22 45.91 (CH_3N); 38.21, 38.05 (CH_2N); 36.40 36.35 (CH_2CO); 35.55–22.69 ($(CH_2)_n$); 14.10 (CH_3).

N^{α}-tert-butyloxycarbonyl-β-benzylester octadecyl-L-aspartamide ***13c***

Procedure identical to that used for the synthesis of compound **13a**. *N-tert*-butyloxycarbonyl-β-benzylester aspartic acid (5.52 g, 17.08 mmol) and octadecylamine (4.59g, 17.08 mmol) afforded **13c** as white crystals (9g, 94%). m.p.: 82–83°C. $[\alpha]_D^{20} = +7.3$ (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, s, Ph); 6.46 (1H, t, N*H*CH_2); 5.68 (1H, d, NHCH); 5.13 (2H, m CH_2Ph); 4.47 (1H, m, CH); 3.22 (2H, q, CH_2NH); 2.90 (2H, 2dd, CH_2Asp); 1.44 (9H s, CH_3tBu); 1.25 (32H, m, $(CH_2)_{16}$); 0.87 (3H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 171.54 (CONH); 170.52 (CO_2Bzl); 155.57 (CO urethane); 135.54 128.53, 128.27, 128.16 (Ph); 80.17 ($C(Me)_3$); 66.67 (CH_2Ph); 50.77 (CH); 39.62 (CH_2NH); 36.42 (CH_2Asp); 31.93–22.69 ($(CH_2)_{16}$); 14.11 (CH_3).

N^{α}-octadecanoyl-β-benzylester octadecyl-L-aspartamide ***14c***

Procedure identical to that used for the synthesis of compound **14a**. Compound **13c** (2 g, 3.58 mmol) afforded **14c** as a white powder (2.37 g, 95%). m.p.: 107–108°C. $[\alpha]_D^{20} = -8.2$ (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.34 (5H, s, Ph); 6.85 (1H, d, NHCH); 6.60 (1H, t, $NHCH_2$); 5.19 (2H, m CH_2Ph); 4.79 (1H, m, CH); 3.19 (2H, q CH_2NH); 2.80 (2H, 2dd, CH_2Asp); 2.19 (2H t, CH_2CO); 1.70–1.10 (62H, m $(CH_2)_n$); 0.87 (6H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 173.44, 171.88 (2CONH); 170.25 (CO_2Bzl); 135.45, 128.61, 128.39, 128.25 (Ph); 66.54 (CH_2Ph); 49.24 (CH); 39.68 (CH_2NH); 36.53 (CH_2CO); 35.90 (CH_2CO); 31.95–22.71 ($(CH_2)_n$); 14.13 (CH_3).

N^{α}-octadecanoyl octadecyl-L-aspartamide **15c**

Procedure identical to that used for the synthesis of compound **15a**. Compound **14c** (2 g, 2.70 mmol) afforded **15c** as a white amorphous powder (1.80g, 97%). m.p.: 118–121°C.
This compound was insoluble in usual deuterated solvents.

N^{α}-dodecanoyl-β-N-(carboxymethyl)amide dodecyl-L-aspartamide **16**

Compound **15a** (1 g, 2.07 mmol) and *tert*-butyl glycinate (272 mg, 2.07 mmol) were dissolved in dichloromethane. After adding DCC (47 mg, 2.27 mmol), the mixture was stirred for 4h at room temperature then concentrated under vacuum. The residual oil was purified by column chromatography on silica gel (ethyl acetate/hexane). The resulting product was dissolved in 50 ml of a mixture of trifluoroacetic acid/dichloromethane (1:1 v/v). The solution was stirred for 1h at room temperature and then concentrated to dryness under reduce pressure. After recrystallization in methanol/ether, pure compound **16** was obtained as white crystals (1.1 g, 80%). m.p.: 174–177°C. $[\alpha]_D^{20} = -12.7$ (c, 1, DMSO).
^{1}H-NMR (DMSO-d_6) δ: 8.65 (1H, t CH_2NHGly); 7.91 (1H, d, CH_2NH); 7.62 (1H, t, CH_2NH); 4.51 (1H, q, CH); 3.72 (2H, d, CH_2NHGly); 3.02 (2H, m, CH_2NH); 2.48 (2H, 2dd, CH_2Asp); 2.10 (2H, t, CH_2CO); 1.50–1.20 (38H, m, $(CH_2)_{10}$); 0.90 (6H, t CH_3).
^{13}C-NMR (DMSO-d_6) δ: 172.45, 171.29 170.80, 169.91 (CONH); 49.84 (CH); 37.34–19.46 ($(CH_2)_n$, CH_2Gly); 13.95 (CH_3).

3.7. Synthesis of glycolipids **17a–25a**

N^{α}-dodecanoyl-β-N-(N- (tris-*(O-(2,3,4,6*-tetra-*O*-acetyl-*β-D- galactopyranosyl)hydroxymethyl)-methyl)carboxamidomethyl)amide dodecyl-L-aspartamide* **17a**

Compound **15a** (153 mg, 0.31 mmol) was dissolved in dichloromethane (20 ml). Successively compound **12** (405 mg, 0.33 mmol), BOP reagent (109 mg, 0.38 mmol) and dimethylaminopyridine (DMAP) (43 mg, 0.35 mmol) were added and the mixture stirred for 2 h at room temperature. After evaporation of solvent under reduced pressure, the resulting oil was dissolved in dichloromethane (50 ml) and washed successively with 1 N hydrochloric acid and saturated sodium hydrogenocarbonate. After drying over sodium sulfate, the solution was concentrated under vacuum and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane 95:5 v/v), then on Sephadex LH 60 ($MeOH/CH_2Cl_2$ 1:1 v/v). Pure compound **17a** was isolated as a white powder (287 mg, 51%). m.p. : 85–86°C.. $[\alpha]_D^{20} = -13$ (c, 1, $CHCl_3$).
Deacetylation of **17a** was performed at room temperature in methanol containing a catalytic amount of sodium methoxide. After treatment with an H^+-type resin (Amberlite IRC 50) followed by filtration and evaporation of the solvent deacetylated compound **17b** was isolated in quantitative yield.
^{1}H-NMR ($CDCl_3$) δ: 7.58 (1H, d, NHCH); 7.00 (2H, 2t, $2CH_2NH$); 6.28 (1H, s, NHtris) 5.38 (3H d, H4); 5.12 (3H, dd, H_3); 5.05 (3H, dd H_2); 4.72 (1H, m, CH); 4.42 (3H, d, H_1); 4.16 (9H, m, $3CH_2OGal$, H_5); 3.93 (5H, m, H_{6a}), CH_2Gly); 3.76 (3H, d, H_{6b}); 3.18 (2H, q, CH_2NH); 3.87 and 2.52 (2H, 2dd, CH_2Asp); 2.23 (2H, t, CH_2CO); 2.17–1.99 (36H, 3s, CH_3CO); 1.62–1.25 (38H, m $(CH_2)_{10}$,$(CH_2)_9$); 0.8 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ: 173.94, 171.75 170.94, 170.44, 170.18, 169.98, 169.55, 168.88 (CONH $COCH_3$); 101.43 (C_1 β anomer); 70.88 (C_5 or C_3); 70.58 (C_3 or C_5); 69.18 (C_2); 68.00 (CH_2OGal); 67.02 (C_4); 61.14 (C_6); 59.51 (CNH); 49.85 (CH); 42.84 (CH_2Gly); 39.71 (CH_2NH); 36.67, 36.50 ($2CH_2CO$); 31.91–22.68 ($(CH_2)_9$, $(CH_2)_{10}$); 20.80, 20.69, 20.63, 20.54 (CH_3CO); 14.10 (CH_3).
FAB$^+$ MS: $m/z = 1151$ $(M + Na)^+$ (deacetylated product).
Anal. calc for $C_{76}H_{120}O_{34}N_4$: C 55.87, H 7.39, N 3.42, O 33.29 (acetylated product); found: C 55.69, H 7.57, N 3.54, O 32.11.

N^{α}-dodecanoyl-β-N-(N-(acetoxymethyl bis-(O-(2,3,4,6-tetra-O-acetyl-β-D- galactopyranosyl)hydroxymethyl)methyl)carboxamidomethyl)-amide dodecyl-L-aspartamide **18a**

Procedure identical to that used for the synthesis of compound **17a**. From **15a** (360 mg, 0.75 mmol) and **10** (473 mg, 0.53 mmol) **18a** was

obtained as a white powder (390 mg, 54%). m.p.: 77–78°C. $[\alpha]_D^{20} = -9.5$ (c, 1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.67 (1H, d, N*H*CH); 7.05 (2H, 2t, 2NHCH_2); 6.47 (1H, s, NHtris) 5.39 (2H d, H_4); 5.08 (2H, dd, H_3); 5.03 (2H, dd, H_2); 4.73 (1H, m, CH); 4.42 (2H, m, H_1); 4.30 (2H, q, CH_2OAc); 4.14 (6H, m, 2CH_2OGal, H_5); 4.05 (4H, m, CH_2Gly, H_{6a}); 3.74 (2H, d, H_{6b}); 3.17 (2H, m CH_2NH); 2.84 and 2.52 (2H, 2dd, CH_2Asp); 2.25 (2H, t, CH_2CO); 2.05 (27H, 3s CH_3CO); 1.61 to 1.25 (38H, m $(CH_2)_9$, $(CH_2)_{10}$; 0.87 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ: 174.06, 169.45 (CONH CH_3CO); 101.38, 101.35 (C_1 β-anomer); 70.89 (C_5 or C_3); 70.55 (C_3 or C_5); 69.05 (C_2); 67.91, 67.58 (CH_2OGal); 67.02 (C_4); 62.75 (*C*H_2OAc); 61.25, 61.13 (C_6); 59.12 (CNH); 49.88 (CH); 42.98 (CH_2Gly); 39.72 (CH_2NH); 36.50 (CH_2CO); 31.92–22.69 ($(CH_2)_n$); 20.79, 20.68, 20.64, 20.54 (CH_3CO) 14.10 (CH_3).
FAB$^+$ MS: $m/z = 1367$ $(M + Na)^+$ (deacetylated product).
Anal. calc. for $C_{64}H_{104}O_{26}N_4$: C 57.13, H 7.78, N 4.16, O 30.91 (acetylated product); found: C 57.17, H 7.96, N 4.33, O 31.01.

N$^\alpha$-dodecanoyl-β-(N-(N-(bishydroxymethyl-O-(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl)hydroxymethyl)methyl)-carboxamidomethyl)amide dodecyl-L-aspartamide **19a**

Procedure identical to that used for the synthesis of compound **17a**. The resulting oil was dissolved in 50 ml of a mixture of acetic acid/water (7:3 v/v). The solution was stirred at 70°C for 2h and concentrated to dryness under reduced pressure. The residue was dissolved in toluene (40 ml) and the solvent removed under vacuum. The operation was repeated twice. The resulting oil was subjected to column chromatography on silica gel (ethyl acetate/methanol 9:1 v/v), then on sephadex LH 60 (methanol/dichloromethane). From **15a** (380 mg, 0.78 mmol) and **6** (452 mg, 0.71 mmol), **19a** was obtained as a white powder (360 mg 90%). m.p.: 92–93°C. $[\alpha]_D^{20} = -17.9$ (c, 1 $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.96 (1H, d, CHN*H*); 7.38 (1H, t, CH_2N*H*); 7.29 (1H, t, CH_2N*H*); 7.01 (1H, s, NHtris); 5.40 (1H, d, H_4); 5.10 (2H, m H_3, H_2); 4.78 (1H, m, CH); 4.53 (1H, d H_1); 4.32–3.53 (11H, m, CH_2OGal CH_2Gly, H_5, H_6); 3.13 (2H, q CH_2NH); 2.78 and 2.51 (2H, 2dd, CH_2Asp); 2.29 (2H, t, CH_2CO); 2.10 (12H, 4s, CH_3CO); 1.63–1.25 (38H, m $(CH_2)_9$, $(CH_2)_{10}$); 0.88 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ : 174.92, 172.93, 172.36, 171.48, 171.05, 170.72, 170.64, 170.52 (4*C*ONH 4$COCH_3$); 102.03 (C_1 β anomer); 71.35 (C_5 or C_3); 69.64 (C_2); 68.52 (*C*H_2OGal); 67.60 (C_4); 63.99, 63.58 (2*C*H_2OH); 62.24 (C_6); 61.68 (CNH); 50.62 (CH); 43.88 (CH_2Gly); 40.41 (CH_2NH); 36.92 (CH_2CO); 32.43–23.19 ($(CH_2)_n$); 21.32, 21.18, 21.14, 21.06 (CH_3CO); 14.62 (CH_3).
FAB$^+$ MS: $m/z = 827$ $(M + Na)^+$ (deacetylated product).
Anal. calc. for $C_{40}H_{76}O_{16}N_4$: C 59.68, H 9.50, N 6.85, O 23.85 (acetylated product); found: C 58.95, H 9.72, N 6.53, O 24.35.

N$^\alpha$-dodecanoyl-β-(N-methyl-N-(N-(O-(2,3,4,6-tetra- O-acetyl-β-D-galacto-pyranosyl)hydroxymethyl-bishydroxymethyl) methyl)carboxamidomethyl)amide dodecyl-L-aspartamide **20a**

Procedure identical to that used for the synthesis of compound **19a**. From **15a** (1.28 g, 2.65 mmol) and 7 (1.49 g, 2.41 mmol) **20a** was obtained as a white powder (1.60 g, 46%). m.p.: 52–53°C. ^{1}H-NMR and ^{13}C-NMR showed the presence of two diastereoisomers.
^{1}H-NMR ($CDCl_3$) δ: 7.92 (1H, d, N*H*CH); 7.21 (1H, t, CH_2NH); 6.87 (1H, s, NHtris); 5.41 (1H d, H_4); 5.18 (4H, d, H_3); 5.04 (1H, dd, H_2); 4.84 (1H, m, CH); 4.56 (1H, d, H_1); 4.22 to 3.48 (11H, m, CH_2OGal, 2CH_2OH, H_5, H_6, CH_2Sar); 3.18 (2H, m CH_2NH); 3.14, 3.02 (3H, 2s, CH_3N); 2.40 (2H, 2dd, CH_2Asp); 2.28 (2H, t, CH_2CO); 2.20–1.99 (12H, 4s, CH_3CO); 1.64–1.25 (38H, m, $(CH_2)_9$, $(CH_2)_{10}$); 0.88 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ: 174.26–168.08 (*C*ONH, *C*OCH_3); 101.55, 101.35 (C_1 β anomer); 70.86, 70.75 (C_5 or C_3); 70.71 70.63 (C_3 ou C_5); 68.96, 68.70 (C_2); 67.90 (CH_2OGal); 67.09, 67.00 (C_4); 64.17, 63.88 (CH_2OH); 63.77, 63.42 (CH_2OH); 61.93, 61.82 (C_6); 61.16 (CNH); 50.18 (CH); 39.93 (CH_2N); 37.02, 36.49 (CH_2CO); 34.57–22.68 ($(CH_2)_n$); 20.77, 20.67, 20.64, 20.54 (*C*H_3CO); 14.10 (CH_3).

FAB$^+$ MS: $m/z = 842$ (M + Na)$^+$ (deacetylated product).
Anal. calc. for $C_{49}H_{86}O_{16}N_4$: C 59.62, H 8.77, N 5.67, O 25.93 (acetylated product); found: C 59.65, H 8.75, N 5.80, O 25.54.

N^{α}-dodecanoyl-β-(N-(N-(O-(2,3,4,6-tetra-O-acetyl-β- D-galactopyranosyl)hydroxymethyl bishydroxymethyl)methyl)carboxamidomethyl)amide-N-methyl dodecyl-L-aspartamide **21a**

Procedure identical to that used for the synthesis of compound **19a**. From **15b** (1.04 g, 2.16 mmol) and **6** (2.3 g, 1.97 mmol) **21a** was obtained as a white powder (1.6 g, 50%). m.p.: 60–61°C. $[\alpha]_D^{20} = -8.9$ (c, 1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.51–7.28 (2H, m, $NHCH_2$, NHCH); 6.99, 6.95 (1H, 2s, NHtris); 5.39 (1H, d, H_4); 5.24 (1H, m, CH); 5.10 (1H, dd H_3); 5.04 (1H, dd, H_2); 4.52 (1H, d H_1); 4.50 et 4.30 (2H, s broadened, 2OH); 4.21–3.59 (11H, m, CH_2Gly, CH_2OGal $2CH_2OH$, H_5, H_6); 3.32 (2H, m CH_2N); 3.08 and 2.90 (3H, 2s, CH_3N); 2.56 (2H, m, CH_2Asp); 2.22 (2H, t, CH_2CO); 2.16 to 1.98 (12H, 4s, CH_3CO); 1.60 to 1.25 (38H, m $(CH_2)_9$, $(CH_2)_{10}$); 0.87 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ: 173.27–169.96 (*C*ONH, *C*OCH_3); 101.48 (C_1 β anomer); 70.86 (C_5 or C_3); 70.54 (C_3 or C_5); 69.10 (C_2); 68.52, 68.36 (CH_2OGal); 67.04 (C_4); 63.83, 63.53 (CH_2OH); 61.71 61.59 (C_6); 61.19 (CNH); 50.11, 48.85 (CH); 46.93 46.53 (CH_3N); 43.54, 43.47 (*C*H_2Gly); 38.91 to 38.36 (CH_2N); 36.48, 35.50 (CH_2CO); 34.17–22.68 ($(CH_2)_n$); 20.84, 20.69 20.64, 20.54 (*C*H_3CO); 14.10 (CH_3).
FAB$^+$ MS: $m/z = 841$ (M + Na)$^+$ (deacetylated product).
Anal. calc. for $C_{49}H_{86}O_{16}N_4$: C 59.62, H 8.77, N 5.67, O 25.93 (acetylated product); found: C 58.80, H 8.54, N 5.56, O 26.10.

N^{α}-dodecanoyl-β-(N-methyl-N-(N-(O-(2,3,4,6-tetra- O-acetyl-β-D-galactopyranosyl)-hydroxymethyl bishydroxymethyl)-methyl)carboxamidomethyl)amide-N-methyl dodecyl-L-aspartamide **22a**

Procedure identical to that used for the synthesis of compound **19a**. From **15b** (706 mg, 1.42 mmol) and **7** (0.8 g, 1.29 mmol), **22a** was obtained as a white powder (672 mg, 52%). m.p.: 52–53°C. $[\alpha]_D^{20} = -6.9$ (c, 1, $CHCl_3$).
^{1}H-NMR and ^{13}C-NMR showed the presence of several diatereoisomers.
FAB$^+$ MS: $m/z = 1024$ (M + Na)$^+$ (deacetylated product).
Anal. calc. for $C_{50}H_{88}O_{16}N_4$: C 59.98, H 8.85, N 5.59, O 25.56 (acetylated product); found: C 60.11, H 8.78, N 5.68, O 25.51.

N^{α}-octadecanoyl-β-(N-(tris-(O-(2,3,4,6-tetra-O-acetyl- β-D-galactpyranosyl)hydroxymethyl)-methyl)carboxamidomethyl)amide octadecyl-L-aspartamide **23a**

Procedure identical to that used for the synthesis of compound **17a**. From **15c** (207 mg, 0.31 mmol) and **6** (0.4 g, 0.34 mmol), **23a** was obtained as a white powder (0.3 g, 52%). m.p.: 94–95°C. $[\alpha]_D^{20} = -14.2$ (c, 1, $CHCl_3$).
^{1}H-NMR ($CDCl_3$) δ: 7.58 (1H, d, CHNH); 7.04 (2H, 2t, $2CH_2NH$); 6.28 (1H, s, NHtris) 5.39 (3H d, H_4; 5.10 (3H, dd, H_3); 5.05 (3H, dd H_2); 4.72 (1H, m, CH); 4.43 (3H, d, H_1); 4.16 (9H, m, $3CH_2$OGal, H_5); 3.78 (5H, m CH_2Gly, H_{6a}); 3.76 (3H, d, H_{6b}); 3.20 (2H, q, CH_2NH); 2.89 and 2.50 (2H, 2dd, CH_2Asp); 2.24 (2H, t, CH_2CO); 2.17–1.99 (36H, 4s, *C*H_3CO); 1.62–1.25 (62H, m $(CH_2)_{15}$, $(CH_2)_{16}$); 0.87 (6H, t, CH_3).
^{13}C-NMR ($CDCl_3$) δ: 173.86, 171.79 170.92, 170.42, 170.17, 169.96, 169.54, 168.82 (*C*ONH CH_3CO); 101.44 (C_1 β anomer); 70.89 (C_5 or C_3); 70.58 (C_3 or C_5); 69.18 (C_2); 67.99 (*C*H_2OGal); 67.02 (C_4); 61.14 (C_6); 59.48 (NHCtris); 49.83 (CH); 42.83 (CH_2Gly); 39.70 (CH_2NH); 36.52 (CH_2CO); 31.93–22.69 ($(CH_2)_n$); 20.80, 20.69, 20.64, 20.54 (*C*H_3CO); 14.11 (CH_3).
FAB$^+$ MS: $m/z = 1823$ (M + Na)$^+$ (deacetylated product).
Anal. calc. for $C_{88}H_{144}O_{34}N_4$: C 58.65, H 8.04, N 3.10, O 30.18 (acetylated product); found: C 58.75, H 8.06, N 3.23, O 30.18.

N^{α}-dodecanoyl-β-(N-(N-(tris-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)hydroxymethyl) methyl)carboxamidomethyl)carboxamidomethyl)-amide dodecyl- L-aspartamide **24a**

Procedure identical to that used for the synthesis of compound **17a**. From **16** (797 mg, 1.47 mmol) and **6** (1.57 g, 1.34 mmol), **24a** was obtained as a white powder (1 g, 44%). m.p.: 87–88°C. $[\alpha]_D^{20} = -18$ (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.98 (1H, d, N*H*CH); 7.81 (1H, t, N*H*CH_2); 7.52 (1H, t, N*H*CH_2); 7.12 (1H, t, N*H*CH_2); 6.49 (1H, s, NHtris); 5.39 (3H d, H_4); 5.12 (3H, dd, H_3); 5.03 (3H, dd H_2); 4.75 (1H, m, CH); 4.45 (3H, d, H_1); 4.35–3.82 (17H, m, C*H*$_2$Gly, C*H*$_2$OGal H_5, H_6); 3.79 and 3.54 (2H, 2dd CH_2Gly); 3.16 (2H, m, CH_2NH); 2.77 and 2.47 (2H, 2dd, CH_2Asp); 2.25 (2H, t, CH_2CO); 2.16–1.99 (36H, 4s, CH_3CO); 1.63–1.25 (38H, m $(CH_2)_9$, $(CH_2)_{10}$); 0.87 (6H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 174.15, 172.62 171.33, 170.44, 170.20, 170.08, 169.99, 169.81, 169.28 (*C*ONH *C*OCH_3); 104.41 (C_1 β anomer); 70.81 (C_5 or C_3); 70.63 (C_3 or C_5); 69.21 (C_2); 68.24 (CH_2OGal); 67.03 (C_4); 61.05 (C_6); 59.23 (CNH); 50.11 (CH); 44.09 (*C*H_2Gly); 42.80 (CH_2Gly); 39.64 (CH_2NH); 36.49 (*C*H_2CO); 31.90–22.67 ($(CH_2)_n$); 20.84, 20.66, 20.62, 20.54 (*C*H_3CO); 14.10 (CH_3).

FAB$^+$ MS: $m/z = 1208$ $(M + Na)^+$ (deacetylated product).

Anal. calc. for $C_{54}H_{99}O_{23}N_5$: C 54.67, H 8.40, N 5.90, O 31.01 (acetylated product); found: C 54.36, H 8.77, N 5.78, O 30.75.

N^{α}-dodecanoyl-β-(N-(N-(acetoxymethyl bis-(O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl) hydroxymethyl)methyl)carboxamidomethyl)-carboxamidomethyl)amide dodecyl-L-aspartamide **25a**

Procedure identical to that used for the synthesis of compound **17a**. From **16** (795 mg, 1.47 mmol) and **10** (1.18 g, 1.34 mmol), **25a** was obtained as a white powder (1.6 g, 90%). m.p.: 90–91°C. $[\alpha]_D^{20} = -12.5$ (c, 1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$) δ: 7.95 (2H, m, CHN*H* CH_2N*H*); 7.47 (1H, t, CH_2N*H*); 7.12 (1H, t CH_2N*H*); 6.64 (1H, s, NHtris); 5.38 (2H, d H_4); 5.10 (4H, m, H_3, H_2); 4.76 (1H, m, CH); 4.50–3.85 (16H, m, 2CH_2OGal, CH_2OAc, H_5, H_1 CH_2Gly, H_6); 3.73 and 3.51 (2H, 2dd CH_2Gly); 3.16 (2H, m, CH_2NH); 2.76 and 2.47 (2H, 2dd, CH_2Asp); 2.25 (2H, t, CH_2CO); 2.17–1.99 (27H, 4s, CH_3CO); 1.62–1.25 (38H, m $(CH_2)_9$, $(CH_2)_{10}$); 0.87 (6H, t, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 174.28, 172.92 171.47, 171.30, 170.46, 170.41, 170.18, 170.01, 169.96, 169.63 (6*C*ONH, 4*C*OCH_3); 101.46, 101.20 (C_1 β anomer); 70.88, 70.83 (C_5 or C_3); 70.61 70.53 (C_3 or C_5); 69.30, 69.07 (C_2); 68.44, 68.03 (CH_2OGal); 67.04, 66.98 (C_4); 63.04 (*C*H_2OAc); 61.08 (C_6); 58.99 (CNH); 50.15 (CH); 44.23, 42.90 (CH_2Gly); 39.64 (CH_2NH); 36.66 (CH_2CO); 35.77–22.68 ($(CH_2)_n$); 20.85, 20.82, 20.66, 20.56 (*C*H_3CO); 14.10 (CH_3).

FAB$^+$ MS: $m/z = 827$ $(M + Na)^+$ (deacetylated product).

Anal. calc. for $C_{68}H_{109}O_{28}N_4$: C 57.09, H 7.67, N 3.91, O 31.31 (acetylated product); found: C 57.09, H 8.34, N 4.37, O 29.80.

Ethanethiol-N-tert-butyloxycarbonyl glycinamide **26**

Tert-butyloxycarbonyl glycine (2g, 11.42 mmol) and aminoethanethiol hydrochloride (1.17 g, 10.38 mmol) were dissolved in dichloromethane. After addition of DIEA (2.5 ml 11.41 mmol), DCC (3.06 g, 14.86 mmol) and HOBT (100 mg), the mixture was stirred for 4 h at room temperature. The solution was dissolved in dichloromethane (150 ml) and washed with 1 N hydrochloric acid and saturated sodium hydrogenocarbonate. After drying with sodium sulfate, the solution was concentrated under vacuum and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane 7:3 v/v). Pure compound **26** was isolated as a colorless oil (900 mg, 34%).

^{1}H-NMR ($CDCl_3$) δ: 6.92 (1H, m, OCON*H*); 5.48 (1H, m, $NHCH_2$); 3.80 (2H, d, $NHCH_2CO$); 3.46 (2H, q, CH_2NH); 2.70 (2H, q, CH_2SH); 1.40 (9H, m, CH_3).

^{13}C-NMR ($CDCl_3$) δ: 169.87 (CH_2CONH); 156.23 (CO urethane); 80.34 (C(Me)$_3$); 44.44 (*C*H_2NHGly); 42.36 (*C*H_2NH); 28.34 (CH_2SH); 24.43 (CH_3).

Telomerization of N-Tris(O-(2,3,4,6-tetra-O-acetyl-β-D- galactopyranosyl)hydroxymethyl)-methyl acrylamide **D** *in the presence of ethanethiol-N-tert-butyloxycarbonyl glycinamide* **26***: telomer* **27**

To a solution of compound **D** (2 g, 1.78 mmol) in anhydrous methanol (50 ml) warmed to 40–50°C under a nitrogen atmosphere, were added the transfert reagent **26** (80.3 mg, 0.34 mmol, $R_o = 0.2$) in methanol (10 ml) and freshly recrystallized AIBN (6 mg, $3.4 \cdot 10^{-2}$ mmol). The mixture was refluxed under stirring and the progress of the reaction monitored by thin-layer chromatography (ethyl acetate/hexane 7:3 v/v). After refluxing for 12 h, the monomer **D** was entirely consumed. The solvent was evaporated and the resulting oil subjected to column filtration on Sephadex LH 60 (dichloromethane/methanol 1:1 v/v) to afford the telomer **27** as a white powder (1.40 g, 70%). The DPn (6.6) was obtained by comparison of the area of *tert*-butyl group signal (singlet $\delta = 1.45$ ppm) with the area of the signals ascribed to protons H_4 ($\delta = 5.40$ ppm) of the galactopyranosyl residues.

Telomerization of N-(bis(hydroxymethyl)-O-(2,3,4,6-tetra-O-acetyl-β-D- galactopyranosyl)hydroxymethyl)methyl acrylamide **C** *in the presence of ethanethiol-N-tert-butyloxycarbonyl glycinamide* ***26****: telomer* ***28***

The procedure was similar to that described above for telomer **27**, starting from monomer **C** (3.60 g, 7.12 mmol), transfert reagent **26** (333 mg, 1.42 mmol, $R_o = 0.2$) and AIBN (12 mg, $7 \cdot 10^{-2}$ mmol). Telomer **28** (2.95 g, 75%) was isolated after purification by gel filtration on Sephadex LH 60. The DPn (6.1) was obtained by comparison of the area of *tert*-butyl group (singlet $\delta = 1.45$ ppm) with the area of the signal ascribed to protons H_4 ($\delta = 5.40$ ppm) of the galactopyranosyl residues.

3.7. Synthesis of amphiphilic telomers ***29***, ***30*** and ***31***

Example: compound ***30***

The same procedure as that used to prepare compound **17** was applied to the synthesis of compound **30**. After deprotection of *tert*-butyl group by a mixture of trifluoroacetic acid/dichloromethane 1:1 v/v, compound **28** (1g, 0.30 mmol) was added to a solution containing **15c** (298 mg, 0.45 mmol), BOP reagent (203 mg, 0.45 mmol) and DMAP (56 mg, 0.45 mmol). Glycolipid **30** was obtained after purification by gel filtration on Sephadex LH 60 (dichloromethane/methanol 1:1 v/v) as a white powder (680 mg 59%).

Acknowledgements

We wish to thank J.P. Rolland for the measurements of the transition temperatures (T_c) and the 'Centre Commun de Microscopie Electronique' of the University of Nice-Sophia Antipolis (Professor G. Nicaise, Dr S. Thirion and J.P. Laugier).

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